The impact of healthcare-associated infections on patient care and the role of diagnostic molecular technology in infection prevention and control practice.

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A thesis submitted for the Degree of Doctor of Medicine

Graduate Entry Medical School
Faculty of Education and Health Sciences
University of Limerick

Supervisors
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Dr Nuala O’Connell

Submitted
October 2016
Declaration of originality

I declare that this thesis, which I submit to the University of Limerick for examination in consideration for the award of MD, is my own personal effort, and was completed with the counsel of my supervisors Professor Colum Dunne and Dr Nuala O’Connell. Where any of the content presented is the result of collaborative research this is duly acknowledged. I have not already obtained a degree in University of Limerick or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed: Ciara O’Connor

Student number: 14081989

Date: 05.10.2016
Thesis abstract

Healthcare-associated infections (HCAIs) are a public health challenge in Ireland and pose a patient safety risk. The emergence of multi-drug resistant (MDR) Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), Panton-Valentine leucocidin toxin-positive *S. aureus* (PVL-SA), and Gram-negative organisms, such as extended-spectrum beta-lactamase (ESBL)-producers and carbapenemase-producing Enterobacteriaceae (CPE), have made HCAIs more complex and diverse. Clinical microbiologists are an integral part of a hospital infection prevention and control (IPC) team, providing clinical guidance and expertise, assisting with the implementation of national and international IPC practices, managing outbreaks, and analysing rates of HCAIs. An efficient microbiology laboratory is an integral component of a hospital’s IPC strategy to facilitate the timely identification of pathogenic organisms from clinical specimens. In order to provide this service, a combination of skilled scientists who can perform traditional ‘bench’ tests and also utilise newer molecular diagnostics is required. Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) has facilitated the identification of bacteria, viruses and fungi in a convenient and time efficient manner and negated the need to employ older methodologies such as biochemical identification techniques. Faster identification of multi-drug resistant organisms (MDROs) is crucial for the timely management of HCAIs. Gradually, more laboratory work is becoming semi-automated and total laboratory automation (TLA) has become reality in many laboratories in Europe. To date, no Irish laboratory has installed a TLA system. Through greater public awareness of HCAIs, patients are more informed than ever regarding the risks associated with the acquisition of a HCAI and the concepts of patient safety and risk
management have become key objectives for hospital management teams. As presented in this thesis, HCAIs have occurred in the Mid-West of Ireland across all age groups, despite the successful implementation of recommended IPC practices. Between 2009 and 2015, two outbreaks of CPE, an ESBL outbreak in a neonatal intensive care unit, the first reported case of neonatal mastitis secondary to PVL-SA, the first Irish outbreak of linezolid-resistant *S. epidermidis* and a rare case of daptomycin and vancomycin resistant enterococcal infective endocarditis have all occurred in the region. The Mid-West of Ireland currently has the highest national rates of CPE and higher than average national rates of ESBL-producing *Escherichia coli* in blood cultures. Future work to track the progression of these trends is needed. Infection prevention and control practices currently employed within the region are in line with national and international guidelines but despite this the rates of HCAIs remain problematic both clinically and practically, with regard to allocation of isolation facilities in acute hospitals. Leadership and support are required from hospital management to implement measures to reduce rates of HCAIs including providing funding for the purchase of laboratory equipment that can facilitate the rapid diagnosis of microorganisms, staff education and training including incentivising and rewarding wards to reduce rates of HCAIs, thoroughly investigating outbreaks as they occur and managing hospital beds in a safe and efficient manner. HCAIs have a negative impact on patient care and staff morale. A hospital-wide approach with input from all key stakeholders is needed for a sustained reduction in HCAI rates to be achieved.
Acknowledgements

I would like to sincerely thank my supervisors, Professor Colum Dunne and Dr Nuala O’Connell, for their guidance, patience, enthusiasm and unwavering belief in this research over the past three years and counting.

To Dr Lorraine Power, Ms Sandra Guilfoyle, the staff of the microbiology laboratory and the infection prevention and control team at University Hospital Limerick, thank you all for your kindness, friendship and practical advice.

I am extremely grateful to the Royal College of Physicians in Ireland for granting me a research year, as part of my higher specialist training programme in clinical microbiology, to facilitate the completion of this MD, and also to the Irish Society of Clinical Microbiologists for the award of a research bursary.

Finally, to my family and Gerard, this thesis is dedicated to you all for the support in so many ways that helped to get this MD completed.
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### Abbreviations

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<td><strong>A</strong></td>
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<tr>
<td>AMR</td>
<td>Antimicrobial resistance</td>
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<tr>
<td>AMRHAI</td>
<td>Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit, London, UK</td>
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<tr>
<td>APIC</td>
<td>Association for Professionals in Infection Control and Epidemiology</td>
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<td><strong>B</strong></td>
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<tr>
<td>BC</td>
<td>Blood culture</td>
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<td>BD</td>
<td>From the Latin “bis die” meaning twice daily</td>
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<td>BSI</td>
<td>Bloodstream infection</td>
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<td><strong>C</strong></td>
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<tr>
<td>CAI</td>
<td>Community-acquired infection</td>
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<td>CA-MRSA</td>
<td>Community-acquired methicillin-resistant <em>Staphylococcus aureus</em></td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CF</td>
<td>Cystic fibrosis</td>
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<td>CFU</td>
<td>Colony forming units</td>
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<td>CIDR</td>
<td>Computerised infectious disease reporting</td>
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<td>CLABSI</td>
<td>Central-line associated bloodstream infections</td>
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<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
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<td>CoNS</td>
<td>Coagulase negative staphylococci</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CPE</td>
<td>Carbapenemase-producing Enterobactericeae</td>
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<td>CPERL</td>
<td>CPE Reference Laboratory, Galway, Ireland</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CTX-M</td>
<td>Abbreviation for ‘active on CefoTaXime, first isolated in Munich’</td>
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<tr>
<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
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<td>ED</td>
<td>Emergency department</td>
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<td>Acronym</td>
<td>Definition</td>
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<td>EPR</td>
<td>Electronic patient record</td>
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<td>ESBL</td>
<td>Extended-spectrum beta-lactamase</td>
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<td>ESKD</td>
<td>End stage kidney disease</td>
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<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
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<td>FTE</td>
<td>Full-time equivalent</td>
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<td>GBS</td>
<td>Group B <em>Streptococcus</em></td>
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<td>GNB</td>
<td>Gram-negative bacillus</td>
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<td>GPC</td>
<td>Gram-positive coccus</td>
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<tr>
<td>HAI</td>
<td>Hospital-acquired infection</td>
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<td>HCAI(s)</td>
<td>Healthcare-associated infection(s)</td>
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<td>HCW(s)</td>
<td>Healthcare worker(s)</td>
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<td>HDU</td>
<td>High dependency unit</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HPSC</td>
<td>Health Protection Surveillance Centre</td>
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<td>HVS</td>
<td>High vaginal swab</td>
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<td>ICU</td>
<td>Intensive care unit</td>
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<td>IMI</td>
<td>Imipenem-hydrolyzing beta-lactamase</td>
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<td>INAB</td>
<td>Irish National Accreditation Board</td>
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<td>IPC</td>
<td>Infection prevention and control</td>
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<td>IV</td>
<td>Intravenous</td>
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<td>KPC</td>
<td><em>Klebsiella pneumoniae</em> carbapenemase</td>
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<td>LRSA</td>
<td>Linezolid-resistant <em>Staphylococcus aureus</em></td>
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<td>LTCF</td>
<td>Long term care facilities</td>
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<td>M</td>
<td>MALDI-TOF MS</td>
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<td>M</td>
<td>MDR</td>
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<td>M</td>
<td>MDRO(s)</td>
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<td>MIC</td>
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<td>P</td>
<td>PVL</td>
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<tr>
<td><strong>PCR</strong></td>
<td>Polymerase chain reaction</td>
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<td><strong>Q</strong></td>
<td>QDS From the Latin “quater die sumendus” meaning four times daily</td>
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<tr>
<td><strong>R</strong></td>
<td>RSV Respiratory syncytial virus</td>
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<tr>
<td><strong>S</strong></td>
<td>SHEA Society for Healthcare Epidemiology of America</td>
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<td></td>
<td>SHV Sulphydryl variable</td>
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<tr>
<td></td>
<td>SSI(s) Surgical site infection(s)</td>
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<td></td>
<td>SSTI(s) Skin and soft tissue infection(s)</td>
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<tr>
<td><strong>T</strong></td>
<td>TAT Turnaround time</td>
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<tr>
<td></td>
<td>TDS From the Latin “ter die sumendum” meaning three times daily</td>
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<tr>
<td></td>
<td>TLA Total laboratory automation</td>
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<tr>
<td><strong>U</strong></td>
<td>UHL University Hospital Limerick</td>
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<td></td>
<td>UK United Kingdom</td>
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<tr>
<td></td>
<td>UMHL University Maternity Hospital Limerick</td>
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<td></td>
<td>ULHG University of Limerick Hospital Group</td>
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<tr>
<td></td>
<td>UTI Urinary tract infection</td>
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<td></td>
<td>US/USA United States/United States of America</td>
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<tr>
<td><strong>V</strong></td>
<td>VRE Vancomycin-resistant Enterococci</td>
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<tr>
<td><strong>W</strong></td>
<td>WCC White cell count</td>
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<td></td>
<td>WGS Whole genome sequencing</td>
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<td>WHO World Health Organisation</td>
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**Summary introduction**

**Personal interest**

My personal interest in completing this thesis for the degree of Doctor of Medicine (MD) arose as a consequence of an interest since medical school in research, having been involved with the Royal College of Surgeons in Ireland (RCSI) Student Medical Journal, and having a personal belief in the importance of lifelong learning. Having commenced higher specialist training in clinical microbiology, I developed an interest in multi-drug resistant organisms (MDROs), in particular those caused by Gram-negative organisms. A stroke of good luck led me to meet my two eventual supervisors, who could also see my vision for this MD, and with their expertise the structure of my proposal came together.

**Clinical and professional thesis outcomes**

This thesis has provided insight into the true scale of the existence of HCAIs and MDROs in the Mid-West of Ireland. The number of individual cases of HCAIs and the number of deaths secondary to these infections identified was alarming, but these findings have also affirmed the value in the completion of this research. Through publishing individual chapters of this thesis, it has enabled us to share with colleagues worldwide our experience of managing challenging MDROs in an environment of limited resources and isolation facilities.

**MD journey and final thesis layout**

The evolution of this thesis over the past three years has been fluid, with an exploration of different types of HCAIs and novel MDROs as they have been identified in the laboratory, and this process has shaped the final appearance of the thesis. Presenting
individual chapters of work for peer-review has been challenging and has forced me to go “back to the drawing board” on multiple occasions, seek advice, face rejections and celebrate final acceptances, and this process has undoubtedly created publications that are far superior to their first drafts. I have been fortunate in working on this research project over the past three years to collaborate with clinical and laboratory colleagues locally, nationally and internationally.

This thesis is presented in the format of a publication-based thesis. Excluding the literature review (chapter 1), chapter 10 and the final discussion (chapter 11), the remainder of the thesis is presented in the format of individual manuscripts, each assigned as individual chapters. These manuscripts represent focussed HCAI research projects completed during my time registered for this MD (May 2014 to May 2016) and are currently either published or in press.

Chapter 1 is a literature review of HCAIs and provides an overview of HCAIs subsequently described in later chapters. It also provides an introduction to the discipline of infection prevention and control and to the clinical microbiology laboratory. Chapter 2 is a published review exploring the role of molecular technology and automation in clinical diagnostics. Chapters 3 (published) and 4 (in press) are a review of work I completed at the University Maternity Hospital Limerick reviewing HCAIs in the neonatal population. Chapters 5 (published), 6 (published) and 7 (published) examine Gram-positive HCAIs while chapters 8 (published) and 9 (in press) review Gram-negative HCAIs. Chapter 10 is an exploration of electronic IPC strategies to manage all HCAIs. Chapter 11 is a composite final discussion. Chapters 5 to 10 represent work completed on-site at University Hospital Limerick.
Future vision for this MD research

Information gathered in the completion of this thesis may be used in the future to plan for the recruitment of additional clinical, laboratory and nursing staff necessary to manage the ever-increasing workload arising from the challenge of managing HCAIs, and also to plan for investment in newer molecular technologies and instruments capable of delivering a rapid turnaround time for clinical samples. Data from this thesis may also be used to assess the need for additional public health resources to assist with increasing numbers of MDRO isolates from the community. Through reviewing this epidemiological data from the Mid-West of Ireland, a clearer understanding of the population that we treat is evident and this will guide us as clinical microbiologists as we update antimicrobial stewardship strategies for the region, design new hospital builds and complete day-to-day clinical duties.
CHAPTER 1

Literature review.
Healthcare-associated infections (HCAIs)

Defining a HCAI

The concept of a healthcare-associated infection (HCAI) was originally proposed in 2002, categorising infections as either community- or hospital-acquired. Community-acquired infection (CAI) is defined as “infection detected within 48 hours of hospital admission in patients without previous contact with healthcare services.” (Friedman et al 2002, Siegman-Igra et al 2002). Hospital-acquired infection (HAI), or healthcare-associated infections, are defined as “an infection present at hospital admission or within 48 hours of admission in patients that fulfil any of the following criteria: (1) received intravenous therapy at home, wound care or specialised nursing care through a healthcare agency, family or friends, or had self-administered intravenous therapy in the 20 days before the infection (patients whose only home therapy was oxygen use are excluded), (2) attended a hospital or haemodialysis clinic or received intravenous chemotherapy in the previous 30 days, (3) were hospitalised in an acute hospital for two or more days in the previous 90 days, (4) resided in a nursing home or long-term care facility” (Friedman et al 2002, Siegman-Igra et al 2002, Chen et al 2008).

Aetiology of HCAIs

The hospital environment and healthcare worker’s (HCWs) hands have been widely implicated in HCAIs and are now considered reservoirs for the emergence, selection and spread of multi-drug resistant organisms (MDROs) that can cause severe clinical syndromes, that are expensive to treat and may potentially become incurable (Best and...
Risk factors for HCAIs

Advances in clinical medicine have resulted in the enhanced likelihood of survival of those born prematurely and critically ill elderly patients, and consequently HCAIs now affect patients of all ages from neonates to end of life adults (National Nosocomial Infections Surveillance System 2004, Posfay-Barbe et al 2008, Foster and Sabella 2011). The elderly are at increased risk of infection as a consequence of physiological and immunological sequelae of ageing, coupled with complex medical and surgical co-morbidities, possibly with medical devices in-situ including, but not limited to, vascular grafts, pacemakers, mechanical valves, left-ventricular assist devices, implantable cardiac defibrillators (Lara et al 2015). HCAIs are increasingly seen amongst residents of long-term care facilities (LTCF) (Koch et al 2008). Obesity, a relatively recent risk factor for the development of HCAIs, and has been shown to be associated with an increased risk of post-operative HCAIs, particularly cardiac, vascular, orthopaedic and gastrointestinal surgery (Dindo et al 2003, Di Leo et al 2009, Huttunen and Syrjänen 2013). Obesity-related immunological dysregulation and co-morbidities, decreased cell-mediated immune responses, respiratory dysfunction, an increased risk of diabetes mellitus and immobility are all proposed to contribute to an overall increased HCAI risk (Falagas and Kompoti 2006, Huttunen and Syrjänen 2010, Huttunen and Syrjänen 2013).
Anatomical distribution of HCAIs

Typical foci of infection in HCAIs are surgical site infections (SSIs), pneumonia, intra-abdominal, infective endocarditis and blood stream infection (BSI) (Cardoso et al 2015).

Prevalence of HCAIs

The United States (US) Surgeon General, Dr William H. Stewart (1965-1969), famously said “It is time to close the book on infectious diseases, and declare the war against pestilence won” (Spellberg 2008). Undoubtedly, he was wrong and the burden of infectious diseases and MDROs is at an all-time high (Harris et al 2008, Humphreys 2009, Rodgers et al 2011, Mutters 2014a). The European Centre for Disease Prevention and Control (ECDC) was established in 2005 to monitor the prevalence of HCAIs and MDROs (Magiorakos et al 2012). The 2013 HCAI prevalence surveys reported European prevalence rates of 6% amongst hospitalised patients and 2.4% in LTCF residents, and determined that the number of patients with a HCAI on any day in a European acute care hospital is 81,000 (European Centre for Disease Prevention and Control 2013). Amongst those presenting for hospital admission, HCAIs can account for 50% of infections admitted from the community (Carratalà et al 2007, Park et al 2012, Cardoso et al 2015).

There are an estimated 177,000 HCAIs in Australia per annum with 1,970,142 lost bed days (Graves et al 2008). On any day in a US acute hospital, figures show that 1 in 25 hospitalised patients have at least one HCAI (Magill et al 2014). Data from a survey of adult patients conducted between February 2006 to May 2006 in acute
hospitals across England, Wales, Northern Ireland and the Republic of Ireland surveyed 75,694 patients; 5743 of these had HCAIs, giving a prevalence of 7.59%. The risk of HCAI varied significantly by hospital type, with the highest prevalence in acute specialist and teaching Trusts and the lowest prevalence in medium and small acute Trusts (Smyth et al 2008).

Geographical distribution of HCAIs

HCAIs are a global problem (Köck et al 2010, Zimlichman et al 2013). 5-10% of hospitalised patients in developed countries get HCAIs but the risk is 2-20 times higher in poorer countries (Burke 2003, Pittet and Donaldson 2005b, Morris 2008, Bates 2009, Harbarth 2013). One meta-analysis demonstrated 3-fold higher rates of infection in the ICUs of developing countries with substantially higher rates of SSI (5.6 vs. 1.2-2.9 per 100 surgical procedures) (Allegranzi et al 2011). The re-direction of financial and human resources to acute services with less investment in infection prevention and control in resource-limited countries is likely a major contributory factors to the increased risk, but also differing surveillance methods, patient case-mix, national reporting requirements and a reliance on traditional infection control practices also impact on rates (Goossens 1998, Harbarth et al 2001, Borg 2012).

The effect of HCAIs on patient morbidity and mortality

HCAIs result in loss of patient confidence in the healthcare system both at an organisational and individual practitioner level (Research Committee of the Society of Healthcare Epidemiology of America (SHEA) 2010). They are a source of significant morbidity and mortality (Oeyen et al 2010) and a patient safety issue (Pittet
et al 2005c). HCAIs are the most frequent adverse event affecting hospitalised patients after medication errors (Januel et al 2010, Vlayen et al 2012) and the fifth leading cause of death in acute care hospitals (Klevens et al 2007). There are an estimated 37,000 deaths per annum in European hospitals as a direct consequence of HCAIs with 16 million extra bed days (European Centre for Disease Prevention and Control 2008) and 75,000 hospital deaths in the US (Magill et al 2014). HCAIs are considered preventable, although some argue that they may not be entirely preventable, (Harbarth et al 2003, Umscheid et al 2011). Reducing preventable HCAIs has been listed as a top priority by the US Department of Health and Human Services in order to reduce rising costs and improve quality of care (Department of Health and Human Services 2013).

The economic cost of HCAIs

Data regarding the annual direct economic costs of preventable HCAIs in Ireland are unknown, but estimates from the US and United Kingdom (UK) exceed $9.8 billion dollars and £1 billion pounds (£1.1 million euros), respectively (House of Commons Public Accounts Committee 2009, Zimlichman et al 2013). HCAI are associated with prolonged length of hospital stay (Graves et al 2010), particularly intensive care unit (ICU)-acquired BSIs (Pittet et al 1994, Dimick et al 2001, Harbarth et al 2002, Laupland et al 2002, Cairns et al 2010).

A matched cohort study conducted in a Canadian ICU compared the outcomes of 144 patients with and without ICU-acquired BSI and found that patients with an ICU-acquired BSI had a significantly increased median length of ICU stay (15.5 days vs
12 days, $P=0.003$) and the median excess hospital cost attributable to an ICU-acquired BSI was $12,231$ per case (Laupland et al 2006). Indirect economic costs associated with HCAIs are substantial including extended sick leave with costs incurred by the State from social welfare benefits, the potential for long-term or permanent unemployment due to incurred disability, childcare costs, deskilling and disruption to public services if the patient is a State employee and absenteeism from work.

*The effect of the global economic recession on HCAI rates*

Continuous investment in acute and community healthcare is essential as productive economies rely upon a healthy workforce (Wanless 2002, O’Riordan and Fitzpatrick 2015). The global environment and financial markets have an effect on HCAI rates in a multifaceted manner secondary to their influence on healthcare funding and staffing, economic emigration of healthcare workers, dwindling numbers of patients with private health insurance who are now reliant on the public health system and the financial ability of patients to spend disposable income on products or services to improve their health (Pittet 2005a, Marcel et al 2008).

Economic crisis disproportionately affects those in lower socioeconomic groups, who carry a greater infectious disease burden (Recehl et al 2011, Suhrcke et al 2011). This has been exemplified in Greece with a recent outbreak of human immunodeficiency virus (HIV) amongst intravenous (IV) drug abusers, in the context of rising IV drug abuse during the recession, felt secondary to unemployment (Paraskevis et al 2013). The economic recession, that began globally in 2007, led to austerity measures and public service cutbacks in Ireland, including in the health sector, with reduced resource
allocation to infection prevention and control (IPC) programmes amongst other hospital services (Rechel et al 2011, Karanikolos et al 2013, O’Riordan and Fitzpatrick 2015). Stagnant hospital infrastructural development, a recruitment embargo, vacant clinical posts at ward level increasing the workload of remaining staff leading to burnout, a reluctance of staff to take sick leave with an increased risk of infection transmission at work and staff salary reductions have all had significant impacts on IPC service provision at an individual and organisational level.

A poll conducted by the Association for Professionals in Infection Control and Epidemiology (APIC) with 2000 respondents demonstrated clearly that the economic recession had directly affected the provision of hospital IPC programmes with 41% of respondents reporting budget cuts for technology, education and staffing in the previous 18 months, 75% reporting reductions in IPC educational activities and 25% reporting a reduction in crucial HCAI surveillance activities (Association for Professionals in Infection Control and Epidemiology 2009). This change in practice due to economic constraints is also mirrored in a recent review demonstrating a trend in IPC publications away from detailing the costs incurred managing HCAI towards studies on value for money in IPC programmes, costs of implementations, costs savings and health benefits accrued (Graves 2014).

**Antimicrobials in clinical medicine**

**Who gets antimicrobials?**

The timely administration of a prescribed antimicrobial is necessary to avoid infection-related morbidity and mortality (Gaieski et al 2010). Research has
demonstrated that 60% of adult and paediatric inpatients receive antimicrobial therapy during their hospitalisation (Pakyz et al 2008, Gerber et al 2010). Despite the importance of antimicrobials, a substantial proportion of antimicrobial use in acute care hospitals may be inappropriate with lack of documented indication or incorrect antimicrobial selection, dosing or duration (Castle et al 1977, Cardoso et al 2013, Fridkin et al 2014, Magill et al 2014). Non-prescription antimicrobial use is also a major stewardship issue and is frequent in low income countries (Morgan et al 2011). The advent of on-line antimicrobial purchasing direct to consumers has also exacerbated this problem.

*How does antimicrobial resistance emerge?*

Figure 1. Clinical and microbiological sequelae following the development of antimicrobial resistance in bacteria. If bacteria are not harbouring resistance mechanisms, a good clinical outcome can be expected when appropriate antimicrobial therapy is prescribed. When antimicrobial resistance is present, patients may experience adverse clinical events, such as non-resolution of symptoms or death, if resistance is not noted and antimicrobial therapy switched to an agent to which the bacteria is susceptible.

The impact of antimicrobial resistance on HCAIs

The incidence of AMR and MDROs in healthcare facilities has quadrupled over the past decade, associated with 23,000 deaths and 2 million infections (Boucher et al 2009, Centres for Disease Control and Prevention 2013a). AMR-related HCAIs pose a considerable healthcare challenge and it is estimated that 10 million people will die worldwide each year by 2050 due to AMR (Hampton 2015). The Lancet Infectious Diseases Commission on antibiotic resistance, marking European Antibiotic Awareness Day in 2013, noted that “antibiotic-resistant infections are increasing at an alarming rate, posing a great threat to human health on every continent” (Earnshaw et al 2013).

50-60% of community-acquired Gram-negative pathogens such as Escherichia coli associated with urinary tract infections (UTIs) have become resistant to common oral antibiotics such as amoxicillin, cefixime and ciprofloxacin (Laxminarayan et al 2013). In Pakistan, the emergence of pan-resistant bacterial isolates such as Acinetobacter spp. and carbapenemase-producing Enterobacteriaceae (CPE) as causes of HCAIs are rendering these infections untreatable (Saleem et al 2010, Perry et al 2011, Khan et al 2010).

Tackling antimicrobial resistance

During the past two decades, Europe has made substantial progress in combating MDROs and in the control of antimicrobial overuse (Boyce et al 2005, Struelens and Monnet 2010a). The agreement on and implementation of consensus guidelines and European-wide strategies has promoted antimicrobial stewardship to reduce
antimicrobial consumption and avoid inappropriate use (Carmeli et al 2010, Wernli et al 2011). Individual European countries have established their own national guidelines and steering groups such as the successful Scottish Antimicrobial Prescribing Group (Nathwani et al 2011). AMR-organisms create a problem for hospitals as those colonised are asymptomatic, and without screening are unidentifiable, thereby facilitating transmission throughout the healthcare environment (Ben-David et al 2010, Landman et al 2012, Kotsanas et al 2013). In March 2015, in recognition of the public human threat and the economic costs of antimicrobial resistance, the Obama administration in the US made a commitment to reduce inappropriate antimicrobial use by 50% in the outpatient settings and by 20% in the inpatient setting by 2020, compared with 2011 (Hwang et al 2015).

**Resistant organisms causing HCAIs**

*Gram-negative organisms*

Multi-drug resistant (MDR) Gram-negative bacteria are defined as having three or more antimicrobial resistance mechanisms affecting different classes of antimicrobials (Wilson et al 2016). Opportunistic Gram-negative bacteria that present increasing resistance issues include Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp.), *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Importantly MDR-Enterobacteriaceae colonise the human gut symptomatically, creating a reservoir for transfer to other body sites and to other patients. The risk of onward transmission is increased with incontinence or diarrhoea, making long-term care facilities reservoirs of antimicrobial resistance, due to the high numbers of incontinent and catheterised patients living in close proximity coupled
with frequent antimicrobial use and its contingent selection pressure (Rooney et al 2009, March et al 2010). Enterobacteriacease have been demonstrated to survive on dry hospital surfaces for months (Jawad et al 1998, Otter and French 2009, Havill et al 2014).

Carbapenemase-producing Gram-negative organisms

Much concern has been raised over the past decade regarding carbapenemase-producing Enterobacteriaceae (CPE), which confer resistance to carbapenem antimicrobials, for example meropenem or ertapenem, traditionally used as ‘last line’ therapy for critically ill patients. It is estimated that CPE is the aetiology for greater than 9000 HCAIs per year in the US (Centres for Disease Control and Prevention 2013b). Studies have demonstrated that 50% of patients who develop a CPE-related BSI will die (Hampton 2014). Patients with a higher risk of mortality include those who are elderly or debilitated, with multiple co-morbidities, burns patients, children, cancer patients, and any patient with frequent hospitalisations and patients undergoing invasive procedures (Perez and Van Duin 2013b). Most cases of infection or colonisation with CPE have occurred in hospital and healthcare settings rather than in the community (Gupta et al 2011, Munoz-Price et al 2013). Within the hospital environment, sharing a room with a colonised patient and ICU admission are risk factors for acquisition of carbapenem-resistant organisms (Hussein et al 2009, Lepelletier et al 2010, Sheng et al 2010).
**Extended-spectrum beta-lactamase-producing Gram-negative organisms**

The acquisition of extended-spectrum beta-lactamase (ESBL)-producing enzymes in Gram-negative organisms confers the ability to hydrolyze penicillins, cephalosporins and aztreonam (Bush and Fisher 2011). Genes for ESBLs are also frequently encoded on transferable plasmids, that encode resistance genes for other classes of antimicrobials, such as beta-lactams, fluoroquinolones, aminoglycosides and sulphonamides, and as a consequence ESBL-producing organisms are frequently resistant to multiple classes of antimicrobials (Magiorakos et al 2012). Originally isolated in the 1980s, ESBL-producing organisms, in particular *E. coli*, are now globally disseminated in both hospitals and the community (Paterson and Bonomo 2005, Ben-Ami et al 2009).

**Gram-positive MDR-organisms**

The incidence of vancomycin-resistance Enterococci (VRE), community-acquired strains of methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are increasing, including strains of Panton-Valentine leucocidin-positive community-associated MRSA (PVL- CA MRSA), identified in previously healthy young individuals with no risk factors for healthcare-associated MRSA (Vandenesch et al 2003, Hsu et al 2006, Tristan et al 2007, Orendi et al 2010, Rasigade et al 2010). Pandemic clones of PVL-MRSA have become a significant cause of spontaneous skin and soft tissue infections (SSTIs) with a high rates of recurrence and inter-household spread (Moran et al 2006).
Mandatory reporting of HCAIs

As of January 2013, 37 out of 52 US states have adopted laws requiring submission of HCAI data (Herzig et al 2016, Wong et al 2005) and pressure is mounting for legislation to be enacted in every state, enabling HCAI rates to be made public (Jarvis 2007). A catalyst for a change in opinion and a new insight into the preventability of HCAIs has been with the success in the prevention of central-line associated bloodstream infections (CLABSIs) in the US (Centres for Disease Control and Prevention 2005, Pronovost et al 2006a, Frieden 2010). Some US health maintenance organisations no longer reimburse costs relating to HCAIs, for example, ventilator-associated pneumonia (Wunderink 2011, Rajaram et al 2015) or costs associated with infections due to vascular or urinary catheters (Pakyz and Edmond 2013, Haas 2014). Consumer-orientated and professional websites are closely tracking state adoption of HCAI reporting (Reagan 2013, McGuckin et al 2013a).

In the UK, mandatory reporting of MRSA BSI for National Health Service (NHS) hospitals was introduced in 2001 followed by mandatory reporting of Clostridium difficile infection in 2004, methicillin-susceptible Staphylococcus aureus (MSSA) BSI in 2011 and followed shortly after by E. coli BSIs (Underwood et al 2011, Johnson et al 2012, Wilson and Kiernan 2012). Since 2007, the incidence of MRSA BSI and C. difficile infections in England have decreased by 59% and 64% respectively (Health Protection Agency 2011). These reductions have been attributed directly to UK Government policy of increased mandatory surveillance, publication of evidence-based guidelines, and the introduction of HCAI reduction programmes.
In Ireland, a national electronic recording system known as ‘CIDR’ (Computerised Infectious Disease Reporting) commenced in 2005 and is compiled by the Health Protection and Surveillance Centre (HPSC) in Dublin. This captures over 80 notifiable diseases in Ireland, including HCAIs such as BSIs. Information from laboratories is entered into CIDR by laboratory scientists/microbiologists. This information is then linked to clinical and epidemiological information provided by public health professionals and clinicians. Weekly and annual reports for the public are published on the HPSC website.

It has not yet been fully established whether public reporting of HCAIs has had a direct effect on numbers of HCAIs (Humphreys and Cunney 2008). A systematic review, on behalf of the US Healthcare Infection Control Practices Advisory Committee, found inconclusive evidence of the effectiveness of public reporting improved healthcare outcomes (McKibben et al 2006) but there has been a correlation identified between public reporting of HCAIs and increased quality improvement initiatives within hospitals (Fung et al 2008).

**Reducing HCAIs**

The prevention and control of HCAIs is fundamental to the provision of safe patient care (Borg 2012) and it has been suggested that HCAI rates can be used as indicators
of healthcare quality (Pittet 2005a). Media interest in reporting HCAIs has fuelled interest from patient and consumer groups (Edmond and Eickhoff 2008). In the last decade, international campaigns have fought to implement hand hygiene practices in an attempt to reduce HCAIs (Sax 2009, World Health Organisation 2009) and studies have demonstrated that improvements in hand hygiene rates reduce HCAIs (Allegranzi and Pittet 2009). Similarly, following a multi-faceted UK education campaign, there was a sharp fall in the incidence of MRSA BSI (Liebowitz 2009) and studies focusing on environmental cleanliness and decontamination have been linked to local reductions in the incidence of HCAI and MDR acquisition rates (Hayden et al 2006, Dancer 2009).

A new challenge in managing HCAIs: medical tourism

Many studies document the role of travellers, including medical tourists, in facilitating the dissemination of bacteria and/or their resistance genes globally (Kumarasamy et al 2010, Struelens et al 2010b, Rogers et al 2011, Wilson and Chen 2012). Travel to another country for medical treatment is defined as “medical tourism” or “cross-border healthcare” (Milstein and Smith 2006, Heible 2011) and usually is undertaken by patients to avoid long delays or costs (Chen and Wilson 2013). Higher rates of infection complicate procedures carried out abroad (Chen and Wilson 2013). The globalisation of medical care has had profound economic, cultural, ethical, legal and HCAI consequences (Hodges et al 2012, Lee and Balaban 2014). Common destinations for medical tourists include India, Thailand, China, Mexico, Latin America, central Europe, the Middle East and Pakistan (Horowitz et al 2007). A major issue with medical tourism in Ireland is the repatriation of these patients who develop
complications post-procedures, with the risks that this entails. The potential for MDRO colonisation following travel to these areas is high with a subsequent risk of spread within the Irish hospital environment on subsequent admissions (Leverstein-Van Hall et al 2011).

Prevalence rates of ESBLs exceed 80% in India. (Tängdén et al 2010, Jean and Hsueh 2011, Molton et al 2013). Faecal samples cultured from tourists before, during and after travel to Mexico showed an increase in resistance in their faecal E. coli even if they took no antimicrobials during their travel (Murray et al 1990). A quarter of MRSA cases from 2000 to 2003 in Sweden were attributed to exposure abroad and the majority were healthcare associated. Ireland and Australia have all reported the transfer of hypervirulent ribotype 027 Clostridium difficile from the US, Belgium and North America (Rogers et al 2011).
Infection prevention and control strategies to manage HCAIs

The evolution of infection prevention and control

Dr Ignaz Semmelweis (1818-1865), the pioneer of antiseptic procedures, has been described as the “saviour of mothers” and the “father of infection control” (Pittet and Boyce 2001a, Harbarth et al 2004, Best and Neuhauser 2004). In 1846, Semmelweis identified that women delivered by obstetricians and medical students had a much higher rate (13-18%) of puerperal fever due to Streptococcus pyogenes compared with women delivered by student midwife or midwives (2%) and initiated a mandatory hand washing policy for medical students and obstetricians leading to a reduction in maternal mortality (Semmelweis 1861, Nuland 2003). In the 1970s and 1980s, the first publications in infection prevention and control (IPC), demonstrating HCAI cause and effect emerged (Shoji et al 1974, Cruse and Foord 1980, Freeman et al 1987, Freeman et al 1988). The publication of the sentinel Study on the Efficacy of Nosocomial Infection Control in the late 1980s demonstrated an overall 32% reduction in HCAI rates associated with adequate human IPC resources, surveillance and infection control educational activities (Haley et al 1985b). In the late 1990s, a consensus panel report from SHEA defined targeted surveillance, detection and control of outbreaks, implementing and auditing written policies and education/training as the key functions of the IPC team in acute care (Scheckler 1998).

Further epidemiological approaches aimed at reducing both the rate and risks of HCAIs came to prominence in the 1990s and 2000s (Harbarth 2000, Harbarth and Pittet 2010, Boyce 2013) and surveillance programmes are now well established (Gastmeier et al 2008, Zingg et al 2009). Numerous change tools have become
available to improve IPC compliance (Dellit et al 2007, Sawyer et al 2010). The implementation of care bundles, wider use of newer cleaning techniques such as hydrogen peroxide vapour and better environmental cleaning, culture-based screening and a renewed enthusiasm and vigour for hand hygiene have improved IPC practices (Gray 2015).

**IPC staffing**

Research has focussed on the effects of IPC nurse staffing levels on HCAIs for over two decades (Kane et al 2007, Shang et al 2015). An IPC staff-to-bed ratio of 1 full-time equivalent (FTE) per 250 beds (0.4 IPC staff per 100 beds) was recommended over 30 years ago (Streeter et al 1967, Thoburn 1968, Eickhoff et al 1969, Wenzel 1970, Haley et al 1985b) but it is widely accepted now that this rate should be one nurse per 100 beds in acute care and one per 150-250 beds in long term care (O’Boyle et al 2002, Rosenthal et al 2003, Weiss et al 2009). Figures regarding the cost of staffing IPC units in Ireland are unknown.

**The modern IPC team**

A collaborative approach between clinical microbiologists, IPC nurses and hospital hygiene managers has been pivotal in this success of HCAI prevention programmes via implementation of recommended strategies from organisations such as the WHO leading hospital quality improvement efforts and championing local infection prevention and control programmes (Carrico et al 2008, Murphy and Resnik 2008, Yokoe and Classen 2008, Murphy et al 2012, Watson and Scales 2013, McNeish et al 2013). Robust organisation management and clear communication strategies have

A consultant who washes his or her hands when appropriate will encourage his or her junior staff to follow same. Ultimately, the emphasis on hand hygiene must begin in medical schools as learning the appropriate behaviours from the beginning of a professional clinical career is crucial as research has demonstrated that professional adults learn and implement new behaviours more easily than unlearning already present practices (Senge 2006). Analogous approaches are necessary across all allied health professional education and training.

IPC nurses play a key role in working with acute ward nurses and building working relationships to reduce HCAIs. Nurses not only provide bedside patient care and act as an advocate for the patient, but are also the link between the IPC team and the clinician caring for the patient and can help to reduce HCAIs (Weaver et al 2014). IPC team members need to develop and foster an organisation culture that is HCAI aware and proactive (Zingg et al 2015). Leadership walk rounds attended by IPC, clinical directors and key hospital management team members can be a key component of a hospital IPC strategy (Singer and Tucker 2014). They are a symbol of management commitment to IPC within the hospital.
The role and scope of the infection control team has evolved significantly beyond the ‘traditional’ hospital acute care setting into the community, liaising with and providing an advisory service to LTCFs, residential care facilities for those with physical or learning disabilities, and public health teams (O’Boyle et al 2002). Unfortunately, despite an increased workload and a diversification of activities, funding for IPC teams, both from a financial and human resources perspective, has been suboptimal (Jarvis 2004, Goldrick 2005). Another major challenge to the expansion of IPC as a specialist nursing area has been that many European countries do not have recognised university IPC qualifications and this was highlighted in the Training Strategy for Infection Control in Europe Project (Brusaferro et al 2010).

Preventing HCAIs

HCAIs are endemic and the majority of HCAIs result from cross-transmission related to inappropriate patient-care practices (Pittet 2004a). It is estimated that 15-30% of all HCAIs could have been prevented if hospital staff had undertaken simple improvements in hygiene practices (Controller and Auditor General 2000, Thompson et al 2009) but critics have argued that 100% prevention is unrealistic (Voss 2009). In January 2015, the Centers for Disease Prevention and Control published the National and State Healthcare-Associated Infections Progress Report which reported that implementation of existing infection prevention strategies could lead to a US$25-31.5 billion saving in medical costs and a 70% reduction in HCAIs (Centers for Disease Prevention and Control 2015).

In-hospital IPC programmes are very broad encompassing hand hygiene, prevention of device-related infections, controlling for opportunistic infections in the
immunocompromised, for example, *Aspergillus* spp., and antimicrobial stewardship in an attempt to reduce, control and ideally eliminate HCAIs (Juan-Torres and Harbarth 2007, Pagani *et al* 2009). The multi-modal approach adopted by IPC teams was aptly described by Carlet *et al* (2009) who wrote that “it is naïve to think that HAIs are preventable by just hand washing or surgical skin preparation”. Cross-infection of patients by the contaminated hands of healthcare workers though remains the major method of spreading infectious agents (Gawande 2004). There has been a movement towards the promotion of bedside, alcohol-based, waterless hand disinfection for non-visibly soiled hands in Europe (Pittet 2001b, Magiorakos *et al* 2009).

*Cleaning, decontamination and disinfection to reduce HCAIs*

Poor hand hygiene is not the sole means by which infections spread; hospital equipment can also pose an infection risk. The added infection risk of increasing amounts of electronic equipment used on the wards creates a substantial HCAI risk. Traditional methods employed to reduce the transmission of airborne pathogens such as fresh air and sunlight penetration in buildings have been replaced by newer methods of whole room decontamination, as an adjunct to standard cleaning, employing biocidal methods such as hydrogen peroxide or ultraviolet-light based systems (Nightingale 1863, Hobday 1997, Li *et al* 2007, Hobday and Dancer 2013).

*Information technology in IPC*

Information technology has played a pivotal role in the expansion of IPC team duties (Goldmann 1986). The employment of electronic surveillance systems to support and
enhance decision-making have played a vital role in changing how surveillance work is conducted (Grota et al 2010, de Bruin et al 2014). Infection control has become more technologically driven in the US compared to most European countries and these programmes are linked to electronic patient records (EPR), fully-automated surveillance programmes that are integrated with data mining systems (Research Committee of the Society of Healthcare Epidemiology of America (SHEA) 2010). Online IPC learning resources and self-learn DVDs for staff are also playing an increasingly important role in IPC education particularly where IPC staff are covering multiple sites (National Health and Medical Research Council 2010). Biostatistical tools have also aided IPC teams to follow trends and identify clusters (Quesenberry 2000).
The future of the clinical microbiology laboratory in the diagnosis of MDROs and HCAIs

Traditional microbiology laboratory work-flow

The clinical microbiology laboratory has been described as having responsibilities ranging from “characterising the causative agent in a patient’s infection to helping detect global disease outbreaks” (Rhoads et al 2014). Clinical microbiology laboratories have historically been a predominantly manual operation, highly dependent on a technically skilled workforce of laboratory scientists to receive, process, and interpret results from a variety of clinical specimens with the aim of providing a rapid diagnosis to clinicians to limit the amount of broad-spectrum empiric antimicrobial therapy given to patients, and reduce the potential for selective antimicrobial pressure and proliferation of MDROs.

Laboratory automation

The concept of laboratory automation was originally developed in Japan and gained increasing popularity in other countries in the early 1990s (Middleton 2000). Automation is a customised process that may range from automation of only of a few steps of the analytical process to a conceptual framework of total laboratory automation (TLA) (Melanson et al 2007). TLA systems have been developed by various companies but none has been installed in hospital laboratories in Ireland to date. Currently three are available, Kiestra TLA (BD Kiestra B.V., Netherlands), Full Microbiology Laboratory Automation (FMLA; bioMerieux, France) and the WASPLab (Copan Diagnostics, California), which all incorporate track systems to move plates, digital cameras to capture plate images and have automated incubators.
Figure 2. An illustrated diagram of a fully automated laboratory - Kiestra TLA (BD Kiestra B.V., Netherlands). The BD Kiestra™ TLA system optimises laboratory workflow enabling automated processing of both liquid and non-liquid bacterial clinical specimens, including the stages of inoculation and incubation. Digital images of growth on the agar plates are then displayed on high resolution monitors and are read by scientists. The system can be integrated with other BD automation modules such as the BD Bruker™ MALDI Biotyper™ automated identification system and the BD Phoenix™ automated antimicrobial susceptibility testing (AST) instruments. There are currently no laboratories in Ireland with this system in operation.

Accessed at: www.bd.com/europe/labautomation/
A combination of robotic arms and fingers manipulates specimens and plates. Conveyers send the inoculated plates to smart incubators. Pipettes or loops make Gram stain slides. After incubation, the systems read and digitise colony growth and may even pick colonies for susceptibility testing. Matrix-assisted laser desorption and/or ionisation time of flight mass spectrometry (MALDI-TOF MS) (Faron et al 2016). Initial published results have demonstrated increased productivity, reproducible standardised results and reduced turnaround times of about 26 hours total from sample receipt to a final authorised result with susceptibility testing (Bourbeau and Swartz 2009, Mutters et al 2014a, Mutters et al 2014b). A major change with this system too is that images of agar plates are stored meaning that retrospectively it is possible to go back and revisit plates again where ordinarily they would have been discarded. TLA can also be set up to act as a virtual lab with links to regional laboratories so that clinical microbiologists there can also review plates.

Laboratory automation affords the opportunity to optimise workflow, reduce costs and expedite diagnoses. In comparison to other pathology specialities, such as clinical biochemistry, clinical microbiology has had a slower transition to automation (Bourbeau and Ledeboer 2013). It has been shown that automated inoculation of samples can be superior to manual inoculation (Mischnik et al 2012) with a reduction in human error.
CHAPTER 2

A commentary on the role of molecular technology and automation in clinical diagnostics.

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A commentary on the role of molecular technology and automation in clinical diagnostics.

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Abstract

Historically, the identification of bacterial or yeast isolates has been based on phenotypic characteristics such as growth on defined media, colony morphology, Gram stain and various biochemical reactions, with significant delay in diagnosis. Clinical microbiology as a medical specialty has embraced advances in molecular technology for rapid species identification with broad-range 16S rDNA polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) demonstrated as accurate, rapid and cost-effective methods for the identification of most, but not all, bacteria and yeasts. Protracted conventional incubation times previously necessary to identify certain species have been mitigated, affording patients quicker diagnosis with an associated reduction in exposure to empiric broad-spectrum antimicrobial therapy and shortened hospital stay. This short review details such molecular advances and their implications in the clinical microbiology setting.

Keywords

Clinical microbiology, MALDI, PCR, patient care, impact.
Introduction

Europe and North America have experienced significant declines in mortality secondary to infectious diseases (Armstrong et al 1999). The incidence of severe sepsis associated with multi-organ failure in the European Union is currently estimated at 90.4 cases per 100,000 population (Daniels 2011). Global developments in education and training for medical students, continuous professional development for qualified physicians, modern cleaner hospitals with single en-suite accommodation, improved infection control practice, newer antimicrobial therapies, advanced molecular technology used in the laboratory, and sepsis management protocols have all contributed to a decline in sepsis-related deaths (Phua et al 2012). Previous studies have shown a direct link between outcomes from infectious illnesses and time to pathogen identification (Yang and Rothman 2004).

Consequently, laboratory testing volumes are increasing by 10-15% per year internationally, driven partly by infection control demands, with enhanced screening for multi-drug resistant organisms (MRDOs) such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum beta-lactamases (ESBLs) and carbapenemase-producing Enterobactericeae (CPE) (Bourbeau and Ledeboer 2013). Clinical microbiology has evolved in response to clinical need and laboratories have adapted to meet demand for testing. The laboratory service expected by physicians and patients has changed dramatically. Most Western tertiary referral hospitals operate a laboratory service capable of providing an on-call 24 hour service enabling clinical teams to obtain results as soon they become available. Patients, many empowered and self-educated through the vast array of
information readily available on the internet, expect rapid reporting of results with minimal delay from time of presentation to diagnosis and discharge. Patients are also increasingly aware of the dangers of acquiring HCAIs, and there are also many added economic and financial pressures being brought to bear on clinical services. As a consequence, approximately 80% of patient management decisions are influenced by laboratory testing, and reducing time to identification within the laboratory is increasingly a priority (Dokouhaki and Blondeau 2012).

The complementary roles of the clinical microbiologist and the microbiology laboratory

The primary roles of the clinical microbiology team are to guide and support physicians in community or hospital settings, to select appropriate diagnostic investigations and antimicrobials, as warranted, and to achieve the best possible outcome for patients. For any patient, an array of samples may be sent for laboratory analysis with the objective of identifying causative pathogens. Specimens may be analysed following sampling from diverse physiological sources including, but not limited to, cerebrospinal fluid (CSF), blood, “sterile” body fluids, tissue, pus, urine, intravascular catheter tips, prosthetic devices and the respiratory tract. Equipped with expertise and experience, clinical microbiologists are responsible for construction of differential diagnoses and provision of advice on required testing. However, skilled medical scientists are critical to the processes of appropriate growth media selection, inoculation and incubation of specimens, analysis and interpretation of complex analytical data.
Patient-Centred Care

Patients presenting for medical attention with signs of infection, particularly evidence of fever, with or without haemodynamic instability, should have at least one set of blood cultures taken using an appropriate aseptic technique. Blood samples forwarded by physicians to the laboratory, that flag as positive when incubated, are typically the first point of contact between clinical microbiologists and patients who may require either commencement or escalation of antimicrobial therapy. However, blood cultures are problematic in diagnosis of sepsis due to the potential presence of unculturable organisms, underfilling of blood collection vessels, protracted incubation times, and false positives caused by leucocytosis or contamination with commensal flora (e.g., coagulase negative staphylococci; commonly referred to as CoNS). In addition, microorganism detection and susceptibility testing may require up to 48 hours, delaying definitive diagnoses for patients.

Why the long delay?

Processing of microbiology samples can be a lengthy process (Figure 1). Once a blood sample is confirmed as positive in the laboratory, preliminary organism identification, usually performed by medical scientists, is determined by Gram stain, traditional culture techniques, and colony morphology. Microscopic examination of stained smears of tissue or biological fluid is relatively quick and requires minimal resource inputs, but analysis is subjective with low sensitivity and specificity. Historically, subjective criteria such as odour and/or colour, and experience of phenotypic patterns derived using a variety of confirmatory biochemical tests performed at the laboratory bench are employed. Subsequently, an authorised clinical diagnosis is reliant on the
organism being culturable on solid media following 24 hours (aerobic) or 48 hours (anaerobic) incubation. Frequently, incubation can prolong the process by 24 to 48 hours.

Figure 1. Conventional laboratory identification process including Gram stain for provisional identification of Gram positive or Gram negative organisms followed by inoculation onto culture plates and incubation for variable periods of time depending on organism type. Biochemical testing for final organism identification can be performed once sufficient growth has occurred on agar plates. Antimicrobial susceptibility testing (AST) requires the set-up of further agar plates onto which antimicrobial impregnated discs are placed. These AST plates generally require overnight incubation.
The duration of processes involved in reaching definitive laboratory diagnoses via these conventional culture methods results in widespread utilisation of empiric broad-spectrum antimicrobial therapy for the stabilisation of deteriorating patients. Clearly, the intention is to administer a broad spectrum of bacteriostatic and/or bacteriocidal agents sufficient to treat the unidentified pathogen but, in reality, this approach is often associated with development of antimicrobial resistance, *Clostridium difficile* overgrowth, and iatrogenic complications for patients (Carleton *et al* 2014, Duszynska 2012). Typically, empiric antimicrobial therapy is rationalised to a narrower spectrum agent once microbiology laboratory results become available.

**Can laboratory results be expedited?**

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has facilitated substantial movement toward same-day clinical decision-making underpinned by molecular technology. (Carbonelle *et al* 2011, Seng *et al* 2010, Neville *et al* 2011, Bullman *et al* 2012). In use, MALDI-TOF MS determines characteristic protein patterns derived from microbe composition and allows interrogation of well-developed databases for identification (Figure 2). This can occur once an incubated sample grows to colony level (approximately $10^5$ cfu) and can be transferred to a slide. Laser analysis then takes less than 10 minutes, significantly reducing organism identification times compared with conventional microbiology.
Figure 2. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry machine commonly referred to as a ‘MALDI-TOF MS machine’. Mass spectrometry is a technique used to identify a molecule and determine its chemical structure by analysing the mass and the charge of its ions and comparing this to a database stored on the machine. Most clinical microbiology laboratories in Ireland now have a MALDI-TOF MS machine in use for the daily identification of bacteria.

Increasingly utilised clinically for the identification of bacteria (Bizzini et al 2010, Loonen et al 2012) and yeasts (Santos et al 2010), MALDI-TOF MS has potential for accurate identification of viruses (Cobo 2013). The utilisation of MALDI-TOF MS in clinical laboratories has resulted in reports of previously undetected organisms, such as new species of anaerobes such as *Prevotella* spp. and *Anaerococcus* spp. (La Scola et al 2011, Barreau et al 2013). However, while MALDI-TOF MS has a reported reliability of >95% of routine isolates grown on solid media in the laboratory (Carbonnelle et al 2012), limitations in clinical practice include an inability to differentiate genetically similar organisms, such as *Streptococcus pneumoniae* from other members of the *Strep. mitis* group, (Werno et al 2012) and *Shigella* species from *E. coli* (Tan et al 2012). Despite this, MALDI-TOF MS analysis can reduce manual workload for medical scientists and improve throughput of laboratory samples (Loonen et al 2012).

Using a MALDI-TOF MS machine, the previously challenging identification of microorganisms from the sputum of cystic fibrosis (CF) patients (Carleton et al 2014) utilising multiple selective media and prolonged incubation, can now be completed within 48 hours of sampling (Baillie et al 2013). A 2012 Canadian study (Lagacé-Wiens et al 2014) reported a mean reduction in turnaround time of 34.3 hours when definitive identification of isolates directly from blood samples was possible on the MALDI-TOF machine using systems such as the MALDI Sepsityper® Kit (Bruker Daltonics 2010). These advances in testing blood culture bottles directly, thereby eliminating incubation times associated with growth on solid media, has generated positive discussion amongst clinical microbiologists (Schieffer et al 2014).
In an attempt to replicate the reductions observed in Canada utilising direct testing from blood culture bottles (described above), 150 blood samples, flagged as positive for presence of microorganisms using the BacT/ALERT® system (bioMérieux®) were analysed at University Hospital Limerick (UHL) over a 6 week period using the MALDI Sepsityper® Kit and conventional microbiological techniques and results compared with respect to turnaround time (TAT) of positive blood cultures (Table 1).

We observed that for polymicrobial blood cultures, MALDI-TOF MS resulted in TAT being reduced by a mean of 17.5 hours; mean reduction of 14.47 hours for Gram-positive cultures and mean reduction of 23.19 hours for Gram-negative cultures.

Figure 3. Conventional use of MALDI-TOF MS utilising colonies of bacteria from agar plates versus MALDI Sepsityper® Kit sampling directly from positive blood culture bottles.
The impact of our study was that, of the patients from whom these blood samples were taken, 15 patients (30.0%) experienced a change of therapy in a more timely manner, 12 patients (24%) were changed to a more appropriate antimicrobial, while one patient (2%) had antimicrobial therapy discontinued.
Table 1. TAT for microbial identification

<table>
<thead>
<tr>
<th></th>
<th>MALDI-TOF</th>
<th>Conventional microbiology</th>
<th>Reduction of mean TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min- Max</td>
<td>Mean</td>
</tr>
<tr>
<td>Gram-positive and</td>
<td>11.12***</td>
<td>2.0-27</td>
<td>28.62</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-positive only</td>
<td>13.41</td>
<td>2.0-27.0</td>
<td>27.88</td>
</tr>
<tr>
<td>Gram-negative only</td>
<td>5.36</td>
<td>2.0-20.0</td>
<td>28.55</td>
</tr>
</tbody>
</table>

*TAT = turnaround time; **Over a 6 week period; 150 blood culture samples at University Hospital Limerick; *** All values are in hours

These changes correspond with findings elsewhere that rapid detection of microbes facilitates the introduction of appropriate narrow spectrum targeted therapy and reduction of patient length of stay or, indeed, avoidance altogether of admission via outpatient antibiotic therapy (OPAT). Related sophisticated use of MALDI-TOF MS further improves antibiotic usage metrics through detection of false positive blood samples associated with environmental or skin contaminants (Tenover 2010). MALDI-TOF MS is both cost and environmentally effective when compared to conventional microbiology. Gaillot et al 2011 demonstrated 89.3% cost savings in the first year of MALDI-TOF use, whereby cost per isolate decreased from $5.80 to $0.50 and waste reduced from >1,400kg to <50kg, in addition to lesser need for DNA/RNA sequencing and, overall, significantly enhanced time to bacterial identification. At the time of this study, microbiological testing at UHL was predominantly based on the bioMérieux® ARIS system costing approximately €3.50 per identification, and with circa €17,000 annual expenditure on supplementary identification methods (bioMérieux® API® and RemelRapID®).
PCR and patients

PCR is used extensively for identification of multiple pathogens, commonly including MRSA, *C. difficile*, *Strep. agalactiae* (Group B *Streptococcus*) and bacterial causes of meningitis. At UHL, PCR is used extensively in testing for *Chlamydia trachomatis* and *Neisseria gonorrhoea*. Between January and December 2013, 13,338 clinical samples (on average 1100 per month) were analysed using PCR for the detection of sexually transmitted infections (STIs) (Table 2).

Table 2. Utilisation of PCR analysis of clinical specimens at UHL over 12 months. a

<table>
<thead>
<tr>
<th></th>
<th>Annual</th>
<th>Monthly</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Total samples tested using PCR</td>
<td>15732</td>
<td>1311</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0-27</td>
</tr>
<tr>
<td>Samples tested positive for potential STI b only</td>
<td>13338</td>
<td>1112</td>
</tr>
<tr>
<td>For Clostridium difficile (toxin) c</td>
<td>1714 d</td>
<td>429</td>
</tr>
<tr>
<td>For Salmonella, Shigella, Campylobacter, VTEC</td>
<td>2680 e</td>
<td>670</td>
</tr>
</tbody>
</table>

a January to December 2013; b STI: sexually transmitted infection, specifically *Chlamydia trachomatis* and *Neisseria gonorrhoea*; c Introduced at UHL September 2013; d Projected annual total based on 4 month analysis (n=5142); e Projected annual total based on 4 month analysis (n=8040).

PCR testing for STIs has facilitated a “same day testing service” with results available for clinicians within hours of a patient’s attendance at an on-site sexual health clinic, facilitating early commencement of directed-therapy and reduced patient anxiety. We also use PCR in evaluating the success or otherwise of antiviral therapies and for long-term follow-up of patients with HIV and hepatitis B and C attending our local services.
Decision making regarding respiratory viruses, for example, respiratory syncitial virus (RSV) in paediatric patients has been reduced at UHL from up to a week to just hours, with significant benefits for hospital bed management and prompt initiation of antiviral therapy as necessary, as reported elsewhere (Steed and Ball 2013). Detection of herpes viruses and a variety of enteroviruses via PCR is performed on all CSF samples received in the laboratory, thus supporting appropriate timely management of common viral central nervous system infections (CNS) (López Roa et al 2013).

Locally, PCR analysis for stool samples has reduced turnaround time for samples by up to 24 hours. *C. difficile* toxin testing via PCR was introduced at UHL in September 2013, with over 400 stool samples now analysed per month. At a practical level, given the transmissibility of *C. difficile*, the need for urgent isolation, commencement of treatment, and the potential for serious complications such as toxic megacolon, the introduction of PCR has considerably improved our patient service. PCR technology continues to evolve, and ready analysis of samples obtained directly from clinical specimens such as pleural fluid, CSF, blood, joint aspirates, heart valves and abscess aspirates is increasingly commonplace (Jensen et al 2014). Regularly, broad-range 16S rDNA PCR is utilised in the context of culture negative samples where clinical suspicion exists but conventional microbiology has not confirmed infection (Duffett et al 2012).
Practical issues associated with molecular microbiology

No diagnostic technology is universally applicable. For example, important pathogens such as *Strep. pneumoniae*, beta-haemolytic streptococci, and enteric pathogens such as *Shigella* spp. and *E. coli* remain difficult to identify using MALDI-TOF MS. For this reason, microbiologists remain reliant on traditional methods of identification and for antimicrobial susceptibility testing. On a financial note, there are also capital funding considerations in adopting molecular technologies. Many clinical laboratories will require refurbishment, or new builds, before being suitable to accommodate molecular equipment such as MALDI-TOF MS or to facilitate total laboratory automation (TLA) suites such as Becton Dickinson’s Kiestra™. Indeed, movement towards sophistication of laboratory methods should ideally be integrated with electronic patient records (i.e., (national) unique patient identification numbers) to avoid duplication of unnecessary tests caused by patients having duplicate or triplicate patient laboratory registration numbers. At least in Ireland, such a system does not yet exist. An equally important consideration is the requirement for up-skilling of staff and maintenance of expensive equipment in the context of stringent laboratory accreditation requirements, such as compliance with ISO standard 17025.

Training in the molecular era

There are challenges for clinical microbiologists and physicians in keeping pace with rapidly changing discoveries in molecular diagnostic techniques, for instance, the potential use of MALDI-TOF MS or high-resolution melting PCR analysis in assessment of antimicrobial susceptibility and isolate profiling (Kostrzewa *et al* 2013, Gabriel *et al* 2012). Training programmes for specialist physicians in clinical microbiology will need to take greater account of this, and allow time for medical
professionals to become and remain familiar with emerging developments, both their advantages and, from a patient safety perspective, their limitations. A further risk to be mitigated is the potential de-skilling of medical scientists with the advent of, and over-reliance on, modern technologies.

**Conclusions**

Clinical microbiology is pivotal to patient outcomes in providing highly accurate diagnostic and supportive advisory services to clinicians across all specialties in acute settings and primary care. The introduction of molecular technology and automation fundamentally changes the way in which laboratory diagnoses are reached, providing superior laboratory services to physicians and patients. Molecular diagnostics afford improved sensitivity and specificity, and the possibility of rapid diagnoses, particularly from blood cultures in paediatric and neonatal patients, with turnaround times of hours rather than days, and subsequent reduced length of hospital stay and mitigated risk of HCAIs. The increase in MDROs globally reinforces the need for more rapid diagnostics, facilitating judicious use of effective antimicrobials and mitigated risk of healthcare-associated infections and, in summary, improving patient-centred care.

**Conflict of interest**

The authors declare that they have no competing interests.

**Funding sources**

None.
CHAPTER 3

Combined education and skin antisepsis intervention for persistently high blood culture contamination rates in neonatal intensive care.

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Combined education and skin antisepsis intervention for persistently high blood culture contamination rates in neonatal intensive care.

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Summary
Contaminated blood cultures represent challenges impacting on diagnosis, duration of hospitalisation, antimicrobial use, pharmacy and laboratory costs. Facing problematic neonatal blood culture contamination (3.8%), we instigated a successful intervention combining skin antisepsis using sterile applicators with 2% chlorhexidine gluconate in 70% isopropanol prior to phlebotomy (replacing 70% isopropanol) and staff education. In the 6-months prior to intervention, 364 neonatal peripheral blood samples were collected. Fourteen (3.8%) were contaminated. In the post-intervention 6-months, 314 samples were collected. Three (0.96%) were contaminated, representing significant improvement (Fisher’s exact test \( P = 0.0259 \)). No dermatological sequelae were observed.

Keywords
Intervention, neonatal, blood culture, bacteria, contamination, chlorhexidine.
Introduction

Use of blood cultures as a basis for diagnostic testing during hospitalisation is ubiquitous. Unfortunately, contamination of blood cultures (i.e., growth of bacteria in blood samples that were not present in patients’ blood during the process of sample collection) with commensal skin microorganisms is relatively common (Dawson 2014) and, due to associated uncertainty regarding “false positive” tests, can cause initiation of empirical antimicrobial treatment, further laboratory testing and lengthened duration of hospital stay (Alahmadi et al 2011, Rowley and Clare 2011). The American Society for Microbiology and The Clinical and Laboratory Standards Institute recommend that an acceptable rate of blood culture contamination should not exceed 3%. There has, therefore, been considerable focus on interventions to reduce contamination of blood cultures, including dedicated education and training (Rowley and Clare 2011), establishing dedicated phlebotomy teams, use of preprepared customised blood culture kits and varying skin antisepsis agents, with generally (but not universally) successful outcomes (Thomas et al 2011). With respect to the latter approach, meta-analysis (Caldeira et al 2011) has demonstrated that alcohol-based approaches were more effective than non-alcoholic, while chlorhexidine plus alcohol performed better than iodine plus alcohol combinations.

In 2013, Washer et al reported a clinical trial of three antiseptic interventions (70% isopropanol followed by 10% povidine iodine, 70% isopropanol followed by 2% iodine tincture, and 2% chlorhexidine gluconate combined with 70% alcohol) in almost 13,000 blood cultures demonstrating no significant difference in contamination rates and recommending that decisions regarding choice of antisepsis be based on cost.
or preference (Washer et al 2013). However, those studies focused on adult patients with relatively little emphasis placed on paediatric, and specifically neonate, patients due to concerns regarding, for example, risk of adverse effects on thin, incompletely keratinised skin or potential anaphylaxis (Ponnusamy et al 2014).

Indeed, when assessments of products such as chlorhexidine have been reported, the described approaches to antisepsis use lower concentration products (e.g., 1% or lower) rather than the 2% chlorhexidine in 70% alcohol combination that has shown efficacy in adults (Nuntnarumit and Sangsuksawang 2013). A notable exception to this is a 2010 report by Marlowe that determined significantly greater efficacy of a 70% alcohol with 3% chlorhexidine gluconate combination versus povidone-iodine alone in reducing blood culture contamination in a paediatric emergency department setting. However, that study did not assess chlorhexidine in children under 2 months (Marlowe et al 2010).

Here, we describe what appears to be the first successful combined intervention, involving both adoption of 2% chlorhexidine in 70% alcohol use and staff education, in a neonatal intensive care unit (including premature and very low-birth-weight newborns (VLBW, <1500g) babies) with persistently high blood contamination rates.
Methods

Setting

This intervention was performed at the neonatal intensive care unit (NICU) with 19 cots in the University Maternity Hospital Limerick, Ireland (UMHL). For context, in 2012, there were 909 NICU admissions (from a cohort of 4905 live births). This intervention was instigated by an audit of NICU records showing blood culture contamination rates of 3.4% in 2009, 3.1% in 2010 and 3.2% in 2011 (unpublished data). In the immediate pre-intervention period (January to July 2012), a total of 364 peripheral blood cultures resulting in 17 positive cultures were detected from 14 patients (10 male and 4 female). Three were considered significant clinical isolates; two *Escherichia coli* and one *Streptococcus bovis*.

A blood culture was considered to be contaminated if at least one of the following organisms, considered representative of skin microflora and most commonly reported contaminants, was identified in at least one of a series of blood cultures: coagulase-negative staphylococcus (CoNS) spp., *Corynebacterium* spp., alpha- or beta-haemolytic streptococci, *Micrococcus* spp., *Bacillus* spp. and *Propionibacterium* spp. in the context of correlated clinical findings (e.g. fever, leukocytosis, blood biochemistry), and time to positivity (van der Heijden *et al* 2011). Thereby, the remaining 14 blood cultures were considered “false positives”, containing CoNS (13 specimens), and mixed CoNS and Diptheroids (1 specimen). This represents a contamination rate of 3.8% (14/364).
**Intervention**

We introduced skin antisepsis using 2% chlorhexidine gluconate in 70% isopropyl sterile applicators (ChloraPrep®, Becton, Dickinson & Co., Franklin Lakes, NJ, USA) (replacing 70% isopropyl alcohol swabs) prior to phlebotomy for all neonates.

![Image of 2% chlorhexidine/70% isopropyl alcohol sterile applicator in use.](image)

**Figure 1.** Still image (posed by anonymised models demonstrating on an adult hand) of a 2% chlorhexidine/70% isopropyl alcohol sterile applicator in use. It is recommended to hold the applicator using the thumb and index finger on the dominant hand for maximal precision in the application of the solution on the skin. Gentle pressure is applied to the applicator leading to the slow release of the solution through the gauze covered outlet.
The antimicrobial efficacy of the chlorhexidine preparation was validated separately in our hospital. Our protocol required that the antisepsis combination remain post-blood collection (leveraging residual chlorhexidine antimicrobial activity). Therefore, in the context of avoiding adverse events for our neonatal patients with potentially fragile skin (particularly premature children), we focused specifically on emergence of any adverse events potentially associated with chlorhexidine use. A concomitant educational programme was provided to NICU staff (consultant neonatologists/paediatricians, doctors in training including registrars and senior house officers, as well as neonatal nursing and midwifery staff) emphasising the importance and opportunities for hand hygiene, detailing the intervention procedures and use of the sterile applicators. This training occurred between July and December 2012 while the introduction of ChloraPrep® use began in January 2013. The intervention was approved by the Ethics Board of the Mid-West Teaching Hospitals (Ireland). Informed consent for participation was obtained from parents of all children.

**Results and Discussion**

We describe the first successful intervention to improve persistently high blood culture contamination rates in a neonatology setting using a combination of 2% chlorhexidine and 70% isopropanol complemented with education of NICU staff. Attendance at the 30 minute training sessions achieved 100% compliance and the intervention was well received. All found the sterile applicator to be user-friendly, did not require unusual storage or handling and with a drying time (an important consideration for NICU staff) of 15-30 seconds being no different to the 70% alcohol swabs previously employed.
In the immediate post-intervention period (January to July 2013), 314 peripheral blood cultures (from children aged less than one day to more than 3 weeks) resulted in 3 contaminated blood cultures (from 3 separate patients), each involving CoNS (a rate of 0.96%; Fisher’s exact test $P = 0.0259$). Although CoNS may cause sepsis in neonates, sepsis was not present. We attribute that improvement to the introduction of 2% chlorhexidine, the applicator system allowing gentle contact with delicate neonate skin, and a greater emphasis on aseptic technique brought about through the provided education programme. We noted no evidence of adverse effects during or following use of the antisepsis combination, thereby providing evidence to support judicious use of chlorhexidine in the neonate setting.

During that period, there were no changes in NICU staffing levels, and no reduction in bed numbers within the unit. Additionally, there were no changes implemented regarding antimicrobial use and no other changes in practice introduced that could have been confounding factors. However, within our NICU, there is increased awareness among staff regarding the procedural skills and expertise necessary for maintaining a sterile field to prevent contamination during taking of blood samples, increased practical phlebotomy training for new staff entering the unit, increased knowledge of the consequences of false positive blood cultures, and a heightened awareness of international best practice guidelines in striving to remain below the recommended rate of $<3\%$ blood culture contamination.
Conclusions
The introduction of staff education and sterile applicators containing 2% chlorhexidine in 70% isopropanol for neonatal skin antisepsis has significantly reduced blood culture contamination, from 3.8% pre-intervention to 0.96% post-intervention. Staff welcomed the training, accepted use of the applicators, neonatal care was not compromised, and no dermatological adverse events were observed. We are unable to determine which element of our intervention was most influential, however, we believe that replication of our combined intervention in larger cohorts or through randomised, controlled trials would have merit. That said, due to our results, we plan to introduce this product for skin antisepsis throughout the University of Limerick Group of Hospitals for medical, surgical and obstetric patients.

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We thank the staff of the University Hospital Limerick microbiology laboratory and the NICU for their continued enthusiasm and commitment to the intervention described here.

Conflict of interest
The authors declare that they have no competing interests.

Funding sources
None.
CHAPTER 4

The first occurrence of a CTX-M ESBL-producing *Escherichia coli* outbreak mediated by mother to neonate transmission in an Irish neonatal intensive care unit.

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The first occurrence of a CTX-M ESBL-producing *Escherichia coli* outbreak mediated by mother to neonate transmission in an Irish neonatal intensive care unit.

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Abstract

Background

*Escherichia coli* (*E. coli*) comprise part of the normal vaginal microflora of women. Transfer from mother to neonate occurs during delivery resulting, sometimes, in neonatal bacterial disease.

Aim

To report the first outbreak of CTX-M ESBL-producing *E. coli* with evidence of mother-to-neonate transmission in an Irish neonatal intensive care unit (NICU) followed by patient-to-patient transmission.

Methods

Investigation including molecular typing was conducted. Infection was defined by clinical and laboratory criteria and requirement for antimicrobial therapy with or without positive blood cultures. Colonisation was determined by isolation without relevant symptoms or indicators of infection.

Results

Index case was an 8-day-old male born at 34 weeks gestation who developed ESBL-producing *E. coli* infections at multiple body sites. Screening confirmed his mother as colonised by ESBL-producing *E. coli*. Five other neonates, in the NICU simultaneously with the index case, also tested positive. Of these, four were colonised while one neonate developed sepsis, requiring antimicrobial therapy. The second infected neonate’s mother was also colonised by ESBL-producing *E. coli*. Isolates from all eight positive patients (6 neonates, 2 mothers) were compared using pulsed-
field gel electrophoresis (PFGE). Two distinct ESBL-producing strains were implicated, with evidence of transmission between mothers and neonates for both strains. All isolates were confirmed as CTX-M ESBL-producers. There were no deaths associated with the outbreak.

Conclusions
Resources were directed towards control interventions focused on hand hygiene and antimicrobial stewardship, which ultimately proved successful. Since this incident, all neonates admitted to the NICU have been screened for ESBL-producers. To date, there have been no further outbreaks.

Keywords
ESBL, outbreak, *Escherichia coli*, Ireland, CTX-M, NICU.
Background

*Escherichia coli* comprise part of the normal vaginal microflora of women. Horizontal transfer from mother to neonate occurs during delivery (Gillespie and Hawkey 2006, Al-Mayahie 2013) resulting, sometimes, in severe neonatal bacterial disease (Wickramasinghe *et al* 2012a, Wickramasinghe *et al* 2012b). Extended-spectrum beta-lactamases (ESBLs) are plasmid-borne beta-lactamases capable of hydrolysing and inactivating beta-lactam antimicrobials with an oxyimino side chain, e.g., cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and the oxyimino-monobactam (aztreonam) (Ena *et al* 2006). TEM-, SHV-, OXA- and CTX-M type beta-lactamases are the types of ESBLs most commonly isolated in the laboratory. First reported in 1989, CTX-M enzymes are the most prevalent ESBL-type worldwide since 2000 (Cantón and Coque 2006, Cantón *et al* 2008). In 2013, it was reported that the incidence of community-associated infections caused by CTX-M ESBL-producing bacteria, particularly urinary tract infections (UTIs) in women of child-bearing age, is increasing (Doi *et al* 2013). ESBL-producing *E. coli* have been reported as a cause of neonatal sepsis and meningitis (Lopez-Cereo *et al* 2008) and mother-to-neonate transmission of ESBL producers has been previously described (Dubois *et al* 2010). The gastrointestinal tract of infected or colonised patients, of all age groups, is the most frequently-reported reservoir of ESBL-producing organisms, and studies have shown that transient carriage of ESBL-producing organisms on the hands of healthcare workers (Paterson and Bonomo 2005) and or on artificial nails may also facilitate transmission (Gupta *et al* 2004).
Neonatal intensive care unit (NICUs) have been described as an interface between the hospital and the community, given the close involvement of parents in the daily care of their neonates while inpatients, which in turn increases the risk of transmission of community-associated multi-drug resistant organisms (MDROs), such as ESBLs (Moissenet et al 2010, Giuffrè et al 2012). NICU stays have become prolonged due to advances in modern medicine, with duration of hospitalisation inversely related to gestational age and with increased risk of healthcare-associated infections (HCAIs) (Crivaro et al 2015). Specifically, risk factors associated with colonisation or infection by ESBL-producers in NICUs include low gestational age, an immature immune system, low birth weight, care in incubators, exposure to third-generation cephalosporins (Linkin et al 2004, Bizarro and Gallagher 2007) and contaminated breastmilk (Rettedal et al 2012). Septicaemia due to ESBL-producing organisms has been associated with a significantly increased mortality rate compared to non-ESBL-producing isolates (Blomberg et al 2005). In general, chemotherapeutic options for dealing with ESBL-related infections are limited, and that challenge is compounded by restrictive prescribing for neonates due to the potential for adverse side-effects. Therefore, when they occur, NICU-associated HCAIs increase hospital costs substantially, with the potential to prolong hospitalisation considerably, and are responsible for 50% of deaths that occur beyond two weeks of age (Polin and Saiman 2003). In addition, there may be disruption of healthcare services due to stringent infection control measures such as restriction of admissions or ward closures.

In this report, we describe the occurrence and outcomes of the first ESBL-producing *E. coli* outbreak in an Irish NICU. In particular, we detail the infection prevention and control interventions that successfully brought the outbreak to an end.
Methods

Setting

The University Maternity Hospital Limerick in Ireland (UMHL) is a tertiary referral centre and includes a NICU with a total of 19 cots. The catchment population of UMHL is approximately 300,000. In the twelve months prior to the outbreak, there were 4905 live births and 909 NICU admissions (of all gestational ages). At the time of this outbreak, the NICU had one intensive care ward consisting of four neonatal intensive care cots, five high dependency cots and two isolation rooms. Two intermediate care rooms, separated from the intensive care ward, contained a further 10 cots. With respect to prevention of nosocomial infection, the NICU intensive care ward provided four washing stations, alcohol hand gels at each bedspace, and a nurse to patient ratio of 1:1 for ICU category cots and 1:2 or 1:3 (depending on staffing levels) for the remainder. A weekly multi-disciplinary NICU ward-round was performed.

Index case identification

The index case for this outbreak was a male infant born (at 34 weeks gestation) in March 2013 via spontaneous vaginal delivery, complicated by premature prolonged rupture of membranes. Antenatal screening had revealed vaginal Group B Streptococcus (GBS) colonisation and intravenous (IV) clindamycin 600mg was administered to the mother during labour. Fifteen minutes after birth, the neonate was transferred to the intensive care ward in the NICU for management of respiratory distress syndrome. A pyrexia of 37.6°C was recorded when the infant was 28 hours old associated with irritability on handling. The neonate was treated with IV benzylpenicillin and gentamicin (dosed as per weight) for 48 hours and a full sepsis
screen performed that included lumbar puncture, white blood count (WCC), C-reactive protein (CRP), blood culture, urine analysis, and chest radiograph. The infant’s WCC and CRP were within normal range. Blood culture samples taken on day of delivery were negative for growth at 48 hours, urine and cerebrospinal fluid cultures remained sterile. Polymerase chain reaction (PCR) testing of blood for GBS proved negative. Antimicrobials were subsequently discontinued.

At 72 hours, while the infant was still in the NICU, a purulent discharge from the right eye and a discrete area of ulceration over the right buttock were noted, from which swabs for culture were taken. A pyrexia of 37.9°C was recorded. Clinically, the neonate appeared septic. WCC and CRP were again within normal range. Gram stain of the right eye pus identified Gram-negative bacilli. IV flucloxacillin and IV gentamicin (both doses as per weight) were commenced empirically. Two separate blood cultures proved negative over the next five days. However, the specimen from the right eye was positive for ESBL-producing *E. coli* resistant to co-amoxiclav, ceftriaxone, aztreonam, ciprofloxacin, gentamicin and piperacillin/tazobactam; sensitive to chloramphenicol, amikacin and meropenem. The specimen from the right buttock ulceration was also positive for ESBL-producing *E. coli* demonstrating the same antibiogram. The infant was placed in an isolation room with contact precautions, chloramphenicol eye drops and IV meropenem (dosed as per weight) administered for seven days. He was discharged home on day 17 after birth without need for further antimicrobials.
Infection control interventions

The isolation of an ESBL-producing *E. coli* from a NICU inpatient triggered initiation of the hospital’s outbreak management protocol, which involved meeting with all key stakeholders, including executive management, nursing administration, infection prevention and control, consultant microbiologists, laboratory managers, bed management, hygiene services, communications team and NICU clinical director. Following a review of related literature, and to be prudent, a decision was made to close the NICU to new admissions from March 22nd 2013, with exception of emergencies, and visiting was restricted to parents of inpatients only. Information leaflets regarding outbreak risks and management were distributed to visiting parents. Appropriate public communication and a press statement were issued by the Clinical Director. Arrangements were made for antenatal inpatients whose neonates might require NICU admission to be referred to other maternity hospitals in Ireland. Empiric IV meropenem (dosed as per weight) was administered for any infant demonstrating signs of sepsis, pending microbiology analysis of urine or rectal swabs. Such signs are typically non-specific and include: reluctance or refusal to feed; irritability on handling; and poor responsiveness to stimuli; amongst others. All infected or colonised neonates were barrier nursed by personnel wearing disposable gowns and gloves. Contact tracing of all inpatients who may have been in contact with the index case while in NICU was conducted using urine samples and/or rectal swabs. In addition, a restriction was placed on the prescription of third generation cephalosporins.

With consent, the mother of the index case and mothers of subsequently positive neonates were screened. Due to availability of only two isolation rooms in the NICU,
the index case and one other neonate infected by ESBL-producers were isolated (Figure 1). The remaining colonised neonates were cohorted in incubators in the main NICU ward, with dedicated single-patient equipment. Given physical environmental constraints, it was not possible to increase the space between cots. Neonates who were fit for discharge were cohorted to a single post-natal maternity ward to minimise cross-transmission.

Figure 1. Visual depiction of the timeline associated with this outbreak. Six positive neonates and two positive mothers were identified as part of the outbreak contact tracing.
With respect to hygiene, enhanced cleaning of the NICU was instigated in parallel with increased auditing. This involved twice-daily cleaning of affected areas and incubators with detergent. Air sampling and environmental sampling were not performed. An intensive targeted educational programme focussed on standard precautions, particularly hand hygiene compliance and on modes of transmission of ESBL-producing *E. coli* transmission was provided to all clinical and administrative staff. Screening of staff for carriage of ESBL-producing *E. coli* was not conducted. Hand hygiene audits were performed with greater frequency in affected areas, which involved twice weekly observational audits at ward level. The last positive isolate was identified on March 25th 2013. The NICU re-opened on March 29th 2013 and weekly multi-disciplinary meetings were held until May 3rd 2013 to discuss and implement hygiene recommendations, at which point the outbreak was declared over. The last known neonate involved in the outbreak was discharged on April 19th 2013.

*Microbiological and molecular detection of ESBL-producing E. coli*

Screening specimens were cultured using ChromID™ ESBL agar (bioMérieux, Marcy l’Étoile, France) and incubated at 37°C aerobically for 18-24 hours. The colour code guide provided by the manufacturer was followed for review of any colonies identified; pink/brown = presumptive *E. coli*, green/blue = presumptive *Klebsiella* species, white = other Enterobacteriaceae. All colonies were identified using MALDI-ToF MS (Bruker Daltonics, Bremen, Germany) as described previously (O’Connor *et al* 2014). Confirmatory testing was performed by disk diffusion on all organisms that warranted further investigation (Thermo Scientific™ Oxoid™ Disks) using a disc dispenser; cefoxitin 30µg (FOX), cefapime 30 µg (FED), ceftazidime 30µg (CAZ) & ceftazidime-clavulanic acid 30/10µg (CAZCV), cefotaxime 30µg (CTX) &
cefotaxime-clavulanic acid 30/10 µg (CTXCV), Muller Hinton agar, 0.5 McFarland inoculum; 35 +/-2°C, ambient air, 16-28 hours. Following incubation, the zone sizes of the cephalosporin disc to that of a cephalosporin plus clavulanic acid combination disc were compared to determine ESBL status. Criteria for positive ESBL disc confirmatory testing on Enterobacteriaceae: a ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone, for example, CAZ zone = 16, CAZ & clavulanic acid =21. Criteria for negative ESBL disc confirmatory testing on Enterobacteriaceae: zone sizes for both cephalosporin and cephalosporin in combination with clavulanic acid that is equal or show no greater difference in diameter than +/- 2mm. Criterion for inconclusive ESBL disc confirmatory testing: difference between matched discs was >2 but <5mm. In 2013, our protocols dictated that any inconclusive disk diffusion result would warrant further confirmatory testing via Etest (bioMérieux, Marcy l’Étoile, France). No isolate from this outbreak required further testing via Etest.

The genetic relationships between the ESBL-producing *E. coli* isolates were determined by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA at the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, Public Health England, London, UK. Electrophoresis was performed on a Bio-Rad CHEF DRII apparatus at 6 V cm⁻¹ for 30 h at 12°C with ramping times of 5 s to 35 s.
Routine breast milk testing involved incubating samples on blood agar (5-10% CO₂) for up to 48 h, blood agar with metronidazole for up to 48 h anaerobically, and MacConkey Agar for up to 48 h aerobically.

Results

Epidemiological features of the outbreak

Following detection of the index case (neonate two, Table 1), between March 16th 2013 and March 27th 2013, a comprehensive screening seven additional screens of two NICU mothers and five neonates proved positive (Table 1). During this outbreak, infection was defined by clinical and laboratory criteria and requirement for antimicrobial therapy, while colonisation was defined by the absence of relevant symptoms. In total, during the outbreak, 86 ESBL screens from 42 individuals were performed. Of these, specimens from six neonates were positive for ESBL-producing *E. coli*: two represented infection and four represented colonisation. The mean gestational age was 33 weeks (range 28 to 36 weeks). There were no bacteraemias due to ESBL-producers.

After the identification of the index case, the neonate’s mother was informed and agreed to participate in screening. A rectal swab, a mid-stream urine and a sample of expressed breast milk all tested positive for ESBL-producing *E. coli*, as shown in Table 2, with the same antibiogram as the isolate from her neonate (detailed earlier). A high vaginal swab was negative for the bacterium. This isolation of an ESBL-producing *E. coli* was the first such result for the patient who had never before had a culture-positive urine test.
One further neonate (neonate six, Table 1) who was in the NICU at the time of the outbreak developed sepsis at 18 days of age, eight days after the first detection of an ESBL-producer in the NICU. While two blood specimens proved negative, ESBL-producing *E. coli* were confirmed from a urine sample and from a rectal swab. An identical antibiogram to that of the index case neonate was noted. He was treated with IV meropenem (dosed as per weight). This neonate’s mother had been admitted at 27 weeks plus three days gestation with premature rupture of membranes and had received erythromycin prophylaxis. With consent, his mother was screened whereupon a high vaginal swab and a sample of expressed breast milk cultured negative, but a rectal swab and a mid-stream urine sample were positive for ESBL-producing *E. coli* demonstrating the same antibiogram as previously found. Again, this second mother had no previous documented urinary tract infections and had never before had a culture-positive urine test.

Of the remaining four neonates, all were clinically stable, underwent weekly surveillance rectal cultures until discharged from the NICU, and did not require treatment with antimicrobials. All mothers of colonised infants underwent screening to determine colonisation or infection and were found to be negative for ESBL-producers (Table 2).
Table 1. Clinical characteristics of the neonates affected in the outbreak. CTX-M was the resistance mechanism detected where specimens were available for molecular analysis. All neonates were male. Two neonates were considered infected with the remaining four colonised with ESBLs.

<table>
<thead>
<tr>
<th>Neonate</th>
<th>Gender</th>
<th>Date of birth</th>
<th>Admission date to NICU</th>
<th>Gestational age at birth (weeks)</th>
<th>Birth Weight</th>
<th>Deliverya</th>
<th>ESBL Source</th>
<th>ESBL resistance mechanism</th>
<th>Days in NICU prior to positive result</th>
<th>Infected/ colonised</th>
<th>Feedingb</th>
<th>Status at time of discharge from NICU</th>
<th>Total length of NICU admission (days)</th>
<th>Repeat ESBL testing post outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>03.02.13</td>
<td>03.02.13</td>
<td>28+2</td>
<td>1.2kg</td>
<td>CS</td>
<td>Rectal</td>
<td>Unknown</td>
<td>46</td>
<td>Colonised</td>
<td>EBM</td>
<td>Alive</td>
<td>75</td>
<td>Not performed</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>13.03.13</td>
<td>13.03.13</td>
<td>34+5</td>
<td>2.38kg</td>
<td>VD</td>
<td>Right buttock, right eye, urine, rectal</td>
<td>CTX-M</td>
<td>8</td>
<td>Infected</td>
<td>EBM</td>
<td>Alive</td>
<td>17</td>
<td>Not performed</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>13.03.13</td>
<td>13.03.13</td>
<td>36+3</td>
<td>1.79kg</td>
<td>CS</td>
<td>Rectal</td>
<td>CTX-M</td>
<td>8</td>
<td>Colonised</td>
<td>EBM</td>
<td>Alive</td>
<td>17</td>
<td>Not performed</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>13.03.13</td>
<td>13.03.13</td>
<td>36+3</td>
<td>2.0kg</td>
<td>CS</td>
<td>Rectal</td>
<td>CTX-M</td>
<td>8</td>
<td>Colonised</td>
<td>EBM</td>
<td>Alive</td>
<td>17</td>
<td>Not performed</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>14.03.13</td>
<td>14.03.14</td>
<td>35+4</td>
<td>2.38kg</td>
<td>CS</td>
<td>Rectal</td>
<td>CTX-M</td>
<td>7</td>
<td>Colonised</td>
<td>Formula</td>
<td>Alive</td>
<td>14</td>
<td>Not performed</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>19.03.13</td>
<td>19.03.13</td>
<td>30+6</td>
<td>1.48kg</td>
<td>VD</td>
<td>Rectal, urine</td>
<td>CTX-M</td>
<td>6</td>
<td>Infected</td>
<td>EBM</td>
<td>Alive</td>
<td>50</td>
<td>ESBL positive rectal swab September 2013</td>
</tr>
</tbody>
</table>

*a* M: male. 
*b* CS: caesarean section, VD: vaginal delivery. 
*c* EBM: expressed breast milk.
Table 2. Microbiology culture results from high vaginal swabs, rectal swabs, mid-stream urine samples and expressed breast milk obtained from mothers of neonates involved in the outbreak.

<table>
<thead>
<tr>
<th></th>
<th>High vaginal swab</th>
<th>Rectal swab</th>
<th>Mid-stream urine</th>
<th>Expressed breast milk</th>
<th>ESBL resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother of neonate 1</td>
<td>negative</td>
<td>negative</td>
<td>Negative</td>
<td>negative</td>
<td>not tested</td>
</tr>
<tr>
<td>Mother of neonate 2</td>
<td>negative</td>
<td>ESBL positive</td>
<td>ESBL positive</td>
<td>ESBL positive</td>
<td>CTX-M</td>
</tr>
<tr>
<td>(index case)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother of neonates 3&amp;4</td>
<td>negative</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
</tr>
<tr>
<td>(twins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother of neonate 5</td>
<td>negative</td>
<td>negative</td>
<td>Negative</td>
<td>not tested</td>
<td>not tested</td>
</tr>
<tr>
<td>Mother of neonate 6</td>
<td>negative</td>
<td>ESBL positive</td>
<td>ESBL positive</td>
<td>negative</td>
<td>CTX-M</td>
</tr>
</tbody>
</table>

Molecular characteristics and antibiogram of the outbreak

Isolates from all eight positive patients were sent to the AMRHAI Reference Unit, Public Health England, for PFGE analysis to determine the genetic relationships (Figure 2). A strain designated LIME04ES-3 was shared between the index case, his mother and four other neonates, indicating cross-transmission. A second strain (LIME04ES-4) was found in both the second infected neonate and his mother. The ESBL isolates from five of the six neonates were confirmed as CTX-M type, identical to that of both colonised mothers. CTX-M subtyping was not performed at the time of the outbreak. All ESBL isolates identified during this outbreak had an identical antibiogram regardless of culture source: resistant to ampicillin/amoxicillin, co-amoxiclav, ceftriazone, aztreonam, ciprofloxacin, gentamicin, cefuroxime, piperacillin/tazobactam, tri/sulfamethoxazole, while susceptible to amikacin, chloramphenicol, ertapenem, and meropenem.
Discussion

Mother-to-neonate transmission of ESBL-producing *E. coli* in a European NICU has been described previously, with a 2010 report from Switzerland of an outbreak that began with transmission from a mother to her newborn twins during vaginal delivery with subsequent spread to other neonates facilitated by HCWs (Tschudin-Sutter et al. 2010). It is noteworthy that a 2013 report traced another NICU outbreak to ESBL-producers originating from a neonate born via caesarean section and exclusively formula-fed (Giuffrè et al. 2013a). Taken together, these reports involving both delivery methods exemplify the potential risk of infection and/or spread regardless of mode of delivery.
This is the first incidence of an ESBL-producing *E. coli* outbreak in a NICU in Ireland mediated by mother-to-neonate transmission that has been confirmed by molecular analysis. The index case’s mother had a rectal swab and a MSU sample that cultured positive for ESBL-producing *E. coli* although the organism was not detected from the HVS. Consensus was that the likely route of transmission was vertical from the mother’s colonised perianal area during delivery. The second ESBL strain (LIM04ES-4), from a separate neonate (neonate six) and his mother, was again thought to have been transmitted vertically during delivery from the colonised perianal area of his mother, who had positive results from a rectal swab and mid-stream urine.

Neither staff nor environmental screening were performed at the time of the outbreak as the limited available resources were prioritised towards coordinating and managing the NICU closure, staff education regarding hand hygiene & transmissibility of ESBL-producers, antimicrobial stewardship for NICU prescribers. By way of comparison, a Greek neonatal outbreak of SHV-5-producing *Klebsiella pneumoniae* in 2012, involving 13 infected and three colonised neonates employed staff and environmental screening and found that both staff and environmental sites were negative with no specific case identified as being the origin of the outbreak (Mavrodi et al 2014). The same outbreak management steps were followed as in our outbreak with the exception of NICU closure, suggesting perhaps that the definitive step of unit closure allowing deep cleaning to occur, reducing incubator/cot occupancy, lowering the nurse: infant ratio, reducing the throughput of clinical staff into the NICU and curtailing the number of antimicrobials in use in the NICU at any one time may have been key in reducing the duration of our outbreak. Additional sequential control measures such as cohort
nursing, a ‘look back’ to identify the outbreak boundaries as well as determining recommendations for adherence regarding physical separation of clean and used equipment added value to our outbreak control.

In the USA, active surveillance strategies have been adopted by many NICUs to detect infants colonised with antibiotic-resistant organisms albeit that the yield, risks, benefits and costs of different strategies have not been fully evaluated (Macnow et al 2013). It is estimated that 21% of UK NICUs undertake routine faecal/rectal swabbing for ESBL-producing Enterobacteriaceae, but there is currently no consensus in Europe with regard to screening in NICUs (Mitra et al 2011). Irish data regarding screening remain unknown (Fernando et al 2008) and a study that reviewed the practices of NICUs from both the UK and Ireland concluded that NICUs “currently lack systematic neonatal infection surveillance” (Anthony et al 2013). A 2014 Swedish study reported that adopting once-a-week screening of all neonates can reduce time from admission to detection by eight days and lead to a substantial reduction in secondary cases and clinical infections (Rybezynska et al 2014). Researchers in the USA determined that the rate of colonisation by antibiotic-resistant bacteria was low, particularly in neonates <7 days old and have recommended that future studies should examine the safety of targeted surveillance strategies focused on older infants (Macnow et al 2013). As a result of the outbreak reported here, and as part of our active surveillance programme, currently all neonates admitted to our NICU are screened for ESBL-producers via a urine or stool sample or rectal swab on arrival. Thereafter, screening is performed if deemed clinically necessary. This contrasts starkly with our practices prior to the outbreak when screening had been
performed only on an ‘as needed’ (and infrequent) basis relating to potential sepsis or maternal ESBL positivity.

Similarly, there is also no consensus with regard to ESBL screening of expectant mothers and, given the mother-to-neonate transmission identified in this Irish outbreak, this may be an area for further research in improvement in reduction of neonatal risk. As a consequence of this outbreak, we conducted a six-week pilot screening programme from June 6th 2013 to July 16th 2013 assessing the prevalence of ESBL-producer carriage. With ethical approval, all women in the third trimester of pregnancy receiving public or private maternity care, who were inpatients or attending any of the outpatient services, were requested to consent for ESBL screening. There was a 100% uptake in the study. In total, 57 women were screened by urine or rectal samples over the six weeks, with only one positive result recorded. Following the pilot study, our current practice is that all pregnant women presenting at their first antenatal appointment have ESBL screening performed via a urine sample.

**Conclusions**

We have learned lessons from our experience of this outbreak of ESBL-producing *E. coli*. In our setting, which may be comparable to many others, once an outbreak was declared, containment and control were achieved via timely closure of the unit to new admissions, staff education, strict adherence to hand hygiene measures with frequent auditing of staff compliance, cohorting infected neonates, screening of all inpatients, enhanced deep cleaning of all equipment within the NICU and utilising of an outbreak management team. We benefited from modification of the antimicrobial policy to move from broad-spectrum antibiotics to those that the outbreak strain was susceptible
to. A multidisciplinary approach was employed incorporating frequent communication with parents while visitor restrictions were enforced. With a deficit in national or international screening guidelines, in the context of a rising national ESBL-producer prevalence in Ireland, both of our screening practices as described here continue, perhaps screening inappropriately large numbers of healthy young women with a low positive predictive value and all NICU admissions, but to date we have not had further ESBL outbreaks on any of our antenatal or postnatal wards due to appropriate early infection control management of newly identified ESBL patients. We hope that others may learn from our experience of successfully managing a neonatal ESBL outbreak.
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Ethical approval
Ethical approval was obtained from the Ethics Committee of the Mid-West Regional Hospital Limerick.

Consent for publication
Not applicable.

Availability of data and materials
No additional data are available.

Competing interests
None.
Authors’ contributions

All authors contributed to this work, read and approved the manuscript.

CO’C: managed the outbreak clinically and drafted the manuscript.

RKP: managed the outbreak clinically and recognised the novelty of the outbreak.

JK: managed the outbreak clinically.

LP: managed the outbreak clinically.

NO’C: managed the outbreak clinically, recognised the novelty of the outbreak and drafted the manuscript.

AO’G: implemented the infection control procedures.

BS: implemented the infection control procedures.

JP: implemented the infection control procedures.

CF: implemented the infection control procedures.

NW: performed laboratory analysis.

JT: performed laboratory analysis.

EMcG: performed laboratory analysis.

CPD: recognised the novelty of the outbreak and drafted the manuscript.

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A case of Panton-Valentine leucocidin toxin-positive *Staphylococcus aureus*-mediated neonatal mastitis.

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**Introduction**

*Staphylococcus aureus* is a commensal skin organism, a major human and veterinary pathogen and is the most common cause of human mastitis (Osterman and Rahm 2000, Peton and Le Loir 2014, Rasigade and Vandenesch 2014, Uhlemann *et al* 2014). As such, considerable emphasis has been placed on elucidating the mechanisms by which the virulence of *S. aureus* strains is mediated (Rasmussen *et al* 2013, Sollid *et al* 2014). Amongst these mechanisms are the horizontal transfer of the chromosomal cassette conferring resistance to methicillin (SCCmec) (Nubel *et al* 2008) and ability to produce toxins such as (but not limited to) PVL-mediating tissue necrosis (Labandeira-Rey *et al* 2007, Diep *et al* 2010). In the context of maternal and infant health, many studies have described the incidence and transmission of community-associated and healthcare-associated strains, in addition to recommended antibiotic regimens, invasive and non-invasive management, and prevention strategies including decolonisation (Saiman *et al* 2003, Fortunov *et al* 2007, Alsubaie *et al* 2012, Jahanfar *et al* 2013, Sheffield 2013).

With respect to mastitis specifically, most clinical reports of mastitis relate to the incidence and treatment of *S. aureus* maternal infection (Branch-Elliman *et al* 2012, Chick *et al* 2012, Iatrakis *et al* 2013, Perez *et al* 2013a) and, to a lesser degree, subsequent transmission of infection to (mostly pre-term and neonatal) infants (Behari *et al* 2004, Gastelum *et al* 2005, Al Ruwaili and Scolnik 2012, Kayiran *et al* 2014). Mastitis in children is less common and is largely related to neonates and pubescent/post-pubescent age groups (Faden 2005). A case series of neonates demonstrated that mastitis occurred most commonly in the fourth and fifth weeks of life, although age
ranged from 12 to 45 days, and that parenteral antibiotics eradicated causative microorganisms (Stricker et al 2005). A larger review of neonatal cases from 2005 to 2011 (in Atlanta, USA) concluded that *S. aureus* was the most common cause of mastitis and that no infant with a positive breast culture had a concordant positive culture elsewhere (Montague et al 2013), arguing that urine, blood and spinal fluid cultures are unnecessary in otherwise well, afebrile infants with mastitis.

PVL-positive clones of *S. aureus* in Ireland are increasing with the number of PVL-positive MRSA isolates rising from 0.2 to 8.8 % between 2002 and 2011 (Shore et al 2014). Despite this, we believe this report to be the first description of neonatal mastitis caused PVL-bearing *S. aureus* and its successful treatment and, as such, will make a contribution to the emerging debate regarding the management of neonatal mastitis (Al Ruwali and Scolnik 2012).

**Methodology**

A retrospective review of previous microbiology results and medical records was performed. Radiology and theatre operative notes were also examined.
Case report

A 20-day-old female infant was admitted to University Hospital Limerick (UHL) on 16 December 2013. She was born at 41 weeks gestation via spontaneous vaginal delivery. At day seven of life, her mother noted that the baby’s right breast was swollen and red. When it failed to settle spontaneously, she brought the baby to her general practitioner who diagnosed right breast mastitis and commenced what proved to be ineffective antimicrobial therapy with oral amoxicillin. The right breast continued to increase in size and the infant’s condition deteriorated, with poor feeding, irritability, lethargy and vomiting. The infant’s parents self-referred to the hospital. On arrival at UHL, examinations revealed discrete areas of erythema suggestive of mastitis on both breasts, more marked on the right. Under the right nipple there was a 4 cm swelling with underlying yellow–green discolouration of the skin. The area was warm and tender to touch, with pain elicited on superficial palpation. There was no obvious pointing over the right abscess. A 1.5 cm swelling was identified under the left nipple with no associated topical changes. The infant was diagnosed with bilateral mastitis, complicated on the right side with early abscess formation. She was apyrexial.

Haematological investigations demonstrated a normal white cell count (16.24 x 10⁹ l⁻¹), elevated neutrophils (8.48 x 10⁹ l⁻¹), mild thrombocytosis (427 x 10⁹ l⁻¹) and normal haemoglobin count (14.4 x 10⁹ l⁻¹). Renal function was normal. CRP was raised at 11 mg l⁻¹. Blood cultures were not completed, while urine was free of pathogens. A superficial swab of the right breast abscess resulted in growth of coagulase-negative staphylococci, but too few to enumerate accurately. Initially, the neonate was treated
conservatively with intravenous flucloxacillin (220 mg four times per day) and IV benzylpenicillin (220 mg three times per day), but exhibited minimal improvement. At 3 days, a small area of necrotic skin developed on the right breast in the periareolar area. IV benzylpenicillin was discontinued, intravenous flucloxacillin was continued and oral clindamycin (25 mg four times per day) was introduced, as (due to increasing prevalence as described above) there was clinical suspicion of PVL toxin-mediated staphylococcal infection.

Subsequently, at 5 days, a 5 ml aspirate was surgically removed from the infant’s right breast abscess, which proved positive for \textit{S. aureus} resistant to amoxicillin and gentamicin and sensitive to flucloxacillin, levofloxacin, linezolid, rifampicin, vancomycin and tetracycline. Intraoperative examination under anaesthesia confirmed a contralateral left breast abscess from which a 2 ml \textit{S. aureus} positive aspirate was removed.

Post-operative antimicrobial therapy involved oral clindamycin and intravenous flucloxacillin. Both isolates of \textit{S. aureus} subsequently tested positive for PVL toxin. Antimicrobial therapy was modified to oral clindamycin for a further 7 days, at which point full resolution had been achieved.

Subsequently, staphylococcal protein A (Spa) typing identified the isolate as being t005 (EMRSA-15) (http://spa.ridom.de/spa-t005.shtml), which has been reported mostly in Europe (including the UK and Ireland), ranked ninth in 2010 with respect to relative incidence (Grundmann \textit{et al} 2010).
Discussion

Reports of neonatal mastitis are relatively uncommon (Stauffer and Kamat 2003) but most frequently (ca. 85 %) caused by *S. aureus* (Holmes and Zadoks 2011). As such, there is some debate regarding best practice in its management (Al Ruwaili and Scolnik 2012), but recommended treatment involves antibiotic therapy followed by surgical incision and drainage or needle aspiration if medical management fails (Sloan and Evans 2003).

In this case, we describe the first incidence of PVL-positive *S. aureus*-caused neonatal breast abscess. Risk factors for neonatal acquisition of PVL positive *S. aureus* are similar to those for other *S. aureus* strains, i.e. ingestion of infected breast milk, impaired skin integrity, or infection of breast tissue (engorged due to maternal hormones) via the nipple (Stauffer and Kamat 2003). The long-term consequences of neonatal mastitis and its treatment were reported by Panteli *et al* (2012), whereby development of the breast was characterised as involving intraductal dilatation, fibrous elements and calcifications or, where surgical incision was required, breast asymmetry and significant reduction in size compared with the uninfected breast. The emergence of PVL toxin-positive *S. aureus* (Labandeira-Rey *et al* 2007, Rasmussen *et al* 2013, Shore *et al* 2014) is adding a new perspective to neonatal mastitis incidence due to its ability for tissue necrosis and the potential damage that this may cause to infected infants (Hsieh *et al* 1999, Nazir 2005).

In this case, specifically, the neonate was breastfed successfully for four days postpartum and subsequently switched to bottle-feeding for convenience. The baby’s
mother did not have any underlying recurrent infective skin or soft tissue infections. This family had not been washing domestic items such as towels used for this neonate separately to those in use for other members of the family. During the course of the neonatal infection no other family member presented with signs or symptoms of a PVL-associated infection. Vaginal carriage of *S. aureus* is possible; however, samples obtained from high vaginal and groin areas proved negative. The potential for zoonosis (Holmes and Zadoks 2011) was eliminated as the family did not have a household pet and did not live on a farm, and there was no other direct contact with animals. As this was an isolated incident, environmental (i.e. delivery room and equipment) and staff screening was considered unnecessary.

The vigilance of the infant’s parents and the relatively early identification of PVL-positive *S. aureus* meant that appropriate targeted (rather than empiric) antimicrobial therapy resulted in eradication of the pathogen. The revision of therapy was influenced in no small part by the availability of aspirated pus, rather than topical swabs, which underwent on-site molecular and conventional susceptibility testing, as described elsewhere (O’Connor *et al* 2014), which reduced time to diagnosis by at least 24 h through avoidance of culture on non-selective solid media. It is debatable whether non-invasive assessment such as sonography would have been useful in this case, although its use has been described in differentiating between neonatal abscesses and mastitis (Borders *et al* 2009)
Conclusion

Neonatal PVL toxin-positive *S. aureus* mastitis and breast abscess formation is uncommon but suspicion should arise with poor response to conventional antimicrobial therapy for mastitis. There is a need to improve awareness of PVL-positive *S. aureus*, particularly among those caring for neonates, as early diagnosis can prevent complications of invasive tissue necrosis and potential for long-term consequences such as abnormal breast development. Sending appropriate samples for testing can expedite diagnosis.
CHAPTER 6

Incidence, management and outcomes of the first $cfr$-mediated linezolid resistant *Staphylococcus epidermidis* outbreak in a tertiary referral centre in the Republic of Ireland.

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Incidence, management and outcomes of the first *cfr*-mediated linezolid resistant *Staphylococcus epidermidis* outbreak in a tertiary referral centre in the Republic of Ireland.

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Summary

Aim

To report the first Irish outbreak of cfr-mediated linezolid-resistant Staphylococcus epidermidis.

Methods

Linezolid-resistant S. epidermidis isolated at University Hospital Limerick from four blood cultures, one wound and four screening swabs (from nine patients) between April and June 2013, were characterised by pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and staphylococcal cassette chromosome (SCCmec) typing. Antibiotic susceptibilities were determined according to the guidelines of the British Society for Antimicrobial Chemotherapy. The outbreak was controlled through prohibiting prescription and use of linezolid, adherence to infection prevention and control practices, enhanced environmental cleaning, isolation of affected patients, and hospital-wide education programmes.

Findings

PFGE showed that all nine isolates represented a single clonal strain determined. MLST showed that they belonged to ST2; and SCCmec typing showed they encoded a variant of SCCmecIII. They were cfr-positive; eight were positive for the G2576T 23S rRNA mutation commonly associated with linezolid resistance. Isolates exhibited multiple antibiotic resistances (i.e., linezolid, gentamicin, methicillin, clindamycin, ciprofloxacin, fuscidic acid and rifampicin). The adopted infection prevention proved effective, with the outbreak limited to the affected intensive care unit.
Conclusions

This is the first documented outbreak of *cfr*-mediated linezolid-resistant *S. epidermidis* in the Republic of Ireland. Despite this, and due to existing outbreak management protocols, the responsible microorganism and source were identified efficiently. However, it became apparent that staff knowledge of antimicrobial susceptibilities and appropriate hygiene practices were lacking and that educational interventions (and reinforcement) are necessary to avoid occurrence of antimicrobial resistance and outbreaks such as those reported here.

**Keywords**

First outbreak, Ireland, oxazolidinone resistance, multi-resistance, 23S rRNA methyltransferase, plasmid-mediated, staphylococci.
Introduction

Linezolid is a bacteriostatic oxazolidinone antibiotic that binds to the 50S subunit of bacterial ribosomes and inhibits protein synthesis (Boak et al 2014). It is licensed for use in 70 countries worldwide, and has been used to treat over four million patients since its introduction in 2000 (Leach et al 2011). Linezolid is currently approved for use in the Republic of Ireland for treatment of multi-drug resistant Gram-positive infections including nosocomial and community acquired pneumonia and skin and soft-tissue infections, including those caused by methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant S. aureus (MRSA), coagulase negative staphylococci (CoNS) and vancomycin-resistant Enterococci (VRE).

Recent surveillance data indicate <1% of S. aureus and 2% of CoNS are resistant to linezolid (Jones et al 2007, Jones et al 2009, Ross et al 2011). Mutations in chromosomal genes encoding the central loop of domain V of the 23S rRNA, with the G2576T substitution are the most frequently reported resistance mechanism (Hong et al 2007). Substitutions for T2500A, T2504A and G2215A have also been identified in some staphylococci from clinical infections, as have mutations in the genes for ribosomal proteins L3, L4 and L22 (Wolter et al 2005, Mendes et al 2010a). In contrast with mutational resistance, the cfr (chloramphenicol-florfenicol resistance) gene encodes a transferable 23S rRNA methyltransferase conferring resistance to linezolid (Shaw and Barbachyn 2011). The cfr gene encodes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antimicrobials (the so-called PhLOPS_A phenotype) and selected macrolides, so conferring multi-drug resistance (Kehrenberg et al 2007). Cfr-positive isolates pose an emerging global
health threat (LaMarre et al 2013) and prompt recognition of this pattern of resistance is needed to prevent the establishment of reservoirs of cfr-positive bacteria in skin and gut flora (Mendes et al 2010b).

Outbreaks of cfr-mediated linezolid-resistant S. aureus (Morales et al 2010, Baos et al 2013) and Enterococcus faecalis (Diaz et al 2012) have been described previously. However, in this paper we describe the molecular epidemiology, management and outcomes of the first documented outbreak of cfr-mediated linezolid-resistant S. epidermidis in the Republic of Ireland.

Methods

Setting

University Hospital Limerick (UHL) is a tertiary referral university teaching hospital with 483 inpatient beds. Patients are admitted from the community and from other hospitals located in the Mid-West of Ireland. The catchment population of the hospital is 400,000. The intensive care unit (ICU) is a medical-surgical unit, which caters for patients over the age of 16 years. At the time of this outbreak, the intensive care unit was a 7-bedded unit (including two isolation rooms). There were three handwashing stations located within the unit, with alcohol hand gels at each bedspace. There were two full-time consultant microbiologists, and two infection prevention and control nurses, employed directly by UHL and working on-site at the time of the outbreak.
The index case for this outbreak was identified as a male in his twenties admitted to UHL in April 2013 following a deliberate self-poisoning. He was diagnosed with an aspiration pneumonia, and antimicrobial therapy was commenced with piperacillin-tazobactam 4.5g TDS intravenous (IV) and clarithromycin 500mg BD IV. *S. aureus* was recovered from peripheral blood samples taken on presentation. A chest x-ray performed demonstrated evidence of a right lower and mid-zone dense consolidation consistent with an aspiration pneumonia. Antimicrobial therapy was modified to flucloxacillin 2g QDS IV, linezolid 600mg BD IV and ciprofloxacin 500mg BD IV. Linezolid was added lest the isolate was proven to be a toxin producer (i.e. Panton–Valentine leucocidin (PVL) positive).

Following 5 days of linezolid therapy, this antimicrobial was stopped as the PVL toxin result, which was sent to a referral laboratory, was negative. Blood samples were taken from a femoral line during a subsequent pyrexial episode and proved positive for CoNS. Paired peripheral blood cultures were not taken at the same time. Routine antimicrobial susceptibility testing demonstrated resistance to linezolid, flucloxacillin and gentamicin, but sensitivity to vancomycin and daptomycin. This isolate was sent to the Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit (AMRHAI), London, UK. The index case was moved into an isolation room within the ICU. The AMRHAI results confirmed *cfr*-positive linezolid-resistant *S. epidermidis*, which also harboured the G2576T mutation (Gu et al 2013) and following confirmation of this result the prescription of linezolid for all other patients within the hospital was prohibited.
Infection control measures

This first case of linezolid resistance coagulase negative staphylococci at UHL was a major cause for concern, in particular as colleagues at AMRHAI had published an alert regarding cfr-mediated resistance which advised of the public health threat associated with same. As a consequence an outbreak management protocol was initiated that involved meeting with all key stakeholders, including executive management, nursing administration, infection prevention and control, consultant microbiologists, laboratory managers, bed management, hygiene services and the communications team. Contact tracing of all inpatients, who may have been in contact with the index case patient while in ICU, was conducted by screening groin and axilla swabs to identify linezolid-resistant CoNS. All CoNS isolates from samples collected from patients who had shared the ICU with our index case, or who had occupied that space at any time up to 14 days after the index case was confirmed as positive, were screened for susceptibility to linezolid.

All affected patients in this outbreak were immediately isolated and standard contact precautions employed. Patients harbouring linezolid-resistant CoNS- were given daily whole-body washes with 2% chlorhexidine gluconate. All patients in the ICU who were fit for discharge were cohorted to one ward to minimise cross-transmission. Any suspect cases that subsequently arose were promptly isolated pending confirmatory results from the laboratory. All patients had axillae and groin screens performed weekly until discharged. Enhanced cleaning of the ICU was instigated in parallel with increased auditing. This involved twice-daily cleaning of affected areas with detergent in addition to a ‘deep clean’, with sodium hypochlorite, to “decontaminate” the area.
on discharge. Hand hygiene audits were also performed with greater frequency in affected areas, which involved twice weekly observational audits at ward level.

Screening of staff for carriage of the organism, air sampling and environmental sampling were not performed due to resource limitations. A further factor influencing the decision not to screen staff was that the ICU is not a closed unit (i.e., as a matter of clinical policy, patients admitted to our ICU remain under the care of their primary consultant rather than the ICU team and not the intensive care team) resulting in considerable traffic of medical and surgical teams to the ICU each day. Instead, a targeted educational programme focussed on hand hygiene and appropriate prescribing was implemented.

*Microbiological and molecular detection of linezolid-resistant S. epidermidis*

Linezolid resistance in staphylococci is defined by both the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as a linezolid minimum inhibitory concentration (MIC) of \( \geq 8 \) mg/L, and this threshold was used to define resistance in this outbreak. In total, eighteen bloodstream isolates were assessed retrospectively for linezolid susceptibility and 187 screen specimens (groin and axilla swabs) were processed in the laboratory. Screening samples were inoculated on Staph/Strep agar (Fannin LIP, Galway, Ireland) with a linezolid disk (10μg) (Oxoid Ltd, Basingstoke, UK). These were incubated aerobically at 35 ± 1 °C for 24h. All suspect linezolid-resistant staphylococcal isolates were subsequently identified using MALDI-TOF MS (Bruker Daltonics, Germany) as described previously (O’Connor et al 2014). MICs of
linezolid were determined using Etest (Etest, bioMérieux, France). All isolates with an MIC of linezolid of ≥8mg/L, as per EUCAST guidelines in use at the time in our laboratory (Mendes et al. 2014, Flamm et al 2013) were referred to AMRHAI for antimicrobial susceptibility testing by agar dilution methodology. Isolates were characterised by SCCmec, pulsed-field gel electrophoresis and multi-locus sequence typing as described previously (Ellington et al 2009, Thomas et al 2007). Isolates were screened by PCR for the cfr gene (Long et al 2006) and by PCR and restriction fragment length polymorphism analysis for the G2576T 23S rRNA mutation associated with linezolid resistance.

**Results**

**Demographics of affected patients**

Between April and June 2013, nine patients (five male, four female) were found to be harbouring linezolid-resistant *S. epidermidis*. The nine affected patients ranged in age from 28 to 83 years. All patients were admitted to UHL via the Emergency Department (ED), and were not transfers from other hospitals. One patient (the index case) was admitted under a medical service. The other eight patients had complex surgical admissions including small and large bowel obstructions, urosepsis secondary to calculus, abdominal aortic aneurysm repair and colorectal malignancy. Eight patients who were deemed to have been contacts of the index case tested positive. Four were identified on screening, and the other four patients were identified from clinical samples (four blood cultures, one wound swab) and their subsequent axilla and groin swabs were positive for linezolid-resistant *S. epidermidis*. CoNS was not isolated from any deep tissue or intraoperative samples. Patient data are shown in Table 1.
Table 1. Epidemiological and microbiological data of nine patients involved in the outbreak. The median age of positive patients was 61 years (range 28-83).

There was one death directly attributed to this outbreak.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Ward</th>
<th>Culture Site</th>
<th>Date of Culture</th>
<th>Linezolid Doses</th>
<th>PHE MIC linezolid</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>M</td>
<td>62</td>
<td>Surgical</td>
<td>Peripheral BC</td>
<td>28/04/2013</td>
<td>112</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient B</td>
<td>M</td>
<td>28</td>
<td>ICU</td>
<td>Femoral line BC</td>
<td>26/04/2013</td>
<td>18</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient C</td>
<td>M</td>
<td>56</td>
<td>ICU</td>
<td>Femoral line BC</td>
<td>10/05/2013</td>
<td>14</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient D</td>
<td>F</td>
<td>65</td>
<td>ICU</td>
<td>Arterial line BC</td>
<td>05/05/2013</td>
<td>12</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient E</td>
<td>M</td>
<td>74</td>
<td>ICU</td>
<td>Wound Swab</td>
<td>19/05/2013</td>
<td>6</td>
<td>≥8</td>
<td>Died</td>
</tr>
<tr>
<td>Patient F</td>
<td>F</td>
<td>76</td>
<td>Surgical</td>
<td>Axilla/Groin Swab</td>
<td>28/05/2013</td>
<td>0</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient G</td>
<td>F</td>
<td>72</td>
<td>Surgical</td>
<td>Axilla/Groin Swab</td>
<td>29/05/2013</td>
<td>28</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient H</td>
<td>F</td>
<td>83</td>
<td>Surgical</td>
<td>Axilla/Groin Swab</td>
<td>29/05/2013</td>
<td>25</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
<tr>
<td>Patient I</td>
<td>M</td>
<td>35</td>
<td>Surgical</td>
<td>Axilla/Groin Swab</td>
<td>03/06/2013</td>
<td>0</td>
<td>≥8</td>
<td>Discharged</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; BC, blood culture; PHE, Public Health England; MIC, minimum inhibitory concentration; M, male; F, female.

Molecular characteristics of linezolid-resistant isolates

All nine isolates were positive for the cfr gene and eight isolates also bore the G2576T 23S rRNA mutation. The linezolid-resistant S. epidermidis strain was detected from four blood cultures: one inpatient on the general surgical ward (from peripheral blood cultures) and three ICU patients (two from femoral line blood cultures and one from arterial line blood culture). All nine isolates represented a single strain as determined by pulsed-field gel electrophoresis (PFGE); by multi-locus sequence typing they
belonged to ST2 and SCC\textit{mec} typing showed that they encoded a variant of SCC\textit{mec}III.

Of the nine patients, only seven had received treatment with linezolid previously. All isolates were resistant to linezolid, gentamicin, methicillin, clindamycin, ciprofloxacin, fusidic acid and rifampicin; teicoplanin resistance was variable. All were sensitive to daptomycin, vancomycin and quinopristin/dalfopristin (Table 2).

Table 2. Antimicrobial susceptibility profiles (minimum inhibitory concentrations, mg/l) from clinical isolates associated with the outbreak.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>PHE MIC</th>
<th>Gent</th>
<th>Ox</th>
<th>Pen</th>
<th>Tei</th>
<th>Van</th>
<th>Clin</th>
<th>Ery</th>
<th>Cip</th>
<th>Moxi</th>
<th>Quin/Dalf</th>
<th>Tet</th>
<th>Dap</th>
<th>Fus</th>
<th>Rif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>M</td>
<td>&gt;8</td>
<td>128</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>4</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td></td>
<td></td>
<td>0.5</td>
<td>8</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient B</td>
<td>M</td>
<td>&gt;8</td>
<td>128</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>4</td>
<td>4</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td></td>
<td></td>
<td>0.5</td>
<td>8</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient C</td>
<td>M</td>
<td>&gt;8</td>
<td>128</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>0.5</td>
<td></td>
<td>&lt;=0.25</td>
<td>16</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient D</td>
<td>F</td>
<td>&gt;8</td>
<td>128</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>0.5</td>
<td></td>
<td>&lt;=0.25</td>
<td>16</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient E</td>
<td>M</td>
<td>&gt;8</td>
<td>128</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>16</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient F</td>
<td>F</td>
<td>&gt;8</td>
<td>256</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>4</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td></td>
<td></td>
<td>0.5</td>
<td>8</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient G</td>
<td>F</td>
<td>&gt;8</td>
<td>256</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td></td>
<td></td>
<td>0.5</td>
<td>8</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient H</td>
<td>F</td>
<td>&gt;8</td>
<td>256</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td></td>
<td></td>
<td>0.5</td>
<td>8</td>
<td>&gt;2</td>
<td></td>
</tr>
<tr>
<td>Patient I</td>
<td>M</td>
<td>&gt;8</td>
<td>256</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>8</td>
<td>2</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>4</td>
<td></td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

PHE MIC, Public Health England minimum inhibitory concentration; Gent, gentamicin; Ox, oxacillin; Pen, penicillin; Tei, teicoplanin; Van, vancomycin; Clin, clindamycin; Ery, erythromycin; Lin, linezolid; Cip, ciprofloxacin; Moxi, moxifloxacin; Quin/Dalf, quinopristin/dalfopristin; Tet, tetracycline; Dap, daptomycin; Fus, fusidic acid; Rid, rifampicin; M, male; F, female.
Discussion

This cfr-mediated linezolid-resistant S. epidermidis outbreak caused great alarm to all UHL staff and was the first such outbreak in the Republic of Ireland. For all clinical microbiologists, CoNS identified in blood cultures are problematic as their clinical significance can be challenging to interpret. It is not unusual for these samples, in our institution as in other centres, to be less than fully characterised with regard to species identification and/or antimicrobial susceptibility testing unless the patient is in critical care or has prosthetic material in situ. This poses an important question as to whether, generally, there may be under-ascertainment of linezolid-resistant CoNS and whether CoNS may be acting as a reservoir of cfr-mediated resistance in our hospitals.

Previous research has demonstrated that the cfr-gene is capable of horizontal transfer between staphylococci (Kehrenberg et al 2007). In Europe, cfr was initially identified on a plasmid from a bovine isolate of S. sciuri in 2000 (Schwarz et al 2000); its occurrence among animal isolates appears to have been influenced by the use of florfenicol in the veterinary industry. Worldwide, the incidence of linezolid-resistant CoNS is currently 28 times that of linezolid-resistant S. aureus (Gu et al 2013).

Clinical reports appear to first date from Toh et al 2007 reported the first cfr-mediated linezolid-resistant clinical isolate of MRSA. Since then, notable reports have included two USA-based cases of cfr-mediated resistance in clinical isolates of S. epidermidis and S. aureus in 2008 (Mendes et al 2008), a 2013 case report of cfr-mediated linezolid resistant MRSA blood culture isolate from an ICU in Barcelona (Sierra et al 2013).
and characterisation of \( cfr \)-mediated MRSA and \textit{S. haemolyticus} with fatal outcomes originating from a German group (Fessler \textit{et al} 2014).

A report detailing a 2008 outbreak of \( cfr \)-mediated linezolid-resistant \textit{S. aureus} (LRSA) in Madrid (Sánchez García \textit{et al} 2010) was particularly similar to our experience during this outbreak, affecting 12 high-risk critically ill ICU patients; two patients infected with LRSA and 3 colonised with LRSA died. The Spanish ICU was not closed to admissions during their 17-week outbreak. Measures to control the outbreak included isolation with contact precautions and restriction of linezolid prescription, as were employed at our hospital. It would have been interesting to compare with our data, but no comment is made in the Spanish paper regarding linezolid consumption prior to their outbreak. However, at UHL, linezolid use has been steadily increasing over the past 2 years. Antimicrobial inpatient consumption at UHL is collated on a quarterly basis with data reported as defined daily doses/100 bed days used (DDD/100BDU). Between 2011 and 2012, there had been a 3\% increase in the prescription of IV linezolid and a 28\% increase in oral (PO) linezolid. Between 2012 and 2013 (when this outbreak occurred), the use of IV linezolid had increased by 11\%. Despite the increases in the use of IV linezolid in UHL between 2011 and 2013, the median level of use in this hospital during that time period was consistently lower than that of other Irish hospitals categorised as having a similar patient mix.
In the UHL outbreak, the authors were unable to perform environmental or staff screening. In the Madrid outbreak, no staff members were found to be colonised with linezolid-resistant *S. aureus*, but 15 of 91 (17%) swabbed environmental surfaces were contaminated by linezolid-resistant *S. aureus* and may have contributed to the prolonged nature of the outbreak. At UHL, staff knowledge of the mechanisms by which antimicrobial resistance emerges was lacking, and an awareness of the transmissibility of Gram-positive organisms via contaminated HCW hands and equipment such as stethoscopes, blood pressures cuffs and intravenous drip stands, etc. was poor. Subsequently, human and financial resources were re-directed to a hospital-wide education programme for all healthcare staff, in liaison with the antimicrobial pharmacist, which targeted management, nursing, medical, household and administration staff. Twelve months since this outbreak, regular education sessions continue to be provided to all staff and a specific lecture on the local antimicrobial resistance patterns is provided to all medical and surgical trainee doctors during induction. Between July 2013 and June 2014, no further instances of *cfr*-mediated linezolid resistance have been identified.

**Conclusions**

This is the first report of *cfr*-mediated linezolid resistance in *S. epidermidis* in the Republic of Ireland. Linezolid is a relatively novel agent and, therefore, an outbreak was unexpected but, in retrospect, was inevitable given the increasing consumption of linezolid in our institution. As an organisation, we have learned from this outbreak and our practice has changed accordingly. Linezolid susceptibility is monitored in all CoNS for ICU patients using a linezolid disk-based process. The judicious use of
linezolid with consultant-only prescribing, the application of strict infection control measures and the isolation of all patients from this outbreak when they have subsequently re-presented for outpatient appointments or for admission, enhanced daily environmental cleaning, a low threshold for characterising CoNS identified in samples such as blood cultures and wound swabs from critical care areas and other high risk hospital patients, and the presence of a visible antimicrobial stewardship team on the hospital wards have been and will continue to be essential for the preservation of linezolid as a valuable therapeutic agent.
Conflicts of interest

None.

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None.

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CHAPTER 7

A case of fatal daptomycin-resistant, vancomycin-resistant enterococcal infective endocarditis in end-stage kidney disease.

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A case of fatal daptomycin-resistant, vancomycin-resistant enterococcal infective endocarditis in end-stage kidney disease.

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Abstract

Introduction

Ireland currently has the highest reported rate in Europe of vancomycin-resistant Enterococcus (VRE) isolated from the bloodstream, but data regarding the prevalence of VRE endocarditis remain scarce. Treatment options for enterococcal-mediated endocarditis are limited and, therefore, daptomycin is commonly used off-license in that setting.

Case

60 year old male with end-stage kidney disease (ESKD) presented with VRE bacteraemia secondary to a gangrenous right foot colonised with VRE. Aortic valve endocarditis was confirmed using transoesophageal echocardiography. Treatment was commenced with linezolid and subsequently modified to combination therapy with daptomycin and rifampicin. High-dose daptomycin therapy was employed unsuccessfully and fatal daptomycin resistance emerged after 20 days of therapy.

Conclusion

The case was ethically challenging and involved a refusal of amputation and, ultimately, any form of treatment by the patient. In summary, however, daptomycin-resistant VRE bacteraemia complicated by daptomycin-resistant VRE endocarditis proved fatal for this patient. Further evaluation of efficacy and safety of high-dose daptomycin for the treatment of VRE infective endocarditis is needed.

Keywords: vancomycin-resistant Enterococcus (VRE), daptomycin, rifampicin, resistance, end-stage kidney disease (ESKD).
Introduction

Enterococci are Gram-positive organisms that form part of the commensal gut flora. A common nosocomial pathogen, enterococci can reside and survive in the clinical environment for prolonged periods with an associated risk of contaminating healthcare workers and the potential for cross-transmission to patients (Biedenbach et al 2004). Enterococci are the second most common healthcare-associated bloodstream pathogen isolated in the United States (US) with the prevalence of VRE in intensive care units (ICUs) currently exceeding 30% (Wisplinghoff et al 2004). Enterococcus faecalis and Enterococcus faecium are the predominant pathogens associated with VRE infections (Carmeli et al 2002). Ireland has the highest proportion of VRE bloodstream isolates in Europe, as reported to the European Centre for Disease Prevention and Control (ECDC). In 2013, 176 VRE were identified among 408 E. faecium isolates from bloodstream infections in Ireland (43.1%). VRE results in significant increases in length of hospital admission and mortality (DiazGranados et al 2005).

For patients aged ≤60 years, enterococci account for up to 10% of cases of native valve infective endocarditis, rising to almost 20% in those over 60 years. In prosthetic valve endocarditis, 5–10% of cases that occur in the first 60 days post-operatively and up to 15% of cases occurring after 60 days are secondary to enterococcal infection (Mylonakis and Calderwood 2001). The first case of VRE infective endocarditis was reported in 1996 (Bishara et al 1999), but subsequent reports in the literature have been rare (Forrest et al 2011). A study involving a review of 107 cases of enterococcal endocarditis noted no cases secondary to vancomycin resistance (McDonald et al 2005). Haemodialysis patients and renal transplant recipients are at relatively high risk of VRE infective endocarditis (Humphreys et al 2004), and rates of intestinal VRE
colonisation have been shown to be higher in these groups than in other patient populations (Patel et al 2001).

There are limited effective antimicrobial treatment options for VRE infections including infective endocarditis. Daptomycin is a concentration-dependent bactericidal lipopeptide antibiotic, licensed in the Republic of Ireland for the treatment of skin and soft tissue infections. It is also licensed for use in the management of *Staphylococcus aureus* bacteraemia, including right-sided infective endocarditis, at doses of 4 and 6 mg/kg of body weight, respectively (Boucher and Sakoulas 2007). Dose adjustment is required for patients with renal impairment. It is used off-label in Ireland to treat patients infected with VRE. It is excreted renally (Hawkey 2008) and is rapidly bactericidal (Mascio et al 2007), and had demonstrated in vitro concentration-dependent killing with a half-life of 8–9 hours (LaPlante and Rybak 2004). The drug acts on the cell membrane causing membrane depolarisation and also inhibits the synthesis of lipoteichoic acid, which is necessary for cell wall synthesis (Enoch et al 2007). Daptomycin displays in vitro activity against >90 % of *Enterococcus* spp., including those resistant to other antibiotics, such as vancomycin, linezolid and quinupristin-dalfopristin (Sader and Jones 2009). Thickening of the cell wall associated with vancomycin intermediate-susceptible *S. aureus* isolates has been shown to decrease susceptibility to daptomycin and, while an exact resistance mechanism in enterococci has not yet been determined, a similar mechanism secondary to reduced daptomycin diffusion through a thickened enterococcal cell wall has been proposed (Cui et al 2006, Kelley et al 2011).
To the best of our knowledge, this is the first report of daptomycin-resistant VRE aortic valve infective endocarditis complicated by end-stage kidney disease (ESKD) in Ireland and one of only nine reports of daptomycin-resistant infective endocarditis globally based on a Pubmed search completed in July 2015. In that search, no reports of such infective endocarditis complicated by kidney disease could be found.

Methodology

A retrospective review of previous microbiology results and medical records was performed. Radiology and theatre operative notes were also examined.

Case Report

The right big toe of a 60-year-old Irish male with ESKD secondary to diabetic nephropathy on haemodialysis became gangrenous secondary to peripheral vascular disease, and he underwent a toe amputation in May 2010. Samples taken from the wound were determined to be positive for VRE (E. faecium) and a surveillance rectal swab detected that the patient was VRE colonised. He was commenced on a 5-week course of IV linezolid 600 mg twice daily followed by 7 days of oral linezolid 600 mg twice daily with limited resolution. A right mid-foot amputation was performed in September 2010 followed by wound debridement in October 2010. The history of this infected foot dated back to 2008 when he had his first episode of cellulitis requiring antimicrobials. With each progressively worsening foot infection, the patient was very reluctant to undergo any surgical procedure to remove the nidus of infection, necessitating prolonged antimicrobial exposure. Between October 2009 and January 2010, a 16-week admission occurred for a Klebsiella pneumoniae and Candida albicans continuous ambulatory peritoneal dialysis-associated peritonitis requiring a
protracted prescription of broad-spectrum bacterial and fungal cover. Ultimately, the patient was commenced on haemodialysis.

On 15 July 2011, he was admitted from the dialysis unit with sepsis. Blood samples were taken from the dialysis permacath, which were positive for VRE. Laboratory testing again demonstrated the presence of \textit{E. faecium}, demonstrating the same antibiogram as the earlier VRE cultured in 2010. He was commenced on intravenous (IV) linezolid 600 mg twice daily. A transthoracic echocardiogram was performed, which did not show any vegetations suspicious for infective endocarditis. Consecutive blood cultures, 48 hours apart, demonstrated evidence of continuous VRE bacteraemia. Sterility of blood was achieved for 22 days until a set of blood cultures taken in early August 2011 again yielded VRE. A transoesophageal echocardiogram identified vegetations on the aortic valve. A decision was made to switch from linezolid 600 mg twice daily to daptomycin 6 mg/ kg IV administered following dialysis sessions. Seven days later, with blood cultures still VRE positive, rifampicin 600 mg twice daily IV was added for additional synergistic bactericidal activity as the patient also had an ongoing active foot infection, for which source control had not been achieved. An interdialytic daptomycin dosing regimen was commenced with daptomycin at 8 mg/kg, 8 mg/kg and 10 mg/kg administered post-dialysis at 48, 48 and 72 hours, respectively (following consultation with the suppliers of the antimicrobial agents and a review of available literature) (Salama \textit{et al.} 2009). The patient consistently declined amputation of his right leg during his final admission. Following 17 days of therapy (29 August 2011), we noted daptomycin resistance (MIC >8 mg/l) during routine in-house susceptibility testing.
In early September 2011, with worsening necrosis and gangrene of his right leg, confirmed aortic valve endocarditis, unresolving VRE bacteraemia and rising CRP, together with refusal to consent to a right leg amputation, the patient undertook his own discharge from hospital with rifampicin 600 mg twice daily orally and daptomycin 6 mg/kg IV once daily to be administered intravenously in the community. He continued to deteriorate with persistent VRE bacteraemia. Dialysis was subsequently withdrawn following consultation with the patient and his family, as there was consensus that, given ongoing sepsis from multiple sources, the patient was too unwell to continue with haemodialysis. Antimicrobial therapy also ceased at this time. Palliative care services reviewed the patient and he was admitted to a local hospice where he died 2 weeks later.

**Discussion**

Patients undergoing outpatient haemodialysis are at high risk of VRE acquisition due to repeated close contact between patients in the dialysis unit, repeated exposure of patients to antimicrobials including vancomycin for treatment of dialysis line infections (at least in Ireland), shared transport to dialysis units and frequent hospital admissions (Kee *et al* 2012). The reliance on central catheters in the dialysis population is thought to be a key factor contributing to the rise of resistant enterococcal isolates (Boucher *et al* 2009). To place this case in context, at the time of writing, the UHL dialysis unit is attended by 67 patients of whom 37 are VRE positive. A further 78 attend a satellite dialysis unit in the nearby city centre of whom 11 are known to be VRE positive.
Mortality rates in patients with VRE bloodstream infections are high, ranging between 20 and 46 % (Han et al 2009, McKinnell et al 2011, Twilla et al 2012), and bacterial endocarditis in dialysis patients is associated with poor prognosis (Leither et al 2013). Surgical management of VRE infective endocarditis is rarely employed, as most patients have significant co-morbidities that would prohibit such invasive intervention (Salgado and Farr 2003). As a consequence, medical management is the preferred treatment approach. The use of daptomycin as monotherapy for the treatment of VRE infective endocarditis is not recommended due to increased risk of resistance (Linden 2007, Schulte et al 2008). Combination therapy of daptomycin with another antimicrobial such as rifampicin, gentamicin, linezolid or a beta-lactam for treatment of VRE endocarditis (Cerón et al 2014) is recommended. Similarly, synergistic therapy with daptomycin and rifampicin has been advocated (Leclercq et al 1991). However, the addition of rifampicin 600 mg twice daily orally proved ineffective in our patient.

Various mechanisms for enterococcal daptomycin resistance have been described including altered cell membrane composition, altered ability of daptomycin to depolarise the cell (Steed et al 2011) and the risk of gene transfer of daptomycin-resistant determinants (Kelesidis et al 2011, Diaz et al 2014). Concern has also been raised as to whether a tendency to provide empiric VRE cover to at-risk patients with daptomycin and linezolid, while awaiting final culture results, may also be contributing to the development of resistance secondary to overuse (Short et al 2014).
The appropriate dose of daptomycin for VRE infective endocarditis has not been defined but, as in vivo and in vitro studies have revealed that using higher doses of daptomycin increases the degree and speed of bactericidal activity due to its concentration-dependent pharmacodynamic mechanism, higher doses than recommended for *S. aureus* bacteraemia (6 mg/kg/day) have been suggested (Cunha *et al* 2007, Hall *et al* 2012). At 6 mg/kg/day, sterility of blood cultures was not achieved. The use of daptomycin at higher doses of ≥8 mg/kg/day (Kullar *et al* 2011, Kullar *et al* 2013) and 14 mg/kg/day (Moise *et al* 2009) for the treatment of infective endocarditis have been demonstrated with no adverse patient outcomes. Reported cases of daptomycin resistance have been associated with complicated infections (e.g. osteomyelitis, medical device infections and endocarditis) at doses of ≤6 mg/kg/day (Kelesidis *et al* 2011). Our attempt at using intradialytic daptomycin at 8 mg/kg, 8 mg/kg and 10 mg/kg post-dialysis at 48, 48 and 72 hours, respectively, also failed. There was no rise in creatinine phosphokinase levels with daptomycin therapy over 20 days, which was a positive finding given that rising creatinine phosphokinase levels can often prohibit the use of daptomycin, particularly in those with renal impairment.

We are unaware of any randomised controlled trials that have evaluated the management of VRE infective endocarditis. Linezolid is a bacteriostatic antimicrobial licensed by the US Food and Drug Administration for treatment in this setting, but cure rates remain disappointing (Birmingham *et al* 2003, Chuang *et al* 2014) and resistance rates of 20 % have been reported (Pogue *et al* 2007). The side-effect profile of linezolid with associated myelosuppression, including neutropenia, thrombocytopenia and anaemia (Hachem *et al* 2003) is particularly problematic in the dialysis population. Tigecycline has been used in combination with other agents
for the treatment of vancomycin-resistant *E. faecium* infections, but it achieves low serum levels and reports of its use are scarce (Florescu *et al* 2008, Schutt and Bohm 2009).

**Conclusion**

In conclusion, VRE infective endocarditis is a very uncommon HCAI but is clinically challenging when it arises, given the lack of licensed treatment options. In this case, the same *E. faecium* isolate persisted from multiple sites despite antimicrobial therapy, having probably initially been a rectal coloniser. High-dose daptomycin is generally well tolerated (and we observed no adverse effects in our patient). Ultimately, without amputation and definitive source control, this patient was unlikely to clear his VRE bacteraemia, affording some potential to cure his infective endocarditis. The ethical issues associated with this case are multi-faceted and deserve discussion elsewhere. The rapid emergence of daptomycin resistance following only 20 days of therapy should heighten the awareness of other centres to this possibility in complicated VRE infections. No linezolid resistance was observed in this case.

We continue to monitor for resistance in all patients receiving treatment with daptomycin. The prescription of daptomycin within our institution remains restricted, and is permissible following consultation with clinical microbiology or infectious diseases staff only. To date, no further cases of VRE infective endocarditis or daptomycin resistance have been identified in our institution.
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CHAPTER 8

Irrepressible carbapenemase-producing Enterobacteriaceae in the Mid-West of Ireland? A retrospective epidemiological and microbiological review of 140 isolates from 2009 to 2015.

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Irrepressible carbapenemase-producing Enterobacteriaceae in the Mid-West of Ireland? A retrospective epidemiological and microbiological review of 140 isolates from 2009 to 2015.

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Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) are prevalent worldwide in all areas of healthcare with isolates and outbreaks reported in acute care facilities (Bratu et al 2005, Schwaber et al 2011, Lemmenmeier et al 2014) including neonatology and paediatrics (Drew et al 2013, Giuffre et al 2013b), respite/convalescence facilities (Ben-David et al 2011) and LTCF (Lin et al 2013). The main subtypes of CPE identified globally are Klebsiella pneumoniae carbapenemase (KPC), New Dehli metallo-beta-lactamase (NDM), IMI (imipenem-hydrolyzing beta-lactamase) and OXA (oxacillin-hydrolysing).

Carbapenem resistance among common Enterobacteriaceae has increased sharply over the past decade. Data from the Centers for Disease Control and Prevention (CDC) published in 2013 highlighted the rising prevalence of CPE in the USA from 1.2% in 2001 to 4.2% in 2011 (Centers for Disease Control and Prevention 2013b). The rising CPE prevalence is emerging amidst an increasing global reliance on carbapenem antimicrobials for the treatment of infections caused by extended-spectrum beta-lactamase producers (ESBLs) (Nordmann et al 2012, Ashiru-Oredore et al 2012). For clinicians and antimicrobial pharmacists, CPE pose a major challenge at the bedside as it significantly limits antimicrobial prescribing (Akova et al 2012). Morbidity and mortality are both increased in CPE infections, particularly bloodstream and respiratory infections (Lee and Burgess 2012), with mortality rates ranging from 38-57% (Carmeli et al 2010), and poor outcomes in survivors (Borer et al 2009, Daikos et al 2009, Tzouvelekis et al 2012).
The first isolate of KPC-producing enzyme was in North Carolina in 1996, although not published until 2001 (Yigit et al. 2001), followed by the first European KPC positive isolate in France in 2005 (Naas et al. 2005). In February 2009, the first Irish KPC isolate was detected in our institution (Roche et al. 2009). Over the past six years the hospital group has been unsuccessful in its control of the spread of CPE, despite a multidisciplinary hospital-wide approach and commitment from all key stakeholders.

Methods

Setting

The Department of Clinical Microbiology at University Hospital Limerick (UHL) provides a centralised microbiology service for six acute hospital sites (800 beds; population circa 400,000 people), known as the University of Limerick Hospitals Group (ULHG).

Microbiological and molecular detection of CPE

CPE surveillance is performed on stool samples or rectal swabs using KPC selective chromogenic agar (CHROMagar™ KPC, Paris, France). MALDI-TOF MS (Bruker Diagnostics) identification is performed on all colonies, as previously described (O’Connor et al. 2014), and non-Enterobacteriaceae are disregarded. Antimicrobial susceptibility testing is performed using broth microdilution (ARIS Sensititre® system (Thermo Fisher Scientific Inc, Massachusetts, USA). Elevated carbapenem minimum inhibitory concentrations (MICs) for meropenem and ertapenem were confirmed by E-test (AB Biodisk, Solna, Sweden) following EUCAST guidelines; ertapenem resistance MIC >1, meropenem resistance MIC >8. Isolates with elevated carbapenem
MICs are further evaluated using the modified hodge test, which is a phenotypic culture-based test for the detection of carbapenemase-producers. It is based on the inactivation of a carbapenem by carbapenemase-producing strains (test isolate) that enable a carbapenem-susceptible indicator strain (*E. coli* ATCC® 25922) to extend growth towards a carbapenem-containing disc along the streak of inoculum of the test strain. Positive test result gives cloverleaf-like indentation. Commercially available diagnostic kits (Rosco Diagnostica A/S, Taastrup, Denmark) consisting of meropenem discs supplemented with β-lactamase inhibitors meropenem + dipicolinic acid, meropenem + boronic acid and meropenem + cloxacillin are used to phenotypically distinguish CPE isolates. Isolates are referred to the National Carbapenemase Producing Enterobacteriaceae (CPE) Reference Laboratory Service (CPERL) at University Hospital Galway, Galway, Ireland for CPE confirmation by molecular methods.

**Study definitions**

Cases were defined as patients with a CPE positive culture from any infected or colonised site during their hospitalisation. An outbreak was defined as at least two CPE cases linked by an epidemiological chain of transmission: an index case followed by one or more secondary case(s). When cross-transmission was suspected, microbiological confirmation was required.

**Study time frame**

Only CPE isolates identified between February 1\(^{st}\) 2009 and December 31\(^{st}\) 2015 were included in this study.
Identifying CPE positive isolates

A manual search of the Laboratory Information Management System was performed to retrieve all positive CPE results from all clinical specimens, irrespective of patient outcome, including blood cultures, line tip cultures, stool samples/rectal swabs, wound swabs, sputum specimens (including bronchoalveolar lavage samples), urine samples (including urinary catheter specimens) and any theatre specimens received. For each positive CPE result, a confirmatory check of molecular results from a reference laboratory was performed. In addition, a manual chart review was completed for all patients involved. The demographic details of all CPE cases were reviewed. Each CPE positive patient was recorded once only during this study, irrespective of whether they repeatedly cultured CPE positive on multiple admissions.

Results

Epidemiological

In 2009 a carbapenemase-resistant *Klebsiella pneumoniae* was identified from a sputum sample at UHL, via an Etest with a meropenem MIC \( \geq 32 \) mg/L, from a 60-year old male admitted with an exacerbation of chronic obstructive pulmonary disease. By the time the isolate had been identified the patient had been successfully treated with piperacillin/tazobactam 4.5g TDS intravenously (IV) for five days and had been discharged home. An earlier sputum sample from the patient during the same admission was culture negative suggesting that the carbapenem-resistant isolate had been nosocomially acquired. The patient had no history of treatment with carbapenem antimicrobials and no significant travel history or hospitalisations abroad. Antimicrobial susceptibility testing showed a high level resistance to \( \beta \)-lactams,
carbapenems, fluoroquinolones and amikacin with reduced susceptibility to tigecycline (4mg/L) but was susceptible to colistin and gentamicin (2mg/L). The isolate was sent to a reference laboratory and PCR amplification using \textit{bla}_{KPC} primers and sequencing showed the isolate carried the KPC-2 gene. This was the first ever case of CPE identified in the Republic of Ireland (Roche \textit{et al} 2009).

Only one other KPC was identified in 2009 at UHL from a catheter urine sample (CSU) of an elderly female admitted from the long term care facility with urosepsis, which had a mixed growth of Enterobacter and \textit{K. pneumoniae}. This patient had numerous previous admissions with urinary infections over the preceding five years and in the twelve months prior to isolation of KPC had been treated with a single course of meropenem 1g TDS IV for seven days as she had previously cultured a multi-drug resistant ESBL \textit{E. coli}.

In 2010 our laboratory retrospectively detected five cases of KPC while a scientist was conducting a Master’s of Science research project. Four of these were detected from urine samples (one dialysis patient, three outpatients) and one from an inpatient sputum sample. The patient reports were amended accordingly and alerts assigned to the patient records for them to be immediately isolated on presentation to the hospital again.

In 2011 there was a dramatic increase in the number of KPC cases at UHL and the first Irish KPC outbreak was notified to the Health Protection Surveillance Centre in Dublin. Between January and March 2011, there had been a reduction of 30% in cleaning staff and supervision, in parallel with less than 50% overall compliance with
hand hygiene protocols. It was to these factors that an outbreak of nine KPC cases were attributed. These occurred in the high dependency unit (HDU: four cases: January and February 2011) and intensive care unit (ICU: two cases: February 2011) and a dedicated surgical ward (five cases: March 2011). All isolates involved were sensitive to gentamicin, colomycin and tigecycline, and were clonally related as demonstrated on pulsed field gel electrophoresis performed at the time. PCR confirmed all carried \textit{bla}_{KPC-2}. There was Irish inter-hospital spread associated to one of our patients from this 2011 outbreak, who self-discharged against medical advice and then represented to a hospital in another Irish city on the east coast (Figure 1). He was admitted to ICU at this hospital and on day 6 of this admission a KPC-producing \textit{K. pneumoniae} was identified (Morris et al 2012).

\textbf{Figure 1. Dendrogram of 2011 KPC outbreak}. Between January and March 2011, 13 \textit{K. pneumoniae} isolates were collected from nine patients at hospital A (University Hospital Limerick) and two patients at hospital B (tertiary referral centre, Dublin). The index case is denoted by number 2261 Hospital A Pt 1 KpB4. Image courtesy of Public Health England.
On performing a chart review of patients involved this outbreak many had multiple risk factors for colonisation or infection with CPE previously described including prolonged hospitalisation, presence of invasive devices such as vascular catheters and urinary catheters, invasive procedures such as surgery, malignancy in particular solid tumours, ICU admission and exposure to broad spectrum antimicrobials including carbapenems (Gasink et al 2009).

Following this outbreak, there was considerable investment in hand hygiene training and surveillance and introduction of a screening policy whereby all HDU and ICU transfers are isolated until determined to be negative for KPC carriage. Despite these measures, control proved to be ineffective as there were simultaneous incidences of seven cases of KPC in June 2012 when a known-KPC positive patient was admitted to a multi-bedded bay.

In total there were eight cases of CPE in 2013 with the isolation of the first Citrobacter freundii KPC and one IMI. In June 2014, the first outbreak of NDM K. pneumonia in Ireland occurred in the region as described in chapter 9 of this thesis. In 2014 and 2015, the largest ever numbers of annual isolates were recorded in the Mid-West causing alarm nationally with concern that CPE was likely now endemic within the region (Figure 2).
Figure 2. Annual number of CPE isolates (n=140) in the Mid West of Ireland from the detection of the first isolate in February 2009 to December 31st 2015 (2009 2; 2010 4; 2011 11; 2012 10; 2013 8; 2014 45; 2015 60).

All 140 cases shown in Figure 3 are centred within a 60 kilometre radius of UHL, where the largest number of positive isolates have been identified (n=104). Twelve positive isolates have been received from four long term care facilities (LTCFs). Despite screening, no positive isolates have been detected to date from the maternity or orthopaedic hospitals. Three CPE positive mid-stream urine samples have been identified from three different general practice clinics in the city. None of these patients were previously known to be CPE positive from rectal swabs or stool specimens. None of these patients required treatment as it was felt that the results reflected asymptomatic colonisation.
Figure 3. Geographical distribution of CPE first isolates in the Mid-West of Ireland with the largest numbers of isolates identified at University Hospital Limerick (UHL). 12 isolates have been received from residents of long term care facilities (public and private). Screening as part of a CPE surveillance programme in a satellite dialysis unit located 5km from (UHL) has yielded 2 positive rectal screens.

The median age of positive patients is 72 years (range 7-94 years). The only paediatric case to date was a non-Irish national living locally who had had recent surgery in her home country (Poland) prior to KPC isolation (Figure 4). The main subtypes of CPE shown in Figure 4, namely, *Klebsiella pneumoniae* carbapenemase (KPC), New Dehli metallo-beta-lactamase (NDM), IMI (imipenem-hydrolyzing beta-lactamase) and
OXA-48 subtype (oxacillin-hydrolyzing). KPC is the most frequently isolated CPE type isolated within the Mid-West region. To date there have been 4 deaths as a consequence of CPE sepsis (intra-abdominal sepsis n=2, skin/soft tissue sepsis n=1); 3 during the 2011 KPC outbreak and 1 death during the 2014 NDM outbreak).

Figure 4. Age distribution of CPE isolates (median age 72 years) and breakdown by four CPE enzyme subtypes with a predominance of KPC-type CPE, which is now considered endemic within the region. CPE sub-types: *Klebsiella pneumoniae* carbapenemase (KPC), New Dehli metallo-beta-lactamase (NDM), IMI (imipenem-hydrolyzing beta-lactamase) and OXA-48 subtype (oxacillin-hydrolyzing).
The first substantial peak in the number of CPE cases occurred in the 2011 during the KPC outbreak, which affected only surgical inpatients. To date there 78% (n=128) of all positive CPE isolates have been received from inpatient in acute hospital beds. From 2012 to 2015, there have been higher numbers of CPE positive isolates received from medical inpatients compared with surgical inpatients (Figure 5).

Figure 5. Distribution of inpatient CPE isolates with more medical patients than surgical patients dominating since 2012.
Seasonality is evident with the highest number of cases identified in the spring and summer months annually (Figure 6). This was an unexpected result from the study as during the summer months there is traditionally a reduction in hospital activity with minimal numbers of elective surgical procedures usually performed due to staff taking annual leave and bed closures as a cost saving measure.

Figure 6. Monthly analysis of CPE isolate detection with seasonal summer peak. The highest number of isolates even detected in a single month was in August 2014 (n=16), which was due to the NDM outbreak and associated screening of known contacts of the index case.
Microbiological

One IMI, 123 KPCs, 13 NDM-1 and three OXA-48s have been identified. *Klebsiella pneumoniae* (n=80), *Klebsiella oxytoca* (n=30) and *Citrobacter freundii* (n=17) account for over 90% of all isolates cultured (Figure 8).

Figure 8. Distribution of CPE isolates by species type. KPC-producing *Klebsiella pneumoniae* and *Klebsiella oxytoca* predominate. CPE sub-types: *Klebsiella pneumoniae* carbapenemase (KPC), New Dehli metallo-beta-lactamase (NDM), IMI (imipenem-hydrolyzing beta-lactamase) and OXA-48 subtype (oxacillin-hydrolyzing).
Rectal swabs account for 74% (n=103) of positive clinical specimens. ‘Rectal swabs’ includes both swabs taken from patients and rectal swabs inoculated by laboratory staff from patient stool samples. Sampling from stool samples is preferred in patients with neutropenic sepsis, for example haematology or oncology patients, in order to prevent translocation of gut flora. There have been three patients with KPC bacteraemias to date (Klebsiella pneumoniae n=1, Klebsiella oxytoca n=1) of whom two patients died as a consequence of fulminant sepsis with multi-organ failure despite antimicrobial therapy. Three intra-abdominal theatre specimens have cultured KPC. Other clinical specimens include sputum (n=3), continuous ambulatory peritoneal dialysis (CAPD) fluid (n=1), mid-stream and catheter urine specimens (n=22), superficial wound swabs (n=2). The distribution of clinical specimens is shown in Figure 9. CPE has never been cultured from cerebrospinal fluid (CSF) or from bone specimens.

Figure 9. Distribution of CPE positive clinical specimens.
Discussion

Screening for CPE

CPE screening, via a rectal swab or stool specimen, is performed for all patients admitted to UHL to the intensive care unit or high dependency unit, any patient transferred from another institution, any patient who has had an acute admission in the past 12 months to any hospital in the Mid-West (except for paediatric, maternity or orthopaedic care) and any patient hospitalised abroad are screened on admission. Haemodialysis patients are screened every three months. Patients in the intensive care unit and the high dependency unit are screened on admission and weekly until discharge. During an outbreak screening of all patients with epidemiological and environmental links to CPE positive patients is carried out immediately once a positive result is confirmed with weekly surveillance for a period of four weeks until no new cases of CPE colonisation or infection is detected in units or wards as per current international best practice (Centers for Disease Control and Prevention 2009). Staff screening has not been performed at UHL as there have been no published reports of staff carriage of CPE as a source of patient colonisation or infection and no evidence that rectal colonisation of healthcare workers contributes to transmission (Dashti et al 2010). In 2011, 2685 screens were processed in the laboratory increasing to 9493 in 2015 (average 792 screens per month), with the number of screens to be completed in 2016 exceeded this again. In the first three quarters of 2015, 20% of the laboratory workload comprised of processing screening specimens.
Carbapenem consumption

Between 2014 and the end of 2015, carbapenem consumption decreased by 21%. This compares very favourably with a 4% increase from 2013 and 2014, 25% increase from 2012 and 2013, 36% increase from 2011 to 2012 (unpublished data). Our carbapenem antimicrobial stewardship programme continues to date. The prescription of carbapenems is not wholly restricted, however measures are taken to limit prescriptions and all those on carbapenems are followed by an antimicrobial pharmacist to encourage a short-course of treatment. All new non-consultant hospital doctors (NCHDs) who commence working within our hospital group are required to attend mandatory training regarding the hospital group antimicrobial guidelines.

Infection prevention and control strategy

Local CPE guidelines have been drafted in line with international best practice (Muto et al. 2003, Strategy for the Control of Antimicrobial Resistance in Ireland 2009, Royal College of Physicians in Ireland 2012, Health Protection Agency 2014) along with the implementation of the Public Health England CRE toolkit (Public Health England 2013). Hand hygiene audits are completed regularly. A hospital-wide audit of 176 staff across all disciplines conducted in 2013 identified 84% hand hygiene observation compliance. At ward level, the maintenance of appropriate hygiene practices when cleaning bed pan washers has been prioritised in the context of repeated KPC outbreaks given the nature of Enterobacteriaceae as commensal gut flora. Long sleeve gowns are used by all staff caring for patients infected or colonised with CPE as previous studies have suggested that long sleeve gowns reduce contamination of health care worker clothing (Puzniak et al 2002). Chlorhexidine
gluconate wash-cloths are used to bathe all patients in critical care areas and are provided to all other colonised or infected patients during their admission.

*Hospital hygiene*

A dedicated hospital hygiene nurse manager has been appointed to monitor the quality of cleaning being performed. The infrastructure of the hospital is in need of reconfiguration with a dependency on 16-bedded medical and surgical wards with limited toilet facilities; on average two toilets per 16-bedded ward. In addition to routine cleaning, hydrogen peroxide vapour decontamination is provided by an external provider and is performed post discharge of any KPC colonised or infected patient. An ultraviolet torch is used to check the quality of cleaning carried out and this has shown variable results. Limited environmental sampling has been performed and to date no CPE has been identified. Air sampling has never been performed.

*Are we missing an agricultural link to our Mid-West CPE problem?*

Agriculture is a significant contributor to the economy of the Mid West of Ireland with many of our patients residing in rural areas with domestic animals. There have been previous reports of NDM-1 (Shaheen et al 2013) and OXA-48 (Stolle et al 2013) isolation in domestic pets. Recently *Klebsiella variicola* producing OXA-181 carbapenemase has been identified in fresh vegetables imported from Asia to Switzerland (Zurfluh 2015). Very little published data exist in Ireland regarding carbapenem consumption in the veterinary industry in comparison to the UK (Veterinary Medicines Directorate United Kingdom, British Small Animal Veterinary Association). Closer collaboration is required with our local veterinarians to gain insight into day-to-day veterinary practices that may be contributing to our CPE rates.
Conclusions

There are many contributing factors to the continued rise in CPE cases including a local reservoir of infected and colonised patients continually re-presenting to the outpatients department or emergency department for admission coupled with overcrowding in 16-bedded wards and too few isolation rooms. Despite these challenges, we continue to implement international best practices including toolkits, hydrogen peroxide vapour cleaning, restricting the prescription of carbapenems, investing heavily in education of prescribers and ward staff, monitoring hand hygiene compliance figures. A national strategy for CPE is needed to avoid replication of the Mid-West KPC endemic. There is a compelling argument for completion of a community and feeder care facility point prevalence study.

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An Irish outbreak of New Delhi Metallo-β-lactamase (NDM)-1 carbapenemase-producing Enterobacteriaceae: increasing but unrecognised prevalence.

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An Irish outbreak of New Delhi metallo-β-lactamase (NDM)-1 carbapenemase-producing Enterobacteriaceae: increasing but unrecognised prevalence.

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Summary
Background
Carbapenemase-producing Enterobacteriaceae (CPE) can cause healthcare–associated infections with high mortality rates. New Delhi metallo-beta-lactamase-1 (NDM-1) is amongst the most recently discovered carbapenemases.

Aim
To report the first outbreak of NDM-1 CPE in Ireland, including microbiological and epidemiological characteristics and assess the impact of infection prevention and control measures.

Methods
Retrospective microbiological and epidemiological review. Cases were defined as patients with a CPE positive culture. Contacts were designated as roommates or ward mates.

Findings
This outbreak involved ten patients, with a median age of 71 years (range 45-90 years), located in three separate but affiliated healthcare facilities. One patient was infected (the index case); the nine others were colonised. Nine NDM-1-producing Klebsiella pneumoniae, a NDM-1-producing Escherichia coli and a K. pneumoniae carbapenemase (KPC)-producing Enterobacter cloacae were detected between week 24 2014 and week 37 2014. Pulsed field gel electrophoresis demonstrated similarity. NDM-1 positive isolates were meropenem resistant with MICs ranging from 12 to 32 µg/ml. All were tigecycline susceptible (MICs ≤1 µg/ml). One isolate was colistin
resistant (MIC 4.0 μg/ml; mcr-1 gene not detected). In 2015, four further NDM-1 isolates were detected.

Conclusions
The successful management of this outbreak was achieved via the prompt implementation of enhanced infection prevention and control practices to prevent transmission. These patients did not have a history of travel outside of Ireland, but a number had frequent hospitalisations in Ireland, raising concerns regarding the possibility of increasing but unrecognised prevalence of NDM-1 and potential decline in value of travel history as a marker of colonisation risk.

Keywords
Carbapenemase-producing Enterobacteriaceae, New Delhi metallo-beta-lactamase-1 (NDM-1), multi-drug resistant organism, outbreak, Ireland
Introduction

Enterobacteriaceae are Gram-negative colonisers of the human gut. Carbapenemase-producing Enterobacteriaceae (CPE) are resistant to most classes of antimicrobials (Gupta et al 2011, Krishna 2010). New Delhi Metallo-beta-lactamase-1 (NDM-1) is one of the most recently discovered carbapenemase enzymes. The \textit{bla}_{NDM-1} gene is thought to have originated in the environment from plant pathogens and is plasmid-mediated (Sekizuka \textit{et al} 2011). NDM-1 confers broad spectrum beta-lactam resistance with the ability to hydrolyse all \beta-lactams antimicrobials with the exception of monobactams, such as aztreonam (Cornaglia \textit{et al} 2011). Many NDM-1-producing bacteria remain susceptible only to colistin, fosfomycin and tigecycline (Livermore 2009, Livermore 2011, Nordmann \textit{et al} 2011a, Nordmann \textit{et al} 2011b). Since first reported as implicated in human disease (Yong \textit{et al} 2009, Castanheira \textit{et al} 2011), NDM-1-producing bacteria have been recovered from numerous infection sites including device-associated infections, intra-abdominal, urinary tract, bloodstream and surgical wounds (Wilson \textit{et al} 2012). Eight variants of the enzyme have been published (NDM-1 to NDM-8), most of which appear to have originated from Asia (Tada \textit{et al} 2013).

Acquisition of NDM-1 has been reported as associated with travel to known reservoirs areas of NDM-producing Enterobacteriaceae, notably the Indian subcontinent (Pakistan, India, Sri Lanka) and the Balkan countries (Mulvey \textit{et al} 2011, Periano \textit{et al} 2011, Tijet \textit{et al} 2011, Khan and Nordmann 2012, Livermore \textit{et al} 2011). The prevalence of community carriage in these primary NDM reservoirs is estimated to be 5-15\% (Perry \textit{et al} 2011, Day \textit{et al} 2013). Global dissemination is facilitated by intercontinental travel (Walsh and Toleman 2011, Walsh and Toleman 2012),
including healthcare tourism (Reed 2008), and migration (Nordmann et al 2011b). International spread has been rapid (Molton et al 2013, Johnson and Woodford 2013).

The eight NDM isolates identified in Ireland prior to this outbreak were isolated or paired cases from a number of hospitals countrywide and generally with an identifiable link with a travel. Dissemination of the \textit{bla}_{NDM-1} gene, like other similar resistance mediators, is facilitated by inadequate infection prevention and control practice in health care settings (Johnson and Woodford 2013), uncontrolled or poorly controlled antimicrobial use (Walsh and Toleman 2012, Bush et al 2011), inadequate practices related to food preparation (Walsh 2011), water treatment and general sanitation (Walsh and Toleman 2011). The largest reported NDM outbreak to date in a non-endemic country was reported from Poland in 2015, where 374 cases of infection or colonisation, with a variety of NDM-producing Enterobacteriaceae, were identified from 40 hospitals over a two-year period (Baraniak \textit{et al} 2015).

In this report, we describe what we believe to be the first outbreak of NDM-1-producing Enterobacteriaceae in Ireland, which occurred in 2014.

\textbf{Methods}

\textit{Setting}

The Department of Clinical Microbiology at University Hospital Limerick (UHL) provides a centralised microbiology service for six acute hospital sites (800 beds; population circa 380,000 people). As an aid to contextualising this outbreak, it is notable that 48 \textit{K. pneumoniae} carbapenemase (KPC) and one imipenem-hydrolyzing
beta-lactamase (IMI)-producing isolates were detected at UHL between February 2009 and May 2015, as previously published (O’Connell et al 2014).

Study definitions
Cases were defined as patients with an NDM-1 positive culture from any site during their hospitalisation. Contacts were designated as roommates or ward mates.

Microbiological and molecular detection of NDM-1
Since 2011, CPE surveillance at UHL had been performed on stool samples or rectal swabs using Klebsiella pneumoniae carbapenemase (KPC)-producing selective chromogenic agar (CHROMagar™ KPC, Paris, France). MALDI-TOF MS (Bruker Diagnostics) identification was performed on all colonies, as previously described (O’Connor et al 2014), and non-Enterobacteriaceae were disregarded. Antimicrobial susceptibility testing was performed using broth microdilution (ARIS Sensititre® system-Thermo Fisher Scientific Inc, Massachusetts, USA). Elevated carbapenem minimum inhibitory concentrations (MICs) for meropenem and ertapenem were confirmed by E-test (AB Biodisk, Solna, Sweden) following EUCAST guidelines; ertapenem resistance MIC >1 g/l, meropenem resistance MIC >8 g/l. Isolates with elevated carbapenem MICs were further evaluated using the modified Hodge test (MHT). Commercially available diagnostic kits (Rosco Diagnostica A/S, Taastrup, Denmark) consisting of meropenem discs supplemented with β-lactamase inhibitors: meropenem + dipicolinic acid; meropenem + boronic acid; and meropenem + cloxacillin were used to phenotypically distinguish CPE isolates. Isolates were referred to the National Carbapenemase-Producing Enterobacteriaceae (CPE) Reference Laboratory Service (CPERLS) at University Hospital Galway, Galway,
Ireland for CPE confirmation by molecular methods. The genetic relationship of NDM-1 isolates was analysed by pulsed-field gel electrophoresis (PFGE) performed at CPERLS to assist with determining the epidemiological relatedness of isolates.

**Identifying NDM-1 positive isolates**

A retrospective chart review assessing clinical and epidemiologic characteristics was completed for all patients involved including: dates of admission, transfers, and hospital discharges; locations within the hospital; procedures and operative notes; use of invasive devices; biochemical and haematological blood test results; antimicrobials received and documentation of a travel history.

**Infection control interventions**

The isolation of a NDM-1-producing *K. pneumoniae* triggered initiation of the hospital’s outbreak management protocol. Rectal swabs or stool samples were obtained from all contacts of the index case. Information leaflets were distributed to all patients and family as appropriate. The Public Health England CPE toolkit was implemented during the outbreak (Public Health England 2013). All infected or colonised patients were barrier nursed with long-sleeved disposable gowns and gloves employed, and single rooms were used when available. Chlorhexidine gluconate wash-cloths were employed for bathing of patients. Dedicated equipment was prioritised for NDM-positive inpatients, both infected and colonised, but was not available for all NDM-contacts. A semi-automated electronic data surveillance system, ICNet™ (Baxter), was used to collate the records of the outbreak meetings and patients involved. All patients identified as CPE positive or as CPE contact were
flagged on the ICNet™ system and their medical charts were assigned a CPE alert sticker, placed on the front cover.

During the thirteen weeks over which this outbreak occurred, there were an additional 13 KPCs and one oxacillin-hydrolysing β-lactamase (OXA) identified. This was the first OXA isolated at UHL. At a practical level, staff were familiar with the term ‘KPC’, but the introduction of the term ‘NDM’ and ‘OXA’ created confusion and the concept of three different types of CPE circulating simultaneously generated alarm amongst clinical staff. Additional education sessions were provided at ward level to nursing staff and healthcare assistants by members of the infection prevention and control team. New CPE posters were designed explaining the different CPE types in simple and clear language, and these were placed in the doctors’ residence (communal living space) and on all wards. Feedback received regarding the posters was positive. An electronic link to the location of the CPE guideline on the hospital intranet was disseminated on a memo to all staff.

Hand hygiene audits were performed with greater frequency in affected areas, which involved twice weekly observational audits at ward level. Enhanced cleaning, twice daily, of all implicated clinical areas and patient equipment was instigated in parallel with increased auditing of cleaning practice. The index cases’ room in the intensive care unit (ICU) and all ward areas, where positive NDM-1 patients had been admitted, underwent routine cleaning followed by hydrogen peroxide vapour decontamination post-discharge. A deep clean of the emergency department (ED) including the waiting room and resuscitation areas was performed as seven patients involved in the outbreak had been admitted via the ED. High-touch surfaces such as door handles, bedside
lockers and chairs and bed rails were emphasised by the hospital hygiene nurse manager for cleaning to reduce cross-transmission. An ultraviolet torch was used to assess the quality of cleaning performed and face-to-face feedback regarding cleaning deficits was provided to cleaning operatives. Environmental sampling was not performed during this outbreak.

A further initiative was introduced involving, on a daily basis, a joint pharmacist/clinical microbiologist handover of all in-house carbapenem prescriptions, and subsequent discussion by the either the microbiology consultant or registrar with clinical teams regarding alternative agents where appropriate.

**Results**

**Index Case**

The index case was a community-dwelling Irish female. In the summer of 2014, she was admitted with sepsis. Blood and peritoneal fluid cultures confirmed *Escherichia coli*. Initial admission was to a six-bed bay in a general medical ward preceding transfer the following day to a single room in the ICU. Admission screens confirmed prior colonisation with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE). A rectal screen at that time did not detect CPE. Empiric therapy utilised intravenous (IV) ceftazidime 2g every 8 hours and gentamicin. As she did not respond to initial therapy, treatment was changed to meropenem 1g IV every 8 hours, metronidazole 500mg IV TDS and vancomycin 1.5g IV every 12 hours but was de-escalated to meropenem monotherapy.
Seven days post admission, a rectal CPE screen (routine for ICU patients) detected a CTX-M extended spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* and NDM-1-producing *K. pneumoniae*. A skin rash developed and diagnostic biopsies were performed on the left forearm. An abscess developed at the skin biopsy site and a CTX-M ESBL-producing *K. pneumoniae* and NDM-1-producing *K. pneumoniae* were isolated from the abscess discharge. IV tigecycline 50mg every 12 hours was initiated and the site was debrided surgically. The patient died 2 months following admission secondary to refractory soft tissue sepsis.

**Microbiological**

Prior to this outbreak, a case of NDM-producing Enterobacteriaceae had not been identified in our laboratory. None of the patients in this outbreak were known to have prior colonisation with extended-spectrum beta-lactamases (ESBLs) based on previous screening. Three patients had been screened for CPE before this outbreak but CPE was not detected on those occasions. In total, nine NDM-1-producing *K. pneumoniae*, a NDM-1-producing *Escherichia coli* and a KPC-producing *Enterobacter cloacae* were detected. Of note, the NDM-1-producing *E. coli* and a KPC-producing *E. cloacae* were identified in one patient. These isolates were detected at UHL and in two regional hospitals affiliated to the hospital group, located 10km and 40km away, respectively. Clinical specimens that were positive for NDM-1-producing isolates included mid-stream urine (MSU) samples (n=2), rectal swabs (n=8) and skin biopsy samples (n=3) from the index case. NDM-1-producing isolates were meropenem resistant with MICs ranging from 12 to 32 µg/ml. All isolates were tigecycline susceptible (MICs ≤1 µg/ml). One isolate was colistin resistant (MIC 4.0
µg/ml; *mcr*-1 gene negative). Pulse-field gel electrophoresis demonstrated that the isolates were closely related. Multilocus sequence typing was not performed.

*The Outbreak: Patient demographics & epidemiological factors*

The patient demographics are summarised in Table 1. The screening policy in operation at the time of this outbreak, and currently, is that all patients are screened on admission if: admitted to the ICU or high dependency unit (HDU) at UHL; transferred from another hospital in Ireland; have had an acute admission in the past 12 months to any hospital within our hospital group (except for paediatric, maternity or orthopaedic); or if hospitalised abroad. Haemodialysis patients are screened every three months. Patients in ICU and HDU are screened weekly until discharge. During an outbreak, screening of all patients with epidemiological and environmental links to CPE positive patients, via rectal swab or stool specimen, is performed weekly. CPE screening is performed for a period of four weeks until no new cases of CPE colonisation or infection are detected.

In June 2014, two contacts of the index case prior to her ICU admission were identified (Patients B, D). Both had been on the medical ward with the index case during her 24-hour admission prior to transfer to ICU. Patient B was screened, identified as NDM-1-producer positive and was isolated. Patient D was discharged prior to CPE screening. She re-presented for admission in July 2014 and was re-admitted to a 6-bedded area on the same medical ward as she had been admitted to previously in June 2014. She had a CPE screen performed and was confirmed as NDM-1-producer positive and she was isolated immediately. Patients E, F and H, all were ward contacts of Patient D and were identified as NDM-1-producer positive when contact tracing
was performed. Patients G and I were identified from routine admission rectal CPE screens performed during the outbreak period. Both had admissions to UHL in the prior 12 months; neither had contact with the index case.

Two additional NDM-1-producer positive patients were identified during the outbreak period but their CPE positivity was not explained by any apparent epidemiological link. These were not contacts of the index case and had no contact with UHL during the defined outbreak period, including the outpatients and the emergency departments. Patient C was identified as CPE positive from a screening rectal swab that was performed at a regional hospital 10km from UHL. This CPE screen was performed as per screening protocol because the patient had been admitted to UHL in the prior 12 months. His last admission to UHL was almost five months before the outbreak was declared. Patient J, identified as being CPE positive following a MSU sample sent for routine culture and sensitivity as the patient had symptoms of cystitis, was located in a regional hospital 40km away from UHL. Notably, this latter isolate was determined by pulsed-field gel electrophoresis (PFGE) (Figure 1) to be the isolate most distantly related to the other outbreak isolates.

Given the identification of NDM-1 at three different associated hospitals, a decision was made to perform contact tracing and screening of all contacts at each site. As a result of that exercise, during the outbreak, 2204 CPE screens, including contact tracing and routine CPE screening, were processed in our laboratory, which in addition to detecting the NDM-1 isolates, also identified 13 new KPCs and one OXA-48 as already discussed earlier in this chapter.
Table 1. Clinical characteristics of the ten patients involved in the NDM-outbreak.

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>M/F</th>
<th>Place of residence</th>
<th>Date admitted</th>
<th>Admitting diagnosis</th>
<th>Treated with meropenem during admission</th>
<th>Specimen</th>
<th>Organism(s) isolated</th>
<th>CPE enzyme detected</th>
<th>Date of culture</th>
<th>Infected/Colonised</th>
<th>Outcome</th>
<th>Previous admission to hospital in past 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44</td>
<td>F</td>
<td>Community</td>
<td>Week 24 (2014)</td>
<td>Peritonitis</td>
<td>Yes</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 25 (2014)</td>
<td>Infected</td>
<td>Died</td>
<td>Yes</td>
</tr>
<tr>
<td>B</td>
<td>78</td>
<td>F</td>
<td>Community</td>
<td>Week 24 (2014)</td>
<td>Streptococcus milleri, bacteraemia</td>
<td>No</td>
<td>Mid-stream urine</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 25 (2014)</td>
<td>Colonised</td>
<td>Discharged to a public LTCF</td>
<td>Yes</td>
</tr>
<tr>
<td>C</td>
<td>61</td>
<td>M</td>
<td>Community</td>
<td>Week 25 (2014)</td>
<td>Urinary retention</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 25 (2014)</td>
<td>Colonised</td>
<td>Discharged to the community</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
<td>65</td>
<td>F</td>
<td>Residential care facility</td>
<td>Week 30 (2014)</td>
<td>Urinary infection</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 32 (2014)</td>
<td>Colonised</td>
<td>Discharged to residential care</td>
<td>Yes</td>
</tr>
<tr>
<td>E</td>
<td>81</td>
<td>F</td>
<td>Public LTCF</td>
<td>Week 30 (2014)</td>
<td>Respiratory infection</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 32 (2014)</td>
<td>Colonised</td>
<td>Discharged to LTCF</td>
<td>Yes</td>
</tr>
<tr>
<td>F</td>
<td>71</td>
<td>F</td>
<td>Community</td>
<td>Week 30 (2014)</td>
<td>Collapse</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 32 (2014)</td>
<td>Colonised</td>
<td>Discharged to a public LTCF</td>
<td>Yes</td>
</tr>
<tr>
<td>G</td>
<td>89</td>
<td>F</td>
<td>Private LTCF</td>
<td>Week 32 (2014)</td>
<td>Congestive cardiac failure</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Escherichia coli, Enterobacter cloacae</em></td>
<td>NDM-1, KPC</td>
<td>Week 33 (2014)</td>
<td>Colonised</td>
<td>Discharged to LTCF</td>
<td>Yes</td>
</tr>
<tr>
<td>H</td>
<td>90</td>
<td>F</td>
<td>Community</td>
<td>Week 30 (2014)</td>
<td>Respiratory infection</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 33 (2014)</td>
<td>Colonised</td>
<td>Died</td>
<td>No</td>
</tr>
<tr>
<td>I</td>
<td>75</td>
<td>M</td>
<td>Public LTCF</td>
<td>Week 35 (2014)</td>
<td>Respiratory infection</td>
<td>No</td>
<td>Rectal swab</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 35 (2014)</td>
<td>Colonised</td>
<td>Discharged to LTCF</td>
<td>No</td>
</tr>
<tr>
<td>J</td>
<td>53</td>
<td>F</td>
<td>Community</td>
<td>Week 37 (2014)</td>
<td>Skin and soft tissue infection</td>
<td>No</td>
<td>Mid-stream urine</td>
<td><em>Klebsiella pneumonia</em></td>
<td>NDM-1</td>
<td>Week 37 (2014)</td>
<td>Colonised</td>
<td>Discharged to the community</td>
<td>No</td>
</tr>
</tbody>
</table>
Carbapenem consumption
Only the index case had been prescribed meropenem during the current admission. This was prior to the isolation of a NDM-positive culture and, in total, the patient was administered five days of meropenem.

NDM-1 isolates identified in 2015
In week 31 2015, a NDM-1 producing *K. pneumoniae* was identified in an MSU sample from an 81-year-old female residing in a private long term care facility (LTCF). This patient was a contact of the index case during the outbreak but CPE was not detected at the time. PFGE demonstrated similarity to the 2014 isolates (Figure 1).
In week 32 2015, a NDM-1-producing *K. pneumoniae* was identified from a rectal swab of a 71-year old public LTCF patient who was known to be previously colonised with a KPC-producing *Citrobacter freundii*. This isolate did not demonstrate similarity to previous isolates. In week 42 2015, a NDM-1-producing *K. pneumoniae* was isolated by rectal swab from an admission CPE screen of a patient repatriated from Bosnia. She had not been admitted to UHL previously and had never had any specimens processed in the UHL microbiology laboratory. This isolate did not demonstrate PFGE similarity. In week 48 2015, a NDM-1-producing *E. coli* was detected in a 60-year patient who had been recently hospitalised in India. Again this isolate did not demonstrate similarity (Figure 2).
Figure 1. Pulsed field gel electrophoresis of 12 *K. pneumoniae* isolates to date (2014 and 2015). ME 140282 MW875001 is the index case (Patient A). Patients B (ME140290), C (ME140289), D (ME140368), E (ME140369), F (ME140372), H (ME140396), I (ME140428), J (ME140453) who all had positive isolates in 2014 are all represented on this dendrogram. ME150339 is an isolate from a patient admitted from a long term care facility in July 2015. This patient had been an inpatient at the time of the NDM outbreak during summer 2014 but screened negative for CPE and demonstrates similarity. ME150339 is an isolate from a patient repatriated from a hospital in Bosnia in September 2015 and displays no similarity to the NDM clinical isolates obtained from patients living within the locality.
Figure 2. Pulsed field gel electrophoresis of two NDM-1 *E. coli* isolates to date (2014 and 2015). ME 140389 represents Patient G who resided locally and had no travel history. ME150567 is an isolate from 2015 with likely origin in India. No similarity between the isolates is demonstrated as expected.

Discussion

The source of the index case patient’s NDM-1-producer acquisition remains uncertain although acquisition during the hospital admission of June 2014 is considered likely. The index case had been on haemodialysis for 16 years and switched to peritoneal dialysis in 2013 (five months prior to NDM-1 detection). In our care, all haemodialysis patients undergo surveillance CPE screening every three months, but the same screening is not conducted for peritoneal dialysis patients. The index case patient was screened for rectal CPE in December 2013, at which time CPE was not detected. Following her transition to peritoneal dialysis, no further CPE screening was performed prior to her transfer to ICU on this final admission to UHL, at which time CPE was likewise undetected. She had never worked in a healthcare setting nor lived with any healthcare workers. She had no known travel to NDM-1 endemic areas.

In this outbreak, international travel was not a recognised factor implying that there may be a hospital and or community burden of *blaNDM-1* than had not been previously
Struelens et al. reviewed 77 NDM-1 producing Enterobacteriaceae reported from 13 European countries from 2008 to 2010 (Struelens et al. 2010). Among 55 of the cases with recorded travel history, 31 had involved travel to, or admission to a hospital in, India or Pakistan, and five patients had been hospitalised in the Balkan region. Possible nosocomial acquisition accounted for 13 of 77 cases (17%). In contrast, our outbreak more closely resembled the outbreak reported by Borgia et al. that occurred in Brampton, Ontario, Canada where five patients were identified as carrying NDM-1–producing *K. pneumoniae*; all of them epidemiologically linked with each other, but none with a relevant travel history (Borgia et al. 2012).

NDM-1-producing *K. pneumoniae* and NDM-1-producing *E. coli* and were identified from one hospital (UHL) and NDM-1 producing *K. pneumoniae* found in the two other hospitals within the region. The predominant species in our NDM-1 outbreak was *K. pneumoniae* (Figure 1). A successfully controlled outbreak in Mexico City reported the isolation of NDM-1 producing *E. coli* and NDM-1 producing *E. cloacae* from the same patient in addition to three NDM-1 producing *K. pneumoniae* isolates derived from three other epidemiologically-related patients. In that outbreak, one plasmid (*IncFII*) was borne by all of the isolates (Torres-Gonzalez et al. 2015). In a large outbreak reported from South Africa (as in our case also from three acute hospitals), which persisted for 16 weeks in 2012, *K. pneumoniae* was also the dominant species, accounting for 28/38 isolates (74%) with *E. cloacae* accounting for the 5/38 (13%) (deJager et al. 2015).

In 2013, Lin et al. reported substantial community reservoirs of CPE in the United States (Lin et al. 2013). Currently, there are no data available in Ireland regarding
national prevalence of CPE in LTCF as a national point prevalence study of LTCF relating to multidrug-resistant organisms has never been performed. However, three of the ten patients in this outbreak were permanent residents of three separate LTCF (two public, one private) and one other patient was a permanent resident in a residential care facility for adults with learning disabilities (Table 1). Such a study is needed, justified by our data and the fact that from 2009 to 2015 CPE strains were isolated from clinical specimens originating in ten local public and two private LTCF (unpublished data).

Influenced by this outbreak, our antimicrobial stewardship has been modified. Overall hospital antibiotic consumption rate in defined daily doses (DDD) per 100 bed days used (BDU) demonstrates a reduction in carbapenem consumption. Between 2014 and the end of 2015, carbapenem consumption decreased by 21% (2014: 4.43 DDD/100BDU, 2015: 3.49 DDD/100BDU). This compares very favourably with a 4% increase from 2013 (4.24 DDD/100BDU) to 2014, 25% increase from 2012 (3.39 DDD/100BDU) to 2013, a 36% increase from 2011 (2.50 DDD/100BDU) to 2012.

**Conclusions**

This outbreak and our other sporadic isolates indicate the changing epidemiology of NDM-1 CPE. In Ireland as elsewhere (e.g. Canada) absence of a history of travel to a known endemic area is of decreasing value in differentiating between those at risk of and those not at risk of colonisation or infection with NDM-1. As the successful management of this outbreak demonstrates, prompt infection prevention and control practices are essential to prevent transmission. No staff or environmental screening was performed but extensive resources directed towards education, hand hygiene
compliance, environmental disinfection, cleaning standards and reducing carbapenemase consumption were successful in controlling rapid in-hospital transmission of NDM-1. The subsequent detection of additional cases, in particular the related isolate from a nursing home resident in July 2015 demonstrates the difficulty of definitely eradicating these organisms once established in the revolving doors systems of nursing homes and hospitals.

Acknowledgements
We thank the staff of the Microbiology Laboratory at UHL for their expertise in successfully dealing with this outbreak, Elaine McGrath for performing PFGE at University Hospital Galway and Sandra Guilfoyle, medical secretary at the UHL Department of Clinical Microbiology, for assisting with obtaining medical charts.

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CHAPTER 10

Identifying and managing healthcare-associated infections with electronic surveillance. Clinical successes and challenges encountered with the introduction of ICNet™ in an Irish hospital group.
Summary

The integration of electronic surveillance in IPC in Ireland is necessary for accurate data surveillance and to ensure patient safety. The aims of this study were to: (1) assess the impact of the introduction of an electronic surveillance system, (2) outline the positive changes to our IPC programme following the introduction of electronic surveillance in our hospital group, (3) detail how ICNet™ has become integrated into our day-to-day IPC practices and (4) highlight issues that we have encountered through its implementation, which will be of benefit to other institutions considering a switch to electronic surveillance.
**Introduction**

An effective IPC programme has the potential to reduce hospital costs (Wenzel 1995) and length of hospital stay (Miller et al 1989), decreases morbidity (Nyström 1994), improves patient survival (Brown et al 2006) and substantially reduces the risk of HCAI acquisition, (Curran et al 2000) which are the most common adverse event during any hospital admission (Burke 2003). Approximately 5-10% of patients acquire a HCAI during their admission, one third of which are considered preventable (Berwick et al 2006). The highest rates of HCAIs are found large in teaching hospitals with complex case-mixes (Cruse and Ford 1980). The economic consequences of HCAIs are substantial with 1.7 million infections per year in the USA and 100,000 deaths (Klevens et al 2002).

The publication of the landmark Study on the Efficacy of Nosocomial Infection Control (Haley 1985b) demonstrated that 32% of HCAIs could be prevented using high-intensity infection surveillance and control programmes (Haley 1986). Surveillance, to be effective, must be reliable, accountable and reproducible with the aim of reducing or eliminating HCAIs and to ease the burden of data management. IPC teams have been reported as spending 45% of their time carrying out surveillance; more time than is spent performing any other task (Schiffman and Palmar 1985), limiting time spent engaging in educational roles, research and performance improvement activities.

Traditionally, IPC surveillance has been performed via individual case identification and clinical patient bed-side review plus manual chart review, employing paper-based
methods for recording and storing data (Nelson et al 2007), but this surveillance methodology is resource intensive and time consuming and associated with a propensity for human error during transcription or inadvertent omissions (Emori et al 1998, Lin et al 2010).

Electronic surveillance is the process of obtaining information from inter-related electronic information databases for identifying infection (Wright et al 2004). It can be semi-automated where the electronic system assists the IPC team in carrying out their duties more effectively, i.e. as a data storage repository that filters data from the laboratory and from patient admissions/discharge systems, or it can be fully automated whereby electronic surveillance systems conduct surveillance entirely independently of IPC involvement following surveillance definitions. Semi-automated systems still require a chart and patient review in most cases. There has been a rapid evolution towards electronic surveillance over the past 15 years (Wright 2008) and despite many hospitals in Ireland moving to electronic surveillance, data has not been published, but anecdotal reports at national meetings have been encouraging. The 2011 National Institute for Health and Care Excellence (NICE) guideline recommended a greater use of technology to support infection prevention (NICE, 2011).

In 2012, ICNet™ (Baxter, UK), an electronic surveillance software system, was introduced to function in a semi-automated manner, replacing previous paper-based manual surveillance.
Materials and methods

The IPC team has four full-time nurses and works closely with the Department of Clinical Microbiology, comprising of two consultant microbiologists and one clinical microbiology specialist registrar. Two surveillance scientists are employed within the microbiology laboratory, who are also daily users of ICNet™. Face-to-face interviews were conducted with all four members of the IPC team and with two surveillance scientists to gain personal insights into how surveillances practices have changed since the introduction of ICNet™. Time was spent shadowing members of the IPC team on the wards and in the laboratory observing how ICNet is utilised for various daily tasks.

Results

IPC practices prior to the introduction of electronic surveillance

Two of the four IPC nurses were working within the department prior to 2012 and were pivotal in leading the switch to electronic surveillance. Another IPC nurse moved into the post in 2014 but had been using ICNet™ in another Irish healthcare institution. Prior to 2012, the IPC team expended much time reviewing electronic laboratory results queues daily to determine what potential IPC issues may be arising in-house. Utilising this method, large volumes of laboratory results were read, the majority of which were of no IPC relevance. This practice occurred as there was no in-built mechanism within the laboratory system to view, for example, ongoing laboratory cultures or finalised laboratory results (microbiology or serology), that had multi-MDROs such as carbapenemase-producing Enterobacteriaceae (CPE), extended-spectrum beta-lactamase (ESBL)-producers, vancomycin-resistant Enterococci (VRE), methicillin-resistant Staphylococcus aureus (MRSA) or other pathogenic organisms that had implications for bed management and isolation such as norovirus,
rotavirus, *Mycobacterium tuberculosis* or varicella. Results for inpatients which needed IPC input were then manually extracted with results kept in paper-copy only. Similarly, results for outpatients, for which follow-up phone calls to general practitioners and/or public health consultants, were also manually extracted with results also kept in paper copy. Vast volumes of paper records were created and required storage.

**Integration and interfacing of ICNet™**

ICNet™ was installed in 2012 using an organisational rather than a definition based approach to IPC practices within the hospital. The system is web-based and can be accessed from any hospital desktop computer, enabling out of office use. In practical terms, the system is integrated seamlessly with the Laboratory Information Management System (LIMS – iLab, CSC Healthcare Group). ICNet™ automatically downloads microbiology results from LIMS as determined by the IPC team, who set specific filters on the system to ensure that only laboratory results that need IPC review are read. The system facilitates regular imports from the laboratory, approximately every 20 minutes, allowing a timely delivery of results and for immediate action if appropriate.

It was also linked to the hospital Patient Administration System (PAS, IMS Maxims), in one of the six hospital sites. Unfortunately, because there were six PAS systems (one for each of the hospitals in the region) there were multiple patient medical record numbers (MRNs) in the LIMS, which led to multiple patient records in ICNet™. Attempts were made to merge these duplicate patient records, but they quickly multiplied beyond the point where this was feasible. The Patient Administration
System was later upgraded in that hospital in 2015 (to iPMS, CSC Healthcare Group), and it is envisioned to migrate the PAS systems of the other five hospitals into this one centralised system later in 2016 or in 2017. This will allow admission, discharge and transfer (ADT) details from all six sites to feed into ICNet™.

The issue of multiple patient records in ICNet™ was significant for a number of reasons, but primarily if an IPC alert (or “flag”) was placed on a patient record, under normal circumstances if this patient is admitted, the flag on ICNet™ would enable the IPC team to quickly arrange suitable isolation facilities reducing the risk of cross-transmission. But because the alert is not automatically assigned to duplicate records there is the potential for the patient to not be isolated correctly on admission.

The system has also been adopted by bed managers, who have no access to clinical notes or laboratory results that filter into the system, but are able to identify patients who have infection control risks or ‘flags’ assigned to their patient profile, which assists in assigning beds to patients especially out of hours. The microbiologist on-call also has access to ICNet™.

**Discussion**

*Positive changes to IPC practices*

The 2014 white paper from The Society of Healthcare Epidemiology of America (SHEA) concluded that electronic surveillance “holds the promise of allowing us to direct ever more of our limited resources towards preventing, rather than counting, infection” (Lin et al 2014). Studies have reported the positive impact of electronic
surveillance on IPC time (Chalfine et al 2006) with one study reporting a 65% reduction in time spent manually inputting and recording surveillance data (Evans et al 1986).

Since its introduction in our hospitals, real-time electronic surveillance has resulted in less “desk time” and more face-to-face ward time engaging with all staff at ward level or more “bench time” for our laboratory scientists. ICNet™ has enabled the timely dissemination of surveillance data, particularly during outbreaks to the assembled outbreak management team (OMT) in our hospitals. Collating data electronically has facilitated analysis of microbiological trends for discussion at weekly infection control meetings, has enabled the rapid identification of outbreaks and sentinel events thereby reducing time-to-response. Importantly, it has also standardised data recording removing all subjectivity that may have previously been recorded via paper-based methods. Patient confidentiality has also been improved as less patient records are being held in storage. Electronic surveillance though has not eliminated the need for all data to be analysed by the IPC team in terms of its clinical context.

Pre-requisites for success with electronic surveillance

In 2013, Freeman et al conducted a systematic review of 44 electronic surveillance programmes for healthcare-associated infections from around the world and concluded that a key aspect of the success of electronic surveillance was the connection and integration of electronic systems with existing electronic patient-record databases within the hospital (Freeman et al 2013). Retrieving this data in real-
time confers substantial benefits particularly the identification a patient who should be isolated pending screening results based on previous results. An integrated system enables patient-specific clinical information, for example microbiology test results, haematological or biochemical blood results, dates of previous admissions, infection control status, serological results to be collated creating an individualised patient profile with relevant infection control alerts. This has been one of the major problems encountered with the introduction of ICnet™ in the absence of a unique patient identifier. The introduction of such a system is an urgent requirement as it poses a patient safety risk.

From experience, we believe that the success of the introduction of an electronic surveillance system is heavily dependent on user involvement and training, communication between all users with regards to how data is inputted and technical support from the software provider. Administrative support from the electronic surveillance software provider is essential to ensure that the most up-to-date software platform, that is affordable for the hospital, is in-use. Additionally in order to ensure that the surveillance system is being utilised to its full capacity and functions, on-site training is essential from the provider. On a day-to-day basis the need for a contact person for minor or major operational malfunctions is needed. The learning curve when switching to electronic surveillance can be challenging for staff if they are not familiar with the type of interface that it presents. The provision of annual capital resources from hospital management to enable attendance at workshops and training sessions is imperative as has been previously documented (Furuno et al 2008).
Future goals with ICNet™

Integrating ICNet™ with patient prescriptions in the future is a key goal for our organisation as we feel strongly that antimicrobial stewardship is pivotal in our infection prevention and control strategy.

Conclusions

In conclusion, the hospital environment is complex and multiple interventions working synchronously and seamlessly are required to achieve a reduction in HCAIs. Technology should be embraced to achieve this as recommended by NICE. Electronic surveillance assists with the collection, analysis and dissemination of data and can help to define and expand the scope of an infection prevention and control team. Improved surveillance alone will not reduce infection rates and electronic surveillance must be integrated within existing structures and adapted for the institutional needs.
CHAPTER 11

Final discussion.
Hospitals have been described as “the most complex human organisation” (Saint and Krein 2015) and the hospital environment and healthcare worker’s (HCWs) hands have been widely implicated in HCAIs and the spread of MDROs (Best and Neuhauser 2004, Pittet et al 2005c, Pittet et al 2006). As presented in this thesis, HCAIs have occurred in the Mid-West of Ireland across all age groups, despite the successful implementation of recommended IPC practices. This patient HCAI demographic will continue into the future as advances in clinical medicine have resulted in the enhanced likelihood of survival of those born prematurely and critically ill elderly patients. Between 2009 and 2015, two outbreaks of carbapenemase-resistant Enterobacteriaceae (CPE) (chapters 8 and 9), an extended-spectrum beta-lactamase (ESBL) outbreak in a neonatal intensive care unit (chapter 4), the first reported case of neonatal mastitis secondary to Panton-Valentine leucocidin toxin-positive Staphylococcus aureus (PVL-SA) (chapter 5), the first Irish outbreak of linezolid-resistant S. epidermidis (chapter 6) and a rare case of daptomycin and vancomycin resistant enterococcal infective endocarditis (chapter 7) have all occurred within the region.

In the midst of these HCAIs, positive changes are occurring in the Mid-West as described in chapter 2 with the introduction of the matrix-assisted laser desorption and/or ionisation time of flight mass spectrometry (MALDI-TOF MS) machine in the laboratory, which has helped to eliminate protracted conventional incubation times previously necessary to identify certain species in samples received from both patients in general practice clinics and from within the hospital. From a clinical perspective the introduction of MALDI-TOF MS has afforded patients quicker diagnosis, associated reductions in exposure to empiric broad-spectrum antimicrobial therapy,
and shortened hospital stay. As laboratory testing volumes are increasing by 10-15% per year internationally, driven partly by infection control demands, with enhanced screening for MRDOs such as MRSA, vancomycin-resistant enterococci VRE, ESBLs and CPE (Bourbeau and Ledeboer 2013), a continued investment in laboratory molecular infrastructure is essential. While total laboratory automation as described in chapter 1 is unlikely to be a realistic prospect in the immediate future in the Mid-West, a move to less manual bench work, such as the use of chromogenic agar plates for the detection of CPE and ESBLs as described in chapters 8/9 and chapter 4 respectively, assisted greatly with the mass screening of patients necessary during these outbreaks and assisted with the rapid detection of patients harbouring MDROs leading to the appropriate isolation of these patients.

A multidisciplinary approach to prevent HCAIs is essential and the success described in chapter 3 of our intervention that led to a reduction in the rate of contaminated blood cultures in the neonatal intensive care unit (NICU) via the introduction of sterile applicators for skin antisepsis plus staff education, cannot be overlooked. This was achieved with no changes in NICU staffing levels, and no reduction in bed numbers within the unit. Most importantly, this positive HCAI intervention has had longer term effects on practices within the NICU including increased awareness among staff regarding the procedural skills and expertise necessary for maintaining a sterile field to prevent contamination during taking of blood samples, increased practical phlebotomy training for new staff entering the unit, increased knowledge of the consequences of false positive blood cultures, and a heightened awareness of international best practice guidelines in striving to remain below the recommended rate of <3% blood culture contamination.
Almost seventy years ago Sir Alexander Fleming not only discovered the world’s first antimicrobial, penicillin, but also described the potential post-antibiotic era we may be facing remarking that “the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistance organism” (Barlett et al 2013). At ward level, the fight against MDROs through antimicrobial stewardship remains a main priority and efforts directed towards reducing carbapenem consumption in the Mid-West, in light of high rates of CPE as described in chapters 8 and 9, continue to be successful with year-on-year reductions.

From a HCW perspective, throughout the course of completing this thesis in talking with both nursing and medical staff, a repeated issue that emerged anecdotally was that of the effect of understaffing and increasing staff workload and a perceived increase in patients developing HCAIs and in the spread of MDROs (particularly CPE). This observation is not incorrect with the transmission of HCAI-causing organisms shown to be higher during periods of understaffing (Kovner and Gergen 1998, Robert et al 2000, Halwani et al 2006). A reliance on agency staffing has also been associated with an increased incidence of HCAIs supported by a belief that these staff members may not be as familiar with MDRO precautions as regular hospital nursing staff (Buerhaus and Staiger 1999, Robert et al 2000, Yang 2003, Alonso-Echanove et al 2003). Into the future as the economy emerges from recession, a recruitment drive for permanent staff will be warranted, including additional infection prevention and control (IPC) staff members, who will remain pivotal in completing electronic surveillance and education activities as described in chapter 10.
The Irish Medical Council’s *Guide to Professional Conduct and Ethics for Registered Medical Practitioners* (2009) promotes the concept of open disclosure whereby “patients and their families are entitled to honest, open and prompt communication with them about adverse events that may have caused them harm”, which includes a requirement to inform patients if they have developed a HCAI and/or acquired an MDRO during their inpatient stay and to document this information in the patient’s chart. This acknowledgement by the Medical Council of the inherent dangers to patient safety posed by HCAIs and MDROs has led to the development of the Royal College of Physicians in Ireland (RCPI) Policy Group on HCAIs and the publication of two documents “*How to Advise Patients with a HCAI- Guidance for Healthcare Workers in Dealing with Patients and Members of the Public*” (2009) and “*Healthcare-Associated Infections and Nursing Homes of Extended Care Settings*” (2010).

At a time when patients are receiving more open information than ever regarding HCAIs and MRDOs, it is acknowledged that patient input into IPC strategies to prevent HCAIs is crucial for the success of an IPC programme (Department of Health 2005, Gudnadottir et al. 2013, McGuckin and Govednik 2013b, Ottum et al 2013, Seale et al 2015a). This was demonstrated in the WHO’s ‘Save Lives: Clean Your Hands’ campaign (2009) which demonstrated that patients improved hand hygiene rates amongst HCWs via encouraging patients to ask HCWs if they had performed hand hygiene prior to the clinical interaction (Pittet et al 2006, Storr et al 2009). At a local level in the Mid-West, the creation of a patient council has given patients more access then ever to hospital management and more information regarding the national
performance of the hospital with regard to infection rates thereby fuelling interest in the area.

In conclusion, HCAIs have a major impact on patient care including mortality and morbidity, length of stay and hospital costs. New and emerging diagnostic molecular testing methods in the laboratory will remain a vital step in expediting diagnoses for clinicians, facilitating effective infection prevention and control practices, distributing scarce isolation facilities appropriately, antimicrobial consumption rationalisation and ultimately better patient outcomes.
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APPENDICES
27th February, 2014.

Dr. Ciara O'Connor,
Clinical Microbiology SpR,
University Hospital Limerick,
Dooradoyle,
LIMERICK.

Re: Protocol Title
Molecular microbiology, the burden of multi-drug resistant organisms and the impact on patient care in UL Hospitals.

Dear Dr. O'Connor,

Thank you for attending the Research Ethics Committee meeting on the 26th February, 2014 in connection with your study.

I wish to advise that the Committee has now approved your study.

However, you should note that your study cannot commence until you also receive Risk Management approval. This approval will be issued to you shortly.

You are obliged to inform us as soon as your study is completed or if it terminates early for any reason.

I wish you every success in your study.

Yours sincerely,

Pat Dillon,
Consultant Anaesthetist,
Chairperson, Research Ethics Committee.
22nd December, 2015.

Dr. Ciara O’Connor,
SpR in Clinical Microbiology,
University Hospital Limerick,
Dooroadoyle,
Limerick.

Re/ Protocol Title
An anonymised stool sampling study assess the community carriage of extended spectrum beta-lactamases (ESBLs) and carbapenemase producing Enterobacteriaceae (CPE) in a sample of the Mid-West population.

Dear Dr. O’Connor,

The Research Ethics Committee at the University Hospital Limerick has received a submission for ethical approval for the above study.

The following documents were reviewed and approved by the Research Ethics Committee:

Application to the Research Ethics Committee
Approved

From an insurance perspective, please note that cover does not extend to those parties not employed by the Health Service Executive (HSE), or non-HSE Institutions.

Yours sincerely,

Brian McKeon,
Planning, Performance & Business Information Manager.
(For and on behalf of the Research Ethics Committee & the QPS Department).
Due to copyright restrictions the full text of the following is not included in the electronic version of this thesis

Bioengineered 5:3, 1–6; May/June 2014;

A commentary on the role of molecular technology and automation in clinical diagnostics

Ciara O’Connor, Marie Fitzgibbon, James Powell, Denis Barron, Jim O’Mahony, Lorraine Power, Nuala H O’Connell, and Colum Dunne

http://dx.doi.org/10.4161/bioe.28599
Short report

**Combined education and skin antisepsis intervention for persistently high blood-culture contamination rates in neonatal intensive care**


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**SUMMARY**

Contaminated blood cultures represent challenges regarding diagnosis, duration of hospitalization, antimicrobial use, pharmacy and laboratory costs. Facing problematic neonatal blood culture contamination (3.8%), we instigated a successful intervention combining skin antisepsis using sterile applicators with 2% chlorhexidine gluconate in 70% isopropanol prior to phlebotomy (replacing 70% isopropanol) and staff education. In the six months prior to intervention, 364 neonatal peripheral blood samples were collected. Fourteen (3.8%) were contaminated. In the post-intervention six months, 314 samples were collected. Three (0.96%) were contaminated, representing significant improvement (Fisher’s exact test: $P = 0.0259$). No dermatological sequelae were observed. The improvement has been sustained.

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**Introduction**

Use of blood cultures as a basis for diagnostic testing during hospitalization is ubiquitous. Unfortunately, contamination of blood cultures (i.e. growth of bacteria in blood samples that were not present in patients’ blood during the process of sample collection) with commensal skin micro-organisms is quite frequent and, due to associated uncertainty regarding ‘false-positive’ tests, may cause initiation of empirical antimicrobial treatment, further laboratory testing and lengthened duration of hospital stay. The American Society for Microbiology and the Clinical and Laboratory Standards Institute recommend that an acceptable rate of blood culture contamination should not exceed 3%. There has, therefore, been considerable focus on interventions to reduce contamination of blood cultures, including dedicated education and...
training, establishing of dedicated phlebotomy teams, use of pre-prepared customized blood-culture kits and varying skin antisepsis agents, with generally (but not universally) successful outcomes.\textsuperscript{5,6} With respect to the latter approach, meta-analysis has demonstrated that alcohol-based approaches were more effective than non-alcoholic, whereas chlorhexidine plus alcohol performed better than iodine plus alcohol combinations.\textsuperscript{5} In 2013, Washer \textit{et al.} reported a clinical trial of three antiseptic interventions (70% isopropanol followed by 10% povidone iodine, 70% isopropanol followed by 2% iodine tincture, and 2% chlorhexidine gluconate combined with 70% alcohol) in almost 13,000 blood cultures demonstrating no significant difference in contamination rates and recommending that decisions regarding choice of antisepsis be based on cost or preference.\textsuperscript{6}

However, those studies focused on adult patients with relatively little emphasis placed on paediatric, and specifically neonatal, patients due to concerns regarding, for example, risk of adverse effects on thin, incompletely keratinized skin or potential anaphylaxis.\textsuperscript{7}

Indeed, when assessments of products such as chlorhexidine have been reported, the reported approaches to antisepsis use lower-concentration products (e.g. $\leq 1\%$) rather than the 2% chlorhexidine in 70% alcohol combination that has shown efficacy in adults.\textsuperscript{5} A notable exception is a report by Marlowe \textit{et al.} that determined significantly greater efficacy of a 70% alcohol with 3% chlorhexidine gluconate combination versus povidone-iodine alone in reducing blood culture contamination in a paediatric emergency department setting.\textsuperscript{8} However, that study did not assess chlorhexidine in children aged $<2$ months.

Here, we describe what appears to be the first successful combined intervention, involving both adoption of 2% chlorhexidine in 70% alcohol use and staff education, in a neonatal intensive care unit (NICU) [including premature and very low birth weight newborns ($<1500$ g) babies] with persistently high blood-contamination rates.

\textbf{Methods}

\textbf{Setting}

This intervention was performed at the NICU with 19 cots in the University Maternity Hospital Limerick, Ireland (UMHL). For context, in 2012, there were 909 NICU admissions (from a cohort of 4905 live births). This intervention was instigated by an audit of NICU records showing blood culture contamination rates of 3.4\% in 2009, 3.1\% in 2010, and 3.2\% in 2011 (unpublished data). In the immediate pre-intervention period (January to July 2012), 364 peripheral blood cultures resulted in 17 positive cultures detected from 14 patients (10 male, four female). Three were considered significant clinical isolates: two \textit{Escherichia coli} and one \textit{Streptococcus bovis}.

A blood culture was considered to be contaminated if at least one of the following organisms (considered representative of skin microflora and most widely reported contaminants) was identified in at least one of a series of blood cultures: coagulase-negative \textit{Staphylococcus} spp. (CoNS), \textit{Corynebacterium} spp., alpha- or beta-haemolytic streptococci, \textit{Micrococcus} spp., \textit{Bacillus} spp. and \textit{Propionibacterium} spp. in the context of correlated clinical findings (e.g. fever, leucocytosis, blood biochemistry), and time to positivity.\textsuperscript{10} Thereby, the remaining 14 blood cultures were considered ‘false positives’, containing CoNS (13 specimens) and mixed CoNS and diphtheroids (one specimen). This represents a contamination rate of 3.8\% (14/364).

\textbf{Intervention}

We introduced skin antisepsis using 2\% chlorhexidine gluconate in 70\% isopropyl sterile applicators (ChloraPrep\textsuperscript{\textregistered}; Becton, Dickinson & Co., Franklin Lakes, NJ, USA) replacing 70\% isopropyl alcohol swabs) prior to phlebotomy for all neonates. The antimicrobial efficacy of the chlorhexidine preparation was validated separately in our hospital. Our protocol required that the antisepsis combination remain post-blood collection (leveraging residual chlorhexidine antimicrobial activity). Therefore, in the context of avoiding adverse events for our neonatal patients with potentially fragile skin (especially premature children), we focused specifically on emergence of any adverse events potentially associated with chlorhexidine use.

A concomitant educational programme was provided to NICU staff (consultant neonatologists/paediatricians, doctors in training including registrars and senior house officers, as well as neonatal nursing and midwifery staff) emphasizing the importance and opportunities for hand hygiene, detailing the intervention procedures and use of the sterile applicators. This training occurred between July and December 2012; the introduction of ChloraPrep\textsuperscript{\textregistered} use began in January 2013.

The intervention was approved by the Ethics Board of the Mid-West Teaching Hospitals (Ireland). Informed consent for participation was obtained from parents of all children.

\textbf{Results and discussion}

We describe the first successful intervention to improve persistently high blood-culture contamination rates in a neonatology setting using a combination of 2\% chlorhexidine and 70\% isopropanol complemented with education of NICU staff. Attendance at the 30 min training sessions achieved 100\% compliance and the intervention was well received. All found the sterile applicator to be user-friendly, did not require unusual storage or handling and with a drying time (an important consideration for NICU staff) of 15–30 s being no different to the 70\% alcohol swabs previously employed.

In the immediate post-intervention period (January to July 2013), 314 peripheral blood cultures (from children aged less than one day to more than three weeks) resulted in three contaminated blood cultures (from three separate patients), each involving CoNS (a rate of 0.96\%; Fisher’s exact test $P = 0.0259$). Although CoNS may cause sepsis in neonates, sepsis was not present. We attribute that improvement to the introduction of 2\% chlorhexidine, the applicator system allowing gentle contact with delicate neonate skin, and a greater emphasis on aseptic technique brought about through the provided education programme. We noted no evidence of adverse effects during or following use of the antisepsis combination.
that could have been confounding factors. However, within our NICU, there is increased awareness among staff regarding the procedural skills and expertise necessary for maintaining a sterile field to prevent contamination during taking of blood samples, increased practical phlebotomy training for new staff entering the unit, increased knowledge of the consequences of false-positive blood cultures, and a heightened awareness of international best practice guidelines in striving to remain below the recommended rate of < 3% blood culture contamination.

In conclusion, the introduction of staff education and sterile applicators containing 2% chlorhexidine in 70% isopropanol for neonatal skin antisepsis has significantly reduced blood culture contamination, from 3.8% pre-intervention to 0.96% post intervention. Staff welcomed the training, accepted use of the applicators, neonatal care was not compromised, and no dermatological adverse events were observed. We were unable to determine which element of our intervention was most influential; however, we believe that replication of our combined intervention in larger cohorts or through randomized controlled trials would have merit. That said, due to our results, we plan to introduce this product for skin antisepsis throughout the University of Limerick Group of Hospitals for medical, surgical, and obstetric patients.

Acknowledgements

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None declared.

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References

A case of Panton–Valentine leucocidin toxin-positive Staphylococcus aureus-mediated neonatal mastitis

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Introduction: Neonatal mastitis is an inflammatory condition of the breast frequently associated with Staphylococcus aureus. While Panton–Valentine leucocidin (PVL), a B-pore-forming cytotoxin, is commonly associated with enhanced virulence in community-acquired methicillin-resistant S. aureus isolates, this is the first report to our knowledge of neonatal mastitis caused by PVL-positive S. aureus.

Case presentation: A 20-day-old full-term female neonate presented with bilateral mastitis, complicated by bilateral abscess formation. PVL toxin-positive S. aureus was cultured from aspirates of both breasts. All family members, none of whom presented with symptoms of infection, and, specifically, maternal vaginal samples proved negative for PVL-positive S. aureus. Successful resolution involved surgical drainage and clindamycin therapy.

Conclusion: While PVL toxin-positive S. aureus has previously been implicated in bovine and ovine mastitis, there may now be a need for vigilance with respect to human incidence. Due to PVL-mediated tissue necrosis, breast abscess formation and poor response to conventional antimicrobial therapy should, perhaps, be a cause for suspicion of PVL-bearing S. aureus and expediting of appropriate therapy to avoid potential for long-term consequences such as abnormal breast development.

Keywords: mastitis; neonatal; Panton–Valentine leucocidin; Staphylococcus aureus.

Introduction
Staphylococcus aureus is a commensal skin organism, a major human and veterinary pathogen, and the most common cause of human mastitis (Osterman & Rahm, 2000; Peton & Le Loir, 2014; Rasigade & Vandenesch, 2014; Uhlemann et al., 2014). As such, considerable emphasis has been placed on elucidating the mechanisms by which the virulence of S. aureus strains is mediated (Rasmussen et al., 2013; Sollid et al., 2014). Amongst these mechanisms are the horizontal transfer of the chromosomal cassette conferring resistance to methicillin (SCCmec) (Nubel et al., 2008) and ability to produce toxins such as (but not limited to) Panton–Valentine leucocidin (PVL) mediating tissue necrosis (Labandeira-Rey et al., 2007; Diep et al., 2010).

Abbreviation: PVL, Panton–Valentine leucocidin.

In the context of maternal and infant health, many studies have described incidence and transmission of community-associated and healthcare-associated strains, in addition to recommended antibiotic regimens, invasive and non-invasive management, and prevention strategies including decolonization (Saiman et al., 2003; Fortunov et al., 2007; Alsubaie et al., 2012; Jahanfar et al., 2013; Sheffield, 2013). With respect to mastitis specifically, most reports relate to incidence and treatment of S. aureus maternal infection (Branch-Elliman et al., 2012; Chick et al., 2012; Iatrakis et al., 2013; Perez et al., 2013) and, to a lesser degree, subsequent transmission of infection to (mostly pre-term and neonatal) infants (Behari et al., 2004; Gastelum et al., 2005; Kayiran et al., 2014). Mastitis in children is less common and is largely related to neonates and pubescent/postpubescent age groups (Faden, 2005). A case series of neonates demonstrated that mastitis occurred most commonly in the fourth and fifth weeks of life, although
age ranged from 12 to 45 days, and that parenteral antibiotics eradicated causative microorganisms (Stricker et al., 2005). A larger review of neonatal cases from 2005 to 2011 (in Atlanta, USA) concluded that S. aureus was the most common cause of mastitis and that no infant with a positive breast culture had a concordant positive culture elsewhere (Montague et al., 2013), arguing that urine, blood and spinal fluid cultures are unnecessary in otherwise well, afebrile infants with mastitis.

PVL-positive clones are emerging as virulent, multidrug-resistant S. aureus in Ireland (prevalence of PVL-positive MRSA increased from 0.2 to 8.8 %, and that of PVL-positive MSSA decreased from 20 to 2.5 % between 2002 and 2011) (Shore et al., 2014). Despite this, we believe this report to be the first description of neonatal mastitis caused by PVL-bearing S. aureus and its successful treatment and, as such, will make a contribution to the emerging debate regarding management of neonatal mastitis (Al Ruwaili & Scolnik, 2012).

Case report

A 20-day-old female infant was admitted to University Hospital Limerick (UHL) on 16 December 2013. She was born at 41 weeks gestation via spontaneous vaginal delivery. At day 7 of life, her mother noted that the baby’s right breast was swollen and red. When it failed to settle spontaneously, she brought the baby to her general practitioner who diagnosed right breast mastitis and commenced what proved to be ineffective antimicrobial therapy with oral amoxicillin. The right breast continued to increase in size and the infant’s condition deteriorated, with poor feeding, irritability, lethargy and vomiting. The infant’s parents self-referred to the hospital. On arrival at UHL, examinations revealed discrete areas of erythema suggestive of mastitis on both breasts, more marked on the right. Under the right nipple there was a 4 cm swelling with underlying yellow–green discoloration of the skin. The area was warm and tender to touch, with pain elicited on superficial palpation. There was no obvious point over the right abscess. A 1.5 cm swelling was identified under the left nipple with no associated topical changes. The infant was diagnosed with bilateral mastitis, complicated on the right side with early abscess formation. She was apyrexial.

Haematological investigations demonstrated a normal white cell count ($16.24 \times 10^9 \, \text{L}^{-1}$), elevated neutrophils ($8.48 \times 10^9 \, \text{L}^{-1}$), mild thrombocytosis ($427 \times 10^9 \, \text{L}^{-1}$) and normal haemoglobin count ($14.4 \times 10^9 \, \text{L}^{-1}$). Renal function was normal. C-reactive protein was raised at $11 \, \text{mg L}^{-1}$. Blood cultures were not completed, while urine was free of pathogens. A superficial swab of the right breast abscess resulted in growth of coagulase-negative staphylococci, but too few to enumerate accurately. Initially, the neonate was treated conservatively with intravenous flucloxacillin (220 mg four times per day) and intravenous benzylpenicillin (220 mg three times per day), but exhibited minimal improvement. At 3 days, a small area of necrotic skin developed on the right breast in the periareolar area. Intravenous benzylpenicillin was discontinued, intravenous flucloxacillin was continued and oral clindamycin (25 mg four times per day) was introduced, as (due to increasing prevalence as described above) there was clinical suspicion of PVL toxin-mediated staphylococcal infection.

Subsequently, at 5 days, 5 ml aspirate was surgically removed from the infant’s right breast abscess, which proved positive for S. aureus resistant to amoxicillin and gentamicin and sensitive to flucloxacillin, levofloxacin, linezolid, rifampicin, vancomycin and tetracycline. Intraoperative examination under anaesthesia confirmed a contralateral left breast abscess from which 2 ml S. aureus-positive aspirate was removed.

Post-operative antimicrobial therapy involved oral clindamycin and intravenous flucloxacillin. Both isolates of S. aureus subsequently tested positive for PVL toxin. Antimicrobial therapy was modified to oral clindamycin for a further 7 days, at which point full resolution had been achieved.

Subsequently, staphylococcal protein A (Spa) typing identified the isolate as being t005 (EMRSA-15) (http://spa.ridom.de/spa-t005.shtml), which has been reported mostly in Europe (including the UK and Ireland), ranked ninth in 2010 with respect to relative incidence (Grundmann et al., 2010).

Discussion

Reports of neonatal mastitis are relatively uncommon (Stauffer & Kamat, 2003) but most frequently (ca. 85 %) caused by S. aureus (Holmes & Zadoks, 2011). As such, there is some debate regarding best practice in its management (Al Ruwaili & Scolnik, 2012), but recommended treatment involves antibiotic therapy followed by surgical incision and drainage or needle aspiration if medical management fails (Sloan & Evans, 2003).

In this case, we describe the first incidence of PVL-positive S. aureus-caused neonatal breast abscess. It seems reasonable that risk factors for neonatal acquisition of PVL-positive S. aureus would be similar to those for other S. aureus strains, i.e. ingestion of infected breast milk, impaired skin integrity, or infection of breast tissue (engorged due to maternal hormones) via the nipple (Stauffer & Kamat, 2003). The long-term consequences of neonatal mastitis and its treatment were reported by Panteli et al. (2012), whereby development of the breast was characterized as involving intraductal dilatation, fibrous elements and calcifications or, where surgical incision was required, breast asymmetry and significant reduction in size compared with the uninfected breast. The emergence of PVL toxin-positive S. aureus (Labandeira-Rey et al., 2007; Rasmussen et al., 2013; Shore et al., 2014)
is adding a new perspective to neonatal mastitis incidence due to its ability for tissue necrosis and the potential damage that this may cause to infected infants (Hsieh et al., 1999; Nazir, 2005).

In this case, specifically, the neonate was breastfed successfully for 4 days postpartum and subsequently switched to bottle-feeding for convenience. The baby’s mother did not have any underlying recurrent infective skin or soft tissue infections. This family had not been washing domestic items such as towels used for this neonate separately to those in use for other members of the family. During the course of the neonatal infection no other family member presented with signs or symptoms of a PVL-associated infection. Vaginal carriage of S. aureus is possible; however, samples obtained from high vaginal and groin areas proved negative. The potential for zoonosis (Holmes & Zadoks, 2011) was eliminated as the family did not have a household pet and did not live on a farm, and there was no other direct contact with animals. As this was an isolated incident, environmental (i.e. delivery room and equipment) and staff screening was considered unnecessary.

The vigilance of the infant’s parents and the relatively early identification of PVL-positive S. aureus meant that appropriate targeted (rather than empiric) antimicrobial therapy resulted in eradication of the pathogen. The revision of therapy was influenced to no small part by the availability of aspirated pus, rather than topical swabs, which underwent on-site molecular and conventional susceptibility testing, as described elsewhere (O’Connor et al., 2014), which reduced time to diagnosis by at least 24 h through avoidance of culture on non-selective solid media. It is debatable whether non-invasive assessment such as sonography would have been useful in this case, although its use has been described in differentiating between neonatal abscesses and mastitis (Borders et al., 2009).

Conclusion
Neonatal PVL toxin-positive S. aureus mastitis and breast abscess formation is uncommon but suspicion should arise with poor response to conventional antimicrobial therapy for mastitis. There is a need to improve awareness of PVL-positive S. aureus, particularly among those caring for neonates, as early diagnosis can prevent complications of invasive tissue necrosis and potential for long-term consequences such as abnormal breast development. Sending appropriate samples for testing can expedite diagnosis.

References


Incidence, management and outcomes of the first cfr-mediated linezolid-resistant Staphylococcus epidermidis outbreak in a tertiary referral centre in the Republic of Ireland


Aim: To report the first Irish outbreak of cfr-mediated linezolid-resistant Staphylococcus epidermidis.

Methods: Linezolid-resistant S. epidermidis isolated at University Hospital Limerick from four blood cultures, one wound and four screening swabs (from nine patients) between April and June 2013 were characterized by pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) and staphylococcal cassette chromosome (SCCmec) typing. Antibiotic susceptibilities were determined according to the guidelines of the British Society for Antimicrobial Chemotherapy. The outbreak was controlled through prohibiting prescription and use of linezolid, adherence to infection prevention and control practices, enhanced environmental cleaning, isolation of affected patients, and hospital-wide education programmes.

Findings: PFGE showed that all nine isolates represented a single clonal strain. MLST showed that they belonged to ST2, and SCCmec typing showed that they encoded a variant of SCCmec III. All nine isolates were cfr positive, and eight isolates were positive for the G2576T 23S rRNA mutation commonly associated with linezolid resistance. Isolates exhibited multiple antibiotic resistances (i.e. linezolid, gentamicin, methicillin, clindamycin, ciprofloxacin, fusidic acid and rifampicin). The adopted infection prevention intervention was effective, and the outbreak was limited to the affected intensive care unit.
Conclusions: This is the first documented outbreak of cfr-mediated linezolid-resistant S. epidermidis in the Republic of Ireland. Despite this, and due to existing outbreak management protocols, the responsible micro-organism and source were identified efficiently. However, it became apparent that staff knowledge of antimicrobial susceptibilities and appropriate hygiene practices were suboptimal at the time of the outbreak, and that educational interventions (and re-inforcement) are necessary to avoid occurrence of antimicrobial resistance and outbreaks such as reported here.

Introduction

Linezolid is a bacteriostatic oxazolidinone antibiotic that binds to the 50S subunit of bacterial ribosomes and inhibits protein synthesis. It is licensed for use in 70 countries worldwide, and has been used to treat over four million patients since its introduction in 2000. Linezolid is currently approved for use in the Republic of Ireland for treatment of multi-drug-resistant Gram-positive infections, including nosocomial and community-acquired pneumonia and skin and soft tissue infections, including those caused by meticillin-susceptible and -resistant staphylococci, coagulase-negative staphylococci and vancomycin-resistant enterococci.

Recent surveillance data indicate that <1% of Staphylococcus aureus and 2% of coagulase-negative Staphylococcus spp. (CoNS) are resistant to linezolid. Mutations in chromosomal genes encoding the central loop of domain V of the 23S rRNA, with the G2576T substitution, are the most commonly reported resistance mechanism. Substitutions for 23S rRNA, with the G2576T substitution, are the most commonly reported resistance mechanism. In contrast with staphylococci from clinical infections, as have mutations in the genes for ribosomal proteins L3, L4 and L22. T2500A, T2504A and G2215A have also been identified in some cfr-resistant staphylococci, coagulase-negative staphylococci and vancomycin-resistant enterococci.

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The cfr gene encodes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antimicrobials (the so-called 'PhLOPSA phenotype') and selected macrolides, thus conferring multi-drug resistance.

Outbreaks of cfr-mediated linezolid-resistant S. aureus and Enterococcus faecalis have been described previously. However, this paper describes the molecular epidemiology, management and outcomes of the first documented outbreak of cfr-mediated linezolid-resistant Staphylococcus epidermidis in the Republic of Ireland.

Methods

Setting

University Hospital Limerick (UHL) is a tertiary referral university teaching hospital with 483 inpatient beds. Patients are admitted from the community and from other hospitals located in the Mid-West of Ireland. The catchment population of the hospital is 300,000. The intensive care unit (ICU) is a medical-surgical unit that cares for patients over 16 years of age. At the time of this outbreak, the ICU had seven beds (including two isolation rooms). There were three handwashing stations located within the ICU, with alcohol hand gels at each bedspace. Two full-time consultant microbiologists and two infection prevention and control nurses were employed directly by UHL, and worked on-site at the time of the outbreak.

Index case identification

The index case patient for this outbreak was identified as a male in his twenties admitted to UHL in April 2013 following a deliberate self-poisoning. He was diagnosed with an aspiration pneumonia, and antimicrobial therapy was commenced with piperacillin-tazobactam 4.5 g TDS IV and clarithromycin 500 mg
BD IV. S. aureus was recovered from peripheral blood samples taken on presentation. A chest X-ray demonstrated evidence of a right lower and mid-zone dense consolidation consistent with an aspiration pneumonia. Antimicrobial therapy was modified to flucloxacillin 2 g QDS IV, linezolid 600 mg BD IV and ciprofloxacin 500 mg BD IV. Linezolid was added lest the isolate was proven to be a toxin producer [i.e. Panton Valentine leucocidin (PVL) positive].

After five days, linezolid therapy was stopped as the PVL toxin result, obtained from a referral laboratory, was negative. During a subsequent pyrexial episode, blood samples taken from a femoral line proved positive for CoNS. Paired peripheral blood cultures were not taken at the same time. Routine antimicrobial susceptibility testing demonstrated resistance to linezolid, flucloxacillin and gentamicin, but sensitivity to vancomycin and daptomycin (Table I). This isolate was sent to the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI), Public Health England, London for further analysis. The index case patient was moved into an isolation room within the ICU. The AMRHAI results confirmed cfr-positive linezolid-resistant S. epidermidis, which also harboured the G2576T mutation. Following confirmation of this result, the prescription of linezolid for all other patients within the hospital was prohibited.

**Infection control measure**

This first case of linezolid-resistant CoNS at UHL was a major cause for concern, particularly as colleagues at AMRHAI had recently issued an alert regarding cfr-mediated resistance that advised of the associated public health threat. As a consequence, an outbreak management protocol was initiated that involved meeting with all key stakeholders, including executive management, nursing administration, infection prevention and control, consultant microbiologists, laboratory managers, bed management, hygiene services and the communications team. Contact tracing of all inpatients who may have been in contact with the index case patient while in the ICU was conducted by screening groin and axillae swabs to identify linezolid-resistant CoNS. All CoNS isolates from samples collected from patients who had shared the ICU with the index case, or who had occupied the space at any time up to 14 days after the index case was confirmed as positive, were screened for susceptibility to linezolid.

All affected patients in this outbreak were isolated immediately and standard contact precautions were employed. Patients harbouring linezolid-resistant CoNS were given daily whole-body washes with 2% chlorhexidine gluconate. All patients in the ICU who were fit for discharge were cohorted to a single ward to minimize cross-transmission. Axillae and groin screens were performed weekly for all patients until discharge. Enhanced cleaning of the ICU was instigated in parallel with increased auditing. This involved twice-daily cleaning of affected areas with detergent, in addition to a ‘deep clean’ with sodium hypochlorite to ‘decontaminate’ the area on discharge. Hand hygiene audits were also performed with greater frequency in affected areas, which involved twice-weekly observational audits at ward level.

Screening of staff for carriage of the organism, air sampling and environmental sampling were not performed due to resource limitations. An additional factor influencing the decision not to screen staff was that the ICU is not a closed unit (i.e. as a matter of clinical policy, patients admitted to the ICU remain under the care of their primary consultant rather than the ICU team), resulting in considerable traffic of medical and surgical teams to the ICU each day. Instead, a targeted educational programme focused on hand hygiene, and appropriate prescribing was implemented.

**Microbiological and molecular detection of linezolid-resistant S. epidermidis**

Linezolid resistance in staphylococci is defined by both the Clinical Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as a minimum inhibitory concentration (MIC) ≥8 mg/l; this threshold was used to define resistance in this outbreak. In total, 18 bloodstream isolates were assessed retrospectively for linezolid susceptibility, and 187 screen specimens ( groin and axillae swabs) were processed in the laboratory.

Screening samples were inoculated on Staph/Strep agar (Fannin LIP, Galway, Ireland) with a linezolid disc (10 μg) (Oxoid Ltd, Basingstoke, UK). These were incubated aerobically at 35 ± 1°C for 24 h. All suspect linezolid-resistant staphylococcal isolates were subsequently identified using MALDI-ToF MS (Bruker Daltonics, Bremen, Germany) as described previously. MiCs of linezolid were determined using E-test (bioMérieux, Marcy l’Etoile, France). All isolates with an MIC of linezolid ≥8 mg/l, as per EUCAST guidelines in use at the time in the laboratory, were referred to AMRHAI for antimicrobial susceptibility testing by agar dilution methodology. Isolates were characterized by staphylococcal cassette chromosome (SCCmec) typing, pulsed-field gel electrophoresis and multi-locus sequence typing as described previously. Isolates were screened by polymerase chain reaction (PCR) for the cfr gene and by PCR and restriction fragment length polymorphism assay for the G2576T 23S rRNA mutation associated with linezolid resistance.

**Results**

**Demographics of affected patients**

Between April and June 2013, nine patients (five males, four females) were found to be harbouring linezolid-resistant S. epidermidis. The nine affected patients ranged in age from 28 to 83 years. All patients were admitted to UHL via the Emergency Department, and were not transfers from other hospitals. One patient (the index case) was admitted under a medical service. The other eight patients had complex surgical admissions including small and large bowel obstructions, urosepsis secondary to calculus, abdominal aortic aneurysm repair and colorectal malignancy. Eight patients who were deemed to have been contacts of the index case tested positive. Four of these patients were identified on screening, and the other four patients were identified from clinical samples (four blood cultures, one wound swab) and axillae and groin swabs. CoNS was not isolated from any deep tissue or intra-operative samples. Patient data are shown in Table II.

**Molecular characteristics of linezolid-resistant isolates**

All nine isolates were positive for the cfr gene, and eight isolates also bore the G2576T 23S rRNA mutation. The linezolid-
resistant *S. epidermidis* strain was detected from four blood cultures: one inpatient on the general surgical ward (from peripheral blood cultures) and three ICU patients (two from femoral line blood cultures, one from arterial line blood culture). Pulsed-field gel electrophoresis showed that all nine isolates represented a single strain. Multi-locus sequence typing showed that they belonged to ST2, and SCCmec typing showed that they encoded a variant of SCCmecII.

Only seven of the nine patients had received treatment with linezolid previously. All isolates were resistant to linezolid, gentamicin, mexitelcalcin, clindamycin, ciprofloxacin, fusidic acid and rifampicin; teicoplanin resistance was variable. All isolates were sensitive to daptomycin, vancomycin and quinupristin/dalfopristin. The full antimicrobial sensitivity testing results are outlined in Table I.

### Discussion

This *cfr*-mediated linezolid-resistant *S. epidermidis* outbreak was the first such outbreak in the Republic of Ireland. Although dissemination to the other ICU patients was rapid and vigorous, multi-disciplinary interventions limited, and led to rapid termination of, the outbreak. While the blood culture of the index case was fortuitously identified as being linezolid resistant, it is not unusual for these samples, in the study institution and others, to be less than fully characterized with regard to species identification and/or antimicrobial susceptibility testing unless the patient is in critical care or has prothetic material in situ. This poses an important question as to whether, generally, there may be under-ascertainment of linezolid-resistant CoNS and whether CoNS may be acting as a reservoir of *cfr*-mediated resistance in hospitals.

Previous research has demonstrated that the *cfr* gene is capable of horizontal transfer between staphylococci. In Europe, *cfr* was initially identified on a plasmid from a bovine isolate of *Staphylococcus sciuri* in 2000; its occurrence among animal isolates appears to have been influenced by the use of florfenicol in the veterinary industry. Worldwide, the incidence of linezolid-resistant CoNS is currently 28 times that of linezolid-resistant *S. aureus*.

Clinical reports of linezolid resistance date from 2007 when Toh et al. reported the first *cfr*-mediated linezolid-resistant clinical isolate of meticillin-resistant *S. aureus* (MRSA). Since then, notable reports have included two US-based cases of *cfr*-mediated resistance in clinical isolates of *S. epidermidis* and *S. aureus* in 2008, a 2013 case report of *cfr*-mediated linezolid-resistant MRSA blood culture isolate from an ICU in Barcelona, Spain, and characterization of *cfr*-mediated MRSA and *S. haemolyticus* with fatal outcomes originating from a German group.

A report detailing a 2008 outbreak of *cfr*-mediated linezolid-resistant *S. aureus* in Madrid, Spain was particularly similar to the UHL outbreak, affecting 12 high-risk critically ill ICU patients; two patients infected with linezolid-resistant *S. aureus* and three patients colonized with linezolid-resistant *S. aureus* died. The Spanish ICU was not closed to admissions during their 17-week outbreak. Measures to control the outbreak included isolation with contact precautions and restriction of linezolid prescription, as were employed at UHL. It would have been interesting to compare linezolid consumption prior to the outbreak between the two studies, but this was not mentioned in the Spanish paper. However, at UHL, use of linezolid has been increasing steadily over the past two years. Antimicrobial inpatient consumption at UHL is collated on a quarterly basis, with data reported as defined daily doses/100 bed-days used.

Between 2011 and 2012, there was a 3% increase in the prescription of intravenous linezolid and a 28% increase in the prescription of oral linezolid. Between 2012 and 2013 (when this outbreak occurred), the use of intravenous linezolid increased by a further 11%. Despite the increase in the use of intravenous linezolid between 2011 and 2013, the median level of use at UHL during this period was consistently lower than that of other Irish hospitals categorized as having a similar patient mix.

In the UHL outbreak, the authors were unable to perform environmental or staff screening. In the Madrid outbreak, no staff members were found to be colonized with linezolid-resistant *S. aureus*, but 15 of 91 (17%) swabbed environmental surfaces were contaminated by linezolid-resistant *S. aureus*, and may have contributed to the prolonged nature of the outbreak. At UHL, staff knowledge of the mechanisms by which antimicrobial resistance emerged was lacking, and awareness of the transmissibility of Gram-positive organisms via contaminated hands of healthcare workers and equipment (e.g., stethoscopes, blood pressure cuffs and intravenous drip stands, etc.) was poor. Subsequently, human and financial resources were mobilized to implement a hospital-wide education programme for all healthcare staff, in liaison with the antimicrobial pharmacist, which targeted management, nursing, medical, household and administration staff. Twelve months after this outbreak, regular education sessions continue to be provided to all staff, and a specific lecture on

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ICU, intensive care unit; BC, blood culture; PHE, Public Health England; MIC, minimum inhibitory concentration; M, male; F, female.
the local antimicrobial resistance patterns is provided to all medical and surgical trainee doctors during induction. No further instances of cfr-mediated linezolid resistance were identified between July 2013 and June 2014.

Conclusions

This is the first report of cfr-mediated linezolid resistance in Staphylococcus epidermidis in the Republic of Ireland. Linezolid is a relatively novel agent and, therefore, an outbreak was unexpected. However, in retrospect, this was inevitable given the increasing consumption of linezolid at UHL. The hospital has learned from this outbreak and practice has changed accordingly. Linezolid susceptibility is monitored in CoNS for all ICU patients using a linezolid disc-based process. The judicious use of linezolid with consultant-only prescribing, application of strict infection control measures, isolation of all patients from this outbreak when they subsequently presented for outpatient appointments or admission, enhanced daily environmental cleaning, a low threshold for characterizing CoNS identified in samples such as blood cultures and wound swabs from critical care areas and other high-risk hospital patients, and the presence of a visible antimicrobial stewardship team on the hospital wards have been, and will continue to be, essential for the preservation of linezolid as a valuable therapeutic agent.

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References


A case of fatal daptomycin-resistant, vancomycin-resistant enterococcal infective endocarditis in end-stage kidney disease

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Introduction: Ireland currently has the highest reported rate in Europe of vancomycin-resistant Enterococcus (VRE) isolated from the bloodstream, but data regarding the prevalence of VRE endocarditis remain scarce. Treatment options for Enterococcus-mediated endocarditis are limited, and therefore daptomycin is commonly used off licence in this setting.

Case presentation: A 60-year-old male with end-stage kidney disease (ESKD) presented with VRE bacteraemia secondary to a gangrenous right foot colonized with vancomycin-resistant Enterococcus faecium. Aortic valve endocarditis was confirmed using transoesophageal echocardiography. Treatment was commenced with linezolid and subsequently modified to combination therapy with daptomycin and rifampicin. High-dose daptomycin therapy was employed unsuccessfully and, after 20 days of therapy, daptomycin resistance emerged, which proved fatal.

Conclusion: The case was ethically challenging and involved a refusal of amputation and, ultimately, any form of treatment by the patient. In summary, however, daptomycin-resistant VRE bacteraemia complicated by recalcitrant daptomycin-resistant VRE endocarditis proved fatal for this patient. Further evaluation of the efficacy and safety of high-dose daptomycin for the treatment of VRE infective endocarditis is needed.

Keywords: Daptomycin; end-stage kidney disease (ESKD); resistance; rifampicin; vancomycin-resistant Enterococcus (VRE).

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Introduction

Enterococci are Gram-positive organisms that form part of the commensal gut flora. A common nosocomial pathogen resistant to many antimicrobials, enterococci can reside and survive in the clinical environment for prolonged periods with an associated risk of contaminating healthcare workers and the potential for cross-transmission to patients (Biedenbach et al., 2004). Enterococci are the second most common nosocomial bloodstream pathogen isolated in the USA with the prevalence of vancomycin-resistant Enterococcus (VRE) in intensive care units currently exceeding 30% (Wisplinghoff et al., 2004). Enterococcus faecalis and Enterococcus faecium are the predominant pathogens associated with VRE infections (Carmeli et al., 2002). Ireland has the highest proportion of VRE bloodstream isolates in Europe, as reported to the European Centre for Disease Prevention and Control. In 2013, 176 VRE were identified among 408 E. faecium isolates from bloodstream infections in Ireland (43.1%). VRE results in significant increases in length of hospital admission and mortality (DiazGranados et al., 2005).

For patients aged ≤ 60 years, enterococci account for up to 10% of cases of native valve infective endocarditis, rising to almost 20% in those >60 years. In prosthetic valve endocarditis, 5–10% of cases that occur in the first 60 days post-operatively and up to 15% of cases occurring after 60 days are secondary to enterococcal infection (Mylonakis and Calderwood, 2001). The first case of VRE infective endocarditis was reported in 1996 (Bishara et al., 1999), but subsequent reports in the literature have been rare (Forrest et al., 2011). A study involving a review of 107 cases of enterococcal endocarditis noted no cases secondary to vancomycin resistance (McDonald...
et al., 2005). Haemodialysis patients and renal transplant recipients are at relatively high risk of VRE infective endocarditis (Humphreys et al., 2004), and rates of intestinal VRE colonization have been shown to be higher in these groups than in other patient populations (Patel et al., 2001).

There are limited effective antimicrobial treatment options for VRE infections including infective endocarditis. Daptomycin is a concentration-dependent bactericidal lipopeptide antibiotic, licensed in the Republic of Ireland for the treatment of skin and soft tissue infections. It is also licensed for use in the management of Staphylococcus aureus bacteremia, including right-sided endocarditis, at doses of 4 and 6 mg (kg body weight)\(^{-1}\), respectively (Boucher and Sakoulas, 2007). Dose adjustment is required for patients with renal impairment. It is used off label in Ireland to treat patients infected with VRE. It is excreted renally (Hawkey, 2008) and is rapidly bactericidal (Mascio et al., 2007), and had demonstrated in vitro concentration-dependent killing with a half-life of 8–9 h (LaPlante and Rybak, 2004). The drug acts on the cell membrane causing membrane depolarization and also inhibits the synthesis of lipoteichoic acid, which is necessary for cell wall synthesis (Enoch et al., 2007). Daptomycin displays in vitro activity against >90 % of Enterococcus spp., including those resistant to other antibiotics, such as vancomycin, linezolid and quinupristin-dalfopristin (Sader and Jones, 2009). Thickening of the cell wall associated with vancomycin intermediate-susceptible S. aureus isolates has been shown to decrease susceptibility to daptomycin and, while an exact resistance mechanism in enterococci has not yet been determined, a similar mechanism secondary to reduced daptomycin diffusion through a thickened enterococcal cell wall has been proposed (Cui et al., 2006; Kelley et al., 2011).

To the best of our knowledge, this is the first report of daptomycin-resistant VRE aortic valve infective endocarditis complicated by end-stage kidney disease (ESKD) in Ireland and one of only nine reports of daptomycin-resistant infective endocarditis globally based on a PubMed search completed in July 2015. In that search, no reports of such endocarditis complicated by kidney disease could be found.

**Case report**

The right big toe of a 60-year-old Irish male with ESKD secondary to diabetic nephropathy on haemodialysis became gangrenous secondary to peripheral vascular disease, and he underwent a toe amputation in May 2010. Samples taken from the wound were determined to be positive for VRE (E. faecium) and a surveillance rectal swab detected that the patient was VRE colonized. He was commenced on a 5-week course of intravenous (i.v.) linezolid 600 mg twice daily followed by 7 days of oral linezolid 600 mg twice daily with limited resolution. A right mid-foot amputation was performed in September 2010 followed by wound debridement in October 2010. The history of this infected foot dated back to 2008 when he had his first episode of cellulitis requiring antimicrobials. With each progressively worsening foot infection, the patient was very reluctant to undergo any surgical procedure to remove the nidus of infection, necessitating prolonged antimicrobial exposure. Between October 2009 and January 2010, a 16-week admission occurred for a Klebsiella pneumoniae and Candida albicans continuous ambulatory peritoneal dialysis-associated peritonitis requiring a protracted prescription of broad-spectrum bacterial and fungal cover. Ultimately, the patient was commenced on haemodialysis.

On 15 July 2011, he was admitted from the dialysis unit with sepsis. Blood samples were taken from the dialysis permacath, which were positive for VRE. Laboratory testing again demonstrated the presence of E. faecium, demonstrating the same antibiogram as the earlier VRE cultured in 2010. He was commenced on i.v. linezolid 600 mg twice daily. A transthoracic echocardiogram was performed, which did not show any vegetations suspicious for infective endocarditis. Consecutive blood cultures, 48 h apart, demonstrated evidence of continuous VRE bacteremia. Sterility of blood was achieved for 22 days until a set of blood cultures taken in early August 2011 again yielded VRE. A transoesophageal echocardiogram identified vegetations on the aortic valve. A decision was made to switch from linezolid 600 mg twice daily to daptomycin 6 mg kg\(^{-1}\) i.v. administered following dialysis sessions. Seven days later, with blood cultures still VRE positive, rifampicin 600 mg twice daily i.v. was added for additional synergistic bactericidal activity as the patient also had an ongoing active foot infection, for which source control had not been achieved. An interdialytic daptomycin dosing regimen was commenced with daptomycin at 8, 8 and 10 mg kg\(^{-1}\) administered post-dialysis at 48, 48 and 72 h, respectively (following consultation with the suppliers of the antimicrobial agents and a review of available literature) (Salama et al., 2009). The patient consistently declined amputation of his right leg during his final admission. Following 17 days of therapy (29 August 2011), we noted daptomycin resistance (MIC > 8 mg l\(^{-1}\)) during routine in-house susceptibility testing.

In early September 2011, with worsening necrosis and gangrene of his right leg, confirmed aortic valve endocarditis, unresolving VRE bacteraemia and rising C-reactive protein, together with refusal to consent to a right leg amputation, the patient undertook his own discharge from hospital with rifampicin 600 mg twice daily per os and daptomycin 6 mg kg\(^{-1}\) i.v. once daily to be administered intravenously in the community. He continued to deteriorate with persistent VRE bacteremia. Dialysis was subsequently withdrawn following consultation with the patient and his family, as there was consensus that, given ongoing sepsis from multiple sources, the patient was too unwell to continue with haemodialysis. Antimicrobial therapy also ceased at this time. Palliative care services reviewed the patient and he was admitted to a local hospice where he died 2 weeks later.

**Discussion**

Patients undergoing outpatient haemodialysis are at high risk of VRE acquisition due to repeated close contact
Daptomycin-resistant VRE infective endocarditis in ESKD

between patients in the dialysis unit, repeated exposure of patients to antimicrobials including vancomycin for treatment of dialysis line infections (at least in Ireland), shared transport to dialysis units and frequent hospital admissions (Kee et al., 2012). The reliance on central catheters in the dialysis population is thought to be a key factor contributing to the rise of resistant enterococcal isolates (Boucher et al., 2009). To place this case in context, at the time of writing, the University Hospital Limerick dialysis unit is attended by 67 patients of whom 37 are VRE positive. A further 78 attend a satellite dialysis unit in the nearby city centre of whom 11 are known to be VRE positive.

Mortality rates in patients with VRE bloodstream infections are high, ranging between 20 and 46% (Han et al., 2009; McKinnell et al., 2011; Twilla et al., 2012), and bacterial endocarditis in dialysis patients is associated with poor prognosis (Leither et al., 2013). Surgical management of VRE infective endocarditis is rarely employed, as most patients have significant co-morbidities that would prohibit such invasive intervention (Salgado and Farr, 2003). As a consequence, medical management is the preferred treatment approach. The use of daptomycin as monotherapy for the treatment of VRE endocarditis is not recommended due to increased risk of resistance (Linden, 2007; Schulte et al., 2008). Various mechanisms for enterococcal daptomycin resistance have been described including altered cell membrane composition, altered ability of daptomycin to depolarize the cell (Steed et al., 2011) and the risk of gene transfer of daptomycin-resistant determinants (Kelesidis et al., 2011; Diaz et al., 2014). Concern has also been raised as to whether a tendency to provide empiric VRE cover to at-risk patients with daptomycin and linezolid, while awaiting final culture results, may also be contributing to the development of resistance secondary to overuse (Short et al., 2014).

A published analysis of infective endocarditis cases warned that daptomycin monotherapy for enterococcal infective endocarditis could not be advocated and recommended combination therapy of daptomycin with another antimicrobial such as rifampicin, gentamicin, linezolid or a β-lactam for treatment of VRE endocarditis (Cerón et al., 2014). Similarly, synergistic therapy with daptomycin and rifampicin has been advocated (Leclerq et al., 1991). However, the addition of rifampicin 600 mg twice daily per os proved ineffective in our patient.

The appropriate dose of daptomycin for VRE infective endocarditis has not been defined but, as in vivo and in vitro studies have revealed that using higher doses of daptomycin increases the degree and speed of bactericidal activity due to its concentration-dependent pharmacodynamic mechanism, higher doses than recommended for S. aureus bacteraemia (6 mg kg⁻¹ day⁻¹) have been suggested (Cunha et al., 2007; Hall et al., 2012). At 6 mg kg⁻¹ day⁻¹, sterility of blood cultures was not achieved. The use of daptomycin at higher doses of 8 mg kg⁻¹ day⁻¹ (Kullar et al., 2011, 2013) and 14 mg kg⁻¹ day⁻¹ (Moise et al., 2009) for the treatment of infective endocarditis have been demonstrated with no adverse patient outcomes. Reported cases of daptomycin resistance have been associated with complicated infections (e.g. osteomyelitis, medical device infections and endocarditis) at doses of ≤6 mg kg⁻¹ (Kelesidis et al., 2011). Our attempt at using intradialytic daptomycin at 8, 8 and 10 mg kg⁻¹ post-dialysis at 48, 48 and 72 h, respectively, also failed. There was no rise in creatinine phosphokinase levels with daptomycin therapy over 20 days, which was a positive finding given that rising creatinine phosphokinase levels can often prohibit the use of daptomycin, particularly in those with renal impairment.

We are unaware of any randomized controlled trials that have evaluated the management of VRE infective endocarditis. Linezolid is a bacteriostatic antimicrobial licensed by the US Food and Drug Administration for treatment in this setting, but cure rates remain disappointing (Birmingham et al., 2003; Chuang et al., 2014) and resistance rates of 20% have been reported (Pogue et al., 2007). The side-effect profile of linezolid with associated myelosuppression, including neutropenia, thrombocytopenia and anaemia (Hachem et al., 2003) is particularly problematic in the dialysis population. Tigecycline has been used in combination with other agents for the treatment of vancomycin-resistant E. faecium infections, but it achieves low serum levels and reports of its use are scarce (Florescu et al., 2008; Schutt and Bohm, 2009).

Conclusion

In conclusion, VRE endocarditis is a very uncommon nosocomial infection but is clinically challenging when it arises, given the lack of licensed treatment options. In this case, the same E. faecium isolate persisted from multiple sites despite antimicrobial therapy, having probably initially been a rectal colonizer. High-dose daptomycin is generally well tolerated (and we observed no adverse effects in our patient). Ultimately, without amputation and definitive source control, this patient was unlikely to clear his VRE bacteraemia, affording some potential to cure his infective endocarditis. The ethical issues associated with this case are multi-faceted and deserve discussion elsewhere. The rapid emergence of daptomycin resistance following only 20 days of therapy should heighten the awareness of other centres to this possibility in complicated VRE infections. No linezolid resistance was observed in this case.

We continue to monitor for resistance in all patients receiving treatment with daptomycin. The prescription of daptomycin within our institution remains restricted, and is permissible following consultation with clinical microbiology or infectious diseases staff only. To date, no further cases of VRE infective endocarditis or daptomycin resistance have been identified in our institution.

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**References**


present. This approach gathers such evidence, using patients as filters to identify those who require further investigation, thus allowing resources to be targeted effectively. Using text messages achieved a PDS rate of 79% and was straightforward and efficient. A website is used to send and receive messages. A template message is stored and is used each month with an Excel file containing the mobile telephone numbers to which the message is to be sent. The responses can be viewed via the website and are sent to a specified e-mail address. In terms of cost, 2000 text messages can be purchased for £120.

A limitation to this approach is the availability of patients’ mobile telephone numbers. Whereas this information should be routinely collected and would therefore require no additional data collection, in reality this does not always happen. This may raise wider questions concerning the ability of healthcare professionals to contact patients for other clinical issues. The availability of mobile telephone numbers can be easily addressed by greater awareness of the importance of recording patient contact details.

A further limitation may be associated with the target population. Text messages were used in this case for caesarean section patients, a population that is highly likely to have a mobile telephone and respond to a text message. PDS on older groups who may not have mobile telephones or may not wish to use text messages may be more difficult.

A text message service for PDS is a cost-effective, efficient, and realistic method of performing PDS and should be considered for future surveillance programmes.

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Against the onslaught of endemic carbapenemase-producing Klebsiella pneumoniae, the war is being lost on the Irish Front

Madam,

In the context of the excellent report of successful control of an outbreak of carbapenemase-producing Klebsiella pneumoniae (KPC) in an Italian neonatal intensive care unit published in this journal, we wish to report the consequences of the first outbreak of KPC in Ireland and how, despite identification of operational factors associated with the incidence and best efforts towards rectifying those, our 410-bed hospital in the West of Ireland is failing to control endemic KPCs.

Globally, there is recognition of the significant morbidity and mortality implications associated with the emergence of carbapenemase-producing bacteria. The resulting vigilance has resulted in enhanced reporting of outbreaks, many being the first of their kind in specific countries, and descriptions of molecular studies to determine incidence and transfer of the carbapenemase-encoding blaKPC-harbouring IncFIA plasmid between clonal variants. With indicative rates of carriage being ~20% internationally (~34% in Ireland as of February 2014), infection control specialists are reacting with novel techniques for microbiological detection and strategies for prevention of nosocomial transmission. Clinical microbiologists are facing therapeutic challenges related to limited, relatively unproven, antimicrobial treatment options.

At our hospital, between January and March 2011, there had been a reduction of 30% in cleaning staff and supervision, in parallel with <50% overall compliance with hand hygiene protocols. It was to these factors that an outbreak of nine KPC cases was attributed. These occurred in high dependency (HDU; four cases: January and February) and intensive care (ICU; four cases: February) units and a dedicated surgical ward (four cases: March). All strains involved were sensitive to gentamicin, colomycin and tigecycline, and were clonally related (using pulsed-field gel electrophoresis). Polymerase chain reaction confirmed that all carried blaKPC-2.

As expected, outbreak management included identification of all affected areas and control of access; an attempt to determine the source; prevention of spread; communication of the risk to all staff, to public health agencies and all receiving hospitals in Ireland. Following the outbreak, which was deemed successfully controlled, there was considerable and rapid investment in on-site molecular technology, enhanced hand hygiene training and surveillance, and introduction of a screening policy whereby all HDU and ICU transfers are isolated until determined to be negative for KPC carriage.

Despite these measures, control has been ineffective and we have experienced simultaneous incidences of seven cases in June 2012, five cases in January 2014, and four cases in April 2014, with significant morbidity and mortality. In light of our inability prevent KPCs, we are debating the value of completing a study of local community carriage and revision of empirical first-line treatments, or even prophylaxis.

Conflict of interest statement
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References
Letter to the Editor

Limerick: forever associated with five lines of rhyme or infamous for irrepressible carbapenemase-producing Enterobacteriaceae for all time?

Sir,

In the context of January’s edition of this journal, focused on multidrug-resistant Gram-negative bacteria (MDRGNB) to coincide with publication of new Joint Working Party Recommendations on the prevention and control of MDRGNB, we wish to report the increasing prevalence of carbapenemase-producing Enterobacteriaceae (CPE) at our 440-bed hospital in Ireland’s mid-west.1,2

The first reported case of CPE in Ireland was in Limerick in 2009, as was the first outbreak of cfr-mediated linezolid-resistant Staphylococcus epidermidis.3,4 We have now identified 140 discrete isolates, each pertaining to a single patient, by retrospective audit of microbiological analyses performed at University Hospital Limerick between February 2009 and December 2015. Despite identification of operational factors associated with the incidence, and best efforts towards rectifying those, there has been an inexorable increase in CPE detection; two were identified in 2009, four in 2010, 11 in 2011, 10 in 2012, eight in 2013, 45 in 2014, and 60 in 2015. Seasonality is evident with highest numbers detected in the spring and summer months.

Of the associated carbapenemases, one was imipenem, three were oxacillin (OXA), 13 were New Delhi metallo-β-lactamase (NDM), and 123 were Klebsiella pneumoniae carbapenemase (KPC). During this period, two outbreaks occurred. In 2011, three out of nine patients affected by KPC-producers died, whereas in 2012, 10 patients were affected by NDM-producers died. Rectal swabs accounted for 74% (N = 103) of our CPE-positive results over this six-year period. This is in contrast to the outcome of an Irish prevalence study performed in 2011 across 40 Irish critical care units (37 adult and three paediatric), which found no CPE carriage.5 However, as CPE was emerging as a global public health challenge at that time, those authors were prescient in recommending the importance of maintaining vigilance for CPE in Ireland.

Our current analysis has identified two fatal KPC bacter-aemias, three intra-abdominal theatre-derived samples positive for CPE, with K. pneumoniae (N = 80), Klebsiella oxytoca (N = 30), and Citrobacter freundii (N = 17) dominant. Indeed, our 2011 outbreak documented transmission of these strains between Irish hospitals.6 Subsequent to this outbreak, CPE screening has been performed, in accordance with national guidelines, via rectal swab or stool specimen, for all patients admitted to our intensive care unit or high dependency unit; similarly for any patient transferred from another healthcare institution, any patient who has had an acute admission in the prior 12 months to any hospital in Ireland’s mid-west (except for paediatric, maternity or orthopaedic care), and for any patient previously hospitalized abroad. In total, 2685 screens were processed in 2011, increasing to 6128 in 2014, and exceeding 9493 in 2015.

We have benefited from considerable investment in on-site rapid molecular laboratory technology. A dedicated hygiene nurse has been appointed to monitor and audit the quality of environmental cleaning. A permanent cohort ward opened in November 2015. Local CPE guidelines have been drafted in line with international best practice along with implementation of the Public Health England carbapenem-resistant Enterobacteriaceae (CRE) toolkit. Hydrogen peroxide vapour decontamination is performed post discharge of any KPC-colonized or -infected patient. Chlorhexidine gluconate wash-cloths are used to bathe all patients in critical care areas, and a recent review of clinical staff has shown 84% hand hygiene compliance. Despite these measures, control has proved ineffective. Reliance on acute 16-bedded Nightingale wards, insufficient isolation rooms, emergency department overcrowding, and lack of unique patient identifiers or of an integrated information technology system are hindering our efforts to contain CPE.

Moreover, due to observed seasonality and the prevalence of agriculture in the Limerick region, we are exploring a sectoral link to our CPE challenge. Measures have been taken in our hospital to limit prescription of carbapenems, thus reducing consumption by 21% in 2015 versus increases of 36%, 25%, and 4% in 2012, 2013, and 2014, respectively. However, in comparison to the UK, there are few published data in Ireland regarding carbapenem consumption in the veterinary industry, whereas previous reports have linked NDM-1 and OXA-48 with close proximity to animals.7

Limerick is known as Ireland’s ‘Treaty City’ due to a Jacobite siege in 1691. We are developing a new siege mentality, and we believe that a national strategy for CPE, akin to that adopted in Israel, is urgently needed to avoid a country-wide replication of the Limerick CPE endemic.8

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An Irish outbreak of New Delhi metallo-β-lactamase (NDM)-1 carbapenemase-producing Enterobacteriaceae: increasing but unrecognized prevalence

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SUMMARY

Background: Carbapenemase-producing Enterobacteriaceae (CPE) may cause healthcare-associated infections with high mortality rates. New Delhi metallo-β-lactamase-1 (NDM-1) is among the most recently discovered carbapenemases.

Aim: To report the first outbreak of NDM-1 CPE in Ireland, including microbiological and epidemiological characteristics, and assessing the impact of infection prevention and control measures.

Methods: This was a retrospective microbiological and epidemiological review. Cases were defined as patients with a CPE-positive culture. Contacts were designated as roommates or ward mates.

Findings: This outbreak involved 10 patients with a median age of 71 years (range: 45–90), located in three separate but affiliated healthcare facilities. One patient was infected (the index case); the nine others were colonized. Nine NDM-1-producing Klebsiella pneumoniae, an NDM-1-producing Escherichia coli and a K. pneumoniae carbapenemase (KPC)-producing Enterobacter cloacae were detected between week 24, 2014 and week 37, 2014. Pulsed-field gel electrophoresis demonstrated similarity. NDM-1-positive isolates were meropenem resistant with minimum inhibitory concentrations (MICs) ranging from 12 to 32 μg/mL. All were tigecycline susceptible (MICs ≤1 μg/mL). One isolate was colistin
Introduction

Enterobacteriaceae are Gram-negative colonizers of the human gut. Carbapenemase-producing Enterobacteriaceae (CPE) are resistant to most classes of antimicrobials.1 New Delhi metallo-β-lactamase-1 (NDM-1) is among the most recently discovered carbapenemase enzymes. The responsible \( \text{bla}_{\text{NDM-1}} \) gene is thought to have originated in the environment from plant pathogens and is plasmid-borne.2 NDM-1 confers broad-spectrum β-lactam resistance mediated by hydrolysis of all β-lactam antimicrobials, with the exception of monobactams, such as aztreonam.3 Many NDM-1-producing bacteria remain susceptible only to colistin, fosfomycin, and tigecycline.4,5 Since first reported as implicated in human disease, NDM-1-producing bacteria have been recovered from numerous infection sites including device-associated infections, intra-abdominal, urinary tract, bloodstream, and surgical wounds.6,7 Publications have described most variants of the enzyme as having originated in Asia.8

Acquisition of NDM-1-producers has been reported as associated with travel to known reservoir areas, notably the Indian subcontinent (Pakistan, India, Sri Lanka) and the Balkan countries, where prevalence of community carriage is estimated to be 5–15%.9–12 Global dissemination is facilitated by intercontinental travel, including healthcare tourism, and migration.13–15 International spread has been rapid.16 The NDM isolates identified in Ireland prior to this outbreak were isolated or paired cases from several hospitals countrywide and generally with an identifiable link with travel. Dissemination of the \( \text{bla}_{\text{NDM-1}} \) gene, like other similar resistance mediators, is facilitated by inadequate infection prevention and control practice in healthcare settings, uncontrolled or poorly controlled antimicrobial use, inadequate practices related to food preparation and water treatment, and poor general sanitation.13,17–19 The largest reported NDM outbreak to date in a non-endemic country was reported from Poland in 2015, where 374 cases of infection or colonization, with a variety of NDM-producing Enterobacteriaceae, were identified from 40 hospitals over a two-year period.20

In this report, we describe what we believe to be the first outbreak of NDM-1-producing Enterobacteriaceae in Ireland, which occurred in 2014.

Methods

Setting

The Department of Clinical Microbiology at University Hospital Limerick (UHL) provides a centralized microbiology service for six acute hospital sites (800 beds; population circa 380,000 people). As an aid to contextualizing this outbreak, it is notable that 48 K. pneumoniae carbapenemase (KPC) and one imipenem-hydrolysing β-lactamase (IMI)-producing isolates were detected at UHL between February 2009 and May 2015, as previously published.21

A CPE screening policy was in place before the outbreak began. Patients in our hospital group were screened on admission for CPE if: admitted to the intensive care unit (ICU) or high dependency unit (HDU) at UHL; transferred from another hospital in Ireland; had an acute admission in the past 12 months to any hospital within our hospital group (except for paediatric, maternity, or orthopaedic); or hospitalized abroad. Haemodialysis patients are screened every three months. Patients in ICU and HDU are screened weekly until discharge.

Study definitions

Cases were defined as patients with a NDM-1 positive culture from any site during their hospitalization. Contacts were designated as room or ward mates.

Microbiological and molecular detection of NDM-1

Since 2011, CPE surveillance at UHL had been performed on stool samples or rectal swabs using KPC-producer selective chromogenic agar (CHROMagar™, KPC, Paris, France). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Diagnostics) identification was performed on all colonies, as previously described.22 Antimicrobial susceptibility testing was performed using broth microdilution (ARIS Sensititre™ system, Thermo Fisher Scientific, Inc., MA, USA). Elevated carbapenem minimum inhibitory concentrations (MICs) for meropenem and ertapenem were confirmed by E-test (AB Biodisk, Solna, Sweden) following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines; ertapenem resistance MIC >1 g/L, meropenem resistance MIC >8 g/L. Isolates with elevated carbapenem MICs were further evaluated using the modified Hodge test (MHT). Commercially available diagnostic kits (Rosco Diagnostica A/S, Taastrup, Denmark) consisting of meropenem discs supplemented with β-lactamase inhibitors (meropenem + dipicolinic acid; meropenem + boronic acid; meropenem + cloxacillin) were used to phenotypically distinguish CPE isolates. Isolates were referred to the National Carbapenemase-Producing Enterobacteriaceae (CPE) Reference Laboratory Service (CPERLS) at University Hospital Galway, Ireland for CPE confirmation by molecular methods. Genetic relationship of...
NDM-1 isolates was determined by pulsed-field gel electrophoresis (PFGE).

**Details of NDM-1-positive patients**

A retrospective chart review assessing clinical and epidemiologic characteristics was completed for all patients involved, including: dates of admission, transfers, and hospital discharges; locations within the hospital; procedures and operative notes; use of invasive devices; biochemical and haematological blood test results; antimicrobials received and documentation of a travel history.

**Infection control interventions**

The isolation of NDM-1-producing *K. pneumoniae* triggered initiation of the hospital’s outbreak management protocol. Rectal swabs or stool samples were obtained from all contacts of the index case. Information leaflets were distributed to all patients and family as appropriate. The Public Health England CPE tool kit was implemented during the outbreak.24 All infected or colonized patients were barrier-nursed using long-sleeved disposable gowns and gloves, and single rooms were used when available. Chlorhexidine gluconate wash-cloths were employed for bathing of patients. Dedicated equipment was prioritized for NDM-positive inpatients, both infected and colonized, but was not available for all NDM contacts. A semi-automated electronic data surveillance system, ICNet™ (Baxter, Gloucester, UK), was used to collate the records of the outbreak had been admitted via the ED. High-touch surfaces where positive NDM-1 patients had been admitted, underwent routine cleaning followed by hydrogen peroxide vapour decontamination post discharge. A deep clean of the emergency department (ED) including the waiting room and resuscitation areas was performed as seven patients involved in the outbreak had been admitted via the ED. High-touch surfaces such as door handles, bedside lockers, and chairs and bed rails were emphasized by the hospital hygiene nurse manager for cleaning to reduce cross-transmission. An ultraviolet torch was used to assess the quality of cleaning performed and face-to-face feedback regarding cleaning deficits was supplied to cleaning operatives. Environmental sampling was not performed during this outbreak.

A further initiative was introduced involving, on a daily basis, a joint pharmacist/clinical microbiologist handover of all in-house carbapenem prescriptions, and subsequent discussion by the either the microbiology consultant or registrar with clinical teams regarding alternative agents where appropriate.

**Results**

During the outbreak, between June and September 2014, nine patients with NDM-1-producing *K. pneumoniae* and one patient with both NDM-1-producing *Escherichia coli* and KPC-producing *Enterobacter cloacae* were detected. The isolates were detected in samples from UHL and from two affiliated regional hospitals, located 10 and 40 km away. Prior to this outbreak, no cases of NDM-producing Enterobacteriaceae had been identified in our laboratory. None of the patients in this outbreak were known to have previous colonization with extended-spectrum β-lactamase (ESBL) producers based on previous screening. Three of the patients had been screened for CPE before this outbreak, and had been found to be negative on those occasions.

Clinical specimens that were positive for NDM-1-producing Enterobacteriaceae included mid-stream urine samples (*N = 2*), rectal swabs (*N = 8*), and skin biopsy samples (*N = 3*). NDM-1-producing isolates were meropenem resistant with MICs ranging from 12 to 32 mg/L. All isolates were tigecycline susceptible (MIC ≤1 mg/L). One isolate was colistin-resistant (MIC 4.0 mg/L; *mcr-1* gene negative). PFGE demonstrated that the *K. pneumoniae* isolates were closely related (Figure 1). Multi-locus sequence typing (MLST) was not performed.

**Index case**

The index case was a community-dwelling Irish female. In the summer of 2014, she was admitted with sepsis. Blood and peritoneal fluid cultures confirmed *E. coli*. Initial admission was to a six-bed bay in a general medical ward preceding transfer the following day to a single room in the intensive care unit (ICU). Admission screens confirmed prior colonization with meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). A rectal screen at that time did not detect CPE. Empiric therapy used intravenous (IV) cefazidime 2 g every 8 h and gentamicin. As she did not respond to initial therapy, treatment was changed to meropenem 1 g IV every 8 h, metronidazole 500 mg IV three times per day and vancomycin 1.5 g IV every 12 h but was de-escalated to meropenem monotherapy.

Seven days post admission, a rectal CPE screen (routine for ICU patients) detected CTX-M extended spectrum β-lactamase (ESBL)-producing *K. pneumoniae* and NDM-1-producing *K. pneumoniae*. The patient subsequently developed a skin rash, and diagnostic biopsies were performed on the left forearm. An abscess developed at the skin biopsy site; CTX-M ESBL-producing *K. pneumoniae* and NDM-1-producing *K. pneumoniae* were isolated from the abscess discharge.
IV tigecycline 50 mg every 12 h was initiated and the site was debrided surgically. The patient died two months following admission secondary to refractory soft tissue sepsis.

Evolution of the outbreak

The patient demographics are summarized in Table I; all were permanent Irish residents. During the outbreak, our routine screening programme was extended to include weekly testing of rectal swabs or stool specimens from patients with epidemiological and environmental links to confirmed CPE-positive patients. CPE screening was performed for a period of four weeks after no new cases of CPE colonization or infection had been detected.

In June 2014, two contacts of the index case prior to her ICU admission were identified (patients B, D). Both had been on the medical ward with the index case during her 24 h admission before transfer to ICU. Patient B was screened, identified as NDM-1-producer positive and was isolated. Patient D was discharged prior to CPE screening. She re-presented for admission in July 2014 and was re-admitted to a six-bedded area on the same medical ward to which she had been admitted in June 2014. CPE screening confirmed that she was NDM-1-producer positive and she was isolated immediately. Patients E, F, and H were ward contacts of the index case, and patients G and I were identified from routine admission rectal CPE screens performed during the outbreak period. Both had been admitted to UHL in the previous 12 months; neither had had contact with the index case.

Two additional NDM-1-producer positive patients were identified during the outbreak period, but that had no apparent epidemiologic link with the outbreak cases. Patient C was identified as CPE positive from a screening rectal swab that was performed at a regional hospital 10 km from UHL. This CPE screen was performed because the patient had been admitted to UHL in the previous 12 months but his last admission to UHL had been almost five months before the outbreak was declared. Patient J was identified as CPE positive from a urine sample collected at a regional hospital 40 km away from UHL: this isolate was determined by PFGE (Figure 1) to be the isolate most distantly related to the other outbreak isolates.

Given the identification of NDM-1 at three different associated hospitals, a decision was made to perform contact tracing and screening of all contacts at each site. As a result of that exercise, during the outbreak, 2204 CPE screens, including contact tracing and routine CPE screening, were processed in our laboratory, which in addition to detecting the NDM-1 isolates, also identified 13 new KPCs and one OXA-48.

Carbapenem consumption

Only the index case had been prescribed meropenem during the current admission; the patient had received five days of meropenem before the isolation of an NDM-positive culture.

NDM-1 isolates identified post outbreak

In week 31, 2015 (i.e. 10 months after the 2014 outbreak ended), NDM-1-producing K. pneumoniae was identified in an mid-stream urine sample from an 81-year-old female residing in a private long-term care facility (LTCF). This patient was a contact of the index case during the outbreak but CPE was not detected at the time. PFGE demonstrated similarity to the 2014 isolates. In week 32, 2015, NDM-1-producing K. pneumoniae was identified from a rectal swab of a 71-year-old public LTCF patient who was known to have been colonized previously with KPC-producing Citrobacter freundii. This isolate did not demonstrate similarity to previous isolates. In week 42, 2015, NDM-1-producing K. pneumoniae was isolated from a mid-stream urine sample of a patient repatriated from Bosnia. She had not had been admitted to UHL previously and had never had any specimens processed in the UHL microbiology laboratory. In week 48, 2015, NDM-1-producing E. coli was detected in a 60-year-old patient who had been recently hospitalized in India (Figure 2). Again, these isolates did not demonstrate similarity to the outbreak strain.

Discussion

The source of the index patient’s NDM-1-producer acquisi- tion remains uncertain although acquisition during the hospital admission of June 2014 is considered likely. The index case had...
### Table I
Clinical characteristics of patients involved in an outbreak of New Delhi metallo-β-lactamase-1 (NDM-1) carbapenemase-producing Enterobacteriaceae

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Place of residence</th>
<th>Date admitted</th>
<th>Admitting diagnosis</th>
<th>Treated with meropenem during admission</th>
<th>Specimen isolated</th>
<th>Carbapenemase enzyme(s) detected</th>
<th>Date of culture</th>
<th>Infected/colonized</th>
<th>Outcome</th>
<th>Previous admission to hospital in previous 12 months</th>
</tr>
</thead>
</table>

LTCF, long-term care facility; KPC, *Klebsiella pneumoniae* carbapenemase.
been on haemodialysis for 16 years and switched to peritoneal dialysis in 2013 (five months prior to NDM-1 detection). In our care, all haemodialysis patients undergo surveillance CPE screening every three months, but the same screening is not conducted for peritoneal dialysis patients. The index case patient was screened for rectal CPE in December 2013, at which time CPE was not detected. Following her transition to peritoneal dialysis, no further CPE screening was performed prior to her transfer to ICU on this final admission to UHL, at which time CPE was likewise undetected. She had never worked in a healthcare setting nor lived with any healthcare workers. She had no known travel to NDM-1 endemic areas.

In this outbreak, international travel was not a recognized factor, implying that there may be a hospital and/or community burden of blaNDM-1 than had not been previously appreciated. Struelens et al. reviewed 77 NDM-1 producing Enterobacteriaceae reported from 13 European countries from 2008 to 2010. 25 Among 35 of the cases with recorded travel history, 31 had involved travel to, or admission to a hospital in, India or Pakistan, and five patients had been hospitalized in the Balkan region. Possible nosocomial acquisition accounted for 13 of 77 cases (17%). In contrast, our outbreak more closely resembled the outbreak reported by Borgia et al. that occurred in Brampton, Ontario, Canada where five patients were identified as carrying K. pneumoniae reported from clinical specimens of which 12 CPE isolates originated (Table I). Such a study is needed, justified by our data and the endemic area is of decreasing value in identifying persons at risk of colonization or infection with NDM-1 producers. As the successful management of this outbreak demonstrates, prompt infection prevention and control practices are essential to prevent transmission. No staff or environmental screening was performed but extensive resources directed towards education, hand hygiene compliance, environmental disinfection, cleaning standards and reducing carbapenem consumption were successful in controlling rapid in-hospital transmission of NDM-1 producers. The subsequent detection of additional cases, in particular the related isolate from a nursing home resident in week 31, 2015, demonstrates both the difficulty of definitely eradicating these organisms once established in the “revolving door” systems of nursing homes and hospitals, and the fact that these bacteria are becoming more prevalent.

In this outbreak, our antimicrobial stewardship has been modified. Overall hospital antibiotic consumption rate in defined daily doses (DDD) per 100 bed-days used (BDU) demonstrates a reduction in carbapenem consumption. Between 2014 and the end of 2015, carbapenem consumption decreased by 21% (2014: 4.43 DDD/100 BDU, 2015: 3.49 DDD/100 BDU). This compares very favourably with a 4% increase from 2013 (4.24 DDD/100 BDU) to 2014, 25% increase from 2012 (3.39 DDD/100 BDU) to 2013, a 36% increase from 2011 (2.50 DDD/100 BDU) to 2012.30

In conclusion, this outbreak and our other sporadic isolates indicate the changing epidemiology of NDM-1 CPE. In Ireland, as elsewhere (e.g., Canada), a history of travel to a known endemic area is of decreasing value in identifying persons at risk of colonization or infection with NDM-1 producers.

Learning from the outbreak

In 2013, Lin et al. reported substantial community reservoirs of CPE in the USA.29 Currently, there are no data available in Ireland regarding national prevalence of CPE in long-term care facilities (LTCFs) as a national point prevalence study of LTCFs relating to multidrug-resistant organisms has never been performed. However, three of the ten patients in this outbreak were permanent residents of three separate LTCFs (two public, one private) and one other patient was a permanent resident in a residential care facility for adults with learning disabilities (Table I). Such a study is needed, justified by our data and the fact that, between 2009 and 2015, 140 CPE isolates were identified from clinical specimens of which 12 CPE isolates originated in local public (N = 10 isolates) and private (N = 2 isolates) LTCFs.30

Influenced by this outbreak, our antimicrobial stewardship has been modified. Overall hospital antibiotic consumption rate in defined daily doses (DDD) per 100 bed-days used (BDU) demonstrates a reduction in carbapenem consumption. Between 2014 and the end of 2015, carbapenem consumption decreased by 21% (2014: 4.43 DDD/100 BDU, 2015: 3.49 DDD/100 BDU). This compares very favourably with a 4% increase from 2013 (4.24 DDD/100 BDU) to 2014, 25% increase from 2012 (3.39 DDD/100 BDU) to 2013, a 36% increase from 2011 (2.50 DDD/100 BDU) to 2012.30

In conclusion, this outbreak and our other sporadic isolates indicate the changing epidemiology of NDM-1 CPE. In Ireland, as elsewhere (e.g., Canada), a history of travel to a known endemic area is of decreasing value in identifying persons at risk of colonization or infection with NDM-1 producers. As the successful management of this outbreak demonstrates, prompt infection prevention and control practices are essential to prevent transmission. No staff or environmental screening was performed but extensive resources directed towards education, hand hygiene compliance, environmental disinfection, cleaning standards and reducing carbapenem consumption were successful in controlling rapid in-hospital transmission of NDM-1 producers. The subsequent detection of additional cases, in particular the related isolate from a nursing home resident in week 31, 2015, demonstrates both the difficulty of definitely eradicating these organisms once established in the “revolving door” systems of nursing homes and hospitals, and the fact that these bacteria are becoming more prevalent.

Acknowledgements

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Conflict of interest statement

None declared.

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References

An optimized work-flow to reduce time-to-detection of carbapenemase-producing Enterobacteriaceae (CPE) using direct testing from rectal swabs


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A commentary on the disparate perspectives of clinical microbiologists and surgeons

Nuala H O’Connell, Ciara O’Connor, Jim O’Mahony, Ronstan Lobo, Maria Hayes, Eric Masterson, Michael Larvin, J Calvin Coffey, and Colum Dunne

http://dx.doi.org/10.4161/bioe.28722
Irrepressible carbapenemase-producing Enterobacteriaceae in the Mid-West of Ireland?

A retrospective epidemiological and microbiological review of 140 isolates from 2009 to 2015.


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick. Infection Prevention and Control Team, University Hospital Limerick, Limerick. Graduate Entry Medical School, University of Limerick, Limerick. Department of Pharmacy, University Hospital Limerick, Limerick.

Irish Society of Clinical Microbiologists Spring Meeting, Dublin, February 2016

The first outbreak of New Delhi metallo-β-lactamase (NDM)-1 carbapenemase producing Enterobacteriaceae in the Republic of Ireland. What did we learn and what would we do differently?


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick. Carbapenemase Producing Enterobacteriaceae (CPE) Reference Laboratory, Department of Medical Microbiology, University Hospital Galway, Galway. Department of Infection Prevention and Control, University Hospital Limerick, Limerick. Department of Clinical
First report of a \textit{cfr}-mediated linezolid resistant \textit{Staphylococcus epidermidis} outbreak in a Republic of Ireland tertiary referral centre.


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Department of Infection Prevention & Control, University Hospital Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick. Antimicrobial Resistance and Healthcare Associated Infection Reference Unit, Public Health England, London, UK.

Irish Society of Clinical Microbiologists Spring Meeting, Dublin, April 2014
Pre-procedure skin antisepsis with 2% chlorhexidine gluconate/70% isopropyl alcohol sterile applicator in the NICU; a positive impact on blood culture contamination rates.


Department of Clinical Microbiology and Infection Prevention & Control Team, University Hospital Limerick. Department of Paediatrics (Division of Neonatology) University Maternity Hospital Limerick.

Irish Society of Clinical Microbiologists Winter Meeting, Dublin, November 2013

Poster Presentations

Unstoppable carbapenemase-producing Enterobacteriaceae in the Mid-West of Ireland? A retrospective epidemiological and microbiological review of 140 isolates from 2009 to 2015.


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick. Infection Prevention and Control Team, University Hospital Limerick, Limerick. Graduate Entry Medical School, University of Limerick, Limerick. Department of Pharmacy, University Hospital Limerick, Limerick.

European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, April 2016
The first incidence of a CTX-M ESBL-producing *Escherichia coli* outbreak mediated by mother to neonate transmission in an Irish neonatal intensive care unit.


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Department of Paediatrics, University Maternity Hospital Limerick, Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick. Infection Prevention and Control Team, University Hospital Limerick, Limerick. Antimicrobial Resistance and Healthcare Associated Infections, Microbiology Services – Colindale, Public Health England, London, UK.

_Irish Society of Clinical Microbiologists Winter Meeting, Dublin, October 2014_

_Healthcare Infection Society (HIS), Lyon, November 2014_

_University of Limerick Research Symposium, Limerick, October 2015_

Too late to win the war? The epidemiology of CRE in the Mid-West of Ireland.


Department of Clinical Microbiology, University Hospital Limerick, Limerick. Department of Infection Prevention & Control, University Hospital Limerick. Centre for Interventions in Infection, Inflammation & Immunity (4i), University of Limerick, Limerick.

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*University of Limerick Research Symposium, Limerick, October 2013*

*Irish Paediatric Association Annual Meeting, Dublin, November 2013*
Dr. Ciara O’Connor  
Department of Clinical Microbiology  
Beaumont Hospital  
Dublin 9  

26th February 2015  

Dear Dr. O’Connor,  

I am pleased to inform you that the Irish Society of Clinical Microbiologists has awarded an ISCM Research Bursary of €50,000 to fund your study "To establish on-site molecular testing for carbapenem resistance mediated by carbapenemases at University Hospital Limerick.”  

If you wish to take up the Bursary, please advise the start and completion dates before March 31st and provide details of an academic or hospital account to which the funds can be transferred. This grant can be taken up at any time between April 2015 and September 2016. A report to the Society on completion of the work will be necessary and for projects extending over two years, a mid-term report will be expected.  

On behalf of the Society I would like to congratulate you on your success. I look forward to hearing from you.  

Yours sincerely,  

________________  
Dr. Susan Knowles  
President of Irish Society of Clinical Microbiologists