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Brief Original Article

New Delhi metallo-β-lactamase in Jamaica

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Abstract

Introduction: The global dissemination of the New Delhi metallo-beta-lactamase (NDM) gene among certain strains of bacteria has serious implications since the infections caused by such organisms pose a therapeutic challenge. Although the NDM gene has been detected in various parts of the world, this is the first report of its detection in the English-speaking Caribbean. The NDM producing *Klebsiella pneumoniae* was isolated from an Indian patient who had recently relocated to Jamaica.

Methodology: Identification and susceptibility testing of the *K. pneumoniae* isolate was performed using the Vitek 2 automated system) in keeping with Clinical and Laboratory Standards Institute (CLSI) standards. It was identified as a metallobetalactamase producer using the Rosco KPC+MBL kit. Genotypic screening for common betalactamase (including carbapenemase) genes, was carried out using two multiplex PCRs: one for SHV-, TEM-, CTX-M-, OXA-1-, and CMY-2-types, and one for VIM-, KPC-, IMP-, OXA-48, GES-, and NDM-types. Strain typing was conducted by pulsed-field gel electrophoresis (PFGE) using *Xba*I and multi-locus sequencing (MLS). Plasmid isolation and analysis was also performed.

Results: *K. pneumoniae* (N11-02395), not previously associated with the dissemination of the NDM in India, Sweden or the UK, was found to harbor the NDM-1 gene on plasmid pNDM112395.

Conclusion: The identification of the NDM-1 gene underscores the need for effective surveillance and infection control measures to identify and prevent spread of multidrug resistant Gram negative bacilli. Strict infection control measures implemented for this patient helped to prevent the spread of this organism to other patients.

Key words: multidrug-resistant; organisms; NDM; Jamaica

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Introduction

The global problems of antibiotic resistance and the spread of multidrug-resistant organisms has been exacerbated by the emergence of metallo-β-lactamases which hydrolyze carbapenems thus removing them from the antibiotic armamentarium reserved for the treatment of serious bacterial infections. One of the most recently discovered metallo-β-lactamases (Ambler class B carbapenemases) is the New Delhi metallo-β-lactamase (NDM) [1]. It hydrolyzes most β-lactams including carbapenems thereby limiting therapeutic options for patients infected with NDM producing organisms [1,2]. NDM was first identified in Sweden in 2008 from a *Klebsiella pneumoniae* isolate obtained from a Swedish national who developed an infection while travelling in India [3]. Follow-up reports have provided strong evidence that the Indian sub-continent was the original source of NDM-1-harboring bacteria [4,5]. Since then NDM-1 has been identified in many parts of the world [1].

There has been very little published data on antibiotic resistance from the English speaking Caribbean. The identification of an extensively drug resistant *K. pneumoniae* isolate from the urine of a 5 month old child who had recently immigrated to Jamaica from India at age 3 ½ months after being hospitalized there from as early as 1 month old, underscores the concept of the world being a global village and highlights the importance of global surveillance.

As is typical for class B metallo-β-lactamases, NDM-1 activity is not inhibited by tazobactam or clavulanic acid but it is inhibited by divalent cation inhibitors such as EDTA and dipicolinic acid. Further, as for other class B enzymes, aztreonam is not efficiently hydrolyzed by NDM-1. Nonetheless, the

broad spectrum of NDM-1 towards virtually all other β-lactams is thought to be due to its large and flexible active site [6]. To date over five point mutation variants of NDM-1 have been described and/or assigned designations (http://www.lahey.org/studies). Further analysis has shown that most isolates carried bla_{NDM-1} on plasmids of multiple incompatibility (Inc) types, which means that the gene is readily transferrable between different plasmid types seen in multiple bacterial species. In addition the plasmids carry other resistance genes including other beta-lactamases and RNA methylases conferring broad aminoglycoside resistance (*rmt*-types, *armA*) [1]. The plasmids located within these organisms have a broad host range and NDM-1 has been detected in many *Enterobacteriacae* such as *Citrobacter, Enterobacter,* and *Providencia*, as well as in other Gram-negative organisms such as *Stenotrophomonas maltophilia* and *Pseudomonas* spp. [1,4]. Although colonized persons may remain asymptomatic, clinical isolates with bla_{NDM-1} have been implicated in urinary tract infections, septicemia, pulmonary infections and peritonitis [1].

In this study, a 5 month old patient was admitted to a tertiary care hospital in Jamaica with a presumptive diagnosis of pulmonary tuberculosis and placed in isolation with strict infection control measures. While in hospital, a urine sample taken within 48 hours post admission as part of a septic screen yielded a doubtful significant growth of a multi-drug resistant (MDR) *K. pneumoniae isolate*. The patient was asymptomatic for a urinary tract infection and repeat urine cultures were negative so the patient received no antibiotics. As a result of the prompt infection control measures that were implemented in keeping with the patient's diagnosis, there was no transmission of this MDR isolate to any other patient in the hospital.

The isolate however was retained for further investigations to:

i) determine the mechanisms of resistance present in this MDR isolate
ii) determine whether the NDM gene was present as a mechanism of resistance to carbapenems
iii) characterize/ elucidate specific resistance genes identified
iv) further characterize the isolate in which the resistance mechanisms were identified.

Methodology

Identification and antimicrobial susceptibility testing of the *K. pneumoniae* isolate were done using the Vitek 2 automated system (bioMérieux, Durham, USA) according to Clinical and Laboratory Standards Institute (CLSI) standards. Phenotypic screening for carbapenemases was conducted by the modified Hodge test and the Rosco KPC+MBL kit (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). Genotypic screening for common β-lactamase (including carbapenemase) genes was done using two multiplex PCRs: one for SHV-, TEM-, CTX-M-, OXA-1-, and CMY-2-types, and one for VIM-, KPC-, IMP-, OXA-48, GES-, and NDM-types [7].

Strain typing was conducted by pulsed-field gel electrophoresis (PFGE) using *Xba*1 and multi-locus sequencing **(**MLS**)** [8]. Plasmid isolation was done

Table 1. Antimicrobial susceptibilities of *K. pneumoniae* N11-02395 and *E. coli* transformant harbouring pNDM112395.

Antimicrobial	***K. pneumoniae* N11-02395**		***E. coli* DH10B [pNDM112395]**	
	MIC (mg/ml)	Interpretation*	MIC (mg/ml)	Interpretation
Amikacin	≥64	R	>64	R
Ampicillin	≥32	R	≥32	R
Amp/Sulbactam	≥32	R	≥32	R
Aztreonam	≥64	R	4	S
Cefazolin	≥64	R	≥64	R
Cefepime	≥64	R	16	I
Ceftriaxone	≥64	R	≥64	R
Ciprofloxacin	≥4	R	≤0.25	S
Ertapenem	≥8	R	≥8	R
Gentamicin	≥16	R	≥16	R
Imipenem	≥16	R	≥16	R
Meropenem	8	R	≥16	R
Nitrofurantoin	32	S	≤16	S
Pip/Tazo	≥128	R	≥128	R
Tobramycin	≥16	R	≥16	R
Trimeth/Sulfa	≥320	R	≤20	S
Tigecycline	≥8	R	≤0.5	S

*According to CLSI except for tigecycline which are as defined by the FDA.

using Qiagen plasmid kits (Qiagen Inc., Toronto, Ontario, Canada) and transformations were conducted using electro-competent *E. coli* DH10B with selection on 0.5 mg/L meropenem. Plasmid restriction fragment length polymorphism analysis was done using *Bgl*II and plasmid Inc grouping carried out by a PCR method [9].

Results

The isolated *K. pneumoniae* was found to be resistant to multiple antibiotics including carbapenems and the glycylcycline, tigecycline (Table 1).

The modified Hodge test done on the isolate was positive suggesting the presence of a carbapenemase. The isolate was identified as a metallo-betalactamase producer using the Rosco KPC+MBL kit (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). PCR and sequence analyses detected NDM-1, SHV-12, TEM-1, CTX-M-15, OXA-1 and CMY-6. The *K. pneumoniae* was identified as N11-02395 and by MLST was ST336 which was not closely related to sequence types associated with the dissemination of NDM-1 in India, Sweden, or the UK [*10*]. Plasmid analysis detected multiple plasmids in *K. pneumoniae* N11-02395. Transformation experiments allowed isolation of a ~110 kb Inc A/C plasmid labeled pNDM11-02395 that harboured $bla_{\text{NDM-1}}$ and $bla_{\text{CMY-6}}$., The transformant was resistant to carbapenems, cephalosporins, and aminoglycosides, but susceptible to aztreonam, ciprofloxacin and tigecycline (Table 1).

Discussion

The identification of the NDM-1 and several other genes that code for resistance to a wide range of antibiotics, provide information on the multidrug resistance profile of the *K. pneumoniae identified* in this patient. The transformant was resistant to carbapenems and cephalosporins, but susceptible to aztreonam (Table 1). This is in keeping with the fact that the plasmid transferred had the NDM gene which causes resistance to virtually all betalactams except aztreonam. The transformant was susceptible to ciprofloxacin and tigecycline while the *K. pneumoniae* isolate being studied was not, suggesting that the genes coding for resistance to these antibiotics were not carried on the pNDM11-02395 plasmid and were therefore not transferred to the transformant. This suggests that this MDR *K. pneumoniae* isolate had several different mechanisms of antibiotic resistance, and not all were being carried on the NDM plasmid, resulting in an extensively drug resistant isolate for which, very few therapeutic options would be available. Although persons colonized with NDM -1 producing organisms may remain asymptomatic, as seen in this patient, clinical isolates with $bla_{\text{NDM-1}}$ have been implicated in urinary tract infections, septicemia, pulmonary infections and peritonitis [1] Such isolates can cause these infections that result in increased morbidity and mortality. The identification of $bla_{\text{NDM-1}}$ in this strain, *K. pneumoniae* N11-02395 ST 336 which is not closely related to sequence types associated with the dissemination of NDM-1 in India, Sweden, or the UK is a novel finding and possibly demonstrates that dissemination of the NDM gene is expanding further among other *K. pneumoniae* strains not usually associated with spread in other parts of the world [10].

In this scenario the patient spontaneously cleared the organism from the urine without the use of antibiotics. This is not unusual and indicates that the patient was only transiently colonized, most likely from the gastrointestinal tract. The fact that no other isolate of this nature has been identified to date in this country makes it very unlikely to be an environmental contaminant.

This case focuses attention on a number of important factors. The first of these is the need to establish global surveillance as a priority issue. The consequences of antibiotic resistance are universal and all the practices that lead to these consequences must be identified and intercepted globally. This case graphically portrays the impact, resistance originating in one part of the world, can have on another very distant part of the world even though prescribing practices may differ. In the English speaking Caribbean, for example, there is a wide range of prescribing practices ranging from countries such as Dominica where one carbapenem is on the formulary and is reserved for life threatening infections and third generation cephalosporins are restricted drugs to countries such as Jamaica and Trinidad, where both of these classes of antibiotics are more freely used [11]. The existence of this discrepancy mandates an informed approach to antimicrobial stewardship tailored to the existing antibiotic landscape of each country.

Although the detection of this carbapenemase producing isolate occurred after over ten years of carbapenem usage in Jamaica, it is interesting to note that its existence was not connected to antibiotic usage in the island but possibly due to the importation of the isolate from India, where the organism is endemic [12]. This patient with a history of recurrent pneumonias from as early as 1 month old, immigrated to Jamaica at age 3 ½ months. He presented to hospital there at 5 months old where he was presumptively diagnosed with tuberculosis and admitted. There have been no other

cases of carbapenem resistant *K. pneumoniae* detected in Jamaica.

Fortunately, the patient's diagnosis required strict infection control measures and these, undoubtedly, helped to prevent spread of this organism in a vulnerable, hospitalized population. The consequences of an outbreak of this organism in Jamaica would have been disastrous as there would have been increased mortality and morbidity because of the lack of effective drugs against carbapenem resistant organisms, certainly in Jamaica. The economic impact of prolonged hospitalization and the additional costs of isolating patients so affected, would have been astronomical considering that with the exception of only a few private rooms, the wards are open, with curtains used to separate patient beds. The social impact of isolation precautions on patients and their relatives is also another unquantifiable factor that should not be overlooked.

Studies detecting the *bla*$_{\text{NDM-1}}$ gene in bacteria such as *K. pneumoniae* and *E. coli* in sewage and tap water in New Delhi, India, [4,5] could easily have unveiled this gene as the poster child for antibiotic resistance, its global spread and the public health consequences of same.

The paucity of many viable options with which to treat infections caused by carbapenem resistant organisms intensifies the threat posed by the spread of antibiotic resistance in the face of a diminishing supply of new antibiotics. The urgent need for increased capacity for global surveillance, even in resource limited countries, the development of antimicrobial strategies appropriate for individual countries and the implementation of appropriate infection control measures to limit the spread of resistant organisms, while seeking to develop new antimicrobial agents, are all critical components of the war against multidrug resistant organisms.

In the absence of demonstrable progress in the above areas however, the increased consumption of antibiotics of all classes will probably continue as desperate doctors grapple with the frustration of an emptying antibiotic quiver.

Conclusion

The detection of NDM-1 *K. pneumoniae* in a patient recently arriving in Jamaica from India, where this organism is endemic, highlights the problem of ongoing antibiotic resistance and its spread. It demonstrates the need for global surveillance to monitor prevailing antibiotic resistance patterns and highlights the effectiveness of prompt infection control measures in preventing the spread of MDR organisms. The novel detection of pNDM11 02395 in *K. pneumoniae* N11-02395 suggests the dissemination of NDM further, among *K. pneumoniae* strains not usually associated with its spread in India and other parts of the world.

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