Emergence of KPC Producing *Pseudomonas aeruginosa* in Trinidad & Tobago

Patrick E. Akpaka¹*, William H. Swanston¹,², Hyginus N. Ihemere², Adriana Correa³, Julian A. Torres³, Jose D. Tafur³, Maria Camila Montalegre³, John P. Quinn⁴, Maria Virginia Villegas³.

1. The Univ. of the West Indies, St. Augustine, Trinidad and Tobago
2. North Central Regional Health Authority (NCRHA), Champ Fleurs, Trinidad and Tobago
3. International Center for Medical Research & Training (CIDEIM), Cali, Colombia
4. Pfizer Global Research and Development, New London, CT

*Room 26, Block 5; Pathology/Microbiology Unit
Eric Williams Medical Sciences Complex
Dept. of Para-Clinical Sciences;
The University of the West Indies
St. Augustine, Trinidad & Tobago
Phone: 868-645-2640 ext 2332 or 868-736-0440
Fax: 868-663-3797; Email: Patrick.Akpaka@sta.uwi.edu;
Web: www.sta.uwi.edu
Abstract

In 2006, the first isolate of KPC-2 producing *P. aeruginosa* in the world was identified in Colombia. Recently, similar strains have been reported from Puerto Rico. We now report KPC-2 producing *P. aeruginosa* in Trinidad & Tobago. Surveillance for similar strains is warranted considering their wide geographic spread and known association with mobile genetic elements.

Case report

A 63-year old male patient was admitted to a hospital in Mount Hope, Trinidad & Tobago with hematuria, dysuria, fever, and chills. No history of travel abroad. Four months prior to his presentation, he had a left hip fracture following a fall and was hospitalized at another regional hospital for ten weeks without any surgical intervention but had conservative care. He had remained bedridden since after the fracture.

On physical examination he appeared chronically ill, was stuporous, febrile (38°C), severely pale, dehydrated, had bedsores on buttocks and sacral area, and had a urinary catheter. He had swelling of the left thigh that was tender and warm to touch with subcutaneous emphysema.

Blood and urine specimens were submitted for culture. Radiological investigations of the pelvis and legs revealed fracture of the neck of the left femur with subcutaneous emphysema and fluid collection along the lateral compartment of the thigh extending to the inguinal region, hip joint and left lower abdominal wall.
He was given gentamicin, ceftazidime and metronidazole. A fasciotomy was performed revealing gas gangrene. Two liters of greenish yellow colored pus in the anterior compartment of the left thigh extending to the left lower abdomen was drained. This pus was cultured.

Blood and urine cultures were negative. However, the culture of the pus from surgery yielded *Pseudomonas aeruginosa*. Antimicrobial susceptibility using the Micro scan Walk Away 96 SI (Siemens USA) revealed that the isolate was resistant to all tested antimicrobials including gentamicin, ceftazidime, ciprofloxacin and carbapenems. Meropenem monotherapy was given despite in vitro resistance while efforts were made to procure polymixin B or colistin; unfortunately these efforts were unsuccessful and he died ten days post admission.

The *P. aeruginosa* isolate was sent to the International Center for Medical Research and Training (CIDEM) Cali, Colombia where the minimal inhibitory concentration (MIC) was determined using Clinical and Laboratory Standard Institute (CLSI) approved broth micro dilution method [1]. Ertapenem, imipenem and meropenem MICs were >128µg/ml. This isolate was also resistant to ceftazidime (128µg/ml), cefepime (>128µg/ml), aztreonam (>128µg/ml), piperacillin/tazobactam (>256/4µg/ml) and ciprofloxacin (>8µg/ml) and remained only susceptible to polymyxin B (2µg/ml).

A three-dimensional (3D) test to screen for carbapenemases was performed as previously reported [9] with some modifications. This test uses a carbapenem-susceptible organism as an indicator for carbapenemases in a cellular extract. To detect the carbapenemase, the extract is placed in a groove made on a Mueller Hinton agar plate inoculated with a susceptible strain with an imipenem disk. Hale distortion indicates presence of the enzyme. The Metallo Beta-lactamase
(MBL) E-strip (AB Biodisk, Solna, Sweden) was used to screen for metalloenzymes. The isolate was positive for the 3D test and negative for MBL E-test, indicating the presence of a carbapenemase but not a metallo-β-lactamase.

The presence of \( \text{bla}_{\text{KPC}} \) in the isolate was initially detected using PCR as described by Yigit H, et al. [15]. For confirmation, sequencing was performed.

**PCR and DNA sequencing of KPC gene:** Identification of \( \text{bla}_{\text{KPC}} \) gene was determined by PCR amplification of a PCR product with the following primers: KPCF: 5'-ATGTCACTGTATCGCCGTCT-3' and KPCR: 5'-TTACTGGCGGTGGAGCGCCA-3'. PCR conditions were as follows: 3 min 94˚C and 30 cycles of 1 min at 94˚C, 1 min at 52˚C, and 1 min at 72˚C which produced a band of 880 bp encompassing the entire KPC coding region. PCR was performed using HiFi Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA) and purified with a PCR purification kit (Qiagen, Valencia, CA). Sequencing of the \( \text{bla}_{\text{KPC-2}} \) gene was carried out at Cogenics (Morrisville, North Carolina) using the following primers: KPCF, KPCR, KPC510R: 5'-CTGGGATGGCGGAGTTCAGC-3' and KPC472R: 5' GCTGAAGGAGTTGGGCGGCC. Sequence analysis was performed online using BLAST (www.ncbi.nlm.gov/BLAST/)

A family of carbapenemases, KPC1-7, is an emerging threat worldwide [8], as they confer resistance to all beta-lactams and may disseminate on mobile genetic elements [5,7]. The first reports were solely in Enterobacteriaceae in the United States followed by reports from France, Israel, Colombia, China, Greece and several other places [2, 3, 4, 6, 11, 12]. In 2006, CIDEIM described in Colombia the first isolation of KPC-2 in \( P. \) aeruginosa in the world [10]. Subsequently from Puerto Rico, two KPC variants KPC-2 and KPC-5 in \( P. \) aeruginosa strains have been reported [13].
The isolation of this organism in Trinidad highlights several key issues. First, the isolate was encountered in a patient who did not have any history of foreign travel. Second, the organism was pan-resistant. Third, as in the cases identified in Colombia, the outcome was fatal.

There are several implications of KPC producing organisms. Most important is the fact that they are difficult to detect with routine laboratory methods, and treatment options are very limited because most isolates are pan-resistant. The use of polymixin B and colistin seems to be alternative choices based on *in vitro* data but this may pose a problem because these drugs may be associated with severe nephrotoxicity and there is paucity of data on clinical outcome [16]. In addition, as exemplified in our case, these agents are not available at all in some areas.

The emergence of *bla*<sub>KPC-2</sub> in different *Enterobacteriaceae*, and their spread to *P. aeruginosa* in different countries emphasizes their potential for dissemination worldwide. In some cases this has been associated with a common transposon, Tn4401 [5]. In the case of KPC-5, a related transposon was noted, with a unique upstream region, with components of both Tn5563 and IS6100A present [14]. Clinicians and microbiologists should be alert to the threat posed by KPC enzymes even in the absence of a history of travel to an area known to be endemic. Additional surveillance for similar strains is warranted considering their wide geographic spread and known association with mobile genetic elements.

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References


