



Case Report of *Enterobacter cloacae* Producing IMP-8 Carbapenemase Isolated from Secretions of Burn Patients and Diabetes Patients with Diabetic Foot

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Abstract

Introduction: With the widespread use of cephalosporins, *Enterobacter cloacae* has become an increasingly important pathogen of nosocomial infections, which causes bacterial infectious diseases involving multiple organ systems. The presence of carbapenem-resistant strains has resulted in problems in the current clinical anti-infective treatment. The current study reports on four cases of carbapenem-resistant *E. cloacae* secretion infection in order to provide suggestions for the detection and treatment of these pathogen infections.

Case Presentation: Investigation of 4 cases was conducted at tertiary care hospitals, and baseline data, treatment and outcomes were collected for patients with carbapenem-resistant *E. cloacae* infection. The strains of burn injury and diabetic foot infection were retrieved from specimens by culture-based methods, and antibiotic sensitivity test was conducted on Vitek 2. All strains showed minimum inhibitory concentration (MIC) values for ertapenem, imipenem, and meropenem of less than 4 $\mu\text{g}/\text{mL}$. The four strains of *E. cloacae* produced IMP-8 type carbapenemase confirmed by PCR and sequence analysis. After the selection of reasonable antibiotic treatment, the patient's condition had improved and they were discharged from the hospital.

Conclusions: Low MIC value makes it difficult to detect IMP-8-harboring strains by traditional susceptibility test; molecular biology techniques may be mandatory for detection of carbapenem resistant isolates. It is very important to treat patients with reasonable antimicrobial based on susceptibility results.

Keywords: IMP-8 Carbapenemase, Secretion Infection, *Enterobacter cloacae*

1. Introduction

Enterobacter cloacae are inhabitants of the intestinal flora and are among the most common human pathogens. The strains could spread easily between humans and acquire antibiotic resistance through horizontal gene transfer, mediated mostly by plasmids and transposons. The infection of carbapenem-resistant isolates has brought great difficulties in clinical treatment, and the main drug resistance mechanism is the production of carbapenemase (1). Carbapenemases are generally categorized to Ambler class A, B, and D β -lactamases. The most common carbapenemases are *Klebsiella pneumoniae* carbapenemase (KPC) belonging to class A, whereas other carbapenemases are relatively rare, such as Metallo-Beta-Lactamases (MBL) and D β -lactamases. IMP of class B MBL is one of the early described carbapenemases in *Enterobacteriaceae*. Since the

first report of IMP-1 in *Serratia marcescens* from Japan in 1991 (2), IMP carbapenemase have been described worldwide, particularly in Asian countries, such as Japan, Indian, and Korea (1, 3). In China, the gene types found in *E. cloacae* are IMP-1, IMP-4, IMP-8 and IMP-26, and carbapenem resistance in *E. cloacae* is becoming a great concern in the region (4, 5).

Patients infected with carbapenem-resistant *E. cloacae* often bring great difficulties to antibiotic decision, and this public health crisis demands redefined and refocused efforts in the diagnosis, treatment, and control of infections in hospitalized patients. The current study describes 4 cases of skin wound infection caused by *E. cloacae* producing IMP-8 type carbapenemase, in order to explore effective way to detect and treat infections. Four cases of infected patients were hospitalized from Liaocheng peo-

ple's hospital, and the diagnosis of wound infection was based on infectious diseases society of America (IDSA) definitions.

All patients had a significant infection in the local wound, and suspected pus or secretions specimens were collected to carry out microscopic examinations and bacterial cultures. The results showed significant phagocytosis of white blood cells, and a single pure isolate. All of the isolated bacteria were identified as *E. cloacae*, and the susceptibility of stains were conducted on Vitek 2, according to clinical and laboratory standards institute (CLSI) breakpoint definitions. The resistance mechanism of strains was the production of IMP-8 type carbapenemase, which was proved by modified Hodge test (MHT) and sequencing as previous studies (6). The Pulsed Field Gel Electrophoresis (PFGE) test revealed that 4 strains had different PFGE patterns.

2. Case Presentation

Case 1 on 7th of January 2016: a 27-day-old neonate was admitted to the department of burns due to deep 2nd degree burn of the right lower limb. Infection was found in burn wounds and secretion was sent to the laboratory for cultivation, identification, and antimicrobial susceptibility testing. Penicillin of 3×10^5 units was administered empirically twice a day for 3 days, but symptoms remained. Then, culture of secretion retrieved *E. cloacae* with elevated MIC for ertapenem. Carbapenemase production was further proved by MHT, and IMP-8 type was confirmed by PCR and sequence analysis. The strain was sensitive to ciprofloxacin and confirmed production of MBL; penicillin was replaced by ciprofloxacin 0.1g a day, for 4 days. Patient's condition improved and he was discharged from the hospital.

Case 2 was a 23-year-old male, who was admitted to the hospital on 7th of July 2016 for infection of deep 3rd degree burn wound in the right foot and secretion could be seen in abscess of the wound. Debridement and disinfection of the wound was performed prior to antimicrobial therapy. Culture of the secretion retrieved *E. cloacae*, which was resistant to almost all β -lactams (Table 1), including carbapenems. However, the strain was sensitive to fluoroquinolones, so 0.2 g of ciprofloxacin was administered twice a day for 7 consecutive days. The infection was finally controlled and the patient was discharged from the hospital after 15 days.

Case 3 was a 62-year-old female with type 2 diabetes, she was admitted to the hospital on 21st of August 2016 due to a diabetic foot. A 0.5 \times 0.5-cm ulceration could be found in the inner side of the first toe of her right foot. While

white seepage of the ulceration was sent to the microbiology lab for culture and identification of the pathogens, cefthiamidine was given empirically with a dose of 2 g twice a day for 4 days. The *E. cloacae* remained sensitive to aminoglycosides and fluoroquinolones yet had elevated MIC for carbapenems (Table 2). Therefore, cefthiamidine was changed to 0.1 g levofloxacin, after 2 days of treatment, and infection of the wound was under control.

Case 4 was a 73-year-old female, who had a 7-year history of type 2 diabetes and was admitted to the hospital on 30th of September 2016 for treatment of diabetic foot, which had lasted for five months. Diameter of the ulceration was found to be 6 cm, purulent exudate could be found at the center of the abscess. Analysis of lower limbs artery by MRA showed bad lower extremity circulation, which indicated a bad prognosis. Culture of the secretion retrieved *E. cloacae* sensitive to imipenem and meropenem but intermediary sensitive to ertapenem, thus 0.5-g meropenem was administered every 8 hours for 7 days. After 24 days of hospitalization, the infection had been well controlled and the patient was discharged from the hospital.

3. Discussion

The IMP-8 type carbapenemase is derived from IMP-2 carbapenemase, and there are only 4 nucleotide differences between the 2 of them, which result in two amino acid differences. However, expression of IMP-2 carbapenemase could result in a significant decrease in susceptibility to a broad array of β -lactams (ampicillin, carbenicillin, cephalothin, cefoxitin, ceftazidime, cefepime, and carbapenems) (7). IMP-8 (AF322577) type carbapenemase was first reported by scientists from Taiwan in 2001; half of these isolates (20 of 40) were recovered from wound specimens and the infected patients had a high mortality rate of 56.3% (9 of 16). All the isolates were resistant to ceftazidime, cefotaxime, and cefoxitin, and most isolates were also resistant to aztreonam (8). In the current study, despite the emergence of drug resistance to cephalosporins, 3 patients chose quinolone antibiotics, according to drug sensitivity and achieved an effective treatment.

The prevalence of IMP-8-producing *E. cloacae* in Taiwan was reported in the following years; all isolates appeared susceptible to imipenem (MICs < 8 mg/L) and meropenem (MICs < 4 mg/L), indicating the difficulty in detection of MBLs in *Enterobacteriaceae* by routine susceptibility testing (9). The current study also confirmed the susceptibility of misdiagnosis, according to drug susceptibility analysis; molecular identification, such as PCR, may be an effective method for the detection of IMP carbapenemase. The current study used VITEK 2 susceptibility test, yet the detected

Table 1. Information of Patients Infected with *Enterobacter cloacae* Producing IMP-8 Carbapenemase

| Patient Number | Gender | Age | Diagnosis | Antibiotic Therapy | Outcome | Hospitalization, d |
|----------------|--------|------|---|--------------------|----------|--------------------|
| 1 | Male | 27 d | Infection of deep 2nd degree burn wound | PEN, CIP | Cure | 10 |
| 2 | Male | 23 y | Infection of deep 3rd degree burn wound | CIP | Improved | 15 |
| 3 | Female | 62 y | Mild Diabetic Foot infection Coronary artery disease | CSU, LEV | Improved | 12 |
| 4 | Female | 73 y | Moderate Diabetic Foot infection Coronary artery disease Retinopathy | MEM | Improved | 24 |

Abbreviations: CIP, ciprofloxacin; CSU, cefathiamidine; LEV, levofloxacin; MEM, meropenem; PEN, penicillin.

Table 2. Susceptibility Profiles of *Enterobacter cloacae* Producing IMP-8 Carbapenemase

| Patient Number | MBL Typing | MIC, µg/mL | | | | | | | | | | | | |
|----------------|------------|------------|-----|-----|-----|-----|-----|------|------|-----|-----|-----|-----|-----|
| | | ETP | MEM | IMP | AMK | GEN | TOB | CIP | LEV | ATM | TZP | CRO | CAZ | FEP |
| 1 | IMP-8 | 2 | 1 | 1 | 2 | 1 | 1 | 0.25 | 0.25 | 64 | 64 | 64 | 64 | 2 |
| 2 | IMP-8 | 4 | 2 | 2 | 64 | 16 | 16 | 4 | 8 | 64 | 64 | 64 | 64 | 64 |
| 3 | IMP-8 | 0.5 | 4 | 4 | 2 | 1 | 1 | 0.5 | 1 | 1 | 4 | 1 | 1 | 1 |
| 4 | IMP-8 | 4 | 1 | 1 | 64 | 16 | 16 | 4 | 8 | 64 | 128 | 64 | 64 | 64 |

Abbreviations: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; ETP, ertapenem; FEP, cefepime; GEN, gentamicin; IMP, imipenem; LEV, levofloxacin; MBL, metallo-beta-lactamase; MEM, meropenem; TOB, tobramycin; TZP, piperacillin-tazobactam.

MIC value appeared to be inaccurate for the detection of carbapenem. However, advanced expert system of VITEK 2 was reliable in clinical identification of IMP type carbapenemase (10).

Wound infection is a common skin infection, and is difficult to treat due to its complicated infection strains. Burn wound and diabetic foot infections are 2 common complex skin wound infections, with a special antibacterial treatment program due to the spread of antimicrobial agents are disturbed. Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following burn injury. It has been found that *E. cloacae* contributes a lot to infection of burn injury (11). Diabetic foot is the long-term complication of diabetes mellitus characterized by ulcerations of the foot, and enterobacteria are the most frequently isolated species among the infection.

Reports of diabetic foot infection showed that 4.7% of *Enterobacteriaceae* were resistant to imipenem, and 3.8% of the isolates produced carbapenemase (12). In the current analysis, 2 cases of burn wound infection and 2 cases of diabetic foot infection were revealed, and 4 different strains of carbapenem-resistant *E. cloacae* were isolated and con-

firmed by PFGE. For such cases complex resistance empiric antibiotic therapy is often ineffective. Nevertheless, quinolones were selected for 3 patients and infection was controlled. Quinolones have a high concentration in tissue, and the drug can achieve effective sterilization concentration at the infected site. From the current analysis, quinolone antibiotics could be used as an effective antimicrobial treatment regimen when exhibiting sensitivity. Simultaneously, serious drug resistance led to prolonged hospital stay.

This research found that patient 2 and patient 4, who had severe symptoms spent a longer time at the hospital, and the strains retrieved were resistant to a greater number of antibiotics. The appearance of multi-resistant strains may have been caused by nosocomial infections or the selection of resistant strains by long-term use of antibiotics. The larger the burn injury or the ulceration, the longer the patient has to stay at the hospital, and thus the opportunity of the patient to become infected by epidemic strains from the environment is greater. Although this study conducted a small amount of carbapenem-resistant *E. cloacae* infection analyses, from the results it could be suggested that the production of IMP-8 type carbapene-

mase detection requires PCR and sequencing analysis. The choice of antimicrobial agents in the infected cases will also provide a theoretical basis for the selection of future antimicrobial agents against such pathogens.

Footnotes

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Conflicts of Interest: The authors declare no conflicts of interest.

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