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ESBL-producing Gram-negative organisms in the healthcare environment as a source of genetic material for resistance in human infections

M. Muzslay [a,*], G. Moore [b], N. Alhussaini [c], A.P.R. Wilson [d]

[a] Environmental Research Laboratory, University College London Hospitals NHS Foundation Trust, London, UK
[b] Biosafety, Air and Water Microbiology Group, Public Health England, Porton Down, UK
[c] Infection and Immunity, University College London, London, UK
[d] Clinical Microbiology and Virology, University College London Hospitals NHS Foundation Trust, London, UK

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SUMMARY

Background: The increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in the healthcare setting and in the community despite established infection control guidelines indicates that these microorganisms may possess survival strategies that allow them to persist in the environment.

Aims: To determine the extent and variation in endemic ESBL-carrying species in different ward environments, and to investigate the potential for cephalosporin resistance to be transferred from environmental isolates to human pathogens.

Methods: Conventional microbiological methods were used to sample 1436 environmental surfaces for ESBL-producing bacteria. Transconjugation assays (broth mating experiments) were performed using environmental ESBL-producing isolates as donors and streptomycin-resistant *Escherichia coli* (NCTC 50237) as the recipient.

Findings: The prevalence of ESBL-producing bacteria on surfaces in a non-outbreak setting was low (45/1436; 3.1%). The sites most likely to be contaminated were the drains of handwash basins (28/105; 26.7%) and floors (14/160; 8.8%). Fifty-nine ESBL-carrying organisms were isolated. Of these, *Klebsiella* spp. (33.9%), *Enterobacter* spp. (20.3%), *Pantoea* spp. (15.3%) and *Citrobacter* spp. (13.6%) were the most common isolates. ESBL determinants were transferred successfully from three representative environmental isolates (*Pantoea calida, Klebsiella oxytoca, Raoultella ornithinolytica*) to the human pathogen *E. coli*.

Conclusion: ESBL-producing Gram-negative isolates were recovered from the hospital environment in the absence of any ESBL infection on the wards. The drains of handwash basins should be considered potential long-term reservoirs of multi-drug-resistant bacteria and drug resistance genes. These genes can reside in various genera of hardy environmental organisms and be a potential source of ESBL for more common human pathogens.

* Corresponding author. Address: Environmental Research Laboratory, University College London Hospitals, Room E-2PT00, EGA Wing Level-2, 235 Euston Road, London NW1 2BU, UK. Tel.: +44 (0) 20 344 76702.
E-mail address: Monika.Muzslay@uclh.nhs.uk (M. Muzslay).

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Introduction

With increasing use of broad-spectrum antimicrobials and seriously ill patients surviving for longer periods, multi-drug resistance (MDR) has increased significantly in the last decade and has emerged as a global concern.

At the same time, fewer new antibiotics are becoming available and the World Health Organization has developed a global action plan to tackle antibiotic resistance.[1] In the UK, as in the rest of Europe, the rapid evolution and spread of MDR Gram-negative bacteria among hospitalized patients is of particular concern.[2,3]

The increased prevalence of Enterobacteriaceae resistant to extended-spectrum beta-lactams has been caused by the circulation of different plasmids containing a diversity of extended-spectrum β-lactamase (blaESBL) genes.[4] The genes encoding for CTX-M type ESBL enzymes are normally found on the chromosomes of *Kluyvera* spp. *Kluyvera* spp. are ubiquitous within the environment (water, soil, hospital sinks) but rarely cause infections in humans. However, they are able to transfer the genes encoding for CTX-M enzymes to other Gram-negative bacteria.[5] Horizontal gene transfer requires DNA from one cell to be physically transferred to another. The DNA must then be incorporated into the recipient's genome so that it can be inherited stably. Horizontal gene transfer by plasmid-mediated conjugation is thought to play an important role in the adaptation of bacteria to various conditions in the clinical environment.[6]

Gram-negative organisms can lose viability rapidly under dry conditions;[7] as such, the role of environmental reservoirs in the transmission of ESBL infection is often overlooked. However, most nosocomial pathogens can persist on inanimate surfaces for many weeks,[8] and prolonged environmental survival of carbapenem-resistant *Klebsiella pneumoniae* and ESBL-producing *Escherichia coli* has been demonstrated.[9,10] The surfaces most likely to be contaminated with MDR Gram-negative organisms are those associated with sinks, toilet and shower facilities.[11] The presence of moisture facilitates bacterial survival, and an increasing number of outbreaks have been linked to hospital sinks and taps.[12–14]

This study seeks to determine the extent and variation in ESBL-carrying Gram-negative species in different types of hospital ward environment, and to demonstrate the potential pathogenic importance of environmental reservoirs of ESBL-producing bacteria in the transmission of Gram-negative infection in the healthcare setting.

Methods

Study setting

A microbiological survey of 13 different ward environments (located in seven different buildings) was carried out at a UK teaching hospital over a four-month period. Patients were not screened routinely for carriage of ESBL. Rooms occupied by patients colonized/infected with ESBL-producing Enterobacteriaceae were not targeted specifically for sampling. There was no outbreak during the study.

Environmental sampling

Sampling focused on those areas considered most likely to harbour Gram-negative organisms (e.g. sinks, showers) in patient rooms, toilets and bathrooms. Sampling was also performed in non-clinical areas including beverage bays, visitor toilets, staff toilets, changing rooms, sluice rooms, corridors and kitchens on different speciality wards.

In total, 1436 surfaces (1006 clinical and 430 non-clinical) were assessed for contamination with ESBL-producing bacteria (Table I).

Each environmental site was sampled using a non-selective blood agar contact plate (diameter 55 mm; Oxoid, Basingstoke, UK). An R2A agar contact plate (supplemented with 0.5% Tween and 0.07% lecithin; diameter 55 mm; Southern Group Limited, Corby, UK) was used to sample an adjacent test area for less fastidious organisms. Cotton swabs, pre-moistened with phosphate-buffered saline (Oxoid), were used to sample two irregular-shaped surfaces (drain and tap aerator). Swabs were plated directly on to Columbia agar with horse blood and R2A agar plates (diameter 90 mm; Oxoid).

Blood and R2A agar plates were incubated aerobically at 37°C for 48 h and room temperature for five to seven days respectively.

Microbiological analysis

Presumptive Gram-negative colonies were Gram stained and tested for their ability to ferment lactose. Presumptive Enterobacteriaceae isolates were screened for resistance to cefpodoxime (10 μg). Resistant isolates (≤19-mm zone diameter)[15] were tested for ESBL and AmpC production (MASTDISCS™ AmpC and ESBL ID set, Mast Group Ltd, Bootle, UK). Organisms demonstrating AmpC activity alone were discarded.

Presumptive *Pseudomonas aeruginosa* isolates were screened for resistance to ceftazidime (30 μg). Resistant isolates were also assessed for ESBL production (MASTDISCS™ ESBL ceftazidime paired ID).

Identification to species level was performed using the oxidase test and either API 20E or API 20NE biochemical test strips (BioMérieux, Basingstoke, UK). Isolates that did not provide an acceptable profile were identified via matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry. All ESBL-producing isolates were subcultured and stored at -20°C on porous ceramic beads.

Conjugation assay

Conjugation assays were performed using a broth mating method[16] to investigate the transferability of genetic material from environmental isolates to a representative human pathogen. Streptomycin-sensitive ESBL-producing isolates recovered from the ward environment were used as donors, and streptomycin-resistant *E. coli* NCTC 50237 (HB101) was used as the recipient. The donor and recipient strains were mixed together at a ratio of 1:5 (1 mL:5 mL of cultures of same concentration), respectively, in nutrient broth, and the suspension was incubated overnight at 37°C. Transconjugants were