Osteomyelitis due to multiple carbapenemase-producing Gram-negative bacteria: The first case report of a GES-13-producing Pseudomonas aeruginosa isolate in Canada

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A case of osteomyelitis in an infant following a burn injury sustained in Pakistan caused by a GES-13-producing Pseudomonas aeruginosa (the first reported in Canada) and an OXA-48 producing Klebsiella pneumoniae is described. The present case serves to highlight the importance of international travel as a risk factor for infection with carbapenemase-producing bacteria and the challenges in the laboratory detection of these organisms.

Key Words: Carbapenemase; Continuous infusion; GES-13; Multidrug-resistant; OXA-48

CASE PRESENTATION

A four-month-old female infant presented to the emergency department of the Children’s Hospital in Winnipeg, Manitoba, with full-thickness burns of the anterior lower limbs. According to the infant’s mother, the burns had been sustained two weeks previously while visiting family in Karachi, Pakistan, when an electronic steamer used to relieve the infant’s nasal congestion slipped and spilled hot water over her legs. She was taken to an emergency department in Karachi, where she was treated with oral amoxicillin-clavulanate and daily dressing changes. Within one week, her wounds became infected. At this time the family left Pakistan without further intervention in the hope of resolving their health concerns in Canada.

On presentation in Winnipeg, the infant’s temperature was 39.1°C, her heart rate was 204 beats/min, her respiratory rate was 36 breaths/min, her blood pressure was 114/64 mmHg and her oxygen saturation was 99% on room air. Examination of her lower limbs revealed 15% body surface area of full-thickness burns, encompassing areas of both legs and feet. The three lateral toes of her right foot were gangrenous. Yellowish discharge was observed from both of her ears.

Initial investigations revealed a white blood cell count of 29×10⁹ cells/L (28% neutrophils and 54% lymphocytes), a hemoglobin level of 102 g/L and a platelet count of 189×10⁹ cells/L. Blood, urine, ear swabs and surveillance swabs for methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci were collected for culture. The patient was taken to the operating room, where her wounds were debrided, the aforementioned gangrenous toes were amputated, and swabs from the left leg and right foot were submitted to the laboratory for microbiological investigations. Antimicrobial therapy was initiated with vancomycin and meropenem. The patient was admitted to the pediatric intensive care unit after her surgery.

Antimicrobial susceptibility testing was performed using the VITEK2 system (bioMérieux, Canada), with minimum inhibitory concentrations (MICs) interpreted using 2013 Clinical and Laboratory Standards Institute (CLSI) breakpoints (1). Colistin MICs were obtained by broth microdilution. Aztreonam and tigecycline MICs were determined by E-test. United States Food and Drug Administration breakpoints were used for tigecycline. Multidrug resistance was defined as resistance to ≥3 antimicrobial classes. Two different strains of MRSA (isolates A and B) were isolated from the nares, as well as the ears, the left leg and the right foot. One isolate was a Canadian MRSA strain type 7 (USA400) according to pulsed-field gel electrophoresis, while the other MRSA had a unique pulsed-field gel electrophoresis pattern that did not match any of the existing Canadian MRSA strain types (2). Multidrug-resistant (MDR) strains of Klebsiella pneumoniae (isolate C) and Pseudomonas aeruginosa (isolate D) were recovered from wounds on the left leg and right foot (Table 1). The K pneumoniae isolate had a relatively elevated meropenem MIC of 1 mg/L, which is at the CLSI defined breakpoint for susceptibility. This prompted a change in meropenem administration from intermittent...
TABLE 1
List of bacteria recovered and their antimicrobial susceptibility patterns

| Isolate designation | A and B | A and B | C | D | E | F |
|---------------------|---------|---------|---|---|---|---|
| Collection date     | December 14, 2012 | December 14, 2012 | December 14, 2012 | December 14, 2012 | December 20, 2012 | December 20, 2012 |
| Specimen source     | Nares swab | Sterile fluid swab | Sterile fluid swab | Sterile fluid swab | Tissue biopsy | Tissue biopsy |
| Site                | Nares | Right foot, left leg, left ear | Right foot, left leg | Right foot, left leg | Right foot first metatarsal | Right foot first metatarsal |
| Organism            | MRSA* | MRSA | Klebsiella pneumoniae | Pseudomonas aeruginosa | Pseudomonas aeruginosa | Providencia stuartii |
| Susceptibility      | Ampicillin | R, R | R | R | R | R |
|                     | Oxacillin | R, R | R, R | R | R | R |
|                     | Piperacillin-tazobactam | R | R | R | S | S |
|                     | Cefazolin | R | R | R | R | R |
|                     | Ceftriaxone | R | R | R | R | R |
|                     | Tobramycin | R | S | S | S | S |
|                     | Meropenem | S* | I | R | S | S |
|                     | Aztreonam | R | R | R | R | R |
|                     | Tigecycline | S* | MIC >8 mg/L | | | |
|                     | Clindamycin | S, R | S, R | | | |
|                     | Linezolid | S, S | S, S | | | |
|                     | Vancomycin | S, S | S, S | | | |
|                     | Trimethoprim-sulfamethoxazole | R, R | R, R | R | R | R |
|                     | Colistin | MIC 0.5 mg/L | S | S | S | S |

All susceptibilities are based on Clinical and Laboratory Standards Institute M100-S23 interpretive breakpoints (1). Minimum inhibitory concentrations (MICs) are reported when no Clinical and Laboratory Standards Institute interpretive breakpoints exist. *Two strains of methicillin-resistant Staphylococcus aureus (MRSA) were isolated from the patient's nares; the same two strains were also isolated from the right foot, the left leg, and the left ear; 1MIC = 1 mg/L; MIC ≤1 mg/L is considered to be susceptible (1); 2Based on Food and Drug Administration interpretive criteria. I Intermediate; R Resistant; S Sensitive

dosing to a continuous infusion. Ciprofloxacin was added to the treatment regimen based on the antimicrobial pattern of the P aeruginosa isolate (Table 1, isolate D). Given the broad-spectrum antibiotics and severe burns, the patient was also given fluconazole prophylaxis.

On day 7 postadmission, the patient's skin grafts were noted to be necrotic in an area of previously exposed tarsal bone. She was taken to the operating room to investigate a potential osteomyelitis. Purulent material adjacent to the first metatarsal bone of the right foot was submitted to the laboratory, and ultimately yielded P aeruginosa and Providencia stuartii on culture (isolates E and F; Table 1). This new P aeruginosa isolate exhibited a different susceptibility pattern compared with the previously isolated P aeruginosa, and was susceptible to amikacin and tobramycin but resistant to meropenem (Table 1). Therefore, amikacin was added to the patient's treatment regimen. Given the presumptively infected bone, the patient completed six weeks of combined antimicrobial therapy (vancomycin, continuous infusion meropenem, ciprofloxacin and amikacin) for osteomyelitis. She improved rapidly following the addition of amikacin and was discharged home with no further complications.

The MDR K pneumoniae (isolate C) and P aeruginosa (isolate D) isolates were forwarded to the National Microbiology Laboratory in Winnipeg, Manitoba, for further evaluation. Two multiplex polymerase chain reactions (PCRs), one for the detection of beta-lactamase genes (SHV, TEM, CTX-M, OXA-1 and CMY-2) and one for the detection of carbapenemase genes (KPC, NDM, IMP, VIM, GES and OXA-48) were used as previously described (3,4). The K pneumoniae isolate (isolate C) was positive for SHV, TEM, CTX-M, OXA-1 and OXA-48, and was considered to be an extended-spectrum beta-lactamase (ESBL) producer and molecular class D OXA-48 carbapenemase producer. The P aeruginosa isolate (isolate D) was positive for GES by PCR and sequencing confirmed that it possessed GES-13, a molecular class A carbapenemase. The carbapenemase PCR was also performed on the P stuartii isolate (isolate F) and the second isolate of P aeruginosa (isolate E); these were both negative.

DISCUSSION
Carbapenemases are beta-lactamase enzymes capable of hydrolyzing carbapenem antimicrobials (5). While some bacteria intrinsically produce a carbapenemase, P aeruginosa and many members of the family Enterobacteriaceae acquire carbapenemases through mobile genetic elements such as plasmids (3). The global dissemination of carbapenemase-producing bacteria is of great concern because these organisms are often MDR, leaving clinicians with few therapeutic options from which to choose (5).

GES beta-lactamases (GES-1 to GES-23; www.lahey.org) are a group of molecular class A enzymes with extended-spectrum properties (6). GES-13 differs from GES-1 by the presence of Lys-170→Asn in the enzyme that produces weak carbapenemase activity (6). GES-13 was first isolated in a MDR strain of P aeruginosa isolate (isolate D) was positive for GES by PCR and sequencing confirmed that it possessed GES-13, a molecular class A carbapenemase. The carbapenemase PCR was also performed on the P stuartii isolate (isolate F) and the second isolate of P aeruginosa (isolate E); these were both negative.
OXA-type carbapenemases are molecular class D enzymes that have been primarily associated with Acinetobacter baumannii (8). In 2001, the first case of an OXA-48-producing K. pneumoniae was reported from Istanbul, Turkey (9,10). For several years, OXA-48 was identified only in patients hospitalized in Turkey or with some link to this country (9,10). However, more recently, the plasmid-mediated OXA-48 and related variants have been detected in Enterobacteriaceae from the Middle East, North Africa, India, Europe and North America (4,9,11,12). Although OXA-48 displays weak activity against carbapenems and extended-spectrum cephalosporins, isolates harbouring this enzyme often produce other ESBLs (4,9,12). Identification of OXA-48-producing Enterobacteriaceae in the clinical laboratory setting is challenging due to the lack of a specific phenotypic test (10,13). Furthermore, these isolates may test susceptible to carbapenems, albeit with relatively elevated MICs. In a recent study by Potron et al (9), 68.6% and 69.5% of 105 OXA-48-producing Enterobacteriaceae were susceptible to imipenem and meropenem, respectively, according to current CLSI breakpoints. Poirel et al (10) have proposed that carbapenemase production among Enterobacteriaceae be suspected for isolates with an ertapenem MIC ≥ 2 mg/L or an imipenem or meropenem MIC ≥ 1 mg/L. Molecular-based techniques (PCR) remain the gold standard for detection of OXA-48 among Enterobacteriaceae (10,14). In our case, the OXA-48-producing K. pneumoniae isolate was resistant to carbapenem (MIC 4 mg/L) and had a relatively elevated meropenem MIC of 1 mg/L (Table 1). On molecular testing, this isolate was positive for SHV, TEM, CTX-M, OXA-1 and OXA-48 by PCR. The CTX-M enzyme was likely responsible for the extended-spectrum cephalosporin resistance demonstrated by the isolate, as has been previously reported (4,9,12).

The present case highlights several of the challenges associated with MDR organisms. As the frequency of worldwide travel continues to increase, clinicians will need to be aware of not only local epidemiology but also global trends in antimicrobial resistance. Treatment of infections caused by MDR organisms may require complicated and potentially toxic antimicrobial combinations. The regimen used in the present case resulted in excellent clinical response but required a prolonged hospital stay for the medications to be effectively delivered. The extended use of an aminoglycoside was well tolerated in our pediatric patient; however, nephrotoxicity from aminoglycosides may be a greater concern in older age groups and/or patients with underlying renal impairment.

Evidence for the use of continuous beta-lactam infusions is limited, particularly in the pediatric population (15-17). Given the K pneumoniae carbapenem MIC of 1 mg/L, found in our patient, combined with abnormal blood flow in a burn setting with infected bone, it was the option pursued due to concern over achieving sufficient drug levels in the targeted tissues. While the need for multiple antibiotics made drawing specific conclusions about the role of the continuous infusion difficult, the clinical success of the approach was notable.

SUMMARY
We report the first case of a GES-13 carbapenemase-producing P aeruginosa in Canada, recovered from the wounds of an infant who sustained burns while travelling in Pakistan. Also recovered from the patient's wounds were two isolates of MRSA, an MDR isolate of P stuartii, and an OXA-48 carbapenemase and CTX-M ESBL-producing K pneumoniae. This case highlights the importance of international travel as a risk factor for infection with carbapenemase-producing bacteria. The travel history here prompted testing for carbapenemase production in the P aeruginosa isolate (isolate D). Without this history, it is likely that molecular testing would not have been performed and the GES-13 enzyme would have gone undetected. This case also illustrates the difficulty faced by clinical microbiology laboratories in detecting OXA-48-producing Enterobacteriaceae because many of these isolates remain susceptible to imipenem and meropenem based on current CLSI breakpoints. Molecular testing remains the gold standard for detection of carbapenemase enzymes among Enterobacteriaceae.

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