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Molecular epidemiology & therapeutic options of carbapenem-resistant Gram-negative bacteria

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***Background & objectives*: The growing incidence and the wide diversity of carbapenemase-producing bacterial strains is a major concern as only a few antimicrobial agents are active on carbapenem-resistant bacteria. This study was designed to study molecular epidemiology of carbapenem-resistant Gram-negative bacterial (GNB) isolates from the community and hospital settings.**

***Methods*: In this study, non-duplicate GNB were isolated from clinical specimens, and phenotypic test such as modified Hodge test, metallo β-lactamase E-strip test, *etc*. were performed on carbapenem-resistant bacteria. Multiplex PCR was performed to identify the presence of bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{OXA48}, bla_{OXA23}, bla_{SPM}, bla_{GIM}, bla_{SIM} and bla_{NDM}. Minimum inhibitory concentration (MIC) of colistin, fosfomycin, minocycline, chloramphenicol and tigecycline was also determined.**

***Results*: Of the 3414 GNB studied, carbapenem resistance was 9.20 per cent and maximum resistance (11.2%) was present at tertiary care centre, followed by secondary care (4%) and primary centre (2.1%). Among the carbapenem-resistant bacteria, overall, the most common isolate was *Pseudomonas aeruginosa* (24%). On multiplex PCR 90.3 per cent carbapenem-resistant isolates were positive for carbapenemase gene. The bla_{NDM} (63%) was the most prevalent gene followed by bla_{VIM} (18.4%). MIC results showed that 88 per cent carbapenem-resistant *Enterobacteriaceae* were sensitive to fosfomycin, whereas 78 per cent of *P. aeruginosa* and 85 per cent *Acinetobacter* spp. were sensitive to colistin.**

***Interpretation & conclusions*: Carbapenem resistance in GNB isolates from the community and hospital settings was found to be on the rise and should be closely monitored. In the absence of new antibiotics in pipeline and limited therapeutic options, prudent use of antibiotics and strict infection control practices should be followed in hospital to limit the emergence and spread of multidrug-resistant bacteria.**

Key words Antimicrobial resistance - Carba NP test - carbapenemase - colistin - multiplex polymerase chain reaction - NDM

Carbapenemase-producing bacteria have become a major concern. Earlier only nosocomial pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* had significant carbapenem resistance, however, the emergence of carbapenemases in *Enterobacteriaceae* is a growing public health problem

worldwide because of their high prevalence, wide range of clinical infections, multidrug resistance and rapid dissemination of plasmid-mediated resistance genes from *Enterobacteriaceae* to other organisms. These enzymes confer resistance to the other β-lactam agents as well and are generally co-associated with resistance genes for aminoglycosides, quinolones and have brought us a step closer to the challenge of extremely drug-resistant bacteria[1,2]. This study was designed to study molecular epidemiology of carbapenem-resistant bacterial isolates from community and hospital settings from north India and further explore therapeutic options for management of infections caused by carbapenem-resistant Gram-negative bacteria (GNB).

Material & Methods

The present study was conducted from August 2014 to July 2016 at the department of Microbiology, Ganesh Shankar Vidyarthi Memorial Medical College (GSVM), Kanpur, India. The clinical specimens were collected from primary Health Centre Kalyanpur, district hospital Kanpur and LLRM Hospital, a tertiary care centre attached with GSVM Medical College, Kanpur. The study was cleared by the Institutional Ethics Committee.

Non-duplicate GNB isolated from various specimens were identified using conventional techniques[3]. Antimicrobial susceptibility was performed by Kirby Bauer disk diffusion method[3] and minimum inhibitory concentration (MIC) breakpoints of carbapenems for the isolates which were resistant by disc diffusion testing was determined by *E*-test (BioMérieux, France). Further to look for treatment options for these carbapenem-resistant isolates MIC of other antibiotics such as fosfomycin, minocycline, chloramphenicol and tigecycline was also determined using E Strip and colistin MIC was determined using broth microdilution method, results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines[4].

The isolation of genomic DNA of carbapenem-resistant bacteria was done by QuiAmp mini DNA extraction kit (Qualigens, Germany) and multiplex PCR was performed to identify the presence of following genes bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{OXA48}, bla_{OXA23}, bla_{SPM}, bla_{GIM}, bla_{SIM} and bla_{NDM} using the primers and protocol described earlier[5]. New Delhi metallo-β-lactamase (NDM) positive amplicons were sequenced and previously published sequences of NDM isolates retrieved from the National Center for Biotechnology (*http://www.ncbi.nlm.nih.gov*) were used as the reference sequence for result interpretation. Phenotypic tests such as modified Hodge test (MHT)[1], metallo-β-lactamase (MBL), *E*-strip test[1], Neo-Sensitabs Test (Rosco Diagnostica, Denmark) and Rapidec Carba NP Test (BioMérieux, France)[6] were performed on carbapenem-resistant PCR-positive bacterial isolates.

Results & Discussion

A total of 8973 samples were processed and 3414 GNB were isolated; of which 312 (9.20%) isolates were carbapenem-resistant. Maximum resistance (11.2%) was present at tertiary care centre, followed by secondary care (4.0%) and primary centre (2.1%). Amongst the carbapenem-resistant bacteria; overall, the most common isolate was *Pseudomones aeruginosa* (24%) followed by *Acinetobacter* spp. (22%) and *Escherichia coli* (16%) (Table I). In a community-based study from south India Sekar *et al*[7] also documented three per cent carbapenem resistance in members of *Enterobacteriaceae*, however in the treatment guidelines document released by the Indian Council of Medical Research[8], surveillance data were collected and compiled from four tertiary care centres in India, and a high meropenem resistance of 42, 47 and 62 per cent was reported among members of *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*, respectively.

On multiplex PCR 282 of 312 (90.3%) isolates were positive f or carbapenemase gene. The bla_{NDM} 178 (63%) was the most prevalent gene followed by bla_{VIM} (18.4%). The bla_{KPC}, bla_{GIM} and bla_{SIM} were not isolated in this study; bla_{NDM} and bla_{OXA48} were co-observed in 20 per cent isolates (Table II). Sequencing was performed on 178 bla_{NDM} positive isolates and 133 (75%) isolates were carrying bla_{NDM-1} and the rest were harbouring bla_{NDM-5} genes. The findings were in concurrence to previously published reports from India[9,10]. Some NDM-positive isolates were earlier screened for the coexistence of ESBL genes, 16s methyltransferase genes determining aminoglycosides resistance and quinolones resistance determinants and it was found that NDM positive isolates were co-harbouring several other resistance determinants[11]. In contrast to western literature[1], bla_{KPC} was not isolated in this study.

Phenotypic carbapenemase detection test was performed on 261 PCR confirmed isolates. Rapidec Carba NP test, Neo-Sensitabs and MHT and showed a sensitivity of 90, 73 and 20 per cent, respectively. MIC of the isolates resistant to carbapenem was determined

Table I. Aetiology of carbapenem resistant bacteria isolated from different healthcare level

Health-care setting	Total GNB grown	Number and per cent of carbapenem resistant bacteria	Aetiology of carbapenem resistant bacteria	n
Primary	237	5 (2.11)	*Escherichia coli*	3
			Pseudomonas aeruginosa	1
			Acinetobacter spp.	1
Secondary	698	28 (4)	*E. coli*	8
			P. aeruginosa	10
			Acinetobacter spp.	8
			Klebsiella pneumoniae	2
Tertiary	2479	279 (9.75)	*P. aeruginosa*	64
			Acinetobacter spp.	62
			E. coli	39
			K. pneumoniae	22
			Enterobacter sp.	16
			Proteus spp.	15
			Citrobacter sp.	18
			Providencia sp.	12
			Morganella morganii	9
			Alcaligenes faecalis	8
			Stenotrophomonas maltophilia	5
			Unidentified	9
Total	3414	312 (9.20)		312

GNB, Gram-negative bacteria

Table II. Molecular epidemiology of carbapenem resistant Gram-negative bacteria

Organism	Total number isolated	Carbapenemase producing gene								
		NDM	OXA_{48}	*VIM*	*IMP*	OXA_{23}	*KPC*	*SPM*	*GIM*	*SIM*
Members of family Enterobacteriacae (n=144)										
Escherichia coli	50	25	11	6	2	0	0	3	0	0
Klebsiella pneumoniae	24	7	8	5	1	0	0	2	0	0
Enterobacter sp.	16	4	6	2	1	0	0	1	0	0
Proteus spp.	15	8	2	3	0	0	0	0	0	0
Citrobacter sp.	18	10	3	4	0	0	0	0	0	0
Providencia sp.	12	6	0	2	0	0	0	0	0	0
Morganella morganii	9	6	0	2	0	0	0	0	0	0
Non-fermenters (n=159)										
Pseudomonas aeruginosa	75	53	0	14	1	0	0	1	0	0
Acinetobacter baumannii complex	71	51	0	12	0	10	0	0	0	0
Alcaligenes faecalis	8	4	0	2	0	0	0	0	0	0
Stenotrophomonas maltophilia	5	4	0	0	0	0	0	0	0	0
Unidentified bacteria (n=9)										
Unidentified bacteria	9	0	0	0	0	0	0	0	0	0
Total	312	178	30	52	5	10	0	7	0	0

Table III. Antimicrobial susceptibility pattern of carbapenem resistant Gram-negative bacteria (GNB)

Tested bacteria	Colistin (per cent sensitive)	Tigecycline (per cent sensitive)	Minocycline (per cent sensitive)	Chloramphenicol (per cent sensitive)	Fosfomycin (per cent sensitive)
Members of *Enterobacteriaceae* (n=161)	-	64	52	35	88
P. aeruginosa (n=75)	78	-	-	-	-
Acinetobacter spp. (n=71)	85	-	70	-	-

for other antibiotics such as chloramphenicol, colistin, fosfomycin, minocycline and tigecycline (Table III).

Carbapenemases are generally encoded by a genetic element found on different plasmids that may jump from bacteria to bacteria easily causing the rapid emergence of multidrug-resistant bacteria[1]. Thus for carbapenem-resistant isolates MIC was also determined for chloramphenicol, colistin, fosfomycin, minocycline and tigecycline. Fosfomycin which was previously used mainly as oral treatment for uncomplicated urinary tract infections, currently attracts clinicians' interest worldwide. Particularly, the reported activity against pathogens with advanced resistance suggests that this antibiotic may provide a useful option for the treatment of patients with these difficult to treat infections[12]. In our study 88 per cent CRE were sensitive to fosfomycin.

Colistin and polymyxin B have recently regained significant interest as a consequence of the increasing incidence of infections due to carbapenem-resistant bacteria and are reconsidered as last-resort antibiotics[13]. Results of this study demonstrated that 78 per cent of *P. aeruginosa* and 85 per cent *Acinetobacter* spp. were sensitive to colistin. Indian data on colistin resistance from ICMR document[8] reported colistin resistance of 10 per cent in *P. aeruginosa* and 22 per cent in *A. baumannii* complex. Another study from north India reported colistin resistance in carbapenem resistance *A. baumannii* as 16 per cent[14]. The use of polymyxins has been challenged by the emergence of the plasmid-borne mobile colistin resistance gene (*mcr-1*)[15]. Since MCR-1 is capable of horizontal transfer between different strains of a bacterial species and after its discovery in November 2015 in *E. coli* (strain SHP45) from a pig in China, it has been found in *E. coli*, *Salmonella enterica*, *Klebsiella pneumonia*, *Enterobacter aerogenes* and *Enterobacter cloacae*[15].

Results of our study show that 70 per cent of *Acinetobacter* spp. and 50 per cent carbapenem-resistant enterobacteriaceae (CRE) were sensitive to minocycline. The study results were in concurrence to other Indian and western literature[16,17]. Tigecycline is a structural analogue of minocycline that was designed to avoid tetracycline resistance mediated by ribosomal protection and drug efflux[18]. It is indicated for the treatment of complicated skin infections, intra-abdominal infections and community-acquired bacterial pneumonia[19]. This study results showed 36 per cent tigecycline resistance in CRE in concurrence with other Indian studies[20,21].

In conclusion, carbapenem resistance in the GNB from the community and hospital settings is on rise and should be closely monitored. In the absence of new antibiotics in pipeline and limited therapeutic options, it is important to prudently use antibiotics and strict infection control practices should be followed in the hospital to limit the emergence and spread of multidrug-resistant bacteria.

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