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Dissemination and spread of New Delhi metallo-beta-lactamase-1 superbugs in hospital settings

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ORIGINAL ARTICLE

Dissemination and spread of New Delhi Metallo-beta-lactamase-1 Superbugs in hospital settings

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Abstract

Objective: To find out frequency of isolation of carbapenem-resistant enterobacteriaceae and the predominantly responsible metallo-beta-lactamasegene in a hospital setting.

Methods: The descriptive, cross-sectional study was conducted from May 2009 to June 2012 at the Aga Khan University Hospital, Karachi, and comprised non-duplicate clinical carbapenem-resistant enterobacteriaceae isolates obtained from different collection units across Pakistan. Kirby-Bauer disk diffusion screening of carbapenem-resistant enterobacteriaceae was confirmed by minimum inhibitory concentration using E-test. Polymerase chain reaction assay was performed to detect blaKPC, blaNDM-1, blaIMP, and blaVIM genes. In addition variable number tandem repeat typing was performed on selected cluster of New Delhi metallo-beta-lactamase-1-positive Klebsiella pneumoniae.

Results: Of the 114 carbapenem-resistant enterobacteriaceae isolates, 104(94%) tested positive for blaNDM-1 gene. At 68(66%), Klebsiella pneumoniae was the most frequent species isolated, followed by E.coli 33(31%). Moreover, 89(78%) of the blaNDM-1 gene positive Klebsiella pneumonia isolates were from the clinical samples of patients admitted to the critical care units and 75(66%) were from neonates and the elderly. Of the 65(67%) patients suffering from bacteraemia and sepsis, 32(57%) had expired, of which 22(60%) were aged <1 month. Variable number tandem repeat analysis of hospital-acquired New Delhi metallo-beta-lactamase-1-positive Klebsiella pneumoniae showed similarities between the isolates.

Conclusion: New Delhi metallo-beta-lactamase-1-positive enterobacteriaceae was found widely disseminated in major hospitals across Pakistan. Patients at extreme ages and those in critical care units were found to be the most affected with fatal outcomes.

Keywords: Carbapenem-resistant enterobacteriaceae, Superbugs, NDM-1, Metallobetalactamase. (JPMA 66: 999; 2016)

Introduction

The frequency of multidrug-resistant (MDR) bacteria is increasing globally. In the developing world, combination of factors such as uncontrolled / inappropriate use of antibiotics, limited laboratory diagnosis and failure of infection-control strategies are commonly implicated as cause of selection and spread of MDR organisms.[1] With ease of international travel, these organisms are moving beyond borders at a rate faster than ever before. More recently, a newly identified mechanism of bacterial resistance to carbapenems, involving a metallo-beta-lactamase (MBL) gene referred as New Delhi metallo-beta-lactamase-1 (NDM-1) has generated a great deal of public alarm owing to its extreme drug resistance.[2] blaNDM-1enterobacteriaceae "new superbugs" first reported in 2010 among strains from India and Pakistan are now recognised as a global threat[3,4] and have been reported from the United States, UK, Sweden and many other countries throughout the world.[5,6] NDM-1-producing strains are of great concern as they exhibit resistance to almost all currently available antibiotics. Therefore, treatment of infections caused by these strains poses a challenge and is associated with increased mortality.[7,8] Moreover, cross-transmission of plasmids carrying resistant gene to other gram-negative bacteria (GNB) endemic in many developing countries (such as Salmonella, Shigella and Cholera) is an impending threat.[9] Recently there has been a concern at international level about increasing number of carbapenem-resistant enterobacteriacea (CRE) isolates, particularly NDM-1 positive strains, from patients returning from hospitals in India and Pakistan.[2]

In Pakistan, carbapenem-resistant (CR) strains of E. coli and Klebsiella pneumonia are emerging fast. This is supported by unpublished clinical laboratory data of the Aga Khan University (AKU), the largest diagnostic centre in Pakistan, which shows an increase in CR rate from zero in 2006 to 2% and 4% in E-coli during 2008 and 2009, respectively.[10] Similarly, CR in Klebsiella pneumonia has risen from 8% in 2010 to 23% in hospitalised patients in

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2011.[10] We have also previously reported emergence and spread of enterobacteriaceae with CTX-M beta-lactamases in Pakistan.[11] These rising trends are alarming and suggest immediate attention to proper surveillance and to ascertaining the mechanism of CR in such isolates. The epidemiological data for NDM-1 and other common MBLs (IMP, VIM, and KPC) in Pakistan is severely limited.

The current study was planned to find out frequency of isolation of CRE and the predominantly responsible MBL gene in our hospital settings. In addition we tried to find out clonality of hospital-acquired Klebsiella pneumonia isolates as well as association between meropenem minimum inhibitory concentration (MIC) and clinical outcome in patients of different age groups.

Materials and Methods

This descriptive, cross-sectional study was conducted from May 2009 to June 2012 at the clinical laboratory of Aga Khan University Hospital (AKUH), Karachi, a 550-bedfacility backed up by a state-of-the-art clinical laboratory. The study comprised clinical isolates of CRE that were screened by disk diffusion method and obtained from clinical samples received for routine culture susceptibility testing.

Extensive research was made pertaining to the prevalence of carbapenemase-producing strains of enterobacteriaceae in published data at national and regional levels. However, the prevalence could not be determined in the absence of sufficient studies, hence the sample size was calculated according to the anticipated frequency of 50%, with 10% of absolute precision, at a significance level of 0.05 (i.e., 5%). The sample size was, therefore, calculated to be 97 samples, which was rounded up to 100.

Samples were collected from collection centres of AKUH in different parts of the country. Only one sample was considered per patient and all duplicates were removed. Patients were contacted via telephone or in person to obtain their informed consent, and complete history and other clinical features were recorded. Isolates were defined as community-acquired after excluding history of exposure to healthcare settings in the last three months in accordance with standard guidelines.[12] The study was reviewed and approved by the institutional ethical committee.

Samples were inoculated on standard culture media and incubated at 37°C.[13] Gram-negative rods (GNR) isolated from samples were identified to species level using API-20 E (bioMérieux, France). Antimicrobial susceptibilities were determined by Kirby-Bauer disk diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) 2011 breakpoints.[14] CR detected on Kirby-Bauer disk diffusion method was confirmed by MIC (µg/ml) using E-test (bioMérieux, Solna-Sweden). Report of CR isolates was promptly communicated to the clinicians for effective patient management.

Polymerase chain reaction (PCR) for detection of blaKPC, blaNDM-1, blaIMP, and blaVIM was performed on CRE isolates using primers as recommended.[15] Variable number tandem repeats (VNTR) typing was performed on NDM-1-positive Klebsiella pneumonia recovered from clinical samples of patients admitted to the intensive care units (ICUs) of different hospitals. The VNTR analysis was performed using eight loci as described previously, with repeat numbers listed in the order (A,D,E,H,I,J,K,L).[16] VNTR on a few extra loci were also performed i.e. N1,N2, N3, N4.

SPSS 19 was used for data analysis. Data was stratified for the known effect modifiers such as age and antibiotic use, and hence analytically controlled. To evaluate statistical relationship between meropenem MIC of the enterobactereaciae isolates and clinical outcome of different age groups, p-value was calculated using Mantel-Haenszel chi-square test.

Results

Of the 114 CRE isolates, 65(57%) were yielded from blood cultures, 32(28%) from urine, while 9(7.9%) and 8(7%) were obtained from pus and tracheal aspirates, respectively. Klebsiella pneumonia was the most commonly isolated species at 73(64%), followed by

Table-1A: Percentage distribution of Enterobacteriaceae species with respect to different Age group studied at Aga Khan University Hospital Karachi 2009-2010.

	Age group	<1 month N=51	1 month-14 years N=7	15-59 years N=33	>60 years N=23	Total N=114
Organism	Klebsiellapneumoniae	40.4%	2.6%	14%	6.1%	63.2%
	E.Coli	2.6%	2.6%	13.2%	13.2%	31.6%
	Citrobacter spp.	-	0.9%	0.9%	-	1.8%
	Enterobacter spp.	0.9%	-	9%	0.9%	2.6%
	Serratia spp.	0.9%	-	-.	-	0.9%

Table-1B: Association between meropenem MIC of the Enterobactereaciae isolates and clinical outcome of different age groupsstudied at Aga Khan University Hospital Karachi during 2009-2010.

MIC ((µg/ml)	Clinical Outcome	Neonate N=40	Child N=5	Adult N=27	Elderly N=16	Total N=88
<=4	Expired	8	1	0	3	12
	Well	9	2	6	3	20
>4	EXPIRED	14	0	6	5	25
	Well	9	2	15	5	31
Statistical significance		0.391	0.414	0.145	1	0.516

MIC: Minimum inhibitory concentration.

Figure: Geographical distribution NDM-1 positive isolates.

Foot note:
Geographical representation of clinical isolates tested NDM-1 positive at the clinical laboratory of Aga Khan University Hospital 2009-2010. Coloured spots represent the AKUH sample collection units from where samples were received, numerical represents number of isolates from that region.
NDM-1: New Delhi Metallo-beta-lactamase-1.

E.coli 35(31%), enterobacterspecies 4(3%), citrobacterspecies 2(2%) and serratiamarcesences 1(1%). Only 7(6%) isolates belonged to patients from community settings (outpatient clinics) while the remainder were identified as hospital-acquired and were from patients admitted to different hospitals in major cities (10 hospitals in Sindh, two in Punjab, and one in Balochistan).Within Sindh, 99(87%) isolates belonged to different hospitals of Karachi (Figure). Moreover, 89(78%) of the blaNDM-1 gene-positive Klebsiella pneumoniae isolates were from the clinical samples of patients admitted to the critical care units (CCUs) and 74(65%) of the blaNDM-1strains were isolated from patients at extremes of ages i.e. 76(67%) from neonates and 36(32%) from the elderly (Table 1A). In this study, 65(57%) patients suffered from bacteraemia and sepsis as evidenced by positive blood culture. As 26(23%) patients were lost to follow-up, outcome analysis was performed on 88(77%). Of them, 37(42%) had expired, of which 22(59%) were aged <1 month (Table-1B). Enterobacteriaceae isolates with meropenem MIC ranges of =<4 µg/ml and >4µg/ml did not show

Table-2: VNTR profile of 28 Klebsiellapneumoniae isolates tested at Aga Khan University Hospital Karachi 2009-2010.

VNTR matching	Isolate No.	Critical Care Setting	VNTR profile (A,D,E,H,I,J,K,L,N_1,$N_2N_3N_4$)
Identical profile	B1	SCU	5387---44413.5
	A1	NICU	5387---44413.5
5 loci matching	A2	NICU	53-1-0-44413.5
	A3	NICU	-------44413.5
	A4	NICU	533401254213.5
	A5	NICU	5386--244413.5
	A6	NICU	5251-9144413.5
	A7	SCU	533-3--42413.5
	A8	NICU	735732-42413.5
	A9	NICU	53510--44-13.5
	A10	NICU	232--6233413.5
	A11	NICU	5387---34413.5
3 loci matching	A12	NICU	233--7-43413.5
	A13	NICU	723530-41413.5
	A14	ICU	5351-2441
	A15	SCU	62-13124441-
	A16	NICU	12--3--7--43.5
	A17	SCU	-4.5
	B2	ICU	53373--4241-
	A18	ICU	-2-----4--4-
	A19	SCU	10.5
	A20	SCU	13313--42-1-
	A21	NICU	12--3--4-443.5
	A22	SCU	10.5
	A23	NICU	-23530143

Isolate number corresponds to A1-23=Hospitals of Karachi, B1-B2= hospitals outside Karachi.
VNTR: variable number tandem repeat.
NICU: Neonatal intensive-care unit.
SCU: Special care unit.
ICU: Intensive care unit.

statistical difference in clinical outcome (p>0.05).

All CRE isolates were extended spectrum beta-Lactamase (ESBL)-positive, hence resistant to penicillin, ampicillin, cephalosporins and aztreonam. Amongst the aminoglycosides, all tested isolates (100%) were resistant to gentamicin and 10(9%) were sensitive to amikacin. Fosfomycin was tested for 29(90.6%) urinary isolates, and was found to be sensitive in 25(86%) of them. All of these CRE strains were found to be susceptible to polymyxin B by disk diffusion method.

Of all the CRE isolates, 107(94%) tested positive for blaNDM-1 gene; 7(6%) NDM-1-negative CRE were also found negative for other carbapenemases (blaIMP, blaVIM, blaNDM-1) gene. Of the 15(13%) of neonatal intensive-care unit (NICU) related isolates which were tested for VNTR profile, 10(67%) displayed either total similarity or profile similarity at 5 loci i.e. showing some degree of relatedness (Table-2).

Discussion

This study indicated NDM-1 to be the commonest cause of CR among the enterobacteriaceae isolates. Both NICUs and adult intensive care units (AICUs) appeared to be the common hub of these highly resistant NDM-1 carrying Klebsiella pneumoniaand E.coli. To confirm this finding, active surveillance-based data is required. Outbreaks of NDM-1 carrying Klebsiella pneumoniae strains in healthcare settings have been reported from other countries as well.[17,19] Recently, two reports of NICU-based NDM-1 producing Klebsiella pneumonia have been published from India and Columbia.[20,21] In our study NDM-1 carrying Klebsiella pneumonia was predominantly from NICUs of different hospitals of Karachi, and their VNTR typing suggested probable outbreak resulting from cross-transmission between neonates of the same as well as NICUs of separate hospitals due to patient transfer. These finding are consistent with literature.[20,21]

Neonatal bacteraemia and sepsis remain a major cause of death in developing countries.[22] Similarly, patients at the other end of the age scale (> 60 years) were also found to be frequently affected. In this group, blaNDM-1 positive E.coli was the commonest cause of urosepsis leading to death. Due to retrospective nature of the study, risk factors for acquisition of CRE have not been evaluated. Other studies done on epidemic spread of MDR organisms (acinetobacterbaumanii and pseudomonas aeruginosa) in ICU settings of Pakistan indicated factors such as mechanical ventilation, catheterisation, prolonged use of antibiotics and long ICU stay[23,24] as possible risk factors.

In the current study, all expired patients were on carbapenem treatment at the time when culture results were communicated to the clinicians. Using carbapenem MIC break point of 4µg/ml (Table-1B), we did not find any statistical difference in clinical outcome of patients. One of the limitations of this study was that statistical analysis of precise carbapenem MIC value of isolate with the clinical outcome was not performed; therefore it was difficult to evaluate its significance. However, keeping in consideration that 65% of isolates had MIC >4 µg/ml (Table-1B) and 30% of isolates had MIC >32 µg/ml, our findings caution against sole use of carbapenem as empirical coverage for suspected gram-negative infections (GNI) in NICUs and AICUs. Treatment options for the infections due to NDM-1 positive isolates are severely limited. In our study polymyxin B was the only antibiotic to which blaNDM-1 positive isolates was susceptible.[25] While use of polymyxins in such a situation is encouraged,

prompt de-escalation is strongly advised to avoid losing this only effective drug currently available in Pakistan.[26]

Seven per cent of the isolates yielded from patients who had not had any exposure to the hospitals in last three months. All of them were residents of Karachi. This is an alarming situation and stresses the need for proactive surveillance.[27] Of particular interest was a cohort of isolates (6%) that were negative for blaNDM-1 and also did not show PCR signals for other enzymes (blaIMP, VIM, NDM-1). Other mechanism of CR such as alteration in outer membrane porins and efflux pumps need to be investigated.

Our findings also highlight the need for rapid methods, for the detection of NDM-1 and other emerging resistance mechanisms in the clinical laboratories. This is essential for effective treatment, infection control measure and active surveillance. Phenotypic screening methods such as Modified Hodge test might miss NDM-1 gene-positive isolates due to variable sensitivity and specificity.[28] While advancedcare-testing methods would be the ultimate turning point in the global control of these superbugs, simple phenotypic methods as opposed to technically-advanced molecular-based tests (difficult to maintain/sustain in developing countries) are the need of time to curtail this global threat.

Small sample size was one of the limitations of this study. Therefore, its VNTR findings cannot be generalised and further studies are warranted.

Conclusion

NDM-1-positive enterobacteriaceae are widely disseminated in the hospitals of major cities of Pakistan. We recommend caution against sole use of carbapenem as empirical coverage for suspected GNIs in NICUs and AICUs. There is a need for rapid methods for the early detection of NDM-1 and other emerging resistance mechanisms to reduce mortality.

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Conflict of Interest: None to declare.

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