Title page

Colonisation with extended-spectrum beta-lactamase (ESBL) not detected in a prevalence study.

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Abstract

Background

The Mid-West of Ireland has higher than average national rates of invasive extended-spectrum beta-lactamase (ESBL) bloodstream infections and carbapenemase-producing Enterobacteriaceae (CPE), with increasing numbers of ESBL isolates detected in community-dwelling patients.

Aims

To conduct a point prevalence study in a convenience sample of the Mid-West population with the aim of determining the extent of ESBL colonisation.

Methods

Utilising anonymised community stool samples that had completed routine analysis, we conducted a point prevalence study over a four-week period on all samples that met defined inclusion and exclusion criteria. Limited epidemiological data was recorded: (1) age of patient, (2) gender, (3) sender location. From these stool specimens, rectal swabs were inoculated (eSwab™ 480CE, Copan, Italy), which were subsequently cultured on selective chromogenic agar (Colorex™ ESBL). Culture plates were incubated aerobically at 37˚C for 24 hours.

Results

Of 195 samples processed, 58% (n=112) were from females. The median patient age was 62.4 years (range 20-94 years). 186 samples (95%) originated from general practitioner clinics. During the study period, only nine eligible stool samples were received from LTCF (6 public). From 195 Colorex™ ESBL chromogenic agar plates cultured, no ESBL-producing organisms were detected.

Conclusions

This community point prevalence study did not identify ESBL-colonisation despite high numbers of patients with invasive ESBL bloodstream infections presenting for admission in our institution. We believe this may be because of our small sample size. Data regarding antimicrobial exposure and other risk factors for ESBL-colonisation was also not available. We remain vigilant for ESBL-producing organisms.

Keywords

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, Ireland, point prevalence study, rectal carriage

Introduction

Extended-spectrum beta-lactamase (ESBL)-producing organisms are a significant cause of nosocomial and community-acquired infections and pose a public health threat [1]. For hospital clinicians, the isolation of an ESBL-producing organism in the laboratory reduces the choice of antimicrobial agents for the patient. The most recent European Antimicrobial Resistance Surveillance System (EARSS) data on antimicrobial resistance in Ireland indicated that invasive ESBL-producing *Escherichia coli infections* (blood or CSF) now account for 10.8% of all invasive *E. coli* infections [2]. However, in the Mid-West of Ireland, this number is substantially higher at 25.4% [2].

ESBLs are transferrable enzymes found in Gram-negative organisms that confer resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect carbapenems (e.g., meropenem or imipenem) [3]. Other anti-microbials, such as piperacillin-tazobactam, cefepime, ciprofloxacin and gentamycin demonstrate limited bactericidal effect against ESBL-producing isolates [4-6]. A consequent reliance on the prescription of meropenem as empiric therapy for critically unwell and unstable patients presenting with Gram-negative bacteraemia is likely a contributory factor to the high rates of CPE within the region [7]. An increase in ESBL-producing isolates has been observed in our laboratory since 2004 (104 isolates) with the number of cases peaking in 2013 (611 isolates) and remaining at a similar level since then (unpublished data). In 2015, 571 new ESBL-producing isolates were identified in our laboratory of which 27% (n=156) were identified in clinical samples submitted from general practitioner clinics and 47% (n=269) from samples obtained from residents of community long term care facilities (LTCF).

Small-scale local prevalence studies have been performed previously in Ireland to assemble data regarding ESBL epidemiology from clinical sites not captured by EARSS but no national ESBL point prevalence study has ever been conducted. In 2005, Ni Chulain *et al.* reported in this journal a prevalence of 0.3% amongst *E. coli* from urine samples (community and hospital) from patients in the West of Ireland during 2002-2003 [8]. The largest Irish observational study of ESBL-producing organisms to date screened all hospital and community samples that cultured Enterobacteriaceae for ESBL production over a four year period (2004-2008). Those samples originated from three separate acute hospitals in close geographical proximity in the West of Ireland, and 974 ESBL producing organisms were identified from 464 patients [9]. A 2012 study conducted in Dublin also found ESBL-producing *E.coli* in both community and acute hospital isolates, raising concern regarding established reservoirs, with dissemination being facilitated via a repetitive cycle of patient admissions and discharges [10]. In 2015, a prevalence study in Dublin sampling immunocompromised patients and those in critical care (n=317), identified 50 patients (15.8%) harbouring ESBL-producing Enterobactericeae [11]. The most recent 2016 point prevalence study conducted in a Dublin maternity hospital by Knowles *et al.* (n=123; antenatal mothers only) used rectal screening for multi-drug resistant organisms (MDROs) and identified two patients (1.63%) colonised with ESBL-producing Enterobacteriaceae [12].

The aim of performing this study was to sample a sub-set of the Mid-West population of Ireland to determine the prevalence of colonisation by ESBL-producing organisms. Broad inclusion criteria were employed to try and capture a typical ‘snapshot’ of community-dwelling patient samples routinely received in the laboratory.

Methods

Setting

The Department of Clinical Microbiology at the University Hospital Limerick (UHL) provides a centralised microbiology service for six acute hospital sites and for general practitioners working within the area. The hospital group serves a population of circa 400,000 people. The laboratory processes approximately 350-400 stool samples per month from inpatients and outpatients, with seasonal variation. Between 2010 and 2015, 2647 new ESBL isolates were detected from clinical specimens in the laboratory, with the largest number of specimens received from LTCFs.

Ethical approval

Ethical approval to complete this study was granted by the Health Service Executive (HSE) Mid-Western Regional Hospital Research Committee in December 2015.

Stool collection

The stool collection period was from January 10th 2016 to February 5th 2016. All stools samples received in the microbiology department of the UHL group during the study collection period were considered for inclusion in the study if they met the inclusion and exclusion criteria outlined below. Only residual stool sample following routine analysis was used.

Inclusion and exclusion criteria

Inclusion criteria: (1) outpatient stool samples from adults ≥18 years, (2) formed, semi-formed and liquid stool including stoma bag samples (Bristol Stool Chart types 1-7) were accepted, (3) ≥ 5mls of stool must have been remaining in the specimen container post completion of the requested stool test. Exclusion criteria: (1) any written information on the sample label/container to indicate known ESBL or other multi-drug resistant organism (MDRO) positivity, (2) stool samples for which the epidemiological data being recorded was not available on the stool sample container.

Stool sample processing

Stool samples (5 mL) were individually decanted into a new numbered universal container (without checking prior microbiology results). No additional information was recorded on the outside of the universal container. Limited epidemiological data were recorded as specified by a local ethics committee for each patient: (1) age of patient, (2) gender, (3) sender location, based on addressograph, recorded as either community or LTCF.

A flocked nylon swab applicator (eSwab™ 480CE, Copan, Italy) was inoculated into each stool specimen and removed once visibly stained with stool. Each eSwab™ specimen transport tube contains 1ml of Liquid Amies medium and swabs were frozen at -20˚C as per manufacturer's instructions.

Culture

Culturing was performed in batches of 30 patient stool samples.. Each numbered eSwab™ was allowed to thaw on ice at room temperature. Once thawed, the eSwab™ tube was vigorously shaken manually for 5 seconds between the thumb and forefinger to release the sample from the swab tip and ensure an even dispersal of the faecal material in the suspension. The tip of the eSwab™ was then removed from the eSwab™ tube. A pipette plus filtered pipette tips were used to transfer 100µl volume of faecal suspension onto the surface of one quadrant of the culture media. Colorex™ ESBL (E&O Laboratories Ltd., Bonnybridge, United Kingdom) chromogenic agar was employed for this study. The agar is formulated to inhibit the growth of non-ESBL isolates.

A sterile 10µl bacteriology loop was then used to streak the primary inoculum across the surface of the second, third and fourth quadrants of the agar plate. Quality control was performed on the culture plates for each new batch of agar plates opened using control strains to ensure integrity. Culture plates were incubated aerobically at 37˚C for 24 hours. If no growth was identified at 24 hours, agar plates were re-incubated for a further 24 hours to assess for any subsequent growth. Any growth was to be analysed by Gram stain, followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)., Antimicrobial susceptibility testing was to be performed if Gram-negative organisms were identified. No further bench work was to be performed on Gram-positive organisms or fungi as neither fell within the study remit.

Results

Epidemiological data

Of 195 samples processed, 58% (n=112) were from females. The median patient age was 62.4 years (range 20-94 years) (Figure 1). 186 samples (95%) originated f.rom general practitioner clinics. During the study period, only nine eligible stool samples were received from LTCF, of which six were public institutions.

Culture results

From 195 Colorex™ ESBL chromogenic agar plates cultured, no ESBL-producing organisms were detected. No growth was identified on 189 plates at 24 and 48 hours following aerobic incubation at 37 ˚C. On five plates at 24 hours incubation, small dull pin-point purple colonies were noted. The colour confirmation on the agar product specification specified growth of *E. coli* as red colonies and *Enterobacter spp., Klebsiella spp.* and *Citrobacter spp.* as metallic blue colonies. A Gram stain was performed of the purple colonies. Gram-positive organisms were identified and no further analysis was conducted. At 48 hours, there was no further growth on these plates.

Discussion

The completion of a point prevalence study in the Mid-West of Ireland was warranted given our higher than average ESBL bacteraemia and CPE rates compared to other similar bed-size and case-mix hospitals in Ireland and we were concerned locally as to whether a community reservoir of ESBLs had become established. The method of ESBL screening to ensure optimal detection of ESBL colonisation via rectal swabbing, stool specimens or PCR-based detection methods, remains topical [13]. For this study, stool specimens were selected for convenience and only one type of ESBL culture agar, Colorex™ ESBL (E&O Laboratories Ltd., Bonnybridge, United Kingdom), was selected for use. PCR-based detection was not available.

The small study size was a limitation of this study. In terms of potentially available stool specimens, on average 350-400 stool specimens per month are received in the laboratory, with a large proportion from inpatients for carbapenemase-producing Enterobacteriaceae (CPE) testing. Inpatient specimens were excluded from the study, which significantly reduced the number of potential patient specimens. The distribution of patients within specified age groups and gender was unbalanced but unintentional, as the design of the study was to include all who met inclusion criteria.

Data regarding recent outpatient hospital visits, hospitalisations or procedures, cumulative antimicrobial exposure, occupational exposures to antimicrobials (the Mid-West has a major agricultural sector), time spent by community-dwellers in long-term care facilities for respite/convalescence, recent foreign travel and/or hospitalisations abroad, background medical/surgical status would have provided useful epidemiological and microbiological information to help identify patients with risk factors for ESBL-colonisation. As the anonymisation of the stool specimens was a prerequisite for gaining ethical approval to proceed, this data could not be collated. Through reviewing patient microbiological records including previous MDRO screens performed, analysis of whether previously known positive patients (infected or colonised) were now ESBL negative would have been useful. Spontaneous loss of ESBL positive-colonisers has been demonstrated in other studies, but is less likely to occur in the context of repeated hospitalisations and antimicrobial prescriptions [14].

The 2016 point prevalence study conducted in a Dublin maternity hospital by Knowles *et al.* regarding colonisation with ESBLs in the Irish maternity population, identified only two women (1.63%) colonised with ESBL-producing Enterobacteriaceae out of 123 antenatal mothers who underwent rectal screening (12). It is notable that while 26/195 (13%) participants in our study were females aged between 20-45 years, all were ESBL free. However, in 2013, a six-week ESBL prevalence study was performed in our 83-bedded maternity hospital. In that study, 57 urine or rectal samples were received from women in the third trimester of pregnancy with only one woman identified as being ESBL-colonised (unpublished data). Although previous studies have reported similarly low rates of ESBL-producing Enterobacteriaceae, the fact that no growth whatsoever occurred on over 95% of the selective ESBL agar led us to question the ability of Gram-negative bacteria to recover after storage at 20 °C with the eSwab™ despite manufacturer's recommendations.

Conclusions

This community point prevalence study did not identify ESBL-colonisation despite high numbers of patients with invasive ESBL bloodstream infections presenting for admission in our institution. We believe this may be because of our small sample size. However, we remain vigilant for ESBL-producing organisms within our laboratory and on the wards in the context of unstable and deteriorating patients not responding to empiric antimicrobial therapy.

Compliance with ethical standards

There are no conflicts of interests to declare. No animal testing was performed. Ethical approval was obtained for this study as outlined in the manuscript.

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