Integrated patient network and genomic plasmid analysis reveal a regional, multi-species outbreak of carbapenemase-producing Enterobacterales carrying both bla\textsubscript{IMP} and \textit{mcr-9} genes

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Abstract

The incidence of carbapenemase-producing Enterobacterales (CPE) is rising globally, yet Imipenemase (IMP) carbapenemases remain relatively rare. This study describes an investigation of the emergence of IMP-encoding CPE amongst diverse Enterobacterales species between 2016 and 2019 in patients across a London regional hospital network.

A network analysis approach to patient pathways, using routinely collected electronic health records, identified previously unrecognised contacts between patients who were IMP CPE positive on screening, implying potential bacterial transmission events. Whole genome sequencing of 85 Enterobacterales isolates from these patients revealed that 86% (73/85) were diverse species (predominantly Klebsiella spp, Enterobacter spp, E. coli) and harboured an IncHI2 plasmid, which carried both blaIMP and the putative mobile colistin resistance gene mcr-9. Detailed phylogenetic analysis identified two distinct IncHI2 plasmid lineages, A and B, both of which showed significant association with patient movements between four hospital sites and across medical specialities.

Combined, our patient network and plasmid analyses demonstrate an interspecies, plasmid-mediated outbreak of blaIMP-CPE, which remained unidentified during standard microbiology and infection control investigations. With whole genome sequencing (WGS) technologies and large-data incorporation, the outbreak investigation approach proposed here provides a framework for real-time identification of key factors causing pathogen spread. Analysing outbreaks at the plasmid level reveals that resistance may be wider spread than suspected, allowing more targetted interventions to stop the transmission of resistance within hospital networks.
Introduction

Infections by carbapenemase-producing Enterobacterales (CPE) pose a substantial clinical, operational, and financial challenge. These organisms are associated with high morbidity and mortality, and therapeutic options are severely restricted. Carbapenemase genes are frequently carried on plasmids, which can easily transfer between bacterial species. CPE outbreaks involving different bacterial species are often unrecognised, as many plasmids are variable in their gene content and have a broad host range. The metallo-β-lactamase – (MBL) Imipenemase gene (IMP)-1, was first described in *Pseudomonas aeruginosa*, whilst IMP genes have spread globally, outbreaks in Enterobacterales species are mostly sporadic and often localised to specific geographical locations. IMP genes are rarely isolated in the UK, but the number of IMP encoding isolates within Enterobacterales species referred to UK Health Security Agency (UK HSA) has been increasing.

Therapeutic options are particularly limited for MBL CPEs, with limited efficacy of the newer beta-lactam combination agents. Colistin and polymyxin B remain the last-line therapeutic agents in most countries, partly due to lack of access to newer agents; yet resistance is increasing globally, particularly through mobilised colistin resistance (*mcr*) genes on plasmids. Since the first discovery of *mcr*-1 in 2016, ten *mcr* genes (*mcr*-1 to *mcr*-10) have been described to date, presenting a substantial global healthcare challenge. Although *mcr* genes are typically associated with phenotypic polymyxin resistance, *mcr*-9, identified in 2019, does not appear to confer direct colistin resistance. It has been detected in clinical isolates co-harbouring different resistance genes in Australia (with *bla*IMP-*a*), South Africa (with ESBLs), Egypt and the Netherlands (with *bla*VIM) and Japan (with *bla*IMP-*d*), and is widespread in isolates from human, animal and environmental origins, in a wide range of bacterial species.

Person-to-person contact is a route of transmission for many infectious diseases. Consequently, understanding the patterns of these contacts, especially in healthcare settings, can offer detailed insight for targeted interventions. Contact patterns, however, become increasingly complex when considering large numbers of interacting individuals over long-time spans, bacterial interactions with the human host and environment, bacterial behaviour under antibiotic and disinfectant pressure, and plasmids that can be shared by multiple bacterial species. Network models provide flexible tools to capture such complex contact patterns, with the availability of...
well-developed analytical toolboxes they offer robust and reproducible methodology that has become widespread across disciplines\textsuperscript{23}. Network models of infectious diseases\textsuperscript{24-26} incorporate both person-to-person transmission through contact networks\textsuperscript{27-30} and spatial spread through networks representing physical locations and their relationships\textsuperscript{31,32}. So far, studies utilising network models of patient contacts in combination with detailed genomic analysis of bacterial isolates have demonstrated their promise through increasing the detail in outbreak characterisation\textsuperscript{33,34}.

Here we describe the emergence of \textit{bla}\textsubscript{IMP} and \textit{mcr}-9 co-harbour by diverse Enterobacterales species that have been isolated from patients across a regional network of London hospitals between 2016 and 2019. We combine in-depth phylogenomic analysis of the IncHI2 plasmid containing \textit{mcr}-9 and \textit{bla}\textsubscript{IMP} with a network model of patient movements to provide novel insight into the spread and dissemination of \textit{bla}\textsubscript{IMP} and \textit{mcr}-9 between bacterial species and patients over 3 years, with implications for how CPE clusters are identified and managed in the hospital setting.

Results

\textbf{Emergence of \textit{bla}\textsubscript{IMP}CPE}

Following the introduction of enhanced CPE screening in 2015\textsuperscript{35}, a CPE-carrying \textit{bla}\textsubscript{IMP} (\textit{bla}\textsubscript{IMP}CPE) was first observed in our hospital group in June 2016 from a routine rectal screening sample. From November 2016, increasing numbers of \textit{bla}\textsubscript{IMP}CPE isolates were identified across diverse Enterobacterales species at three of the acute hospital sites (representing 64\% of all inpatient beds at our institution) (\textbf{Figure 1a}), and simultaneously at two other external hospitals in the regional network, all served by the same microbiology diagnostics laboratory, and closely connected by regular inter-hospital patient transfers. The highest incidence of \textit{bla}\textsubscript{IMP}CPE cases occurred in January and April 2019 (\textbf{Figure 1b}). In total, \textit{bla}\textsubscript{IMP}CPE were isolated from screening or clinical samples from 116 patients who were admitted to our hospital group up until the end of November 2019, when new cases rapidly dropped, despite no targeted changes in infection control interventions. However, only two clusters of cases, (totalling 5 out of 116 cases), fitted the real-time infection control outbreak definition, i.e. two or more cases of the same bacterial species with the same resistance mechanism overlapping in time and space. Pulsed Field Gel Electrophoresis (PFGE) typing
performed at the public health laboratories for those 5 cases had confirmed similarity, suggesting cross transmission events had occurred. To reveal the operational burden of blaIMP-CPE, the daily number of occupied beds was computed for all colonised patients. The analysis revealed a continuous burden of patients colonised with blaIMP-CPE which was far higher than expected from the new case incidence, particularly for those colonised with *Enterobacter* species (Figure 1a,b), with 424 total inpatient bed days per month at the peak (March 2019), across the hospital network (Figure 1c).

**Figure 1:** Characteristics of confirmed blaIMP-CPE patient cases and distribution of CPE species. (A) Total number of days patients (rows labelled by CPE isolate identifier) were present in a hospital ward before confirmed to be colonised/infected with blaIMP-CPE (*in-hospital infectious period*) and infection prevention and control measures were taken. Additionally, patients with known carriage of blaIMP-CPE but without sequenced isolates are shown as unlabelled rows. Patients with paired isolates (IMP 22/24, IMP 25/33, IMP 96/97, IMP 100/101) are on adjacent rows. (B) Monthly total number of confirmed blaIMP-CPE isolates detected in patients during the study period from 2016 to 2019. (C) Weekly cumulative number of bed days of patients with confirmed carriage of blaIMP-CPE before they identified as colonised with blaIMP-CPE over their infectious in-hospital period. Colours in each of the panels indicate the genus of CPE identified in the study.
Contact network of blaIMP-CPE patients

A detailed patient contact network for all 116 blaIMP-CPE colonised/infected patients who were identified in our hospital group, including data from their admissions at the other regional hospitals, confirmed that 77/116 (66%) patients were in contact with at least one other blaIMP-CPE colonised/infected patient (Figure 2). The analysis showed that the median number of contacts was two per patient, however, contact numbers displayed substantial variation. For example, one patient (Case 85, cluster 1.5) had 10 contacts with 9 other patients which linked them to 8% of all cases (Table S1) and therefore identified the patient as a potential ‘super-spreader’ in the outbreak.

Across all blaIMP-CPE patient contact pairs, 57/96 (59%) pairs differed in bacterial species (8 species identified carrying blaIMP), explaining why these contacts were not identified as outbreak-related using the clinical infection control outbreak definition, which was reliant on the same bacterial species rather than resistance mechanism. The network of patient contacts split patients into 12 separate clusters, with interactions occurring across hospitals, as patients were transferred between wards and sites (Figure 2). The largest cluster (Cluster 1) contained 45 patients and was partitioned into a further seven subclusters, labelled 1.1 to 1.7, that comprised 13, 12, 2, 6, 5, 5 and 2 patients, respectively (Figure 2). The analysis of blaIMP-CPE patient contacts, at regional, individual hospital and ward level, suggested that different species
of blaIMP-CPE were involved in transmission events (Figure 2). This multi-species involvement in the ongoing outbreak prompted genomic analysis of the available blaIMP-CPE isolates.

Figure 2. Patient contact network of interactions among blaIMP-CPE positive patients.

Each node of the network represents a patient (coloured according to blaIMP-CPE isolate species) and each edge represents a contact between two patients. Edges represent recorded contacts between confirmed blaIMP-CPE cases (patients present on the same ward on the same day according to their electronic health records). The patient contact network identified 12 distinct clusters (each shaded in a different colour) based on disconnected components of patient contact. Cluster 1, the largest cluster consisting of 45 patients, was further partitioned into 7 subclusters using community detection with edges weighted by duration of contact (see Supplementary methods – Community detection). Six patient contacts re-occurred over different wards, indicated by additional edges connecting the same patients.

Genomic and phenotypic characterisation of blaIMP-CPE isolates

A total of 85 bacterial isolates from 82/116 blaIMP-CPE colonised patients were sequenced for genomic analysis (Table S2). The blaIMP gene was detected in 13 different bacterial species, most commonly in members of the Enterobacter cloacae complex species (55/85), K. pneumoniae (16/85) and E. coli (10/85) (Table S2). Most patients harboured only one blaIMP
encoding species, but there were four patients who were colonised with two different bacterial species that carried \( \text{bla}_{\text{IMP}} \) (Table S2).

Genomic analysis identified that two alleles, \( \text{bla}_{\text{IMP}-1} \) (75/85) and \( \text{bla}_{\text{IMP}-4} \) (10/85), co-occurred with two groups of plasmids: IncHI2 plasmid was identified in 72/85 isolates and IncN3 in 11/85 (Figure 3). One isolate of \( K. \ aerogenes \) (IMP79) carried both plasmids, and two isolates (IMP47, IMP76) carried a different plasmid co-occurring with \( \text{bla}_{\text{IMP}-1} \). As expected, all \( \text{bla}_{\text{IMP}} \) CPE isolates had carried multiple \( \beta \)-lactam resistance genes in addition to other antimicrobial resistance genes (Table S3), though only one isolate (IMP89) had another carbapenem resistance gene, \( \text{bla}_{\text{OXA-48}} \) (Table S3).

Figure 3. Phylogenetic tree of 85 Enterobacterales genomes in this study. A neighbour-joining phylogenetic tree of 85 Enterobacterales genomes was constructed using whole-genome FastANI distances. The colour at the end of each branch indicates the species identity for that isolate. The rings are as follows: the innermost ring indicates the type of plasmid detected; the middle ring indicates the allelic variant of the \( \text{bla}_{\text{IMP}} \) gene detected, and the outer ring indicates the presence or absence of the \( \text{mcr}-9 \) gene. NF = not found. The scale bar indicates the average nucleotide distance (in percent) per site.
The multi-species distribution of the 72 IncHI2 plasmids among contact network linked patients, was highly suggestive of plasmid mobility. The mcr-9 gene was detected in 81% (69/85) of all blaIMP CPE isolates, all of which harboured the IncHI2 plasmid (94% 69/72 of IncHI2 plasmid carrying isolates; none of IncN3 plasmids had mcr-9). The mcr-9 specific LAMP assay confirmed the presence of mcr-9 with 100% concordance (Table S4) as previously reported. MALDIxin (which detects modifications to the lipid A of the lipopolysaccharide as mediated by mcr1-5 and mcr-8) did not detect any Lipid A modifications attributable to the mcr-9 gene in this study. Altogether, 12/84 isolates tested (all Enterobacter species) were resistant to colistin with MICs > 2 μg/ml (range 4 to >64 μg/ml) (Table S4), and 5 isolates demonstrated a skipped-well phenomenon suggestive of colistin heteroresistance. However, determining the contribution of mcr-9 or other mechanisms of resistance to this was beyond the scope of this study, and will be the subject of future investigations.

Population structure of IncHI2 plasmids
Phylogenetic analysis of the 72 IncHI2 plasmids (where reads were mapped to complete genomic sequence of plasmid pKA_P10 (NZ_CP044215.1)) identified two major lineages (A and B) and four sublineages (A.1, A.2, B.1 and B.2, Figure 4). While all the isolates were carrying multiple antimicrobial resistance (AMR) genes and multiple plasmids, only plasmids of the B.1 lineage carried blaIMP-4 and were present in isolates that also exclusively saw co-occurrence of tet(D), qnrB2, blaOXA-1 and mph(A) (Figure 4). Comparison of 233 plasmid core-genome single-nucleotide polymorphisms (cgSNPs) showed some congruency of cgSNPs with the plasmid sequences (Figure S1). Furthermore, this analysis grouped the IncHI2 plasmids into 15 polytomic clades (with identical SNP profiles), of which 12 showed identical cgSNPs after masking recombination regions (Table S5 and Figure S2).

Figure 4. Dated phylogenetic tree showing the relationship between 72 IncHI2 plasmids identified in CPE isolates with blaIMP. The complete genome of IncHI2 plasmid pKA_P10 was used as the reference for variant calling. The tree was generated and rooted using BactDating. Branch colours denote major plasmid lineages, which are supported by bootstrap values of 100. At the tips of the tree, circles represent plasmids from routine rectal screening samples, and squares represent plasmids from clinical samples. Colours of the tip symbols indicate species. Lineage identifiers are labelled above lineage-specific root branches. The
The column panel to the right of the phylogenetic tree indicates presence-absence of mobile antimicrobial resistance genes and plasmid replicons. NA: information was not available.

The root of the plasmid tree was dated in 1949 (95% CI: 1927-1971), with the most recent common ancestors of lineages A and B dated in 1965 (95% CI: 1943–1988) and 1967 (95% CI: 1945–1988), respectively (Figure S3). These estimates suggest that not only IncHI2 plasmids have been circulating amongst Enterobacterales for many decades, but also that they have undergone extensive evolution in the antibiotic era.

**Plasmid lineages and patient cluster comparisons**

To investigate if the identified IncHI2 plasmid lineages A and B were responsible for the emergence of \textit{blaIMP}/CPE in our hospital network, plasmid genomic data was overlaid onto the
patient contact network (Figure 5, Figure S4). IncHI2 plasmid lineage A (3/72 IncHI2 plasmids) was exclusively linked to patients in cluster 5 (Figure 5), with contacts confined to the same hospital site, highly suggestive of a patient-to-patient or common-source transmission event. The predominant IncHI2 plasmid clade B, representing 69/72 plasmids sequenced and 60% of all cases identified in the study, was distributed widely across the hospital network, dominated Cluster 1 of patient contact clusters, and showed particularly strong association with contacts at hospital site 3 (Figure 5). The IncN3 plasmid (9.5% of overall cases) displayed strong association with patients in network Cluster 9, with contacts occurring at three different hospitals (hospitals 1, 2 and 3) (Figure 5). Although 3 of the IncN3 cases in cluster 9 had been identified as an outbreak (Cases 80, 81 and 82) at the time of initial isolate identification, 5 other linked cases have now been revealed by this combined network and plasmid analysis, that were previously missed. Similarly, Cases 67 and 71 (Cluster 1.2), identified real-time as related cases, are revealed by this combined analysis to be linked to multiple (n=34) other cases across clusters 1.1, 1.2, 1.3 and 1.4, with the IncHI2 plasmid lineage B.2.

Figure 5. Patient contact network of interactions among patients focussed on specific locations of contact and plasmid information. Each node represents a patient, edges represent recorded ward contacts between confirmed blaIMP CPE cases. The edges are coloured according to the hospital site (site 1-4) and the width of the edge is proportional to the duration of the contact. Cluster nodes are coloured according to the lineage of the IncHI2 plasmid identified in whole genome sequenced blaIMP CPE isolates; patients with isolates lacking the IncHI2 plasmid are coloured in white; patients with no whole genome sequenced blaIMP CPE isolates are displayed as labels without a circle. The presence of an IncN3 plasmid in the blaIMP CPE isolates is denoted by dark-grey borders surrounding the nodes. Contact network clusters are highlighted with individual colour shading as in Figure 4.
Bacterial sequence data was missing from some cases that could have clarified links between and within plasmid lineages A and B, as suggested by the contact network analysis. Similarly, data was not available for potentially linked cases identified at other regional sites but not transferred to our hospital network. Despite missing hospital network data, clear associations between plasmid clades and patient pathway network clusters are evident, supporting the hypothesis of plasmid spread throughout the course of this outbreak, particularly at hospital 3. When reviewing the cumulative burden of the plasmid clades over time, it becomes apparent that most of the cumulative bed days of patients shown in Figure 1 are due to patients colonised with clade B2 (Figure S5).

**Spatial and temporal analysis**

Having identified four plasmid lineages among the 72 *bla*\text{IMP}-CPE IncHI2 plasmids, a network was constructed capturing the structure of patient transfers among hospital wards and used to investigate the spatial distribution of the four major plasmid lineages (Figure 6). The smaller plasmid lineages A.1 and A.2 were present in only two and one patients, respectively, and hence, were locally constrained by a smaller set of patient movements. Plasmid lineage A.1 was localised within hospital 3 renal services and had no recorded patient inter-hospital
movement. Plasmid lineage A.2 was localised to a single patient movement within hospital 4, then an inter-hospital movement to hospital 2. Patients with isolates in plasmid lineage B.1 were recorded on wards from hospital sites 1, 2, 3 and 5. However, inter-hospital transfers of these patients were only recorded between hospitals 2 and 5, and hospitals 3 and 5. It is thus likely that some transmission of plasmid lineages B.1 was missed, either through patients who were not detected on screening, or these were patients whose isolates were not available for sequencing. Plasmid lineage B.2 was spatially more distributed with recorded multiple bi-directional inter-hospital transfers among hospital sites 1, 2, 3 and 5. Looking at the association between plasmid lineage clusters and ward/specialties over the study period the most common associations across all specialities were critical care and renal services (Table S6). The only exception was general internal medicine and general surgery predominated in plasmid lineage B.1 at hospital site 5, which has more general wards and less specialist services than the other hospital sites in the network. Despite the predominance of cases being identified in specialties with high risk for invasive disease, only 4 clinical infections were identified during the study, and no blood stream infections (Table S2).

**Figure 6. Spatial analysis representing the pathways of patient transfers among wards and hospitals.** Movement of patients carrying *bla*IMP-CPE are represented by arrows between wards (nodes) and the five hospitals (dotted ovals). For hospital site 5, only hospital-level data was available, therefore, only intra-hospital movement is shown. Repeated transfers of patients between wards are aggregated into edges with proportionally greater edge width. Plasmid clusters, based on plasmid lineage (A.1, A.2, B.1 and B.2) are coloured accordingly. For patients with multiple CPE isolates, transfers are duplicated to match each isolate uniquely in the visualisation.
Although the first case was identified in 2016 (IMP 51/Case 91), a temporal network analysis indicated it played only a minor role in onward transmission, due to the small number of reachable patients over time (Figure S6). This may suggest that previous/subsequent patients were not identified or point to the existence of an environmental reservoir. Considering the timing and ordering of all contacts, IMP79 could have seeded up to 27 subsequent cases (restricted to those in Cluster 1 in Figure 2), either directly or indirectly, via possible transmission routes. The patient carried the isolate that co-harboured both IncN3 and IncHI2 plasmids, potentially suggesting multiple transmission opportunities to, as well as, from, this patient. There were 14 patients (15 isolates) from the 72 isolates carrying an IncHI2 plasmid, which had a proportionately larger number of reachable time-ordered contacts, suggesting that these patients may also have contributed substantially to transmission (Figure S6).

These integrated geno-spatio-temporal representations show that the spread of plasmids containing blaIMP followed patient movements for specialty care around the region, with possibly multiple introductions and re-introductions of the plasmid at different points during the patient journeys, and multiple chances for cross transmission events to have occurred.
Discussion

Following the detection of a new mechanism of resistance, investigation of its origin and mode of transmission is challenging, especially in healthcare settings where investigations usually focus on single species cross transmissions. With confounding factors such as multiple bacterial species and spread over different hospital locations, new methods to investigate potential outbreaks are much needed. The incorporation of plasmid genomics and patient networks into our analysis changed the way the emergence of \textit{bla}IMP\textsubscript{CPE} was visualised and produced a clearer understanding of the high-risk ward locations and pathways for potential cross transmission in our regional healthcare system. As patients were found to follow common routes, with regular re-encounters, this information can provide dynamic risk assessments to be introduced along those pathways, to prevent future cross transmission events of any healthcare-associated pathogen from occurring\textsuperscript{39}. Detailed genomic analysis of plasmids enhanced our understanding of the relatedness of different patient isolates to the network analysis. It moreover revealed concerning information about unsuspected resistance mechanisms, with potential for antibiotic treatment failures that were missed on conventional laboratory susceptibility testing. In this study, we characterized IncHI2 plasmids as the main vehicle in horizontal transfer of the metallo-\(\beta\)-lactamase gene \textit{bla}IMP. A dated phylogeny of the IncHI2 plasmid carrying \textit{bla}IMP, indicated that this plasmid underwent a clonal expansion around 2008, about 8 years before the onset of the outbreak described in our study. IncHI2 plasmids are common, large plasmids with a wide host range, that has been reported globally\textsuperscript{19}, and more recently have been reported to be associated with colistin resistance genes \textit{mcr-1} and \textit{mcr-3}\textsuperscript{40,41}. Although in our study we detected \textit{mcr-9} in 81\% of the isolates tested, we did not find evidence of phenotypic expression of this gene, in line with previous observations\textsuperscript{7,42}. We identified the predominant IncHI2 plasmid in multiple different bacterial species across linked patients, highlighting the need for integration of genomics into routine clinical practice. Although this study focussed on the emergence of the \textit{bla}IMP carbapenemase gene in our hospital network, it supports the concept that plasmid analysis across different resistance mechanisms as well as among different species should be the standard for investigations in the future. The small number of clinical infections from this outbreak in comparison to other CPE outbreaks from our hospital network\textsuperscript{43} and other reports of \textit{bla}IMP CPE\textsuperscript{7,19} is noteworthy, and poses questions about the wider importance of this plasmid and the resistance mechanisms revealed in this study. This observation reinforces the argument that screening for silent carriage of CPE in hospitals is key to preventing spread\textsuperscript{44,46}, and cautious antimicrobial
stewardship is essential to prevent expression of hidden resistance mechanisms\textsuperscript{47}. The association of IMP carbapenemases and drainage\textsuperscript{48,49}, leads to speculation that the acquisition of this plasmid may provide an environmental survival advantage, enhancing the potential for spread between human hosts through a poorly maintained plumbing environment\textsuperscript{50,51}.

We acknowledge some limitations of our study. Firstly, long-read data was available only for two isolates, pKA\textsubscript{P10} and pEB\textsubscript{P9} (NZ\textunderscore CP043767.1)\textsuperscript{10}, and though both were tested as references, assumptions had to be made for in-depth genomic analysis of our plasmids. As a result, our plasmid tree may omit some similarities and differences between identified IncHI2 plasmids. Secondly, full pathway data across the hospital during the three years of the outbreak was only available for identified positive cases, not for all patients. It was therefore not possible to fully establish potential missed cases flagging as close contacts but with potential for missed screening or false negative results. Full pathway movement data for all positive cases identified within our hospital network was available, yet neither pathway details nor genomic data were available for other bla\textsubscript{IMP}\textsubscript{CPE}-positive cases identified in the two other regional hospitals who did not visit our institution, thus reducing the understanding in our analysis. Interactions at other potential hospital locations such as interventional imaging or endoscopy were not examined in this study, nor was environmental sampling performed, which could inform future studies on modes of transmission. Nevertheless, this study highlights a previously unidentified extent of transmission and thus provides valuable new insights into the spread of an emerging resistance mechanism. Moreover, our novel multi-layered methodology, incorporating plasmid phylogeny with contact network analysis, provides invaluable tools for outbreak investigation that can be generalised to a wide range of scenarios.

Materials and Methods

Clinical Setting

This study was carried out using data from a regional network of London hospitals, comprising of 7 hospital sites with a total of 2000 inpatient beds, with managerial responsibility assigned to 2 different NHS Trusts. Cases of colonisation/infection with bla\textsubscript{IMP}\textsubscript{CPE} were identified at 3 hospital sites from one NHS trust (referred to as our Institution in the results) and 2 hospital sites from the other. All hospitals in the network are are served by the same centralised
microbiology laboratory and patients are frequently transferred between hospitals in the regional network for specialist investigations and treatment.

**Sampling, microbiology, antimicrobial susceptibility testing and detection of mcr-9**

*bla*<sub>IMP</sub>-encoding Enterobacterales isolates were cultured from rectal screens or clinical samples as part of routine diagnostic testing between June 2016 and November 2019. Rectal screens were cultured on chromogenic media (Colorex™ mSuperCARBA™, E&O Laboratories, UK), and species identification was performed using Biotyper MALDI-TOF MS (Bruker Daltonics, Germany) according to the manufacturer’s protocol. Antimicrobial susceptibility testing was performed according to EUCAST guidelines.<sup>52</sup> Colistin susceptibility testing was performed by broth microdilution on all viable isolates according to manufacturer’s instructions (MICRONAUT, BioConnections, UK).<sup>52</sup> The identification and detection of carbapenemase genes was by PCR (Xpert® Carba-R, Cepheid Inc, USA). After February 2018, all isolates were tested using the RESIST 3 O.K.N. K.SeT (Coris BioConcept, Belgium), and negative isolates had the carbapenem inactivation method (CIM) performed, and CIM positive isolates then had PCR (Xpert® Carba-R, Cepheid Inc, USA) performed as previously described.<sup>35</sup> *bla*<sub>IMP</sub> positive isolates with suspected links were sent to UK Health Security Agency (UK HSA) reference laboratories for typing by Variable Number Tandem Repeat VNTR (*Klebsiella* sp.) or Pulsed Field Gel Electrophoresis PFGE (all other species).

Loop-mediated isothermal amplification (LAMP) was used to examine the prevalence of *mcr-9* amongst *bla*<sub>IMP</sub>-CPE isolates as described previously.<sup>53</sup> MALDIxin testing was conducted to confirm phenotypic colistin resistance, as previously described on isolates IMP 1-58 only.<sup>37</sup>

**Whole-genome sequencing**

Bacterial isolates were grown on Columbia Blood Agar (Oxoid Ltd, UK), incubated aerobically at 37°C overnight before DNA extraction. Genomic DNA was extracted using GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, USA) following manufacturer instructions.

Multiplexed DNA libraries were generated with Nextera XT (Illumina, USA) and were sequenced for a minimum of 100-fold average coverage on Illumina HiSeq 4000 systems under a 150-bp paired-end layout. Seven isolates (IMP09/CB_P14_L5_12.18, IMP29/KP_P12_L5_11.18, IMP33/EB_P9_L5_03.19, IMP40/EB_P8_L5_01.19, IMP41/KQ_P11_L5_04.19, IMP42/KA_P10_L5_03.19, IMP45/ EB_P13_L5_04.19) were also sent to UK HSA for MinION (Oxford Nanopore, UK) long-read sequencing.<sup>10</sup>
Sequence data processing and analyses

Short-read sequence reads were quality assessed using FastQC v0.11.6\(^{54}\). Raw reads were trimmed using Trimmomatic v0.39\(^{55}\) and were de novo assembled using SPAdes v3.11\(^{56}\) and annotated with Prokka \(^{57}\). Species identification was confirmed with KmerFinder 3.2\(^{58}\).

Multilocus sequence typing (MLST) was performed with the MLST v1.8 online server\(^{59}\) using the Achtman MLST scheme for *E. coli* curated on PubMLST and EnteroBase\(^{60}\) and the Pasteur MLST scheme for other *Enterobacterales* isolates. AMR genes, and plasmid typing (Table S7) were done using ResFinder v3.2\(^{61}\), and PlasmidFinder v2.1\(^{62}\), respectively. A neighbour-joining tree of CPE isolates used in this study was generated from assemblies using FastANI v1.3 based on whole-genome average nucleotide identities \(^{63}\).

Phylogenetic analysis of IncHI2 plasmids

A reference complete genome of IncHI2 plasmids was selected from three candidates: pKA_P10 (NZ_CP044215.1; 334 kbp) from *Klebsiella aerogenes*, pEB_P9 (NZ_CP043767.1; 331 kbp) from *Enterobacter hormaechei* isolate\(^{10}\) and R478 (NC_005211.1; 275 kbp) identified in *Serratia marcescens*\(^{64}\). The selection aimed to maximise: (i) overall coverage of each reference genome by reads, (ii) the number of plasmid core-genome SNPs identified from the read alignment, and (iii) bootstrap values supporting major lineages in the plasmid phylogenetic tree reconstructed using the same approach based on each reference. Eventually, plasmid pKA_P10 was determined as the reference for the rest of analysis (Figures S7).

Repetitive regions (≥90% nucleotide identity) in the reference plasmid genome were identified using NUCmer of MUMer v3.1\(^{65}\). Processed short reads of 73 IMP isolates harbouring IncHI2 plasmids were further filtered for a minimum length of 50 bp using Trimmomatic for improving mapping specificity, given estimated read depths >100 fold per bacterial genome before and after the filtering. Filtered reads were mapped to the reference pKA_P10 using Snippy (github.com/tseemann/snippy) with parameters set to as follows: mapping quality ≥ Q60, base quality ≥ Q20, base coverage ≥10 folds, and variant-call quality ≥ Q100. Plasmid pIMP06 was excluded from further steps as its reads covered only 27% of the reference genome. Core-genome SNPs (cgSNPs, sites presented in all genomes) outside of repetitive regions were identified and a whole-sequence alignment of remaining 72 plasmids against the reference was generated using Snippy. A neighbour-joining tree were generated from recombination-free cgSNPs using pairwise SNP counts as distance metrics (namely, SNP distances) (Figure S8).
Maximum-likelihood phylogenetic reconstruction from the whole-genome plasmid alignment (which includes invariant sites, gaps, and masked regions) was performed using IQ-Tree v1.6.12\textsuperscript{66}, with the best-fit model “HKY+F+R2” (determined through extended model selection and full-tree search implemented in IQ-Tree), 10 independent runs (for tree selection), and 500 bootstrap replicates (for branch supports). The resulting unrooted tree was corrected for recombination using ClonalFrameML \textsuperscript{67} with its default parameters and the whole-genome alignment of plasmids. Major plasmid lineages were identified from topology of the corrected tree. Detected recombination regions and reconstructed ancestral sequences were drawn against the corrected tree using the script cfml_results.R from ClonalFrameML. Sample information and AMR gene content of IMP isolates harbouring these plasmids were drawn against the plasmid tree using iTOL v5\textsuperscript{68}. The dating of internal nodes of the plasmid tree was inferred by BactDating using the mixed-Gamma model, root-finding algorithm, $5 \times 10^4$ random walks of Markov Chain Monte Carlo (MCMC), and accounting for recombination identified by ClonalFrameML. To evaluate the robustness of tree reconstruction, a cgSNP tree was generated by IQ-Tree (best-fit model: “TIM3e+ASC”; other parameters were the same as reconstructing the whole-genome tree) and corrected for recombination using ClonalFrameML (with default parameters). Tree comparisons (Figures S9–S12) were performed in R v3.6.3 with function tanglegram in package dendextend\textsuperscript{69}.

**Patient contact network analysis**

Ward locations and timings were extracted from the Electronic Health Record (EHR) data for \textit{bla}\textsubscript{IMP}-CPE positive patients to establish patient movement history. Movement to other locations for procedures during an inpatient episode, such as endoscopy, interventional radiology or operating theatres, was not examined. To reveal potential transmission events structure, a patient contact network was constructed from patient movement history, which included information on when and where patients were on the same ward (for more details please see Suppl. Methods). Time aggregated patient contacts were subsequently clustered to reveal groups of patients more closely linked together using the Walktrap community detection algorithm\textsuperscript{70}. In this framework, contacts were also weighted by the duration of time spent together across their hospital episode, capturing that the probability of transmission increases with the amount of time two patients spend together\textsuperscript{71}. Moreover, a temporal analysis of patient interactions was performed over the time ordered sequence of contacts between patients\textsuperscript{72} and used to assess patient roles and position in transmission (for more details please see Suppl. Methods).
For insights into the spatial distribution of $bla_{IMP}$CPE, a spatial network of ward/hospital network, connected by the same $bla_{IMP}$CPE patient movement was generated. From this spatial network, the following was calculated: total number of days patient has spent on each ward over the time before they were known to be colonised with $bla_{IMP}$CPE and infection prevention and control measures were taken (their infectious in-hospital period); network structure to determine ward/hospital spread of $bla_{IMP}$CPE; highly visited wards from patients of each specific genomic sub-cluster were also determined (using PageRank$^{73,74}$) and contrasted for evidence of genomic localisation to hospital spaces. All network analysis and visualisation was completed in Python, R, and Cytoscape$^{75}$.

Data availability

Illumina reads and draft genome assemblies of 85 isolates were deposited in European Nucleotide Archive (ENA) under the BioProject accession PRJEB38818.

Ethics

This study was carried out in accordance with ethics reference 21/LO/0170 (279677), protocol 21HH6538 Investigation of epidemiological and pathogenic factors associated with infectious diseases.

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Transparency declarations

JT holds some shares in Oxford Nanopore Technologies. All other authors have nothing to declare.

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C, AS, GL-M, JR-M. Methodology: FD, AM, AW, SM, YW, EJ, BA, XD, MB, JO, KH, ME, JT, MG. Project administration: FB, AH, FD, EJ. Resources: FD, AH, AE, HD, KH, JT. Supervision: FD, EJ, AH, MB. Validation: KH, JT, ME, HD, AW, SM. Visualization: AB, AM, YW, EJ, FD. Writing – original draft and further drafts: AB, AM, YW, EJ, FD, FB. Writing – review & editing: All authors.

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