Emergence of New Delhi Metallo-Beta-Lactamase (NDM-1) and Klebsiella pneumoniae Carbapenemase (KPC-2) in South Africa

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This report documents emergence of New Delhi metallo-beta-lactamase (NDM-1) and Klebsiella pneumoniae carbapenemase (KPC-2) in K. pneumoniae and Enterobacter cloacae in South Africa. NDM-1 producers have not been described in South Africa, and this is the first instance that KPC producers have been identified in Africa. The two patients infected with these carbapenemase-producing bacteria demised.

CASE REPORTS

On 1 August 2011, an 86-year-old male patient was admitted to an orthopedic ward in a private hospital in Johannesburg, South Africa, with a right hip fracture and a right-sided pleural effusion. Comorbidities included diabetes mellitus and hypertension, and he was in chronic renal failure on hemodialysis. On 3 August, the patient underwent open reduction and internal fixation of the right hip and had a right-sided thoracostomy for the pleural effusion. He was subsequently admitted to the high-care unit (HCU), where admission urine and blood cultures were negative. Single doses of intravenous (i.v.) ciprofloxacin (400 mg) and vancomycin (1 g) were administered. On 20 August, the patient was discharged to the renal ward for ongoing hemodialysis, but 2 days later he developed PCR-confirmed Clostridium difficile diarrhea, and treatment with metronidazole (400 mg per os [p.o.] every 8 h) was commenced for a week. On 26 August, the patient became delirious with fever and an increased C-reactive protein and white cell count. Empirical therapy with meropenem (500 mg i.v. every 8 h) and linezolid (600 mg p.o. every 24 h) was initiated. Blood and urine cultures taken at the same time revealed no growth, and the meropenem and linezolid were discontinued on 31 August and 7 September, respectively.

On 5 September 2011, while still on linezolid, he became hypotensive, and a urine microscopy, culture, and sensitivity (MCS) grew a Klebsiella pneumoniae strain that, according to automated susceptibility testing (Vitek2; bioMérieux, Johannesburg, South Africa), was resistant to most commonly used antibiotics (i.e., amino-penicillins, β-lactam/β-lactamase inhibitors, aminoglycosides, fluoroquinolones, cephalosporins, tigecycline, and carbapenems). Subsequent disc susceptibility testing showed that fosfomycin and colistin, according to the Clinical and Laboratory Standards Institute (CLSI) (2) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (4), respectively, were the only active agents. MICs were determined by E-tests (AB bioMérieux, Johannesburg, South Africa) on Mueller-Hinton agar at 37°C and interpreted according to CLSI standards (2), except those for tigecycline, for which the U.S. Food and Drug Administration recommendations were applied (≤2 μg/ml, susceptible; ≥8 μg/ml, resistant), and for colistin, for which EUCAST clinical breakpoints for Enterobacteriaceae (≤2 μg/ml, susceptible; >2 μg/ml, resistant) (4) were applied. Based on MIC testing, colistin was the only agent active against this pathogen (Table 1). Utilizing the method described by Zarfel et al. (16), the isolate was genotypically analyzed to detect the presence of carbapenemase genes, and sequencing confirmed the presence of the blaNDM-1 gene. The patient demised, however, on 9 September 2011 without having received antibiotics and prior to all the tests being completed.

The 2nd patient was a 37-year-old female with ulcerative colitis, admitted on 25 March 2011 to an HCU in a private hospital in Pretoria, South Africa, with deep-vein thrombosis of the left leg and a wound on the right ankle. On 28 March, a pus swab of the wound grew a Pseudomonas aeruginosa strain susceptible to all antipseudomonal agents, but no immediate therapy was given, as it was deemed not to be a significant pathogen. On 29 March, an inferior vena caval filter was inserted, and on 3 April, monotherapy with doripenem (500 mg every 8 h as a 30-min bolus infusion) was commenced for 10 days. On 5 April, following a respiratory arrest, the patient was transferred to the intensive care unit (ICU), where she was resuscitated and placed on a mechanical ventilator.

On 7 April, while the patient was on doripenem, a tracheal aspirate grew a strain of Enterobacter cloacae that, according to Vitek2, was resistant to amino-penicillins, β-lactam/β-lactamase inhibitors, cephalosporins, and carbapenems but susceptible to the aminoglycosides (aminoglycosides, gentamicin, and tobramycin), ciprofloxacin, and tigecycline. MICs were subsequently performed and interpreted as described above (Table 1). Only ciprofloxacin and tigecycline were in the susceptible range, whereas the
The presence of \( \text{bla}_{\text{KPC}} \) was confirmed by multiplex and real-time PCR (6).

On 11 April, the patient was extubated. A sputum sent for MCS cultured a strain of \( K.\ pneumoniae \) resistant only to ampicillin, which was regarded as a colonizer. On 18 April, the patient again became hypotensive and was reintubated and resuscitated, and antibiotic therapy with tigecycline (100 mg loading dose followed by 50 mg every 12 h) and ciprofloxacin (400 mg every 8 h) was commenced. Despite this, the patient demised. Blood cultures, central venous catheter tip, and a tracheal aspirate taken at the time of her deterioration all grew \( K.\ pneumoniae \) resistant to carbapenems, several studies have shown that prior carbapenem therapy is not a prerequisite for acquisition (9). The genes conferring such resistance usually reside on large plasmids, which frequently carry additional resistance determinants that confer cross-resistance to the fluoroquinolones and aminoglycosides. As a consequence, prior use of any of these antibiotic classes may select for carbapenemase-producing GNB. It also appears that not only is prior exposure a risk factor but also the risk increases with increasing duration of prior treatment. Kritsotakis et al. (8) recently demonstrated that this was the case for \( \beta \)-lactam–\( \beta \)-lactamase inhibitor combinations (odds ratio [OR], 1.15 per day increase; \( P = 0.001 \)) and also for the fluoroquinolones, where increased duration of treatment amplified the effect of exposure to carbapenems (and vice versa) (OR, 1.02 for interaction term; \( P = 0.0009 \)).

The recognition of carbapenemase expression is the key to appropriate treatment of CRE. However, due to heterogeneous expression of resistance, clinical laboratories may encounter difficulties when trying to detect carbapenemase production during routine diagnostic procedures. This is particularly the case when using automated susceptibility testing, as highlighted by Woodford et al. (15), where the sensitivity and specificity for the identification of a carbapenemase with Vitek2 were only 74% and 38%, respectively. Unusually elevated MICs arouse suspicion but do not prove resistance, as was the case in these patients. Therefore, since 1 March 2010, screening for both \( \text{bla}_{\text{NDM}} \) and \( \text{bla}_{\text{KPC}} \) was performed on any carbapenem-nonsusceptible \( \text{Enterobacteriaceae} \) isolate referred to the Ampath National Referral Laboratory. Prior to the confirmation described in the case reports, 181 carbapenem-nonsusceptible isolates (mostly ampicillin resistant) had been screened and were negative. Routine testing for CREs should be extended to all bacterial species reported from any confirmed patient. If this had not been done in the 2nd case, the \( \text{bla}_{\text{KPC}} \)-positive \( K.\ pneumoniae \) isolate would have gone unnoticed. Screening should also be promptly extended to include patients hospitalized in the same unit, which was not done in this case, as the patients died before the results of molecular investigations were known.

This report documents the emergence of NDM and KPC among clinical isolates of \( K.\ pneumoniae \) and \( E.\ cloacae \) in South Africa and, for the first time, KPC in Africa. Rapid routine molecular detection is essential to optimize therapy, improve outcomes, and limit the spread of such resistance through aggressive infection control measures, including the screening of potentially colonized high-risk patients.

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**TABLE 1** MICs for \( \text{bla}_{\text{NDM}} \) \( K.\ pneumoniae \) and \( \text{bla}_{\text{KPC}} \) \( E.\ cloacae \) and \( K.\ pneumoniae \)

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \text{bla}_{\text{NDM}} ) ( K.\ pneumoniae ) (n = 1 isolate from urine)</th>
<th>( \text{bla}_{\text{KPC}} ) ( E.\ cloacae ) (n = 1 isolate from tracheal aspirate)</th>
<th>( \text{bla}_{\text{KPC}} ) ( K.\ pneumoniae ) (n = 3 isolates from blood culture, tracheal aspirate, central venous catheter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERT</td>
<td>≥32</td>
<td>≥32</td>
<td>≥32</td>
</tr>
<tr>
<td>IPM</td>
<td>≥32</td>
<td>≥32</td>
<td>1</td>
</tr>
<tr>
<td>MER</td>
<td>≥32</td>
<td>≥32</td>
<td>0.5</td>
</tr>
<tr>
<td>DOR</td>
<td>4</td>
<td>≥32</td>
<td>0.5</td>
</tr>
<tr>
<td>CAZ</td>
<td>≥256</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>FEP</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
</tr>
<tr>
<td>TZP</td>
<td>≥256</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>AMX</td>
<td>≥256</td>
<td>≥256</td>
<td>≥256</td>
</tr>
<tr>
<td>CIP</td>
<td>8</td>
<td>0.5</td>
<td>0.032</td>
</tr>
<tr>
<td>AMK</td>
<td>≥256</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>TGC</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>COL</td>
<td>0.25</td>
<td>≥256</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* ERT, ertapenem; IPM, imipenem; MERO, meropenem; DOR, doripenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; AMX, amoxicillin-clavulanate; CIP, ciprofloxacin; AMK, amikacin; TGC, tigecycline; COL, colistin.

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Since the first \( \text{Klebsiella pneumoniae} \) carbapenemase (KPC) producers were reported in the United States in 1996 and diverse New Delhi metallo-beta-lactamase (NDM-1) producers were reported in India in 2008, both have rapidly emerged as important causes of extreme drug resistance worldwide (5, 7, 9, 11, 12). Carbapenem-resistant \( \text{Enterobacteriaceae} \) (CRE) have become an international health issue and pose a major threat to the viability of currently available antibiotics (10). Numerous epidemics have been reported, CRE have become endemic in several institutions, and increased mortality has been ascribed to both KPC-producing \( \text{Enterobacter} \) spp. and \( K.\ pneumoniae \), as well as metallo-\( \beta \)-lactamase (MBL)-producing Gram-negative bacilli (GBN) (1, 7, 9, 10). In South Africa, \( K.\ pneumoniae \) with reduced susceptibility to carbapenems due to CTX-M extended-spectrum beta-lactamases (ESBLs) in conjunction with porin loss has previously been described (3, 14). Although NDM-1 has recently been reported in \( K.\ pneumoniae \) from Kenya and Morocco (12, 13), NDM producers have not been detected in South Africa, nor has the emergence of KPC in Africa been described.
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REFERENCES