Carbapenem Resistance in *Klebsiella pneumoniae* Due to the New Delhi Metallo-β-lactamase

Hanna Sidjabat,1 Graeme R. Nimmo,1,2 Timothy R. Walsh,5 Enzo Binotto,2,3 Anthony Htin,2 Yoshiro Hayashi,1 Jian Li,4 Roger L. Nation,4 Narelle George,2 and David L. Paterson1,2

1University of Queensland Centre for Clinical Research, and 2Pathology Queensland, Royal Brisbane and Women’s Hospital Campus, Brisbane, 3Cairns Base Hospital, Cairns, and 4Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia, and 5Cardiff University, Cardiff, United Kingdom

(See editorial commentary by Bronomo, on pages 485–487.)

Carbapenem resistance in *Klebsiella pneumoniae* is most notably due to the *K. pneumoniae* carbapenemase (KPC) β-lactamase. In this report, we describe the occurrence of a newly described mechanism of carbapenem resistance, the NDM-1 β-lactamase, in a patient who received medical attention (but was not hospitalized) in India.

The emergence of carbapenem resistance in *K. pneumoniae* has become a substantial clinical problem, most typically attributed to production of KPC [1]. KPC-producing organisms are most frequently found in the United States, but sizeable outbreaks have also occurred in Israel and Greece [2, 3]. Numerous other countries have now also been affected by KPC-producing organisms. KPC-producing *K. pneumoniae* cause considerable clinical problems because they are multidrug resistant, lacking susceptibility to β-lactam antibiotics, fluoroquinolones, and aminoglycosides [4]. Thus, therapy for clinically significant isolates rests on the use of tigecycline or polymyxins, both of which have been associated with development of resistance during treatment [5]. In addition, a dominant strain of KPC-producing *K. pneumoniae* (sequence type 258, as determined by multilocus sequence typing [MLST]) accounted for 70% of isolates in one study [6], suggesting some particular adaptive-ness of this very resistant strain for the health care setting.

Carbapenem resistance in *K. pneumoniae* may be due to other causes; these include combinations of outer-membrane permeability loss and β-lactamase production [7] and the production of metallo-β-lactamases, such as those of the IMP or VIM groups [8]. With the exception of Greece [9], most countries have been spared the widespread occurrence of IMP- or VIM-producing *K. pneumoniae*. In this Brief Report, we review the latest cause of carbapenem resistance in *K. pneumoniae* to be described—the New Delhi metallo-β-lactamase enzyme, NDM-1. There is emerging evidence that NDM-1–producing *K. pneumoniae* is destined to create clinical issues at least as substantial as those caused by KPC-producing strains. By virtue of its epicenter in the huge population of India, the number of individuals affected by NDM-1–producing *K. pneumoniae* may already exceed that of KPC-producing *K. pneumoniae*.

**CASE REPORT**

An 87–year-old woman was brought to a hospital in Australia directly from the airport, immediately after arriving from India. The patient was an Australian resident of Indian origin who had visited Khanna, in the state of Punjab, from November 2009 to January 2010. While in India, she developed a chronic draining foot ulcer. She was treated in India with an unknown intravenous antibiotic, which she administered at home. She was never hospitalized in India.

At the time of arrival, she developed fever (temperature, 38.9 degrees), dysuria, and suprapubic pain. Urine culture grew *K. pneumoniae* and *Escherichia coli* resistant to multiple antibiotics. The *K. pneumoniae* isolate was resistant to ertapenem, imipenem, meropenem, ceftazidime, cefotaxime, cefoxitin, piperacillin–tazobactam, ticarcillin-clavulanate, nalidixic acid, ciprofloxacin, amikacin, gentamicin, and trimethoprim-sulphamethoxazole, as determined on the basis of CLSI standards [10]. The organism was susceptible to aztreonam, chloramphenicol, colistin (minimum inhibitory concentration [MIC], 0.25 μg/mL), and tigecycline (MIC, 1 μg/mL). The MICs of meropenem and doripenem were >32 μg/mL. Empirical treatment was given with intravenous ticarcillin-clavulanate. Despite the lack of susceptibility to this combination treatment, the patient’s symptoms resolved, and she was discharged from hospital. Both the *K. pneumoniae* and *E. coli* isolates were also...
Table 1. Comparison of the 2 Most Common Causes of Carbapenem Resistance in Enterobacteriaceae: *Klebsiella pneumoniae* Carbapenemase (KPC) and New Delhi Metallo-β-Lactamase (NDM) Type β-Lactamases

<table>
<thead>
<tr>
<th>β-lactamase type</th>
<th>KPC</th>
<th>NDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambler cass</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Most commonly affected species</td>
<td><em>K. pneumoniae</em></td>
<td><em>K. pneumoniae</em></td>
</tr>
<tr>
<td>Other species commonly affected</td>
<td><em>E. coli</em>, <em>E. cloacae</em></td>
<td><em>E. coli</em>, <em>E. cloacae</em></td>
</tr>
<tr>
<td>Common MLST types</td>
<td>ST258</td>
<td>Variable</td>
</tr>
<tr>
<td>Geographic epicentre</td>
<td>NE USA</td>
<td>India, Pakistan</td>
</tr>
<tr>
<td>β-lactam antibiotics affected</td>
<td>Penicillins, cephalosporins, carbapenems</td>
<td>Penicillins, cephalosporins, carbapenems</td>
</tr>
<tr>
<td>Phenotypic Detection</td>
<td>Modified Hodge Test (MHT) positive</td>
<td>Unknown (positive MHT likely)</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Boronic acid</td>
<td>EDTA</td>
</tr>
</tbody>
</table>

Legend: EDTA = Ethylenediaminetetraacetic acid

grown from a rectal swab specimen, which was plated on MacConkey agar that contained 8 μg/mL gentamicin.

Phenotypic detection of a metallo-β-lactamase was made in the *K. pneumoniae* isolate by using inhibition of the enzyme by EDTA [10]. Polymerase chain reaction (PCR) and sequencing for antibiotic-resistance genes was positive for bla*NDM-1*, bla*CMY-6*, bla*SHV*, and bla*OXA*. The isolate was also positive for *aac-6'-1b* and *rmtC* [11–13]. PCR for the detection of *bla*NDM-1 was performed using forward primer (5'-GGGCCGTATGAGTGA-3') and reverse primer (5'-GAAGCTGAGCACCGCATTAG-3'), which amplifies a 758-bp fragment.

Transferability of *bla*NDM-carrying plasmids to laboratory strains of *E. coli* was conducted by transformation of extracted plasmids [12, 13] into Top10 *E. coli* (Invitrogen) and by conjugation with rifampin-resistant *E. coli K-12*. Transformants and transconjugants were selected on Luria-Bertani agar supplemented with ceftazidime, 2 μg/mL. *bla*NDM-1 together with *bla*CMY-6, *aac-6'-1b*, and *rmtC* were successfully transferred by transformation and conjugation, which were confirmed by PCR and sequencing. To differentiate the successful transconjugants from the donor (NDM-1 producing *K. pneumoniae*), *E. coli* species-specific PCR was performed for the transconjugants [14]. The size of the plasmid was ~70kb. The transformants and transconjugants were resistant to ertapenem, meropenem, imipenem, ceftazidime, cefotaxime, cefoxitin, amikacin, and gentamicin. The MICs in the transformants to meropenem and doripenem were 32 and 24 μg/mL, respectively.

MLST was performed as described on the MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). Allelic numbers were obtained on the basis of sequences of 7 housekeeping genes [6]. According to this typing scheme, the *K. pneumoniae* isolate was ST147.

**DISCUSSION**

Antibiotic-resistant *K. pneumoniae* has been a notable hospital pathogen for ≥4 decades. Sequentially, aminoglycoside resistance in *K. pneumoniae* in the 1970s, third-generation cephalosporin resistance by way of extended-spectrum β-lactamases in the 1980s and 1990s, and then carbapenem resistance in this century have been major problems. KPC-producing *K. pneumoniae* has become a substantial international issue [1]. The presence of KPC producers increases reliance on polymyxins or tigecycline as “workhorse” therapy. Increased use of any antibiotic hastens development of resistance to that class. Numerous reports of polymyxin- or tigecycline-resistant *K. pneumoniae* now exist [15–24]. Given the lack of new antibiotics active against multidrug-resistant gram-negative bacilli, a potential now exists for *K. pneumoniae* resistant to all commercially available antibiotics. The emergence of NDM-producing *K. pneumoniae* heightens this risk.

The first isolate from India known to produce NDM was retrospectively found in a survey of isolates from 2006 [25]. A number of reports now document the widespread occurrence of NDM-producing *K. pneumoniae* in India and Pakistan. NDM producers have been detected in ≥10 major population centers in India, traversing both the north and south of the country [25, 26]. Of consecutive carbapenem-resistant Enterobacteriaceae collected in 3 months in late 2009 in a single hospital in Mumbai, 91.7% were NDM producers [25]. Although surveillance studies are lacking, it is known that at least 10% of *K. pneumoniae* in some hospitals in India are carbapenem resistant (T.R.W.; unpublished data). Similar problems exist in Pakistan, where NDM producers have been documented in at least 8 cities [26]. Given the populations of India (1.184 million) and Pakistan (170 million), it can be appreciated that NDM producers may already be creating a massive problem in this region. Enterobacteriaceae other than *K. pneumoniae*, such as *E. coli* and *Enterobacter cloacae*, are also affected [26].

Since the first report of NDM-producing *K. pneumoniae* from Sweden in December 2009 (the patient had received medical care in New Delhi) [27], NDM producers have been detected in the United Kingdom [26, 28], the United States [29], Kenya [30], Japan [31], Canada [32], Belgium [31], the Netherlands [33], and Taiwan [34].
Pakistani diaspora is also considerable, estimated to number
United Kingdom, and Canada) have nonresident Indian pop-
million: 11 nations (including the United States, Saudi Arabia,
United Kingdom, and Canada) have nonresident Indian pop-
ulations exceeding 1 million (http://indianadiosporanic.in). The
Pakistani diaspora is also considerable, estimated to number ≥7
million individuals (http://www.opf.org.pk). In addition, “medical
tourism” to India is increasingly popular. A recent case of
NDM-producing Providencia rettgeri in an Australian who
received elective plastic surgery in India is illustrative of this
phenomenon [33]. Current data would suggest that recent
hospitalization in India or Pakistan greatly increases the risk
that an individual is colonized with an NDM-producing strain.
Strong consideration should be given to screening patients who
have undergone recent hospitalizations in India or Pakistan for
carbapenem-resistant organisms and for preemptively using
contact isolation precautions.

The isolate recovered from the patient who we describe was
K. pneumoniae ST147. The only previous report using MLST
showed an NDM-producer that was ST14 [27]. The vast
majority of KPC-producing K. pneumoniae isolates are ST258,
although occasional KPC-producers are ST14 [6]. In a recently
published evaluation, 26 NDM-producers from a single
institution in Haryana, India, belonged to a single pulsed-field
gel electrophoresis profile implying clonal spread [26].
However, isolates from another Indian institution showed no
similarity with each other [26]. British isolates have also been
quite diverse [26]. There is clear evidence from the Haryana
molecular epidemiology that institutional outbreaks can
occur. However, global spread appears to be due to a wide
diversity of strains, indicating that a wide variety of NDM
producers are circulating in India. Thus far, 2 NDM-
producing E. coli isolates (1 from Canada and 1 from Australia) were ST101 [34, 35].

Although the isolate we characterized was susceptible to col-
listin, the full threat of NDM producers was illustrated by a re-
cent report in which 1 NDM-producing K. pneumoniae isolate
had a colistin MIC > 32 mg/L [26]. Without doubt, NDM
producers are destined to provide problems at least as great as
KPC producers (Table 1). That the full extent of this impending
catastrophe may be played out in developing nations should not
reduce the need for urgent intervention.

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against blaKPC-containing Klebsiella pneumoniae isolates, including


