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An outbreak of double carbapenemase-producing *Klebsiella pneumoniae*, harbouring NDM-5 and OXA-48 genes, at a tertiary hospital in Canberra, Australia

Malizgani Mhango, Frances Sheehan, Alexandra Marmor, Callum Thirkell, Karina Kennedy

# Abstract

In July 2023, a carbapenemase-producing *Klebsiella pneumoniae* (CPKP) with New Delhi metallo-beta-lactamase (NDM-5) and oxacillinase (OXA-48) carbapenemase genes was detected in the urine sample of a patient. A similar CPKP organism had previously been isolated from a surveillance rectal swab of an admitted patient, prompting an outbreak investigation. A confirmed case was defined as any suspected case in which a species of Enterobacterales was isolated from a clinical or surveillance specimen (infection or colonisation) exhibiting an NDM-5 or OXA-48 CPE gene or both, irrespective of phenotypic susceptibility. A descriptive epidemiological investigation was conducted to describe the investigation, infection prevention and control responses, and public health intervention carried out. Three confirmed cases of CPKP were identified, including the index case; 62 contacts were identified, of which 13 contacts were screened. CPKP transmission occurred between two patients on contact transmission-based precautions in separate single ensuite rooms. Despite being in the same ward, the patients did not share medical teams but shared nursing teams and ancillary staff.

This study emphasises the importance of strict adherence to infection prevention and control practices and contact transmission-based precautions for patients admitted with carbapenemase-producing Enterobacterales.

Keywords: carbapenemase-producing Enterobacterales; dual carbapenemase-producing organisms; infection control; hospital-acquired infections; antimicrobial resistance; multidrug resistance; whole-genome sequencing; phylogenomic analysis

# Introduction

Carbapenem antibiotics are commonly used to treat severe infections caused by multidrug-resistant (MDR) Gram-negative organisms;1 their increased usage has led to the emergence of carbapenemase-producing Enterobacterales (CPE) and carbapenem-resistant Enterobacteriaceae (CRE).2,3 Owing to the limited therapeutic options to treat CPE globally, infections with these microorganisms lead to high mortality rates, particularly in immunocompromised patients.4,5 Additionally, containment of CPE involves high costs due to the additional length of hospitalisation, enhanced screening, staff time spent on contact tracing and outbreak management, contact transmission-based precautions (isolation), bed closures, and sometimes infrastructural improvements.6

CPE is considered a significant public health concern in Australia. Data submitted to the National Alert System for Critical Antimicrobial Resistances (CARAlert) showed a 37.4% increase in CPE in 2022 compared to 2021; CARAlert data showed that CPE was the most critical reported antimicrobial resistance in 2021 and 2022.7 In Australia, CPE are usually detected in patients who have received healthcare services overseas, except for imipenemase-4 (IMP-4), which is now considered endemic in some states.8–10 The annual incidence of CPE in the Australian Capital Territory (ACT) ranged from 9 in 2019 to 13 in 2022.7

Here, we describe the investigation of an outbreak of carbapenemase-producing *Klebsiella pneumoniae* (CPKP), exhibiting double carriage of the NDM-5 and OXA-48 CPE genes, at a teaching tertiary hospital (The Hospital) in Australia, including infection prevention and control measures enacted as part of the outbreak response.

# Methods

## Setting

The Hospital is a public tertiary teaching hospital in Canberra, Australia. It is the largest hospital in the region, with approximately 672 beds, catering to a population of approximately 459,000 people. The hospital also serves New South Wales’s southeastern region.

## Definitions

Guided by the Australian Commission for Safety and Quality in Healthcare (ACSQHC) recommendations,11 we developed the following definitions for cases and contacts:

Suspected case: Any person admitted to or who underwent a procedure at The Hospital from May 2023, from which Enterobacterales with a meropenem minimum inhibitory concentration (MIC) > 0.125 mg/L was isolated from a clinical or surveillance specimen (infection or colonisation).

Confirmed case: As per the ACSQHC recommendations,11 a confirmed case was any suspected case in which a species of Enterobacterales was isolated from a clinical or surveillance specimen (infection or colonisation) exhibiting an NDM-5 or OXA-48 CPE gene or both, irrespective of phenotypic susceptibility.

Contact: Any person who may have shared a room, bathroom, or toilet facility with a confirmed case for greater than 24 hours.11

# Description of the outbreak

On 14 May 2023, a surveillance swab for a patient (Case 1) yielded a CPKP, and the patient was admitted to a single, separate ensuite room (Room 10). On 20 May 2023, another patient (Case 2) with vancomycin-resistant Enterococci (VRE) was admitted to Room 9, an ensuite room next to Room 10. The two patients were in adjacent rooms for four days; Case 1 was moved from Room 10 on 24 May. On 12 June, Case 3 was admitted into Room 5; a day later, Case 2 was moved into Room 4, sharing a bathroom and toilet with Room 5. On 28 June, Case 2 was moved into Room 10. On 7 July 2023, a urine culture from Case 2 yielded CPKP exhibiting the same phenotypic antimicrobial resistant patterns as CPKP isolated from a surveillance swab from Case 1 (Figure 1). Genotypic testing confirmed that the isolates belonged to the same genus and species carrying the same two CPE genes, and an outbreak was declared per the ACSQHC recommendations for CPE control.11 The Hospital’s infection prevention and control unit (IPCU) investigated the outbreak.

# Investigations

## Records review

The Hospital’s digital health record (DHR) system was reviewed to identify medical device use by patients, ward admission dates, bed admission dates, and contacts.

## Contact tracing

Contacts who were still inpatients were notified of their exposure to CPE, and verbal consent was sought to collect three surveillance rectal swabs 24 hours apart. For contacts who had been discharged, we identified upcoming medical appointments within the ACT Health Services, contacted appropriate teams to request swabs, and documented in their records to have surveillance swabs taken upon future contact with the Canberra Health Services.

## Environmental investigation

Environmental samples were collected from rooms occupied by confirmed Cases 1 and 2, and their bathrooms. Swabs were also collected from bathrooms and toilets shared by Cases 2 and 3 when they were in rooms 4 and 5, respectively. Using the wet sampling method, swabs were taken from inside toilet bowls, shower drain holes, sink holes in the bathroom basins, hand hygiene basins in the rooms, and hand hygiene basins outside the rooms. No other parts of the rooms were sampled.

## Laboratory investigation

Clinical, environmental and surveillance samples were cultured, and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltronics, Bremen, Germany) was used to identify Enterobacterales species in the samples. Susceptibility testing was performed using Vitek 2 (bioMérieux, Marcy l’Etoile, France). Isolates with a meropenem MIC greater than 0.125 mg/L were screened for carbapenemase production using either the double disc diffusion test, Carba NP, or carbapenem inactivation method. The genotypes of the isolates with a positive carbapenemase screening test and those with a meropenem MIC > 4 mg/L were characterised using a commercial assay (Cepheid Xpert® Carba-R, Sunnyvale, USA).

Whole-genome sequencing (WGS), multilocus sequence typing (MLST), and phylogenetic analyses were conducted at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) in Melbourne, Victoria. The maximum likelihood method, based on the core genome alignment of all three isolates, was used to determine pairwise single nucleotide polymorphism (SNP) distances, to conduct cluster analysis, and to develop phylogenetic trees.

# Ethical approval

The study was covered under the Australian National University’s Health Research Ethics waiver of consent for using data in research for the Master of Philosophy (Applied Epidemiology) project approval for 2023 Reference 2017/909 and as a public health response under the *ACT Public Health Act 1997*.

# Results

## Overview of the outbreak

We conducted an outbreak investigation between July and August 2023. Figure 1 shows the timeline of the significant outbreak events.

Contact tracing detected an additional case (Case 3) who shared a bathroom and toilet were shared with Case 2. The characteristics of the three cases are listed in Table 1.

Cases 1 and 2 were in single ensuite separate rooms next to each other under different medical specialties, with shared nursing and allied health services, and no shared medical devices. These two patients were also put under contact transmission-based precautions in single rooms for different reasons. Case 1 was found to have CPKP from a surveillance swab taken on the day of admission, and Case 2 was colonised with VRE before admission. Assessment of the structural outlay revealed that the two rooms housing Cases 1 and 2 shared a wash basin outside the room, where staff washed their hands after contact with the patients. Additionally, both rooms shared space for donning and doffing personal protective equipment (PPE). One contact of Case 2, from whom surveillance swabs were taken after sharing a room, bathroom, and toilet for 16 days, had all three swabs test negative for CPE.

Sixty-two contacts were identified, 13 of whom were screened for CPE. The remaining patients were either discharged and could not be contacted for screening or died.

Figure 1: Timeline of significant events in the outbreak investigation

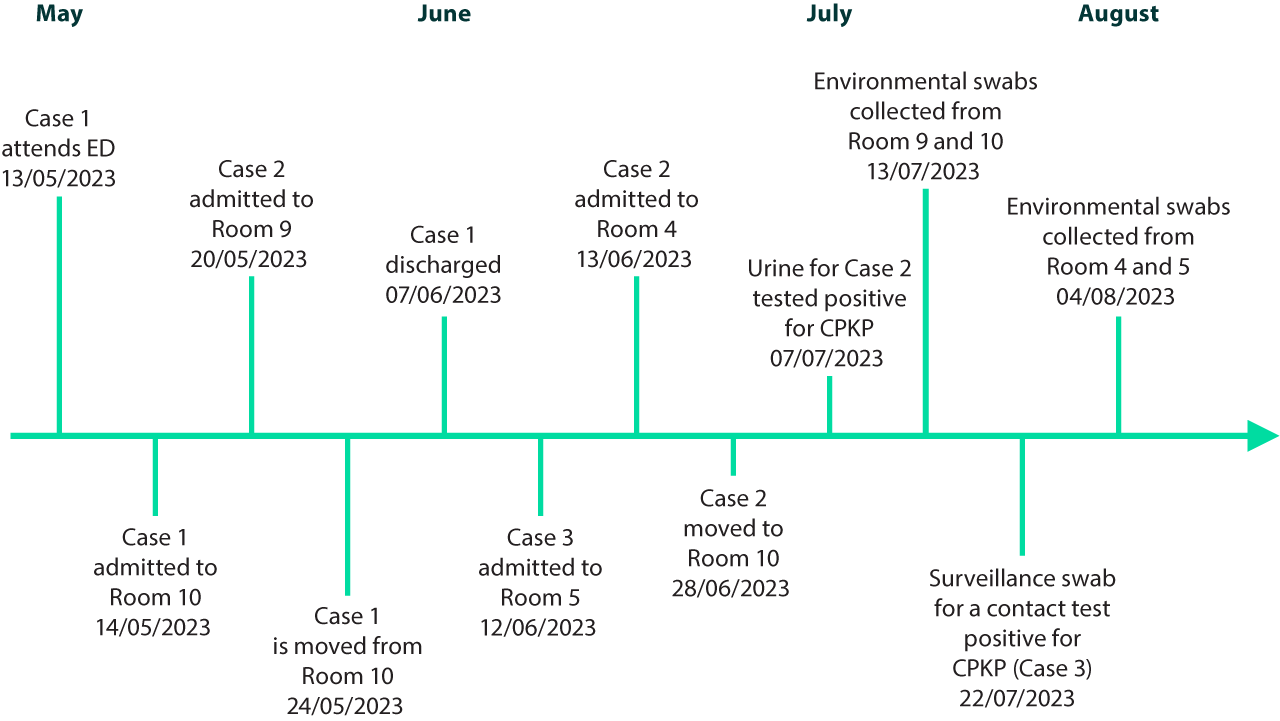


Table 1: Description and characteristics of cases

| Demographics and clinical characteristics | Case 1 | Case 2 | Case 3 |
| --- | --- | --- | --- |
| Age group (years) | > 65 years | > 65 years | 45–55 years |
| Vancomycin-resistant Enterococci (VRE) status | Negative | VRE VAN B | VRE VAN B |
| Total hospitalisation days (at the time of investigation) | 25 | 92 | 72 |
| Comorbiditiesa | Multiple comorbidities | Multiple comorbidities | Multiple comorbidities |
| Medical procedures in the last 12 months | Liver biopsy in Africa | None recorded | Orthopaedic surgery in Australia |
| Other risk factors for acquisition | Recent procedures in Africa |  |  |
| Discharge status | Discharged | Deceased (cause not specified) | Deceased (cause not specified) |
| Reason to testing | Routine surveillance (admission screen) | Testing after a fever | Contact tracing |
| Specimen type | Rectal swab | Midstream urine | Rectal swab |
| Number of contacts identified | 6 | 32 | 26 |
| Number of contacts screened | 2 | 6 | 4 |

a Comorbidities included liver adenocarcinoma, COVID-19, chronic cardiac failure, chronic kidney disease, type 2 diabetes mellitus, hypertension and chronic obstructive pulmonary disease.

## Laboratory and environmental investigation

The *K. pneumonia*e implicated in this outbreak was resistant to penicillins, quinolones, aminoglycosides, cephalosporins, and carbapenems, with high resistance to meropenem (MIC > 32 mg/L). Isolates from all three cases exhibited a dual carriage of the NDM-5 and OXA-48 genes, with a ribosomal methyl transferase gene (*arma*). MDU PHL performed MLST and found that three isolates were *K. pneumoniae* ST11.

Twelve environmental swabs were collected from sinks, bathrooms, and toilets. Of these, eight were collected from hand hygiene and bathroom hardware associated with Rooms 9 and 10. Four were collected from the sink and bathroom in front of Rooms 4 and 5. Of the 12 swabs, one collected from the sink in the Room 10 bathroom was positive for the *Enterobacter cloacae* complex harbouring IMP-4. The four environmental swabs collected from the bathroom shared by Rooms 4 and 5 were negative for CPE and other MROs.

## Whole genome sequencing

Phylogenetic analysis was conducted on three CPKP isolates harbouring the NDM-5 and OXA-48 genes, and publicly available *Klebsiella pneumoniae* ST11 isolates from MDU PHL (n = 24). The isolates from the three cases had pairwise SNP distances ranging from 0 to 4 SNPs. The next most closely related isolate was greater than 100 SNPS from this cluster.

## Infection control measures

In the weeks following outbreak detection, the IPCU implemented several measures. The IPCU team informed medical staff, nurses, and cleaning personnel about the outbreak and about the outbreak response measures being undertaken to prevent further transmission. The team conducted educational sessions with nursing and ancillary staff on CPE in the wards where CPE-positive patients were admitted. The education focused on the importance of strict infection prevention and control measures, including correct hand hygiene and contact transmission-based precautions.

# Discussion

Here, we describe an investigation of a small outbreak of CPKP at a tertiary hospital in Canberra, Australia. The findings from this investigation revealed the transmission of CPKP, even though the cases were under contact transmission-based precautions in separate ensuite rooms. This highlights the need for careful infection prevention and control practices when dealing with CPE. In addition, the organism implicated in this outbreak harboured two CPE genes, NDM-5 and OXA-48.

Case 2 spent four days in a room adjacent to the room occupied by Case 1, sharing nursing and ancillary staff. Thirty-four days later, Case 2 was transferred to a room previously occupied by Case 1. This gave rise to the initial hypothesis that Case 2 was infected through contact with an already colonised room, which prompted an environmental investigation. Because sinks, bathrooms, and drains have been previously implicated in CPE outbreaks in hospital settings,10,12,13 it is notable that the limited environmental investigation in this study failed to link the environment as a mode of CPE transmission.10,14 However, it should be noted that environmental sampling has low sensitivity, and several MROs (including CPEs) may survive in biofilms that are difficult to culture.14 Environmental contaminants or chemicals can affect the ability of environmental organisms to produce successful cultures. For these reasons, negative environmental swab cultures should not provide a sense of security. Therefore, the environment must be treated as if it is contaminated, and cleaning and disinfection should be performed appropriately, as was done in this study.

WGS revealed that the three CPKP isolates from these cases were highly related, consistent with direct transmission among patients or with acquisition from a common source (the environment or a person). Such transmission appears feasible in this instance. Furthermore, all three confirmed cases in this study had a double carriage of CPE genes (NDM-5 and OXA-48), a recent phenomenon that is becoming common in Australia. Multiple carbapenemase genes in Gram-negative bacteria in hospitalised patients are problematic,5,15 because they put newer antibiotics at risk and threaten the usefulness of these antibiotics in treating patients.16

The patients in this outbreak had risk factors associated with CPE acquisition in Australia9 which included chronic conditions, history of receiving medical treatment overseas, and lengthy hospital admission history, which has been found to predispose patients to CPE colonisation or infection.17,18 This is also supported by the studies of Segagni et al. and Mariappan et al, which revealed higher odds of CPE acquisition for patients admitted to a hospital for more than 20 days than for those admitted for a shorter time.19,20 Chronic conditions such as renal failure, cardiac failure, kidney disease, type 2 diabetes mellitus, renal cell adenocarcinoma, and osteomyelitis have all been implicated in patients investigated in CPE outbreaks;10,12 all three cases had one or more of these chronic conditions.9,21

The cases in this investigation were being treated under different medical specialties; we could not identify any shared equipment among the three cases. Despite adherence to infection prevention and control (IPC) protocols for screening and transmission-based contact precautions, Case 2 (who had been initially screened for MROs on admission) acquired CPE during their hospital stay. During the investigations, it was found that Cases 1 and 2 never shared a room or bathroom but were housed in separate rooms with ensuites meant for isolation and contact transmission-based precautions. Thus, a possibility of transmission through contaminated patient-facing and non-patient-facing healthcare workers could be possible in this study. The donning and doffing of PPE were performed in a single confined area, which could have possibly led to contamination, which concomitantly contaminated the treating teams and nursing staff involved in both Cases 1 and 2. The IPC guidelines require separate areas for donning and doffing PPE.22 It is important to note that for all single-ensuite rooms used for isolation and contact transmission-based precautions, the design must prioritise infection prevention, including segregation and containment. This was revealed in a review by Budhram et al., which supported the need for a proper structural outlay to minimise contact between patients and staff with contaminated material.12

Evidence shows that the risk of being admitted to the same room as a previously known case with an MRO increases the risk of CPE infection.18 Case 2 was moved to the room formerly occupied by Case 1 and stayed there for 20 days, which is a potentially significant time for CPE acquisition in colonised rooms,10 unless there was evidence of shared equipment (which we failed to establish during the review of records) or of environmental colonisation (which we could not establish). Contrary to the notion that the greater the contact, the higher the chance of acquiring CPE, we found one patient who had spent 16 days sharing a bathroom and nursing staff with Case 2, but all their three screening swabs were negative for CPE. This reinforces the idea that, in some instances, not only one risk factor is sufficient for transmitting CPE.

In this outbreak, the NDM-5 gene is a concerning development in carbapenem resistance and can result in severe consequences and restricted treatment options.16 This is particularly important because *K. pneumoniae* is implicated as an entry point for antimicrobial resistance in the Enterobacteriaceae family, and the presence of NDM-5 amplifies the potential for transmission.23 Thus, CPKP, as in this study, must be managed and controlled to limit further spread, as it might transmit resistance to other Enterobacteriaceae species existing in the hospital environment. Recommendations to limit CPE transmission in healthcare facilities are based on early detection of asymptomatic carriers, implementation of contact transmission-based precautions, and isolation in a single room with a dedicated bathroom. Strategies that combine various interventions are employed in hospitals based on risk assessment and available resources. For example, Case 1 was identified through screening upon admission, and Case 3 was identified during contact tracing of a positive CPE.

The findings of this outbreak investigation underscore the importance of screening for MROs, including CPE, in patients with risk factors upon admission and following the necessary IPC precautions. Based on the WGS phylogenomic analysis and SNP results, on the presence of the same CPE genes, and on antimicrobial susceptibility testing (AST) patterns for Cases 2 and 3 which were similar to Case 1, we suspect that Cases 2 and 3 likely acquired their infections from Case 1. Epidemiological and environmental investigations suggest that transmission occurred from Case 1 to Case 2, as gastrointestinal colonisation was confirmed using a surveillance swab collected from Case 1 upon admission. Case 2 tested positive for CPE 55 days later. Case 3 shared a bathroom and toilet with Case 2, and may have shared nursing staff, which may have been the mode of CPE transmission between the two cases.

This investigation was limited because healthcare workers were not screened for CPE to ascertain whether there was transmission from Case 1; our case definition was limited to only patients. Second, we could not obtain rectal swabs from all contacts because most were discharged before the outbreak was detected. Some cases were probably undetected, leading to an underestimation of the actual number of cases, as well as the lack of swabbing of the broader environment, such as trolleys, bedside commodes, doorknobs, and computer workstations, which may have limited the isolation of CPE.

# Conclusion

In conclusion, using traditional epidemiological methods and IPC procedures, we investigated the local transmission of CPKP harbouring two CPE genes (NDM-5 and OXA-48). Although no confirmed transmission mode was identified, the study revealed the importance of consistent screening for MROs in patients with risk factors upon admission; following this investigation, all unscreened contacts had notes in their records for the need to have surveillance swabs taken when they accessed ACT health services. We highlight the importance of WGS in supplementing epidemiological, laboratory, infection, and prevention control processes in outbreak investigations of healthcare-acquired infections. This study also emphasises the importance of strict adherence to infection prevention and control practices and to contact transmission-based precautions for patients admitted with MROs organisms.

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