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 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 SCCmecII. 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.
Introduction

Linezolid is a bacteriostatic oxazolidinone antibiotic that binds to the 50S subunit of bacterial ribosomes and inhibits protein synthesis.\(^1\) It is licensed for use in 70 countries worldwide, and has been used to treat over four million patients since its introduction in 2000.\(^2\) 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.\(^3,5\) 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.\(^6\) Substitutions for T2500A, T2504A and G2215A have also been identified in some commonly reported resistance mechanism.\(^6\) Substitutions for 23S rRNA, with the G2576T substitution, are the most

Outbreaks of *cfr*-mediated linezolid-resistant *S. aureus*\(^13,14\) and *Enterococcus faecalis*\(^15\) 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 caters 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

### Table I

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<th>Cln</th>
<th>Ery</th>
<th>Cip</th>
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PHE MIC, Public Health England minimum inhibitory concentration; Gent, gentamicin; Ox, oxacillin; Pen, penicillin; Tei, teicoplanin; Van, vancomycin; Cln, clindamycin; Ery, erythromycin; Lin, linezolid; Cip, ciprofloxacin; Moxi, moxifloxacin; Quin/Dalf, quinupristin/dalfopristin; Tet, tetracycline; Dap, daptomycin; Fus, fusidic acid; Rif, rifampicin; M, male; F, female.
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 (AMRHI), Public Health England, London for further analysis. The index case patient was moved into an isolation room within the ICU. The AMRHI 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 AMRHI 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 inititiated 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. Microdilution MICs of linezolid were determined using E-test (bio-Mérieux, 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 AMRHI for antimicrobial susceptibility testing by agar dilution methodology. Isolates were characterized by staphylococcal cassette chromosome 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 analysis 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, mexitcillin, clindamycin, ciprofloxacins, 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 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 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 be 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 and 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 emerges 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
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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Conflict of interest statement

None declared.

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None.

References


