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Letter to the Editor

Gram-negative bacteria resistant to commonly used antibiotics from wastewater within a hospital building

Sir,

Patient colonisation by antibiotic-resistant Enterobacterales is linked to hospital wastewater sources such as sink traps. Wastewater systems become colonised when used by colonised patients. There are then routes for bacteria to escape, e.g., drain blockages and aerosolisation, into the ward environment, facilitating transmission to patients [1].

Most analyses of hospital wastewater as a source of Enterobacterales transmission have been completed after extended-spectrum beta-lactamase-/carbapenemase-producing Enterobacterales (ESBL/CPE) outbreaks in intensive care units (ICUs) [2]. There have been limited studies on Enterobacterales with resistance to commonly used antibiotics, such as amoxicillin-clavulanic acid, outside ICUs. If Enterobacterales with resistance to commonly used antibiotics are present, we may not be preventing their transmission to patients.

We carried out an environmental study at Leeds Teaching Hospitals NHS Trust in July 2024 to investigate whether multidrug resistant-Gram negative bacilli (MDR-GNB) resistant to commonly used antibiotics were present in our hospital's healthcare wastewater outside ICUs, based on previously reported methods [3]. Samples were collected throughout a single hospital building, from inpatient wards (renal/general surgery and urology), an outpatient department and a food processing area. Samples were from patient areas, e.g., rooms with patient beds, and non-patient areas, e.g., kitchen areas. A total of 50 mL of wastewater was aspirated from wastewater traps using a sterile syringe (Appleton Woods-BD587) and Ryles tube (Pennine Healthcare-AERT2314). Samples were collected from wastewater and handwash basin wastewater traps, plate washing basins, sluices and hot water geysers. Basins sampled were the domestic type, with drain outlets in the floor of the basin. If sink-trap aspiration did not result in sufficient volume, water from the tap corresponding to the location being sampled re-filled the water trap to allow sample collection. Samples were transported in a 50-mL Falcon Tube (Sarstedt 62.559). Ten microlitres of uncentrifuged sample were cultured in air on Chromogenic Coliform Agar (E&O laboratories) for 24 h at 37°C. Gram-negative bacteria were speciated by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF; Bruker microflex) analysis. Antimicrobial susceptibility disc diffusion testing was performed by European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods/zone sizes. EUCAST expert rules were not applied.

Of 40 locations sampled, 35 samples were collected from inpatient wards (renal/general surgery/urology), three from an outpatient department and two from a food-processing area, in a single hospital building. Samples were collected from handwash and plate washing basins ($N = 33$), four sluices and three geyser drains.

Of the 40 samples five were not collected on the first attempt, but after running the locations tap for 30 s. Bacterial growth was detected in 30 samples. No growth from samples was potentially because of prior recent disinfection by cleaning staff, though this was not formally assessed. From the 30 samples with bacteria detected, 40 bacterial isolates were recovered as shown in Table I. In the 40 isolates, antibiotic resistance in *Escherichia coli* was detected to co-amoxiclav (1/8, 12.5%) with no resistance detected to cefuroxime, ciprofloxacin, or piperacillin-tazobactam. Antibiotic resistance in non-*E. coli* Enterobacterales (NECE; Citrobacter, Enterobacter, Klebsiella) was detected to co-amoxiclav (16/20, (80%)), cefuroxime (6/20 (30%)), ciprofloxacin (6/20, 30%), and piperacillin-tazobactam (5/20, 25%). One *Pseudomonas aeruginosa* was detected, which was carbapenem resistant.

We therefore demonstrated the almost universal detection of Enterobacterales, in our hospital wastewater as reported previously [4]. We demonstrated antibiotic-resistant NECE (Citrobacter, Enterobacter, Klebsiella) in healthcare wastewater. Resistance for NECE was common, at 25—80%, for antibiotics such as co-amoxiclav and cefuroxime, antibiotics frequently used as first-line treatment for a wide range of infections such as hospital-acquired pneumoniae. We also found Enterobacterales that have two susceptibility patterns, *E. coli* with low antibiotic resistance, and antibiotic-resistant NECE (Citrobacter, Enterobacter, Klebsiella). The pattern raises the question, is the epidemiology of these two populations (*E. coli* vs NECE) in hospital wastewater different? In environmental microbiology *E. coli* is an indicator of recent faecal contamination, whereas *Klebsiella* spp. are able to persist in water environments [5,6]. Our antimicrobial resistance data are consistent with a hypothesis that *E. coli* is a non-permanent resident in wastewater in healthcare, with NECE being permanent residents. Permanent residents would be more exposed to antibiotics, which are present in wastewater traps, and thus antibiotic resistance may be selected and promoted [7]. Temporary residence by *E. coli* is biologically plausible, as human adapted *E. coli* is adapted for survival at 37°C and for

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Table I
Summary microbiology results of 40 hospital wastewater samples from the hospital built environment including the bacterial species identified and antibiotic resistance detected

Species identified	*Escherichia coli* antibiotic resistance	Antibiotic resistance in non-*E. coli* Enterobacterales (Citrobacter, Enterobacter, Klebsiella)
Acinetobacter spp., $N = 4$	Co-amoxiclav 12.5% (1/8)	Co-amoxiclav 80% (16/20)[a]
Aeromonas spp., $N = 4$	Cefuroxime 0% (0/8)	Cefuroxime 30% (6/20)[a]
Citrobacter spp., $N = 9$	Ciprofloxacin 0% (0/8)	Ciprofloxacin 30% (6/20)
E. coli, $N = 8$	Piperacillin-tazobactam 0% (0/8)	Piperacillin-tazobactam 25% (5/20)
Enterobacter spp., $N = 5$	Meropenem 0% (0/8)	Meropenem 0% (0/20)
Klebsiella spp., $N = 6$		
Pseudomonas aeruginosa, $N = 1$		
Environmental species, $N = 3$		

[a] Intravenous EUCAST break points used.

limited periods of time at room temperature. This contrasts to species such as Klebsiella, environmental bacteria adapted for prolonged survival in water [8].

This study was limited, as it did not assess whether the antimicrobial-resistent GNBs detected in this study were being transmitted to patients and the small number of samples means our interpretation of the results is hypothesis-generating only.

Given these findings we believe wastewater in hospitals is a risk for transmission of Enterobacterales resistant to commonly used antibiotic therapy, and wastewater interventions, based of fundamental principles of infection prevention and control, should be considered for routine implementation.

CRediT authorship contribution statement

S. Brown: Writing — review & editing, Writing — original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **E. Best:** Writing — review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **A. Kirby:** Writing — review & editing, Writing — original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethics statement

Not required.

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Conflict of interest statement

None declared.

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