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Tracing the transmission of carbapenem-resistant *Enterobacterales* at the patient: ward environmental nexus

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Abstract

Introduction Colonisation and infection with Carbapenem-resistant Enterobacterales (CRE) in healthcare settings poses significant risks, especially for vulnerable patients. Genomic analysis can be used to trace transmission routes, supporting antimicrobial stewardship and informing infection control strategies. Here we used genomic analysis to track the movement and transmission of CREs within clinical and environmental samples.

Methods 25 isolates were cultured from clinical patient samples or swabs, that tested positive for OXA-48-like variants using the NG-Test® CARBA-5 test and whole genome sequenced (WGS) using Oxford Nanopore Technologies (ONT). 158 swabs and 52 wastewater samples were collected from the ward environment. 60 isolates (matching clinical isolate genera; *Klebsiella, Enterobacter, Citrobacter* and *Escherichia*) were isolated from the environmental samples using selective agar. Metagenomic sequencing was undertaken on 36 environmental wastewater and swab samples.

Results 21/25 (84%) clinical isolates had >1 bla_{OXA} gene and 19/25 (76%) harboured >1 bla_{NDM} gene. Enterobacterales were most commonly isolated from environmental wastewater samples 27/52 (51.9%), then stick swabs 5/43 (11.6%) and sponge swabs 5/115 (4.3%). 11/60 (18%) environmental isolates harboured >1 bla_{OXA} gene and 1.9% (1/60) harboured bla_{NDM-1}. bla_{OXA} genes were found in 2/36 (5.5%) metagenomic environmental samples.

Conclusions Potential for putative patient-patient and patient-ward transmission was shown. Metagenomic sampling needs optimization to improve sensitivity.

Keywords Antimicrobial resistance, OXA48, NDM, Plasmids, Transmission, Environment, Hospital, Metagenomics, Oxford Nanopore, Carbapenem-resistant *Enterobacterales*, CRE

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Introduction

Colonisation and subsequent healthcare associated infection (HCAI) with multi-drug resistant organisms (MDROs) is a concern for vulnerable patient groups, such as the elderly, or immunocompromised, within the hospital setting. Many of these HCAI incidents could be preventable with enhanced infection and control (IPC) measures [1]. The first line therapy for use against MDROs are the carbapenems, and infection with carbapenem-resistant Enterobacterales (CRE) are associated with high patient mortality rates [2]. CREs are difficult to treat as carbapenemase enzymes can hydrolyse almost all β-lactam antibiotics [3].

Oxacillinase-48-type carbapenemases (OXA-48) and New Delhi metallo-β-lactamase (NDM) are common CRE resistance mechanisms, which are now found globally, are highly mobile and no longer confined to the original bacterial species that they were characterised from [2, 4]. CRE resistance is most often transferred between bacterial isolates on mobile genetic elements, including plasmids, and a wide range of plasmid types have been seen in CRE organisms [4]. CREs are often associated with other genes that confer β-lactam resistances, such as bla_{SHV} and bla_{CTX}, as they can be found on the same plasmids, complicating detection [5].

CREs have been found in both community settings and hospital environments around the globe [6]. Whilst colonised patients do not need antibiotic therapy, they still pose a transmission risk, and so both colonisation and infection must be considered when undertaking IPC. CREs have been isolated from high-touch surfaces, such as door handles, medical equipment trolleys, as well as bed sheets and rails, and also from hospital wastewater, including sink U-bends [2, 7–10]. The colonisation of hospital wastewater by CREs and other MDROs may be particularly problematic, as this water will pass out of the hospital and into the general wastewater system. Without adequate processing at wastewater treatment plants, and the risk of sewage being released into water systems, this may lead to the distribution of these MDROs and their AMR genes back into environmental and community settings [11, 12]. This is especially likely to be problematic in low- and middle-income settings, where the treatment of wastewater may not be adequate to remove these organisms [13].

Surveillance is crucial for the containment of pathogen and AMR outbreaks, especially in hospital settings with vulnerable patients and multiple-occupancy bed bays [14]. The current standard testing for CREs is culture and phenotypic antibiotic sensitivity testing (AST), and molecular methods such as PCR or rapid diagnostic tests (RDTs), such as lateral flow devices (including the NG-Test CARBA-5) [15].

As the COVID-19 pandemic showed with real-time tracking of variants, genomic analysis provides vital enhanced surveillance of transmission patterns [16]. Phenotypic and other molecular tests for resistance genes cannot identify the genetic relatedness of isolates, and thus cannot accurately track potential transmission. The use of whole genome sequencing (WGS) and metagenomics in the diagnosis and surveillance of MDROs can identify phylogeny, novel drug resistance mutations and inform design of targeted diagnostics [17].

bla_{OXA}- and bla_{NDM}-mediated CRE colonisations and infections were detected in several patients occupying single-bed rooms and multiple-occupancy bed bays on wards at a North London tertiary referral hospital between 2022 and 2023. Temporal and spatial associations between patients indicated the possibility of ongoing transmission events but routine phenotypic and molecular testing was unable to pinpoint transmission routes. In this study we aimed to use genomic analysis to build a picture of the movement and transmission of CRE species, AMR and plasmids within clinical pathogens and environmental species found in the ward environment.

Methods

Sample collection

Environmental sampling was requested by the hospital Trust and IPC lead for investigation as part of the extended standard of care. Further characterisation by molecular typing of the CREs that were part of the outbreak was included as an extension of the routine standard of diagnostic care pathway. Patient metadata was obtained through the electronic clinical infection database (elCID). AST profiles of the clinical bacterial pathogens isolated relevant to the CRE surveillance cases were undertaken following the EUCAST Clinical breakpoints (v12.0) for Gram Negative bacteria [18].

Twenty-eight clinical isolates were obtained from 20 patients with a positive NG-Test® CARBA-5 (NG Biotech Laboratories) immunochromatographic lateral flow test for bla_{OXA} and/or bla_{NDM} resistance on multiple wards between 7 February 2022 and 20 January 2023 [19]. Four were from infection sites and 24 were CRE screen samples. These CRE screens were rectal swabs, which were plated onto speciation agar, any suspected to be CREs were tested using the CARBA-5 lateral flow test, as per Health Services Laboratories 'healthcare associated infection detection of carbapenemase producing organisms by culture' standard operating procedure. Phenotypic pathogen species data collected from patient samples was obtained using the MALDI-TOF (Bruker). MALDI-TOF-MS of bacterial isolates was undertaken from pure isolates no older than 24 h from culture. Isolates were spotted in duplicate and identifications with

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corresponding Log scores ≥ 2.0 "high-confidence to the species level" were considered only, and reported in the results. Patient spatio-temporal metadata was collected, see supplementary materials S1. For ward specialties, see supplementary materials S2.

For environmental sampling, a site visit to ward 7D was undertaken prior to collection, to evaluate ward layout and staff and patient routes of travels and sampling locations. Samples were taken from every multiple-occupancy bed bay and single-bed room, covering affected patient areas and non-affected areas. From each bay and room every sink and drain was sampled (in both bed and bathroom areas) and shower drains sampled. Non-clinical rooms were also covered, such as the shared use pantry, staff toilets, nurses station, storage rooms, sluice and workstations on wheels. For the layout of ward 7D see supplementary materials S3.

Two hundred μL of wastewater samples were collected into sterile sample containers pre-dosed with 1 mL of a neutralising buffer comprising: 3% (w/v) Tween 80, 0.3% (w/v) Lecithin, 1.0% (w/v) Sodium thiosulfate, 1.5% (w/v) K_2HPO_4, KH_2PO_4 0.05% (w/v), 1% (w/v) Poly-[sodium-4-styrenesulfonate], 0.1% (v/v) Triton$^®$ × 100 (Sigma-Aldrich, UK) and prepared in Phosphate-buffered saline (PBS) solution (Oxoid, UK) as previously described [20]. Samples were refrigerated (2–8 °C) within 2 h of collection and processed within 24 h. Aliquots (0.5 mL, 0.1 mL) from the neat and serial 1/10 and 1/100 dilutions from the original wastewater samples were surface-plated onto selective agars: Colorex™ mSuperCARBA™ (EO Labs, UK), Brilliance CRE, *E. coli* Coliform, *Pseudomonas* CN (Oxoid, UK) and non-selective Columbia Blood Agar (Oxoid, UK). Plates were incubated aerobically at 37 °C for 72 h and inspected daily. A further 45 μL of the original samples were vacuum-filter concentrated as previously described [21] via 47 mm diameter nitrocellulose membranes (0.45um pore size) and the membrane transferred to selective agars used above prior to incubation.

Cotton-tipped stick swabs (SS352, Appleton Woods) and sponge swabs (TS/15-B, Technical Service Consultants Ltd.), both pre-moistened with a neutraliser buffer were used to collect samples from difficult-to-access areas, as described above. Stick swab samples were transferred to sterile universal tubes containing 9 mL of diluent buffer (saline), 1 mL of neutralising buffer and 3–5 glass beads. The swab contents were released by bead-washing (vortex mixing) for 30 s. Aliquots and serial dilutions of the resulting homogenised suspension was plated on selective and non-selective agars as above.

Suspect colonies were harvested for streak-purification onto non-selective agars and confirmed by MALDI-TOF mass spectrometry (MS) (Maldi-TOF Biotyper IVD system Bruker Daltronics). The remaining portion of the environmental samples were preserved in 500 μL 1 × DNA/RNA Shield (Zymo Research Corporation).

DNA extraction and quantification

Clinical and environmental isolates were grown on Columbia horse blood plates (Oxoid Limited), then the DNA extracted using the DNeasy Blood & Tissue Miniprep Kit (Qiagen), following manufacturer's instructions [22]. Metagenomic DNA from a subset of environmental samples, due to limited resources, was extracted directly from the environmental swab and water samples using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corporation), following manufacturer's instructions [23]. A sample of ZymoBIOMICS™ Microbial Community Standard (Zymo Research Corporation) was included, following manufacturer's instructions [24]. DNA was assessed for concentration using the Qubit™ dsDNA BR Assay Kit (Thermo Fisher). Molecular weight and DNA integrity was confirmed using the Genomic DNA ScreenTape and reagents on the TapeStation 4150 (Agilent Technologies Inc.).

Library preparation

Clinical and environmental isolate DNA libraries were prepared using the Rapid Barcoding Kit 96 (SQK-RBK110.96) with a DNA input of between 50 and 200 ng, following manufacturer's instructions [25]. For the environmental swab samples, metagenomic DNA libraries were prepared using the ONT Rapid PCR Barcoding Kit (SQK-RPB004) with a DNA input of 1–5 ng and following the manufacturers' instructions [26]. ZymoBIOMICS™ Microbial Community DNA Standards (Zymo Research Corporation) were included, following manufacturer's instructions [27].

Sequencing and basecalling

Up to 24 barcoded clinical and environmental isolate samples, or 12 barcoded environmental metagenomic samples were run together on a flow cell version R9.4.1 (Oxford Nanopore Technologies) using a MinION device for 72 h, using the default parameters on the MinKNOW software (v23.04.6). Basecalling was performed either by the MinKNOW software alongside sequencing or using the Guppy basecalling software (v6.5.7) [41], using the flip-flop high accuracy algorithm, with a minimum Q score of 8 and minimum depth of 40x.

Data analysis

Fastq files were quality checked (QC) using FastQC (v0.21.1) and MultiQC (v1.15) and those with a read depth of > 40x were included in further analysis [28, 29]. 25 clinical isolates, 60 environmental isolates, 36 metagenomics environmental samples were included

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in this analysis. Barcodes were trimmed from the reads using Guppy. All clinical and environmental isolates, and environmental metagenomic samples were analysed for the presence of AMR genes using KmerResistance 2.2 [30, 31] and for species using KmerFinder 3.2 (v3.0.2) [30, 32, 33]. Both programmes match query sequences to k-mer databases, for resistance genes and species respectively. Plasmids were identified using PlasmidFinder 2.1 (v2.0.1) [30, 34]. MLST types were identified using MLST (v2.0) [30, 35–40].

Clinical and environmental isolate fastq files were aligned to the reference genome for their species (*C. freundii*: GCF_003812345.1, *C. portucalensis*: GCA_023374935.1, *C. youngae*: GCF_015139575.1, *E. cloacae*: GCF_905331265.2, *E. bugandensis*: GCF_020042625.1, *E. hormaechei*: GCF_024218835.1, *E. coli*: GCF_000005845.2, *K. pneumoniae*: GCF_000240185.1, *K. michigenensis*: GCF_015139575.1) using MiniMap2 (v2.26) [41], then sorted and indexed using Samtools (v1.17) [42]. Alignments were visualised using Artemis (v18.1.0) [43]. Depth and coverage were calculated using Samtools. Consensus fasta files were created using Samtools and then dendograms for each species (with three or more isolates) were created using Parsnp (utilising maximal unique matches) (v1.7.4) [44–47] and visualised using iTOL (v6) [48].

Speciation for the results and discussion were as per the genomic speciation. Sequence data were deposited under BioProject PRJEB76684 on the European Nucleotide Archive and outlined in supplementary materials S4, S5 and S6.

Results
MALDI-TOF vs WGS for isolate speciation
All 25 clinical and 48 environmental isolates were subjected to both MALDI-TOF and WGS analysis. Non-concordant *C. freundii* by MALDI-TOF were speciated as *C. portucalensis* (one clinical isolate and one environmental isolate) or *C. youngae* (three environmental isolates) in WGS. One MALDI-TOF call of *C. braakii/freundii* was called as *C. youngae* using WGS. One environmental isolate that MALDI-TOF identified as *C. freundii* was speciated as *P. mirabilis,* possibly a mixed culture in which the *P. mirabilis* may have been present on the original selective plate, but then grew on the non-selective plate used for DNA extraction and sequencing. Non-matching *E. cloacae* were speciated as *E. asburiae* (one environmental isolate) and *E. hormaechei* (nine, including all five of the clinical isolates and four environmental isolates). All MALDI-TOF and WGS speciation for *E. coli* and *K. pneumoniae* was concordant. All the MALDI-TOF-called *K. oxytoca* were speciated as either *K. michigenensis* (4/5 isolates) or *K. grimontii* (1/5 isolates) using WGS. See Table 1 and supplementary materials S5.

Environmental sampling
210 environmental samples were collected, including 43 stick swabs, 115 sponge swabs and 52 wastewater samples. In total, 195 bacteria were isolated from 36 (16.9%) environmental samples, 76 isolates were Enterobacterales: 6 from stick swabs, 5 from sponge swabs and 65 from non-potable water samples. There was a significant difference in the proportion of samples from which Enterobacterales species were isolated from, when the one-way ANOVA was applied ($p = \leq 0.0001$). When swab types were compared using Tukey's multiple comparisons test, there was no significant difference between stick and sponge swabs ($p = 0.4286$), but a significant difference between stick swabs and wastewater ($p = \leq 0.0001$) and sponge swabs and wastewater ($p = \leq 0.0001$). Enterobacterales were isolated from 51.9% of water samples, compared with 11.6% of stick swabs and 4.3% of sponge swabs (see Table 2).

Enterobacterales were isolated from 36 different environmental samples (mean = 2.2 isolates per site, SD = 1.4). See Table 3. Table 4 details the sample sites from which each species was isolated, Table 5 describes the ward areas in which they were isolated. For full data see supplementary materials S6.

Table 1 Concordance of MALDI-TOF speciation vs WGS for both clinical and environmental isolates. All non-concordant samples were either of the *C. freundii* or *E. cloacae* complex

	Clinical isolates	Environmental isolates	Combined clinical and environmental isolates
C. freundii/portucalensis	0/1 (0%)	16/21 (76%)	16/22 (73%)
E. cloacae/hormaechei	0/5 (0%)	3/8 (38%)	3/12 (25%)
E. coli	9/9 (100%)	11/11 (100%)	21/21 (100%)
K. pneumoniae	10/10 (100%)	8/8 (100%)	20/20 (100%)

Note that 48/60 environmental isolates are included in this table, the rest were different species. The numerator is the number of isolates that were concordant, the denominator is the number of total isolates of that species

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Table 2 Environmental sample types collected, and the number of each sample type from which Enterobacterales were isolated

Environmental sample type	Total number of samples	Number from which Enterobacterales were isolated	Number from which no Enterobacterales were isolated	Significance (ANOVA)
Stick swab	43	5 (11.6%)	38 (88.3%)	$p = \leq 0.0001$
Sponge swab	115	5 (4.3%)	110 (95.6%)	
Water	52	27 (51.9%)	25 (48.1%)	
Total samples	210	37 (17.6%)	173 (82.3%)	

Significance was calculated using one-way ANOVA

Table 3 Number of each species isolated from each different type of environmental sample

	Stick swab	Sponge swab	Water sample	Total environmental isolates
C. freundii	2 (15%)	5 (38%)	6 (46%)	13
C. portucalensis	1 (50%)	0 (0%)	1 (50%)	2
C. youngae	0 (0%)	3 (43%)	4 (57%)	7
Citrobacter sp.	0 (0%)	0 (0%)	1 (100%)	1
E. asburiae	0 (0%)	1 (33%)	2 (66%)	3
E. cloacae	0 (0%)	1 (33%)	2 (66%)	3
E. hormaechei	0 (0%)	3 (60%)	2 (40%)	5
E. roggenkampii	0 (0%)	0 (0%)	1 (100%)	1
E. coli	1 (9%)	4 (36%)	6 (55%)	11
K. grimontii	0 (0%)	1 (100%)	0 (0%)	1
K. michigenensis	1 (25%)	1 (25%)	2 (50%)	4
K. pneumoniae	0 (0%)	2 (25%)	6 (75%)	8
P. mirabilis	0 (0%)	0 (0%)	1 (100%)	1

Table 6 details the environmental sample swab types, total number of bacterial species, and the total number of reads obtained from each of the 36 metagenomic sequencing sample sites that Enterobacterales were also present. There was no significant difference in the number of sequencing reads obtained, when comparing the swab type, or when comparing the swab location type. When comparing rooms, there were no significant differences, apart from Bay (beds 1–4) and Bay bed 20 ($p = 0.0321$). All tests were undertaken using One Way ANOVA and Tukey's multiple comparison test. Speciation was based on genomic data, so only those that passed QC are included in the analysis.

AMR genes
Clinical isolates
Of the clinical isolates, bla_{OXA-48} was identified in 2 of the total 28 (7.1%) isolates (one *C. portucalensis* and one *K. pneumoniae*). bla_{NDM-1} was identified in nine (32.1%) clinical isolates (one *C. portucalensis*, five *E. hormaechei*, one *E. coli* and two *K. pneumoniae*). bla_{OXA} genes on the CARBA-5 panel were found in 84% of clinical isolates and bla_{NDM} genes on the CARBA-5 panel were found in

76% of clinical isolates, see Table 7. See supplementary materials S7 for full list of genes found.

Environmental isolates
Of the environmental isolates, bla_{OXA-48} was identified in 11 of the total 60 (18%) isolates (See Table 8). bla_{NDM-1} was identified in one (1.7%) environmental isolate, a *K. michigenensis*. See supplementary materials S8 for full list of genes found.

Metagenomic environmental samples
bla_{OXA-48} was identified in 1/36 (2.8%) metagenomic environmental sample, and three other bla_{OXA} genes on the CARBA-5 panel ($bla_{OXA-204}$, $bla_{OXA-370}$ and $bla_{OXA-515}$) were found together in 1/36 (2.8%) other metagenomic environmental sample. No bla_{NDM} genes were identified in any of the 36 metagenomic environmental samples.

Plasmids
Clinical isolates
The clinical isolates had a mean number of 5.6 plasmids (SD = 2.3) and a total of 20 different plasmids were identified. Table 9 describes the plasmid types and species

Table 4 Environmental isolates present divided by sample site and type depicting an environmental reservoir density by location

Sample site and type	C. freundii	C. portucalensis	C. youngae	Citrobacter sp.	E. asburiae	E. cloacae	E. hormaechei	E. roggenkampii	E. coli	K. grimontii	K. michigensis	K. pneumoniae	P. mirabilis	Total species found per site
Bathroom HWB drain	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
Bathroom HWB DWT	1 (17%)	0 (0%)	2 (33%)	1 (17%)	1 (17%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)	7
Bathroom toilet bowl	1 (25%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	4
Bathroom toilet floor	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Medication Cupboard exterior surface	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
Domestic Waste skip lid	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Drinks Cooler mixed Pre-Flush	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Bay HWB DWT	9 (26%)	0 (0%)	5 (14%)	0 (0%)	1 (3%)	3 (9%)	0 (0%)	1 (3%)	6 (17%)	0 (0%)	2 (6%)	7 (20%)	1 (3%)	35
Office HWB DWT	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
Sink drain U bend exterior	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Sink DWT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)	1 (25%)	0 (0%)	0 (0%)	4
Staff toilet HWB DWT	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
WoW Keyboard and Mouse	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Total isolates of each species present	13	2	7	1	3	3	4	1	11	1	4	8	1	

HWB hand wash basin, *DWT* drain waste trap, *WoW* workstation on wheels

Table 5 Environmental isolates present divided by room indicating the environmental reservoir densities by room-type

Sample site and type	C. freundii	C. portucalensis	C. youngae	Citrobacter sp.	E. asburiae	E. cloacae	E. hormaechei	E. roggenkampii	E. coli	K. grimontii	K. michigenensis	K. pneumoniae	P. mirabilis	Total species found per site
Bay (Beds 1–4)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (20%)	1 (20%)	5
Bay (Beds 15–18)	1 (8%)	0 (0%)	2 (17%)	1 (8%)	0 (0%)	3 (25%)	0 (0%)	0 (0%)	2 (17%)	0 (0%)	2 (17%)	1 (8%)	0 (0%)	12
Bay (Beds 21–24)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	2
Bay (Beds 29–32)	4 (67%)	0 (0%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (17%)	0 (0%)	6
Bay (Beds 5–8)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)	0 (0%)	4
Bed 9	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	4
Bed 10	2 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4
Bed 19	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	5
Bed 20	1 (25%)	0 (0%)	2 (50%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4
Drug prep room	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Office	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4
Pantry (Staff & Patient use)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	1 (20%)	0 (0%)	1 (20%)	1 (20%)	1 (20%)	0 (0%)	0 (0%)	5
Sluice	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Staff toilet	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Work-station on wheels	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1
Total isolates of each species found	13	2	7	1	3	3	5	1	11	1	4	8	1	

Percentages describe the proportion each species makes up per location

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Table 6 Environmental swab metagenomic samples, from which Enterobacterales were isolated, showing which type of sample was taken (sponge swab, stick swab or wastewater sample), number of bacterial species identified (including Gram negatives and Gram positives), and number of reads obtained from metagenomic sequencing

Environmental sample number	Room	Swab location	Total species found	Swab type	Total reads
15	Sluice	Domestic Waste skip, lid	163	Sponge swab	2,613,760
35	Pantry (staff & patient)	Drinks Cooler, pre-Flush	53	Wastewater	1,032,506
41	Drug Prep Room	Sink drain, U bend exterior	44	Sponge swab	790,760
60	Workstation on wheels	Keyboard and Mouse	34	Sponge swab	864,837
71	Bay (Beds 1–4)	Bathroom HWB Drain	74	Stick swab	1,388,557
74	Bay (Beds 1–4)	Bathroom toilet bowl	48	Stick swab	1,037,570
75	Bay (Beds 1–4)	Bathroom toilet floor	22	Sponge swab	581,158
84	Bay (Beds 5–8)	Bay Medication Cupboard	98	Sponge swab	1,597,559
97	Bed 9	Bathroom HWB Drain	74	Stick swab	1,201,404
102	Bed 9	Bathroom toilet bowl	118	Stick swab	2,036,935
142	Bay (Beds 15–18)	Bathroom toilet bowl	70	Stick swab	1,315,258
158	Bay (Beds 21–24)	Bathroom toilet bowl	145	Stick swab	2,429,559
176	Staff toilet	Staff toilet HWB DWT	290	Wastewater	4,156,799
177	Staff toilet	Staff toilet HWB DWT	75	Sponge swab	1,279,241
178	Pantry (Staff & patient)	Sink DWT	93	Wastewater	1,818,705
179	Pantry (Staff & patient)	Sink DWT	56	Sponge swab	1,284,616
182	Room 33	Office HWB DWT	328	Wastewater	4,703,504
183	Room 33	Office HWB DWT	177	Sponge swab	2,537,593
184	Bay (Beds 1–4)	HWB DWT	63	Wastewater	1,083,094
185	Bay (Beds 1–4)	HWB DWT	39	Sponge swab	654,450
186	Bay (Beds 5–8)	HWB DWT	440	Wastewater	6,622,675
187	Bay (Beds 5–8)	HWB DWT	126	Sponge swab	2,021,118
190	Bed 9	HWB DWT	263	Wastewater	6,456,693
191	Bed 9	HWB DWT	111	Sponge swab	2,078,635
194	Bed 10	HWB DWT	51	Wastewater	795,058
195	Bed 10	HWB DWT	88	Sponge swab	1,573,130
198	Bay (Beds 15–18)	HWB DWT	171	Wastewater	11,334,805
199	Bay (Beds 15–18)	HWB DWT	53	Sponge swab	9,700,533
202	Bed 19	HWB DWT	160	Wastewater	2,521,155
203	Bed 19	HWB DWT	41	Sponge swab	13,997,999
206	Bay (Beds 29–32)	HWB DWT	255	Wastewater	4,058,517
207	Bay (Beds 29–32)	HWB DWT	37	Sponge swab	789,087
208	Bay (Beds 29–32)	Bathroom HWB DWT	448	Wastewater	12,313,813
209	Bay (Beds 29–32)	Bathroom HWB DWT	14	Sponge swab	210,049
212	Bed 20	HWB DWT	409	Wastewater	6,070,475
213	Bed 20	HWB DWT	43	Sponge swab	30,494,541

HWB hand wash basin, *DWT* drain waste trap

they were found in. Col440II was the only plasmid found across all clinical isolate species. IncFIB(AP001918) was the most commonly identified plasmid.

Environmental isolates

The environmental isolates had a mean number of 4.3 plasmids (SD = 2.3) and a total of 42 different plasmids were identified. Supplementary materials S9 describes the plasmids and species they were found in. Col(IRGK) was the most commonly identified plasmid in 35/60 (59.3%) isolates, no plasmid was ubiquitous across all environmental isolate species.

Metagenomic environmental samples

Plasmids for Enterobacterales were found in 24/36 (66.6%) environmental metagenomic samples (see

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Table 7 Clinical isolates with bla_OXA-48, at least one bla_OXA (on the CARBA-5 panel), total number of isolates with any bla_OXA gene, bla_NDM-1, at least one bla_NDM (on the CARBA-5 panel) and total number of isolates with any bla_NDM gene

Species	Total isolates	bla_{OXA-48}	bla_{OXA}	Any bla_{OXA}	bla_{NDM-1}	bla_{NDM}	Any bla_{NDM}
C. portucalensis	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
E. hormaechei	5	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (100%)	5 (100%)	5 (100%)
E. coli	9	0 (0.0%)	8 (89%)	8 (89%)	1 (11%)	1 (11%)	1 (11%)
K. pneumoniae	10	1 (10%)	9 (90%)	9 (90%)	2 (20%)	10 (100%)	10 (100%)
Total	25	2 (8.0%)	21 (84%)	21 (84%)	9 (32%)	19 (76%)	19 (76%)

Table 8 Environmental isolates with bla_OXA-48, at least one bla_OXA (on the CARBA-5 panel), total number of isolates with any bla_OXA gene, bla_NDM-1, at least one bla_NDM (on the CARBA-5 panel) and total number of isolates with any bla_NDM gene

Species	Total isolates	bla_{OXA-48}	bla_{OXA}	Any bla_{OXA}	bla_{NDM-1}	bla_{NDM}	Any bla_{NDM}
C. freundii	13	5 (39%)	5 (39%)	5 (39%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
C. portucalensis	2	2 (100%)	2 (100%)	2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
C. youngae	7	1 (14%)	1 (14%)	1 (14%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Citrobacter sp.	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. asburiae	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. cloacae	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. hormaechei	5	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. roggenkampii	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. coli	11	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
K. grimontii	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
K. michigenensis	4	1 (25%)	1 (25%)	1 (25%)	1 (25%)	0 (0.0%)	1 (25%)
K. pneumoniae	8	2 (25%)	1 (13%)	2 (25%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
P. mirabilis	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	60	11 (18%)	10 (17%)	11 (18%)	1 (1.7%)	0 (0.0%	1 (1.7%)

Supplementary materials S10). The mean number of plasmids was 3.1 (SD = 3.6).

Metagenomic species

When species were analysed in the metagenomic samples, reads mapping to the Enterobacterales order were found in 64%-75% of sites (see Table 10). When analysed, there was no significant difference in the number of reads that mapped to *Citrobacter, Enterobacter, Escherichia* or *Klebsiella* genera, using One Way ANOVA and Tukey's multiple comparison test.

Transmission and patient metadata

One large cluster of *C. freundii* isolates that mapped to strain N16-03880 were all from the same room Bay (beds 29–32) but came from both the bay HWB DWT as well as the bathroom HWB DWT samples, suggesting cross-contamination of the two sinks (see Fig. 1A, *C. freundii* cluster #1). These, along with a toilet bowl swab from Bay (beds 15–18) were the only *C. freundii* found to harbour bla_{OXA-48} genes (see Fig. 1A). All the *C. youngae* environmental isolates mapped to strain CF10 (see

Fig. 1B, *C. youngae* cluster #1). The *C. portucalensis* isolates all mapped to different strains. The clinical isolate (patient Z1) mapped mainly (85% query coverage) to strain PNUCL1, and the environmental isolates mapped to FDAARGOS_617 and SWHIN_111. All harboured bla_{OXA} genes, and the patient isolate additionally harboured bla_{NDM} genes (see Fig. 1C).

The *E. asburiae* environmental isolates all mapped to different strains: A2563, 2497 and RHBSTW-00542 (see Fig. 1D). The *E. cloacae* environmental isolates all mapped to strain GGT036. Neither species were found to harbour any bla_{OXA} or bla_{NDM} genes (see Fig. 1E). All of the *E. hormaechei* clinical isolates mapped to strain SH19PTE2 and were found to harbour bla_{NDM} genes, whereas the environmental isolates each mapped to a different strain: Y323, F2, AR_0365 and RHBSTW-00086, none of which harboured either bla_{OXA} or bla_{NDM} genes (see Fig. 1F).

Except patient Z2, all patient *E. coli* isolates mapped most closely to strain 035152. The 035152 isolates had a similar AMR profile, with all (9/9) harbouring $bla_{OXA-181}$ and 7/9 harbouring $bla_{OXA-484}$. Patient Z2, which mapped

Table 9 Plasmid types found in percentage of clinical isolates

Target plasmid	C. portucalensis	E. hormaechei	E. coli	K. pneumoniae	Total no. isolates found in
Col(pHAD28)	0 (0.0%)	5 (100%)	0 (0.0%)	0 (0.0%)	5 (17.9%)
Col156	0 (0.0%)	0 (0.0%)	9 (90.0%)	0 (0.0%)	9 (32.1%)
Col440I	1 (100%)	0 (0.0%)	2 (20.0%)	12 (100%)	15 (53.6%)
Col440II	1 (100%)	1 (20.0%)	2 (20.0%)	12 (100%)	16 (57.1%)
ColKP3	1 (100%)	0 (0.0%)	1 (10.0%)	10 (83.3%)	12 (42.9%)
IncC	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.6%)
IncFIA	0 (0.0%)	0 (0.0%)	9 (90.0%)	11 (91.7%)	20 (71.4%)
IncFIB(AP001918)	0 (0.0%)	0 (0.0%)	9 (90.0%)	9 (75.0%)	18 (64.3%)
IncFIB(K)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	1 (3.6%)
IncFIB(pQil)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (91.7%)	11 (39.3%)
IncFII	0 (0.0%)	0 (0.0%)	9 (90.0%)	4 (33.3%)	13 (46.4%)
IncFII(K)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (91.7%)	11 (39.3%)
IncHI1A(NDM-CIT)	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.6%)
IncHI1B(pNDM-CIT)	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.6%)
IncHI2	0 (0.0%)	0 (0.0%)	1 (10.0%)	1 (8.3%)	2 (7.1%)
IncHI2A	0 (0.0%)	0 (0.0%)	1 (10.0%)	1 (8.3%)	2 (7.2%
IncL	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.6%)
IncR	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	1 (3.6%)
IncX3	0 (0.0%)	5 (100%)	0 (0.0%)	11 (91.7%)	16 (57.1%)
p0111	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	1 (3.6%)

Col440II was the only plasmid found across all clinical isolate species. IncFIB(AP001918) was the most commonly identified plasmid

most closely to *E. coli* VREC0864, clustered with EI106, an environmental isolate from a water sample from the pantry sink's drain waste trap, which also mapped most closely to VREC0864 (see Fig. 1G, *E. coli* cluster #1).

The environmental isolates that mapped to *K. michigenensis* mapped most closely to strains RHB20-CO2, K518 and RHBSTW-00409 (two isolates from the same HWB DWT in Bay (beds 15–18). Only one was found to harbour bla_{OXA} and bla_{NDM} genes (see Fig. 1H). Except Z5 (strain RHBSTW-00113), all of the *K. pneumoniae* clinical isolates mapped most closely to strain B5617 (see Fig. 1I, *K. pneumoniae* cluster #2). Patient isolates Z3, Z4, Z5, Z6, Z17, Z18 and Z19 (all strain B5617) showed a similar AMR profile, all harbouring $bla_{OXA-181}$, $bla_{OXA-232}$ and $bla_{OXA-484}$, and bla_{NDM-5}. There was a cluster of environmental isolates that mapped most closely to *K. pneumoniae* strain 3987, all isolated from hand wash basin drain waste traps, but in differing patient bed areas around the ward (see Fig. 1I, *K. pneumoniae* cluster #1).

9/20 (45%) patients were housed on ward 7D during their time as an inpatient and 9/9 (100%) patients had stayed in Bay (beds 15–18), Bay (beds 29–32) or in one of the single-occupancy bed rooms, each of which were noted to have had higher numbers of Enterobacterales isolated from the environmental samples. 4/9 (44%) of these patients had been housed in more than one of the bays, one patient had been on both Bay (beds 15–18), bay (beds 29–32) and Bay 19.

Fig. 1. Phylogram of CRE species isolated in this study and metadata including location (of either sample collection for environmental isolates, or beds inhabited for clinical isolates), strain and presence or absence of bla_{OXA} and bla_{NDM} genes. A) *C. freundii*, B) *C. portucalensis*, C) *C. youngae*, D) *E. asburiae*, E) *E. cloacae*, F) *E. hormaechei*, G) *E. coli*, H) *K. michigenensis* and I) *K. pneumoniae*. Branch lengths indicate number of substitutions divided by the length of the genome sequence. HWB=hand wash basin, DWT=drain waste trap, WoW=workstation on wheels. bla_{OXA}=bla_{OXA} gene on CARBA-5 panel, bla_{NDM}=bla_{NDM} gene on CARBA-5 panel, EI=Environmental isolate. The top branch of each phylogram is the

Table 10 Number of metagenomic samples sites for which reads mapped to Enterobacterales genus sequences

Enterobacterales genera	Number of swab locations found in	Mean number of reads (SD)
Citrobacter	26 (72%)	1,229,432 (4,492,888)
Enterobacter	23 (64%)	122,971 (316,369)
Escherichia	27 (75%)	171,588 (749,207)
Klebsiella	26 (72%)	47,032 (71,755)

A *C. freundii*

Dendrogram leaves (top to bottom): ASM381234v1, EI115, EI134, EI165, EI061, EI136, EI166, EI121, EI083, EI022, EI174, EI160, EI155, EI157

Branch values: 0.030239, 0.032347, 0.000012, 0.000019, 0.032596, 0.101552, 0.024715, 0.034485, 0.007703, 0.023551, 0.489205, 0.008932, 0.031744, 0.000137, 0.008621, 0.0323, 0.000071, 0.00001, 0.00003, 0.000068, 0.000038, 0.00001, 0.000022, 0.010442, 0.004951, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
PSV	Beds 1-4	HWB DWT	□	□	□	□
PSV	Beds 1-4	HWB DWT	□	□	□	□
IDR1900015725-01-02	Bed 10	HWB DWT	□	□	□	□
RHBSTW-00119	Beds 1-4	Bathroom toilet floor	□	□	□	□
M92	Beds 5-8	HWB DWT	□	□	□	□
FDAARGOS_549	Bed 10	HWB DWT	□	□	□	□
FDAARGOS_549	Bed 20	HWB DWT	□	□	□	□
RHBSTW-00830	Bed 9	Bathroom HWB Drain	□	□	□	□
N18-04078	Beds 15-18	Bathroom toilet bowl	■	■	□	□
N16-03880 (*C. freundii* cluster #1)	Beds 29-32	Bathroom toilet bowl	■	■	□	□
	Beds 29-32	Bathroom HWB DWT	■	■	□	□
	Beds 29-32	HWB DWT	■	■	□	□
	Beds 29-32	HWB DWT	■	■	□	□

B *C. youngae*

Dendrogram leaves: CPO452, EI184, EI172.2, EI170, EI195, EI138, EI120, EI127

Branch values: 0.081316, 0.470653, 0.01372, 0.161683, 0.000036, 0.000469, 0.000107, 0.000115, 0.000279, 0.00027, 0.000028, 0.002072, 0.000089, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
CF10 (*C. youngae* cluster #1)	Bed 19	HWB DWT	■	□	□	□
	Beds 15-18	Bathroom HWB DWT	□	□	□	□
	Bed 19	HWB DWT	□	□	□	□
	Beds 15-18	HWB DWT	□	■	□	□
	Beds 29-32	HWB DWT	□	□	□	□
	Bed 20	Bathroom HWB DWT	□	□	□	□
	Bed 20	HWB DWT	□	□	□	□

C *C. portucalensis*

Dendrogram leaves: CF7303, EI027, Patient Z1, EI113

Branch values: 0.722049, 1.750034, 0.003375, 0.373715, 0.001443, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
FDAARGOS_617	Beds 21-24	Bathroom toilet bowl	■	■	□	□
PNUCL1	Not housed on sampled ward		■	■	■	■
SWHIN_111	Office	HWB DWT	□	□	□	□

D *E. asburiae*

Dendrogram leaves: 17NKHM, EI101, EI193, EI122

Branch values: 0.328204, 0.299744, 0.013884, 2.011847, 0.01269, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
RHBSTW-00542	Bed 10	HWB DWT	□	□	□	□
A2563	Pantry	Sink DWT	□	□	□	□
2497	Bed 20	Bathroom HWB DWT	□	□	□	□

E *E. cloacae*

Dendrogram leaves: GGT036, EI183, EI148, EI171.1

Branch values: 7.261367, 0.000347, 0.00043, 0.000211, 0.000095, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
GGT036 (*E. cloacae* cluster #1)	Beds 15-18	HWB DWT	□	□	□	□
	Beds 15-18	HWB DWT	□	□	□	□
	Beds 15-18	HWB DWT	□	□	□	□

F *E. hormaechei*

Dendrogram leaves: VKH10, EI145, EI008, EI055, EI038, EI004, Patient Z15, Patient Z15, Patient Z16, Patient Z15, Patient Z15

Branch values: 0.047788, 0.047881, 0.00012, 0.000051, 0.056576, 0.540401, 0.049064, 0.109391, 0.14433, 0.110435, 0.074927, 0.00001, 0.000015, 0.000047, 0.000006, 0.000096, 0.000036, 0.000108, 0.00003, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
EB_P9_L5_03.19	Bed 10	HWB DWT	□	□	□	□
F2	Drug Prep	Sink U bend exterior	□	□	□	□
RHBSTW-00086	WoW	Keyboard & Mouse	□	□	□	□
AR_0365	Pantry	Drinks Cooler	□	□	□	□
Y323	Sluice	Domestic Waste lid	□	□	□	□
SH19PTE2 (*E. hormaechei* cluster #1)	Bed 7, 8, 30, 18, 9		□	□	■	■
	Bed 7, 8, 30, 18, 9		□	□	■	■
	Bed 24		□	□	■	□
	Bed 7, 8, 30, 18, 9		□	□	■	□
	Bed 7, 8, 30, 18, 9		□	□	□	□

G *E. coli*

Dendrogram leaves: K12, EI168, EI154, EI129, EI131, EI097, EI096, EI111, EI167, EI190, EI071, Patient Z2, EI106, Patient Z9, Patient Z9, Patient Z10, Patient Z7, Patient Z13, Patient Z14, Patient Z12, Patient Z11

Branch values: 0.146741, 0.000038, 0.044579, 0.000013, 0.051041, 0.000046, 0.051237, 0.000131, 0.000216, 0.023071, 0.000278, 0.08399, 0.020205, 0.017423, 0.000791, 0.02634, 0.0002, 0.043466, 0.750391, 0.014199, 0.138884, 0.01366, 0.031016, 0.000043, 0.030644, 0.000081, 0.000205, 0.000006, 0.140034, 0.000006, 0.000001, 0.000001, 0.000005, 0.000001, 0.000009, 0.000001, 0.000019, 0.000001, 0

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
CT02	Bed 19	HWB DWT	□	□	□	□
CT02	Bed 19	HWB DWT	□	□	□	□
A29	Office	HWB DWT	□	□	□	□
A29	Office	HWB DWT	□	□	□	□
ECONIH4	Bed 9	Bathroom HWB Drain	□	□	□	□
0H23	Staff toilet	HWB DWT	□	□	□	□
VREC0761	Office	HWB DWT	□	□	□	□
ABWA45	Beds 15-18	HWB DWT	□	□	□	□
ABWA45	Beds 15-18	HWB DWT	□	□	□	□
MSB1_6C	Beds 1-4	Bathroom toilet bowl	□	□	□	□
VREC0864	Bed 15, 2, 20		□	□	■	□
VREC0864	Pantry	Sink DWT	□	□	□	□
35152 (*E. coli* cluster #1)	Not housed on sampled ward		□	■	□	□
	Not housed on sampled ward		□	■	□	□
	Not housed on sampled ward		□	■	□	□
	Not housed on sampled ward		□	■	□	□
	Not housed on sampled ward		□	■	□	□
	Not housed on sampled ward		□	■	□	□
	Bed 25, 4		□	■	□	□
	Not housed on sampled ward		□	■	□	□

Fig. 1 Dendograms for species sequenced in this study

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H *K. michigenensis*

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
RHB20-CO2	Beds 21-24	Bathroom toilet bowl	■	■	■	□
K518	Pantry	Sink DWT	□	□	□	□
RHBSTW-00409	Beds 15-18	HWB DWT	□	□	□	□
RHBSTW-00409	Beds 15-18	HWB DWT	□	□	□	□

I *K. pneumoniae*

Strain	Location	Sample site	bla_{OXA-48}	bla_{OXA}	bla_{NDM-1}	bla_{NDM}
3987 (*K. pneumoniae* cluster #1)	Beds 1-4	HWB DWT	□	□	□	□
	Beds 29-32	Bathroom HWB DWT	□	□	□	□
	Beds 15-18	HWB DWT	■	□	□	□
	Bed 19	HWB DWT	■	■	□	□
RHBSTW-00610	Bed 9	HWB DWT	□	□	□	□
RHBSTW-00113	Bed 15, 2, 20		□	■	■	■
RHBSTW-00995	Beds 5-8	HWB DWT	□	□	□	□
RHBSTW-00995	Beds 5-8	HWB DWT	□	□	□	□
FDAARGOS_1315	Beds 5-8	HWB DWT	□	□	□	□
	Bed 7, 31		■	■	■	■
	Bed 31, 4, 7, 32		□	■	□	■
	Bed 29		□	■	□	□
B5617 (*K. pneumoniae* cluster #2)	Not housed on sampled ward	□	■	□	■
	Bed 32, 11, 7, 33, 31		□	■	□	■
	Bed 5, 14, 24, 25, 31, 16, 19		□	■	□	■
	Bed 5, 14, 24, 25, 31, 16, 19		□	■	□	□
	Bed 16, 22, 9		□	■	□	□
	Bed 27, 4, 30		□	■	□	□

Fig. 1 continued

reference genome (e.g. HS11286 for *K. pneumoniae*), against which the clinical and environmental isolates were mapped.

Discussion

There was evidence for the potential for patient-patient transmission for *E. hormaechei*, *E. coli* and *K. pneumoniae*. This conclusion is supported by the patient spatiotemporal data collected (supplementary materials S1). This study also identified environment-patient transmission. The *E. coli* isolate from patient Z2 clustered with EI106, an environmental isolate from a wastewater sample taken from the communal pantry sink's DWT, although they did not share the same plasmids. Whilst the clinical isolate was found to harbour the bla_{NDM-1} gene, no bla_{OXA} or bla_{NDM} genes were found in the environmental isolate. This may have been a loss or gain of function; bacteria found within the environment are less likely to encounter antibiotics or their residues compared with those in a hospitalised patient, so that isolate may have lost the plasmid containing these resistance genes. Conversely, they may gain plasmids from commensal organisms within the host [49].

It was noted that Enterobacterales were commonly isolated from environmental samples in Bay (beds 15–18), Bay (beds 29–32) and the single-occupancy bays. All the patients in this study located on ward 7D had been housed in at least one of these bays and 44% had been housed in more than one of these bays, suggesting potential host-related reservoirs. One report suggests that patients are on average 73% more likely to acquire a HAI if the patient previously occupying their room was colonised or infected, [50]. This suggests enhanced location-specific IPC would be beneficial when colonised or infected patients have been identified, and that isolation of these patients may not be enough. Enterobacterales were also isolated from environmental samples in non-clinical areas such as the shared-use pantry and the staff office. Studies have shown that HCWs can be colonised when handling patients and infected materials, it's also possible that colonised patients or HCWs may have caused reservoirs in the pantry due to transmission of organisms via the faecal-oral route [51].

It is well known that it can be difficult to standardise environmental sampling, and that the recovery rate can vary between sampling tools. In this study, multiple environmental surfaces were swabbed with both cotton and sponge swabs and then isolates of interest grown on selective agar to minimise selection bias and maximise the chance of isolating a CRE. In this study, there was a much greater recovery from wastewater. For recovery from dry surfaces, sponge swabs appeared to isolate more Enterobacterales than cotton swabs, perhaps due to the larger surface area for absorption (surface area of swab site remained the same).

Even with comprehensive patient metadata, such as bed movements, it can be difficult to confidently infer transmission of a clonal isolate. Environmental isolates are especially complex, as the bacteria may have been present for long periods, for example by forming hard to remove biofilms in U-bends, which is in itself difficult to monitor and identify provenance [52]. For human pathogens, the ward environment may not be optimal for growth, so their doubling time may be slower.

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Genetic-relatedness cutoffs to determine phylogeny tend to be calculated depending on the sample number, type and environments, as well as the species, as some have faster molecular clocks than others, and so the number of SNPs difference does not necessarily reflect the closeness of two isolates. These data are often missing for isolates extracted from the environment [53].

Whilst all clinical isolates tested positive for OXA-48-like or NDM-like variants using the CARBA-5 test, only 84% of the clinical isolates were found to harbour bla_{OXA} genes that the CARBA-5 panel tested for when whole genome sequenced. Studies have shown the specificity of the CARBA-5 test to vary from 96% (from blood cultures) to 100% from isolates and rectal swabs [54–57]. The clinical sites that the swabs were taken from in this study varied and the majority came from CRE screens, usually rectal, rather than sites of infection. It is possible that the CARBA-5 panel picked up bla_{OXA} genes from other colonising species present in CRE screening samples, as these were not sterile sites. Most of the clinical and environmental isolates found to harbour the bla_{OXA-48} gene also had other bla_{OXA} genes present, as well as other resistance genes, such as bla_{CTX} and bla_{SHV}. These are often found together on the same plasmids and are likely to be transferred between bacteria collectively [4]. Indeed, all of the *C. portucalensis* isolates identified in this study were found to harbour bla_{OXA} genes, with the clinical isolate also containing bla_{NDM-1}.

Whilst Col440II, which does not carry resistance or virulence genes, was the only plasmid found across all clinical isolate species, IncFIB(AP001918), the most commonly identified plasmid, is linked with resistance to several antimicrobial classes, including β-lactams, aminoglycosides, sulfonamides and tetracyclines, but was less commonly identified in environmental isolates [58, 59]. The prevalence of IncFIB(AP001918) in clinical samples suggests that genomic analysis of plasmids as well as isolates is important for enhanced IPC surveillance.

In this study, the initial MALDI-TOF speciation for *C. freundii*, *E. cloacae* and *K. oxytoca* isolates did not all match with the WGS speciation. Both methods were more consistent when comparing environmental rather than clinical isolates. All three species complexes contain multiple closely related species, which can make it difficult to fully resolve using conventional methods. *C. portucalensis* is a relatively newly described clinical pathogen but has the capacity to harbour and transmit AMR genes, thus identifying it to species level may be important in the future [60].

The use of genomic analysis in enhanced outbreak surveillance technologies provides greater detail on the potential transmission of MDROs and their associations with patients, HCWs and the ward environment.

Sequencing platforms such as ONT shows promise, especially for use in low- and middle-income countries, as long read sequencing enables read lengths of thousands, rather than hundreds, of base pairs which is especially useful when resolving speciation in metagenomic samples.

Conclusions

Understanding the resistance genes, plasmids and sequencing types present in an environment can provide greater resolution than phenotypic and other molecular methods, helping to identify targeted IPC interventions in outbreak situations. As a result of the evidence from this study, highlighting the presence of CREs in wastewater, the hospital estates team has since replaced all of the sink U-bends on the sampled ward, as well as reviewed and revised ward IPC practices to reduce potential transmission risks. Due to the number of colonised patients found, this study also recommends the use of more widespread CRE screening for hospitalised patients, to enable interventions to reduce the risks from human and environmental reservoirs and therefore reduce risks to vulnerable patients.

Overview:

- Putative patient-patient and patient-ward transmission was identified, utilising WGS and patient metadata
- CREs were more commonly isolated from wastewater samples than either stick swabs or sponge swabs
- All patients in this study tested positive for OXA-48-like carbapenemase variants using the NG-Test® CARBA-5 rapid diagnostic test, 84% of patient isolates harboured a bla_{OXA} gene present on the CARBA-5 panel
- bla_{OXA} and bla_{NDM} genes were identified in fewer environmental CRE isolates, compared with clinical isolates, suggesting either different populations, or a loss/gain of plasmids or genes
- ONT sequencing can expedite clinical decisions whilst awaiting reference laboratory results, providing economic and patient care benefits.

Abbreviations

AMR	Antimicrobial resistance
AST	Antibiotic sensitivity test
CRE	Carbapenem-resistant Enterobacterales
DWT	Drain waste trap
HCAI	Healthcare associated infection
HWB	Hand wash basin
IPC	Infection prevention and control
MDRO	Multi-drug resistant organism
NDM	New Delhi metallo-beta lactamase
ONT	Oxford Nanopore Technologies
RDT	Rapid diagnostic test

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SD   Standard deviation
WoW   Workstation on wheels

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00762-8.

> **Supplementary materials 1.**

Author contributions
EW, LE, TM and DM designed the study. EW oversaw the clinical aspects of this project and coordinated the collection and processing of the clinical isolates. SQ, JH, VP, LC, CO were instrumental in the ward environmental analysis and collection of the environmental samples. AW, SA, OY, EC, IW, MM, SY, KK, ES undertook phenotypic analysis on the environmental isolates. LE undertook clinical and environmental isolate and metagenomic DNA extraction, WGS and metagenomic sequencing, with advice from SR. LE was responsible for data analysis and the writing of this manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All datasets have been made freely available and can be found within the supplementary materials. Further patient metadata are available from the corresponding author upon reasonable request. Sequence data were deposited under BioProject PRJEB76684 on the European Nucleotide Archive.

Declarations

Ethical approval and consent to participate
This diagnostic service evaluation was undertaken at the request of the Division of Nursing in support of the Infection Prevention and Control team as part of enhanced standard of care practices for IPC outbreak investigation, in accordance with the Declaration of Helsinki. This project is under HRA approval for ELCID and registered with Health Services London. IRAS project ID: 283,831, National HRA REC reference: 20/HRA/4928, Local REC: RFL R&D ref: 134,895, HSL Project number: 1529.

Consent for publications
Not applicable.

Competing interests
The authors declare no competing interests.

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