Interspecies Transfer of \( \text{bla}_\text{IMP-4} \) in a Patient with Prolonged Colonization by IMP-4-Producing Enterobacteriaceae

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A patient was colonized by IMP-4-producing Enterobacter cloacae and Escherichia coli strains for 7 months. IMP-4-producing \( E. \) cloacae strains were first and last isolated at day 33 and at 8 months after admission, respectively. IMP-4-producing \( E. \) coli strains were first and last isolated at days 88 and 181 after admission, respectively. The \( E. \) cloacae and \( E. \) coli isolates shared identical genetic features in terms of \( \text{bla}_\text{IMP-4}, \text{bla}_\text{TEM-1}, \text{qnrB2}, \text{aacA4}, \) HI2 plasmids, and ISCR1. This study shows the first prolonged colonization with in vivo interspecies transfer of \( \text{bla}_\text{IMP-4} \).

CASE REPORT

A 52-year-old Australian female patient suffered 60% burns with inhalational injury in May 2013. The patient underwent multiple debridements and allografts with several episodes of sepsis. She was hospitalized continuously for 8 months. Multiple susceptible organisms, including Enterococcus faecalis, Serratia marcescens, Escherichia coli, and Pseudomonas aeruginosa, were isolated from the patient. Additionally, Stenotrophomonas maltophilia strains were isolated from this patient. (Consent to use all isolates for molecular analysis was provided by the patient.) The \( S. \) maltophilia strains were resistant to \( \beta \)-lactam antibiotics, including carbapenems; however, they remained susceptible to trimethoprim-sulfamethoxazole. The patient received various antibiotics, including amikacin, cefepime, ciprofloxacin, co-trimoxazole, gentamicin, meropenem, piperacillin-tazobactam, and vancomycin as prophylaxis at the time of debridement as well as for the treatment of sepsis.

At 33 days after hospitalization, a meropenem-resistant \( E. \) cloacae strain was isolated from the tracheal aspirate. The meropenem resistance of \( E. \) cloacae was determined with the Vitek 2 system (bioMérieux, Victoria, Australia). Meropenem-resistant \( E. \) cloacae strains were isolated on 10 occasions during a 7-month hospitalization period from tracheal aspirates, rectal swabs, and wound specimens, four of which were available for analysis. Simultaneous meropenem-resistant \( E. \) cloacae isolates were obtained from rectal swabs and wounds during sepsis episodes at the fourth month of hospitalization.

On the 88th day of hospitalization, a meropenem-resistant \( E. \) coli strain was isolated from urine. Meropenem-resistant \( E. \) coli strains were isolated on nine consecutive occasions from rectal swabs and urine from day 88 until day 181 of hospitalization. Four \( E. \) coli isolates from 1 urine specimen and 3 rectal swabs were available for analysis. A total of 30 meropenem-resistant \( P. \) aeruginosa strains were isolated from different body sites, including blood, rectal swabs, wound swabs, and tracheal aspirates. Seven \( P. \) aeruginosa strains were included for analysis. The first meropenem-resistant \( P. \) aeruginosa strain was isolated on day 28 after admission. \( P. \) aeruginosa and \( C. \) albicans were isolated from blood during sepsis episodes in June and August 2013.

Antibiotic susceptibility testing of all \( E. \) cloacae, \( E. \) coli, and \( P. \) aeruginosa strains by the Vitek 2 system (bioMérieux, Victoria, Australia) showed resistance to ertapenem, meropenem, imipenem, ceftazidime, cefotaxime, cefoxitin, cefepime, pipercillin-tazobactam, and ciprofloxacin according to the EUCAST standard (1). In addition, the \( E. \) cloacae and \( E. \) coli strains were also resistant to gentamicin and tobramycin. They remained susceptible to amikacin. The \( E. \) cloacae and \( E. \) coli strains showed carbapenemase production on a modified Hodge test using carbapenem disks and by the Carba NP test (2, 3). The MICs of meropenem tested by Etest (bioMérieux) ranged from 2 to 8 mg/liter for \( E. \) cloacae and from 4 to 8 mg/liter for \( E. \) coli.

Four \( E. \) cloacae, four \( E. \) coli, and seven \( P. \) aeruginosa strains were analyzed for clonal relatedness using appropriate kits of the Diversilab repetitive-sequence-based PCR (rep-PCR) system (bioMérieux) following the manufacturer’s instructions (4). The \( E. \) cloacae strains were clonally related, showing >97% similarities (Fig. 1A). All \( E. \) coli strains were also clonally related, with >96% similarities (Fig. 1B). All \( P. \) aeruginosa strains from various specimens were identical by Diversilab rep-PCR (data not shown).

Phenotypic and genotypic characterization was used to determine the antibiotic resistance mechanisms. PCR and sequencing were performed to determine the genes encoding resistance to \( \beta \)-lactams and aminoglycosides (5–7). Plasmid-mediated quinolone resistance (PMQR) was determined as previously described (8). The plasmid replicon and the insertion element associated with the carbapenemase-encoding gene were also identified by previously described methods (9, 10). PCR and sequencing determined the presence of \( \text{bla}_{\text{IMP-4}}, \text{bla}_{\text{TEM-1}}, \text{qnrB2}, \text{aacA4}, \) and ISCR1 in all meropenem-resistant \( E. \) cloacae and \( E. \) coli strains isolated from this patient. None of the \( P. \) aeruginosa strains was positive for \( \text{bla}_{\text{IMP-4}} \). HI2 plasmids were detected in \( E. \) cloacae and \( E. \) coli. Plasmid multilocus sequencing was performed for both HI2 plasmids using the scheme from http://pubmlst.org/plasmid/. The sequence type of both HI2 plasmids was ST1. The sequence results using primers to target the entire integron class 1 showed that the
blaIMP-4 gene and aacA4 (aminoglycoside resistance gene) were located inside the class 1 integron (11). The composition of the sequence inside this class 1 integron was 5′-intI1-blaIMP-4-aacA4-3′. This blaIMP-4 genetic region in our isolates was similar to the previously reported blaIMP-4 region from Taiwan (12). Conjugation experiments were performed using the IMP-4-producing E. cloacae isolate (EclI) and E. coli isolate (Ec1), the first two IMP-4-producing Enterobacteriaceae isolates from this patient. The blaIMP-4-harboring H12 plasmids were transferred into an E. coli K-12 strain by liquid mating and screening on MacConkey agar supplemented with 150 μg/ml ampicillin. The blaTEM-1, aacA4, and qnrB2 genes were located in the blaIMP-4-harboring H12 plasmids. The identical key genetic features of IMP-4-producing E. cloacae and E. coli strains showed that the in vivo transfer of the blaIMP-4-carrying plasmid had occurred from E. cloacae to E. coli. This horizontal plasmid transfer was potentially induced by prolonged antibiotic treatment. The MICs of meropenem and ciprofloxacin of the E. coli K-12 strains acquiring blaIMP-4-harboring plasmids from the two donors were both 4 and 0.5 mg/liter, respectively. The initial MICs to meropenem and ciprofloxacin of the E. coli K-12 strain prior to the conjugation experiment were 0.02 and 0.004 mg/liter, respectively. The increase in the meropenem and ciprofloxacin MICs of the transconjugants was due to the acquisition of plasmids harboring blaIMP-4 and qnrB2.

The sequence type of IMP-4-producing E. coli was determined using the scheme from http://mlst.warwick.ac.uk/mlst/dbs/Ecoli: the ST was ST744. The IMP-4-producing E. coli belonged to phylogenetic group A, the commensal group of E. coli (13). This ST744 was also identified as phylogenetic group A (14). To confirm whether the patient was still colonized with the IMP-4-producing Enterobacteriaceae, a rectal swab and a fecal specimen were collected in the 8th month after admission (day 238 of hospitalization), prior to patient discharge. An IMP-4-producing E. cloacae strain was isolated from feces. No IMP-4-producing E. coli strain was isolated from the final rectal swab and fecal specimen. The IMP-4-producing E. cloacae strain was isolated from feces (Ec4) was identical to other previous E. cloacae isolates from this patient, as determined by Diversilab rep-PCR (Fig. 1A). The patient was still colonized in the gastrointestinal tract with IMP-4-producing E. cloacae, but at low numbers, as evidenced by the fact that the organism was not detected by rectal swab.

IMP-producing Enterobacteriaceae are the most commonly reported carbapenemase-producing Enterobacteriaceae (CPE) in Australia (15–17). The emergence of IMP-4-producing Enterobacteriaceae in both sporadic and outbreak-like situations has been reported in Australia over the past few years (15–17). Outbreak-like situations caused by IMP-4 producers have been reported due to contaminated equipment or environment (18). The mechanism of spread of IMP-producing Enterobacteriaceae in sporadic cases has not been elucidated. Espedido and colleagues had shown in vitro transfer of plasmids harboring blaIMP-4 from various strains of Enterobacteriaceae into laboratory strains of E. coli (15). We report an interspecies transfer of the blaIMP-4 plasmid.
from *E. cloacae* into *E. coli* within a patient in Australia. In total, this patient had colonization by two species of IMP-4 producers for 7 months.

Most of the reported cases or outbreaks by IMP-producing *Enterobacteriaceae* in hospitals in Asia were by *Klebsiella pneumoniae* and *Enterobacter cloacae* in China (19, 20). In China and Taiwan, IMP producers were initially more prevalent than other types of CPE. Recently KPC-producing *K. pneumoniae* have been reported to be more prevalent and to be causing outbreaks in these two countries (19, 21).

Following the identification of IMP-producing *Enterobacteriaceae* in this patient, rigorous screening for CPE was performed on other patients within the same ward and hospital environment. No CPE was found from other patients or the hospital environment. Therefore, the initial source of IMP producers in this patient remained unknown. Strict infection control measures have been consistently adhered to in this hospital. Examples of these preventive measures include use of isolation rooms for burn patients, thorough regular cleaning of the burn unit, and strict cleaning of the bathroom after each patient. This case illustrates further emergence of IMP-4-producing *E. cloacae* and the potential spread of IMP-4 to *E. coli*.

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**REFERENCES**


