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Community prevalence of carbapenemase-producing organisms in East London

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SUMMARY

Background: Over the last decade there has been a rapid, worldwide increase in carbapenem resistance, which is of growing concern. The main protagonists, the carbapenemases *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase β-lactamase 48 (OXA-48), imipenemase metallo-β-lactamase (IMP), Verona integron-borne metallo-β-lactamase (VIM), and New Delhi metallo-β-lactamase (NDM) have also been reported across the UK. However, these reports are derived from a combination of reactive screening, outbreak control, inpatient surveillance, and diagnostic samples. Therefore, the true community prevalence is unknown.

Aim: To determine the community prevalence of carbapenemase-producing organisms (CPOs) in the area served by Barts Health NHS Trust.

Methods: Active screening of 200 non-duplicate community stool samples was performed. Patient demographics and foreign travel history were extracted from the laboratory information management system to identify potential risk factors for carriage of CPOs.

Findings: Patients in this study were aged from one to 93 years and were evenly distributed between male and female. Foreign travel in the last year was listed for 46 out of 200 (23%) patients, with the most commonly visited countries including Bangladesh (4%), India (2.5%), Morocco (2%), and Turkey (1.5%). However, only one patient tested positive for a CPO, an NDM-producing *Pseudomonas aeruginosa*, and this patient had travelled to the Caribbean.

Conclusion: To date, there have been no studies investigating the prevalence of CPOs in the UK community. Given the high-risk patient population served by Barts Health NHS Trust, it is reassuring that the prevalence observed here was low. However, it should be highlighted that travel to countries not previously categorized as high risk may also pose a threat.

Introduction

Carbapenemase enzymes are typically produced by a range of Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [1]. Many carbapenemases have been discovered, but the most frequently

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described, also known as the 'big five', are New Delhi metallo-β-lactamase (NDM) *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase β-lactamase 48 (OXA-48), imipenemase metallo-β-lactamase (IMP), and Verona integron-borne metallo-β-lactamase (VIM) [2]. These enzymes confer resistance to the carbapenem class of antibiotics, which are currently reserved for life-threatening or multidrug-resistant infections among the critically ill [3]. Carbapenemase-producing organisms (CPOs) have been reported around the world, with 13 out of 38 European countries reporting increased cases of carbapenemase-producing Enterobacteriaceae (CPE) in 2015, compared with only six out of 38 in 2013 [4].

Members of the KPC family of serine carbapenemases are reported to be the most prevalent worldwide, with only two states remaining in the USA that have not reported a case of KPC-producing CPE [5]. Italy and Greece have the highest rates of isolates producing KPC enzymes within Europe, reporting between 25% and 50% and >50% of invasive infections caused by CPE [6]. NDM-producing isolates are reported mostly across Asia, particularly India. In Europe inter-regional spread of NDM has been documented in Romania, Poland, and Denmark. Spain, Italy, and Hungary are the predominant European countries where VIM are reported. OXA-48-like β-lactamase producers are frequently isolated from patients within the UK and Mediterranean countries, with the first report documented in Turkey [6]. The Asia-Pacific countries, such as China, Taiwan, and Australia, are frequently associated with IMP produced by *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *E. coli* [7].

The European Antimicrobial Resistance Surveillance Network (EARS-Net) reported that, between 2014 and 2018, carbapenem resistance was most frequently encountered in *K. pneumoniae*, with some countries reporting resistance rates of >10%. However, carbapenem resistance in both *P. aeruginosa* and *Acinetobacter* spp. was reported to be much higher compared with *K. pneumoniae*, whereas infections caused by carbapenem-resistant *E. coli* were low at <1%. Countries reporting high levels of carbapenem resistance also reported the highest levels of extended-spectrum β-lactamases (ESBLs) and resistance to all other antimicrobial groups, including aminoglycosides [6]. However, this prevalence is thought to be underestimated, as not all clinical laboratories routinely screen or investigate for CPOs and current data typically comprise reports from outbreaks, reactive screening, inpatient surveillance and diagnostic specimens [6]. Therefore, the steady global increase and limited laboratory detection significantly hinders the infection control teams, doctors, and the scientists trying to care for patients carrying or infected with these organisms [8].

In London, there have been outbreaks with CPOs at various hospitals, including at Barts Health NHS Trust (BHT) [3,9–11]. BHT covers an area of East London encompassing ~2.5 million people, of which 5–36% of the local community are of Bangladeshi, Pakistani, or Indian origin [12]. This background automatically categorizes these patients as suspected carriers of carbapenem-resistant organisms (CROs) [13]. Other groups that national guidance advises should be considered as suspected cases are those previously testing positive for a CPE and/or those who have been inpatients in a hospital overseas, or in a UK hospital with reported cases of CPEs, within the last 12 months [13]. Nevertheless, our current approach to CPO screening at BHT is reactive. Therefore, it was important to determine which patients need to be investigated for CPOs based on the local prevalence, to prevent carriage becoming an infection and to prevent any subsequent outbreaks.

In the absence of any UK community studies on CPO prevalence, we sought to determine the CPO prevalence of our community population.

Methods

Patient samples

A total of 200 non-duplicate community stool samples received sequentially by the Microbiology Laboratory at BHT were included in this study. The sample size was determined using the equation described by Naing *et al.*, based on an expected proportion of 3.1% (point prevalence study at Royal London Hospital), a 95% confidence interval, and a precision level of 5% [14]. The calculated sample size was 73, which was further increased to 200 stool samples, to gain a more representative proportion of the BHT community CPOs.

Patient age, sex, ethnicity and foreign travel history were extracted from the laboratory information management system (LIMS), enabling the identification of potential risk factors for CPO carriage. All patient identifiable information was removed from the specimen and samples anonymized, along with the associated LIMS data.

CPO screening

A pea-sized portion of stool was emulsified in 3 mL nutrient broth (Oxoid, UK) and incubated aerobically, overnight, at 37°C. The broth was sub-cultured on to mSuperCARBA (E&O Laboratories Ltd, Bonnybridge, UK) selective medium and incubated aerobically for a further 18–24 h at 37°C. All colonies were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Germany), with a score >1.8 accepted for species-level identification. All identified Enterobacteriaceae, *Acinetobacter* spp., and *Pseudomonas* spp. underwent antibiotic susceptibility testing (AST) by disc diffusion against meropenem, ertapenem, fosfomycin, mecillinam, amikacin, temocillin, and piperacillin–tazobactam according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [15].

All antibiotics were supplied by Oxoid (Basingstoke, UK). Each isolate, regardless of the AST results, was tested for possession of the 'big five' carbapenemase genes, using a modified version of a published reverse transcription–polymerase chain reaction (RT-PCR) assay [16].

Ethics

This project was reviewed by the Joint Research Management Office at Barts Health Trust. The Integrated Research Application System (IRAS) application was submitted as a proportionate review, prior to full Health Research Authority approval being received (IRAS 219422).

Results

The study population ranged from one to 93 years of age, with a median age of 33 years. Females comprised 51.5% of participants and males 48.5%. The nationality of patients was extracted from the LIMS: 9% were British, 6.5% Asian, 1% Caribbean, 1% African, and 5.5% listed as 'other'; the remaining 77% had no nationality documented. No travel history was detailed for 15.5%, and a further 60.5% documented that they had not travelled abroad in the last 12 months. Of the remaining 24%, countries visited included: Turkey (3), Morocco (4), India (5), Bangladesh (8), Bali (1), Brazil (1), Caribbean (2), Egypt (1), Estonia (1), France (1), Germany (1), Gran Canaria (1), Granada (1), Greece (1), Hungary (1), Lithuania (1), Mexico (1), Nepal (2), Nigeria (1), Philippines (1), Portugal (1), Romania (1), Spain (2), Sri Lanka (2), Thailand (1), and Vietnam (1). A total of 32% of study participants have travelled to countries deemed as high risk for CPO prevalence (Bangladesh, Greece, India, North Africa, South East Asia, South/Central America, and Turkey) within the last year [13].

Twenty out of 200 (10%) isolates were culture-positive, and subsequently tested for the presence of a carbapenemase by RT-PCR. Eleven of the 20 (55%) isolates demonstrated reduced susceptibility (resistant or intermediate) to ertapenem and to piperacillin–tazobactam, four (20%) to meropenem, and 12 (60%) to temocillin (Supplementary Table I).

From the 20 isolates tested by RT-PCR, only one patient tested positive for a CPO: an NDM-producing *P. aeruginosa*. This was a 32-year-old male of uncertain ethnic origin who had travelled to the Caribbean. The strain was resistant to ertapenem, piperacillin–tazobactam, mecillinam, and temocillin, but tested susceptible to meropenem (Supplementary Table I).

Discussion

The steady global increase in CPOs and the need for improved laboratory detection methods are some of the current challenges facing infection control teams, clinicians, and the scientists identifying these organisms. Public Health England (PHE) has reported a rapid increase in the number of infections involving CPOs in the UK since the early 2000s and now strongly recommends screening for them using either rectal swabs or stool samples [2]. The exact prevalence of CPOs worldwide is unknown; however, increased screening and local awareness is providing further insight into the current global problem [5,6].

Public Health England has produced guidance to assist with the management of CPEs and prevent further spread. According to their acute trust toolkit, on admission to hospital of any suspected or confirmed CPO-positive patient, (1) the patient should be isolated, (2) strict infection control procedures should be enforced, (3) contacts should be identified and screened, (4) CPO status should be recorded on the patient's notes, and (5) a discharge summary to their general practitioner detailing their CPO status should be provided. Patients should be notified of their status and, importantly, good communication should be used throughout with all healthcare staff and facilities involved with patient care. The toolkit also stipulates a suspected carrier as a patient who has previously tested positive for CPE or, within the last 12 months, has either been an inpatient in a hospital abroad, or in a UK hospital with reported cases of CPEs [13]. Following these guidelines, 32% of our study participants could be considered high risk, as they have listed previous foreign travel to a high CPO prevalence country and should, therefore, be screened for a CPO upon admission to hospital. However, our study demonstrates that foreign travel may not be the most significant risk factor contributing to the increased spread of CPOs worldwide, and additional factors — such as pre-existing medical conditions, previous hospitalization, and stay on an intensive care unit — may play a larger role in their distribution. However, as this study anonymized patient samples, this additional demographic information cannot be determined.

Of the 20 isolates tested by RT-PCR, a significant proportion (11 out of 20, 55%), were resistant or intermediate to at least one carbapenem by AST. National PHE guidelines suggest meropenem as the antibiotic of choice for CPE screening, alongside resistance to piperacillin–tazobactam for *Pseudomonas* and *Acinetobacter* spp. [2]. However, strictly following these guidelines would have led to just two out of 20 (10%) of these isolates undergoing further investigation for carbapenemase production, ignoring the remaining (18 out of 20) isolates, including the NDM-producing *Pseudomonas aeruginosa*. These AST results demonstrate that carbapenem resistance alone cannot be solely used as a tool for detecting CPOs, as reported in previous studies, where OXA-48-producing isolates, especially, demonstrate varying sensitivity patterns to carbapenems [17].

At BHT, a laboratory two-pronged algorithm identifies potential CPEs depending on whether they are resistant to both ertapenem and meropenem or resistant to piperacillin–tazobactam and temocillin (OXA-48 only) [11]. For *Acinetobacter* and *Pseudomonas* spp., resistance to meropenem, ertapenem, piperacillin–tazobactam, ceftazidime, ciprofloxacin, and gentamicin must be observed, due to the possession of intrinsic mechanisms [18]. ESBL production, AmpC β-lactamase production, upregulation of efflux pumps, and porin loss can all give the appearance of CPO but actually be CROs, with an alternative resistance mechanism other than carbapenemase production [19]. In this study, just two out of 20 isolates would have been referred to the reference laboratory for further investigation according to our algorithm. Both of these isolates were resistant strains (Supplementary Table I) and there is currently no routine in-house methodology to differentiate between CRO and CPO. Referral to confirm the mechanism of resistance would be necessary, and a review of the BHT screening algorithm is not recommended based on these findings.

Alternative algorithms have been devised which could overcome these issues and reduce the volume of isolates being referred to the reference laboratory. For example, breakpoints for ticarcillin–clavulanate and cefepime have demonstrated 100% sensitivity and 68.1% specificity for the detection of carbapenemases, which increased further by the addition of either imipenem (76.1%) or meropenem (78.8%) [20]. Furthermore, a surveillance programme in Québec reported varying carbapenem resistance rates in their CPEs: to ertapenem (99%), imipenem (95%), and meropenem (87%) [21]. Although ertapenem is reported to have the highest sensitivity when detecting CPOs, EUCAST recommend the use of meropenem as an indicator for carbapenem resistance, due to the poor specificity of ertapenem [2]. By using both ertapenem and meropenem resistance, as done here, a good compromise is

achieved between the higher sensitivity of ertapenem and the increased specificity of meropenem.

The decision to test all culture isolates by PCR was done to allow complete identification of all potential CPOs and not restrict data based on the locally derived algorithm. It also enabled our algorithm to be interrogated and to determine whether it was still fit for purpose. The NDM-producing *P. aeruginosa* detected in this study did not fit our algorithm and, therefore, would have gone undetected, as it was a relatively susceptible strain for a CPO. The meropenem zone size of this NDM producer was large, according to EUCAST guidelines, suggesting that monotherapy with a carbapenem could achieve clinical cure in this case [22]. This raises the question as to whether CPOs that appear susceptible to carbapenems could be considered low risk, as treatment with a carbapenem may still be successful.

UK guidelines state that patients from high-risk geographical locations, including Bangladesh, India, South East Asia, Italy, Turkey, Greece, and Israel are at risk of CPO carriage and infection [13]. In this study, 19% of our study population originated from, or had visited, these high-risk countries within the last 12 months. However, just one CPO was detected in our community study, resulting in a local prevalence of 0.5%. Furthermore, the CPO-positive patient had travelled to the Caribbean and not one of the high-risk countries listed by the PHE toolkit. A surveillance study conducted in Belgium also found a high CPE burden in countries not deemed high risk, with travel to the African continent (45%), as well as Asia (28%) and Europe (28%) reported [23]. This suggests that with the increased spread of CPOs observed worldwide, the countries considered high risk need to be revised.

A limitation of this study was that the stool samples were from patients presenting with a suspected gastrointestinal infection. This could lead to an imbalance of bacteria and, therefore, not be truly representative of the normal gut microbiome of that individual. When bacteria are causing a gastrointestinal infection they are frequently present in large numbers and this imbalance of bacteria could lead to the underdetection of CPOs. To overcome this, future studies should include otherwise healthy patients for a more representative sample of the community population. UK guidelines also list previous hospitalization as a risk factor for CPO carriage, but due to the anonymization process, these data were not collected.

The inoculation of stool using nutrient broth is a non-selective enrichment for the recovery of CPOs and could lead to overgrowth of other non-resistant bacteria; this has the potential to lead to false negatives, which could be overcome by the addition of a carbapenem disc, therefore being selective for carbapenem-resistant bacteria. This was not performed in our study, as, during an initial evaluation, isolates testing positive for a carbapenemase enzyme by RT-PCR were found to be sensitive by disc diffusion to ertapenem and meropenem.

In conclusion, it was reassuring that the community CPO prevalence was $<1\%$, especially given the large number of 'high-risk' patients served by our trust. Importantly, this study indicates that risk could potentially be determined by more than just travel to or birth in a particular country, and that the list of high-risk countries included in UK guidelines may need updating. Furthermore, this study highlights the importance of determining the local CPO prevalence prior to establishing the CPO screening/detection protocol in a diagnostic laboratory.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2019.04.014.

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