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CARBAPENEMASE PRODUCING BACTERIA IN CLINICAL ISOLATES FROM TERTIARY CARE UNIVERSITY HOSPITAL IN BANGLADESH

Economics

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ABSTRACT

Carbapenem-resistant bacteria are concerned for increased outbreaks of resistant infections and mortality rates across the world. Carbapenemase-producers are particularly important because of location of their enzyme-coding gene in mobile genetic elements, facilitating dissemination of resistance in different bacteria. We studied 1212 bacterial isolates in clinical samples in Bangladesh, where 600 were multi-drug resistant (MDR) and 114 (114/600,19%) were resistant to carbapenem. Forty-eight isolates (48/600,42.1%) were producing blaNDM, blaVIM and blaOXA -type carbapenemase. Many of them were also co-harboring extended spectrum b-lactemase (ESBL)-producing genes. This study introduced several novel bacteria carrying resistant genes and variants of NDM and OXA-type in Bangladesh. Evident burden and distribution of MDR bacteria in Bangladesh indicates that the developing regions in Asia are potential resource for surveillance of unrecognized threat to global health and thus this region should be thoroughly monitored to prevent spreading of antibiotic resistant infections across the globe.

KEYWORDS

Carbapenemase; blaNDM; blaOXA

INTRODUCTION:

Infections caused by carbapenemase-producing Gram negative bacteria pose major challenges for current available antimicrobials. Indeed, these enzymes are worrying as they compromise the efficacy of almost all b-lactams (except aztreonam), including carbapenems. Emergence of resistance by production of carbapenemase enzyme is particularly important since the carbapenemase-gene are located in mobile genetic elements, facilitating the dissemination of resistance among different bacteria (1-2).

There is a huge burden of Gram-negative resistance in Asia, particularly in south and south-east Asia (3). A study from Bangladesh showed 55 Gram-negative organisms, out of 403 (13.6%) clinical isolates, were imipenem resistant and majority of these organisms were *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumoniae* (4). We aimed to evaluate the prevalence of carbapenemase producing bacteria and their enzyme variants in clinical isolates collected from the largest tertiary care university hospital in Dhaka, Bangladesh.

METHODS AND MATERIALS:

Clinical samples were cultured on blood agar media and yielded growth were identified by chemical tests and their susceptibility to antibiotics were determined in Bangabandhu Sheikh Mujib Medical University (BSMMU) laboratory by disc-diffusion method following Clinical and Laboratory Standards Institute (CLSI) protocol (5). The organisms were defined multi drug resistant (MDR) according to an international expert panel opinion (6), and identified MDR bacteria were preserved in casitone medium. Thus, MDR bacteria isolated between Augusts to October, 2015 were send to our laboratory in Tokyo. Here, we cultured the isolates in selective agar medium (CHROMagar mSuperCARBA, France) to identify carbapenem resistant bacteria. Carbapenemase producing capacity among carbapenem-resistant bacteria were determined by carbapenemase-inactivation method (CIM) (7, 8). Identification of selected bacteria were further determined in Japan laboratory by AutoSCAN-4, USA and concomitantly, MIC of imipenem and meropenem were determined by E-test (BioMérieux, France). Their DNA were harvested using kits (QIAGEN, Germany) and each DNA was amplified in presence of selective primers (Table 1). DNA of Enterobacteriaceae were further amplified by multiplex PCR in presence of mixed CTX-M primers. The amplicons of those bacteria producing NDM and OXA-48-like type of carbapenemases, were purified by QIAquick PCR purification kit (QIAGEN, Germany) and sequenced to compare with associated sequences in the BLAST database (http://blast.ncbi.nlm.nih.gov).

Table 1: Sequences of primers used for antimicrobial resistance gene detection

Target	Primer	Oligonucleotide sequence (5'–3')	Size (bp)	Tm (oC)
blaKPC	KPC-F	ATGTCACTGTATCGCCGTCT	887	60
	KPC-R	TTTTCAGAGCCTTACTGCCC		
blaOXA-48-like	OXA-48A-F	TTGGTGGCATCGATTATCGG	743	60
	OXA-48B-R	GAGCACTTCTTTTGTGATGGC		

blaIMP	IMP-uni-F	GAATAGRRTGGCTTAAYTCTC	188	52
	IMP-uni-R	CCAAACYACTARGTTATC		
blaVIM	VIM-uni-F	GTTTGGTCGCATATCGCAAC	382	56
	VIM-uni-R2	AATGCGCAGCACCAGGATAG		
blaNDM-Full	NDM-F	ATG GAA TTG CCC AAT ATT ATG CAC	813	62
	NDM-R	TCA GCG CAG CTT GTC GGC		
blaOXA-like (Multiplex)				
OXA-23-like	OXA-23-F	GATCGGATTGGAGAACCAGA	501	52
	OXA-23-R	ATTTCTGACCGCATTTCCAT		
OXA-24-like	OXA-24-F	GGTTAGTTGGCCCCCTTAAA	246	52
	OXA-24-R	AGTTGAGCGAAAAGGGGATT		
OXA-51	OXA-51-F	TAATGCTTTGATCGGCCTTG	353	52
	OXA-51-R	TGGATTGCACTTCATCTTGG		
OXA-58-like	OXA-58-F	AAGTATTGGGGCTTGTGCTG	599	52
	OXA-58-R	CCCCTCTGCGCTCTACATAC		
blaCTX-M (Multiplex)				
Group-1	CTX-M-1-F	GCGTGATACCACTTCACCTC	260	55
	CTX-M-1-R	TGAAGTAAGTGACCAGAATC		
Group-2	CTX-M-2-F	TGATACCACCACGCCGCTC	341	55
	CTX-M-2-R	TATTGCATCAGAAACCGTGGG		
Group-8/25/26	CTX-M-8-F	CAATCTGACGTTGGGCAATG	207	55
	CTX-M-8-R	ATAACCGTCGGTGACAATT		
Group-9	CTX-M-G-9	ATCAAGCCTGCCGATCTGGTTA	293	55
	CTX-M-9-R	GTAAGCTGACGCAACGTCTGC		

RESULTS:

A total of 12075 clinical samples were cultured, from where 1212 bacteria were isolated. Susceptibility test revealed that 600 (49.5%) isolates were MDR and among those 114 (19%) isolates yielded growth in Chrom-Carba medium. CIM detected 48 (42.1%) isolates were producing carbapenemase (Table 2) and those were collected from urine (19/48, 39.6%), tracheal aspirate (12/48, 25%), blood (11/48, 22.9%), pus (4/48, 8.4%) and sputum (2/48, 4.2%) samples.

PCR revealed total 3 types of carbapenemases in 48 isolates (Table 3) and those were mostly NDM type (33/48, 68.7%). Other type of carbapenemase includes OXA (13/48, 27.1%) and VIM (3/48, 6.25%) types and no isolate was positive for KPC and IMP. All E.coli, C. freundii and E. cloacae under the list of carbapenem resistant in this study were found exhibiting their resistance by NDM-type carbapenemase production. All OXA producing bacteria were either K. pneumoniae or A. baumannii and VIM was produced by 2 P. aeruginosa and 1 A. baumannii. Among these bacteria only one P. aeruginosa which was collected from urine samples found producing both VIM and OXA-type carbapenemase. Further selection of variants in NDM and

Table 2: MDR bacteria and their carbapenemase producing genes

MDR Isolates (n, %)	Carbapenem resistant (n)	Carbapenemase producer (n)	Encoding genes (n, %)
K. pneumonia (79, 13.2)	34	21	blaNDM (17, 81), blaOXA (4, 19)
E. coli (291, 48.5)	38	11	blaNDM (11, 100)
A. baumanii (79, 13.2)	11	9	blaVIM (1, 11.1), blaOXA (9, 100)
C. freundii (9, 1.5)	5	3	blaNDM (3, 100)
P. aeruginosa (41, 6.8)	24	2	blaVIM (2, 100)
E. cloacae (8, 1.3)	2	2	blaNDM (2, 100)
Others (93, 15.5)	0	0	-
Total (600)	114	48	

MDR; multi-drug resistant, n; number, %; percentage, others include *Salmonellae* spp., *Enterobacter* and *Proteus* spp.

OXA-48 like carbapenemase by gene sequencing and comparison of sequence with BLAST database identified -1, -4, -5, and -7 variants of NDM and -48, -181 variants of OXA-type carbapenemase in isolates. All A. baumannii were carrying OXA-23 like carbapenemase. In 48 carbapenemase producing isolates 37 (77.1%) were belonged to Enterobacteriaece family and among them 32 isolates (66.6%) were co-harboring CTX-M-1 b-lactamase. Interestingly while most prevalent isolates were K. pneumoniae among carbapenemase producing organisms (21/48, 43.8%), there was no KPC-producing bacteria.

K. pneumoniae or A. baumannii and VIM was produced by 2 P. aeruginosa and 1 A. baumannii. Among these bacteria only one P. aeruginosa which was collected from urine samples found producing both VIM and OXA-type carbapenemase. Further selection of variants in NDM and OXA-48 like carbapenemase by gene sequencing and comparison of sequence with BLAST database identified -1, -4, -5, and -7 variants of NDM and -48, -181 variants of OXA-type carbapenemase in isolates. All A. baumannii were carrying OXA-23 like carbapenemase. In 48 carbapenemase producing isolates 37 (77.1%) were belonged to Enterobacteriaece family and among them 32 isolates (66.6%) were co-harboring CTX-M-1 b-lactamase. Interestingly while most prevalent isolates were K. pneumoniae among carbapenemase producing organisms (21/48, 43.8%), there was no KPC-producing bacteria.

Table 3: Carbapenemase producing bacteria, their antimicrobial susceptibility pattern and resistant genes

ID (TMUS)	Specimens	Strains	MIC (mg/ml)		CIM (mm)	Serine-b-lactamase			Metallo b-lactamase							
			Imipenam	Meropenam		*bla*$_{KPC}$	*bla*$_{OXA-48}$ like	*bla*$_{OXA-181}$	*bla*$_{IMP-uni}$	*bla*$_{VIM-uni}$	*bla*$_{NDM}$	*bla*$_{OXA-23}$ like	*bla*$_{OXA-24}$ like	*bla*$_{OXA-51}$ like	*bla*$_{OXA-58}$ like	*bla*$_{CTX-M}$
3480	Urine	*E. coli*	>32	>32	31	-	-	-	-	-	-5					M-1 group
3482	Pus	*E. coli*	>32	>32	31	-	-	-	-	-	-5					M-1 group
3483	Urine	*E. coli*	32	32	31	-	-	-	-	-	-5					M-1 group
3500	Urine	*E. coli*	>32	>32	31	-	-	-	-	-	-5					M-1 group
3509	Urine	*E. coli*	>32	>32	31	-	-	-	-	-	-4					M-1 group
3510	Urine	*E. coli*	>32	>32	31	-	-	-	-	-	-7					M-1 group
3523	Urine	*E. coli*	>32	>32	29	-	-	-	-	-	-7					M-1 group
3545	Urine	*E. coli*	>32	>32	32	-	-	-	-	-	-7					M-1 group
3569	Urine	*E. coli*	>32	>32	30	-	-	-	-	-	-5					M-1 group
3570	Tracheal Aspirate	*E. coli*	8	>24	30	-	-	-	-	-	-5					M-1 group
3573	Urine	*E.coli*	>32	>32	30	-	-	-	-	-	-4					M-1 group
3581	Sputum	*K. pneumoniae*	8	>32	30	-	+	-	-	-	-					M-1 group
3587	Sputum	*K. pneumoniae*	>32	2	30	-	-	-	-	-	-1					M-1 group
3589	Blood	*K. pneumoniae*	>32	4	30	-	-	-	-	-	-1					M-1 group
3594	Urine	*K. pneumoniae*	16	8	30	-	-	-	-	-	-1					M-1 group
3602	Urine	*K. pneumoniae*	8	12	32	-	-	-	-	-	-1					M-1 group
3603	Urine	*K. pneumoniae*	16	>16	33	-	-	-	-	-	-1					M-1 group

3605	Pus	*K. pneumoniae*	>32	>32	32	-	-	-	-	-	-5					M-1 group
3606	Blood	*K. pneumoniae*	6	6	32	-	-	-	-	-	-1					M-1 group
3607	Urine	*K. pneumoniae*	12	8	31	-	-	-	-	-	-1					M-1 group
3608	Blood	*K. pneumoniae*	6	6	31	-	-	-	-	-	-1					M-1 group
3610	Blood	*K. pneumoniae*	4	4	31	-	-	-	-	-	-7					M-1 group
3612	Blood	*K. pneumoniae*	8	>32	30	-	-	-	-	-	-1					M-1 group
3613	Urine	*K. pneumoniae*	12	8	30	-	-	-	-	-	-1					M-1 group
3617	Blood	*K. pneumoniae*	>32	1.5	31	-	-	-	-	-	-1					M-1 group
3621	Urine	*K. pneumoniae*	>32	>32	30	-	-	-	-	-	-1					M-1 group
3622	Blood	*K. pneumoniae*	6	4	30	-	-	-	-	-	-1					M-1 group
3632	Pus	*K. pneumoniae*	>32	>32	30	-	+	+	-	-	-					-
3633	Tracheal Aspirate	*K. pneumoniae*	>32	16	30	-	+	+	-	-	-					-
3636	Pus	*K. pneumoniae*	16	>32	30	-	+	+	-	-	-					M-1 group
3658	Tracheal Aspirate	*P. aeruginosa*	>32	>32	30	-	-	-	-	VIM	-					
3688	Urine	*A. baumannii*	>32	>32	13	-	-	-	-	VIM	-	+	-	+	-	
3689	Tracheal Aspirate	*A. baumannii*	>32	>32	30	-	-	-	-	-	-	+	-	+	-	
3690	Tracheal Aspirate	*A. baumannii*	>32	>32	30	-	-	-	-	-	-	+	-	+	-	
3691	Tracheal Aspirate	*A. baumannii*	>32	>32	30	-	-	-	-	-	-	+	-	+	-	
3692	Tracheal Aspirate	*A. baumannii*	>32	>32	15	-	-	-	-	-	-	+	-	+	-	
3693	Tracheal Aspirate	*A. baumannii*	>32	>32	31	-	-	-	-	-	-	+	-	+	-	
3694	Tracheal Aspirate	*A. baumannii*	>32	>32	10	-	-	-	-	-	-	+	-	+	-	
3695	Tracheal Aspirate	*A. aumannii*	>32	>32	16	-	-	-	-	-	-	+	-	+	-	
3696	Tracheal Aspirate	*A. baumannii*	>32	12	12	-	-	-	-	-	-	+	-	+	-	
3699	Urine	*E. cloacae*	>32	8	30	-	-	-	-	-	-5					-
3707	Urine	*E. cloacae*	24	0.5	30	-	-	-	-	-	-1					M-1 group
3711	Urine	*C. freundii*	32	32	30	-	-	-	-	-	-7					M-1 group
3717	Blood	*C. freundii*	8	0.5	30	-	-	-	-	-	-1					M-1 group
3718	Blood	*C. freundii*	2	2	30	-	-	-	-	-	-1					M-1 group
3775	Tracheal Aspirate	*P. aeruginosa*	>32	>32	30	-	-	-		VIM	-					
3779	Blood	*K. pneumoniae*	4	3	30	-	-	-	-	-	-1					-
3782	Blood	*K. pneumoniae*	6	6	30	-	-	-	-	-	-1					-

DISCUSSION:

Gram-negative bacteria producing NDM-1 has been reported in clinical samples (4, 9), fecal samples (10) and in wastewater (hospital-adjacent and community) samples (11) from Bangladesh. In these studies, isolated bacteria mainly causing infections and producing NDM-1 includes K. pneumoniae, P. aeruginosa and A. baumannii. Islam et al. reported that common NDM-1 producing organisms among clinical isolates in Bangladesh were K. pneumoniae and A. baumannii (11). As first, we are reporting NDM-4, -5 and -7 producing bacterial isolates in clinical samples in Bangladesh and these variants were commonly seen in E. coli isolates. A very recent study reported NDM-3 and -4 variant in E.coli in water samples from Bangladesh (12). Our study is also the first evidence of NDM-1 producing pathogenic C. freundii and E. cloacae in Bangladesh. Concomitantly, NDM-7 producing C. freundii and NDM-5 producing E. cloacae isolation from Bangladesh patients are also our novel findings. Moreover, we first reporting K. pneumoniae producing NDM-5 and 7 variants of NDM in Bangladesh. VIM was reported from Bangladesh along with A. baumannii and Providencia rettgeri (11), and we, as first, reporting this in P. aeruginosa.

OXA-48 type mainly observed in Enterobacteriaceae which is increasingly reported with outbreaks and case reports across the world (13). In Singapore, OXA-181, were isolated from two Bangladeshi patients (14). To our knowledge, no report about isolation of OXA-48 and/or -181 producing organisms in Bangladesh is available. In this study we first identified OXA-48-type carbapenemase in 4 K. pneumoniae isolates in 4 individual samples obtained from patients in Bangladesh and among them 3 were producing OXA-181-type carbapenemase. We found A. baumannii isolates from Bangladesh patients were concomitantly harboring plasmid mediated OXA-23-like gene and chromosomally encoded OXA-51-like gene, suggesting possible wide distribution of these enzymes producing organisms in this country.

We observed that majority of isolated Enterobacteriacae are concomitantly producing carbapenemases and ESBL (Table 3). A recent review explaining treatment options for carbapenemase producing bacterial infections noted that currently available antibiotics may not be sufficiently effective for the treatment of all types of carbapenemase producers by monotherapy, indicates selection of antibiotics for organisms producing both carbapenemase and ESBL are extremely complicated and it is currently more concern by the emergence of KPC-producing K. pneumoniae resistant to colistin (15). Irrational prescription practice and over-the-counter availability of antibiotics in developing countries confers abuse of drugs which is crucial for developing new mechanisms of protection in organisms against available antibiotics (15). Because of several

limitations in our study like, unavailability of total patient information from where 12075 samples were collected, selection of hospital where patients are charged for their laboratory tests and inclusion of both in- and out-patients from only one hospital, although we can't conclude our result as a true prevalence rate of MDR bacteria in Bangladesh, but collection of 600 MDR isolates from a referral hospital (1500 bed hospital) within 3 months reflects the possible burden of resistant bacteria in this region. Moreover, isolation of MDR bacteria from BSMMU hospital, where patients are drained from every corner of Bangladesh, suggesting risk of wide dissemination of resistant gene across the country. Nationwide resistant-bacteria surveillance program is not available in Bangladesh where developed countries can contribute in this regard by sharing their experiences and technologies. It would be worth to conduct multi-center AMR bacteria surveillance programs in south-east Asian region to facilitate development of effective antibiotics and prevention of emerging antibiotic resistant infections across the globe.

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