The Evaluation Of Antibiotic Susceptibility Among Carbapenem Resistant Gram-Negative Bacilli In A Tertiary Care Hospital Of Delhi, India

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1. INTRODUCTION

Antimicrobial resistance is a public health emergency at the global level with the situation being critical in developing countries like India (Kumar et al., 2013). Particularly overwhelming is the challenge posed by multidrug resistant Gram-negative bacilli (GNB) belonging to the family *Enterobacteriaceae, Pseudomonas* species and *Acinetobacter* species (Giske, Monnet, Cars, & Carmeli, 2008; Viswanathan et al., 2012). The carbapenems are β-lactam antibiotics. Those are administeredfor the therapy of infections caused by extended spectrum beta-lactamases (ESBL) producing Gram-negative bacteria (GNB); which causedisorders like meningitis, pneumonia, sinusitis, etc.(Papp-Wallace, Endimiani, Taracila, & Bonomo, 2011). A carbapenemase is an enzyme, which acts as a tool of resistance used by bacteria to defend themselves against various types of antibiotics like imipenem, meropenem, ertapenem, doripenem known as the carbapenem group (Nordmann, Naas, & Poirel, 2011). Their origins are from β -lactamase enzymes, which usually provide resistance against β-lactam antibiotics *viz.* penicillin, cephalosporin, etc. Found in Gram-negative bacilli with usual occurrence of sepsis. It is also associated with other types of resistance like ESBL mechanisms, providing resistance to several other antibiotics like quinolones and aminoglycosides (Zhang et al., 2014). It is very difficult to deal with the infections ofcarbapenemase-producing bacteria; which causes a high mortality rate worldwide.(Khan, J. A., et al 2018) Thus, a rise and worldwide spread of carbapenemase producing GNB, especially Enterobacteriaceae is a major concern because they are often resistant to all β-lactam and other antibiotics (Sun et al., 2016). Majority of carbapenemasesare the members of 3 classes of β-lactamases, namey Ambler class A, B [metallo-betalactamases (MBL)] and D. Molecular studies have determined that Ambler class A and class D are serine enzymes possessing a serine moiety at their active site and Ambler class B or MBLs require a divalent cation as a metal cofactor, usually zinc (Sun et al., 2016, Ahmad, A. et al 2020)). Continuous mutations among the genes encoding β-lactamases and natural selection due to high use of antibiotics results in the development of newer beta lactamases. Among these, transferrable MBLs are of major concern as they hydrolyse almost all drugs in the class (Pasteran, Mendez, Guerriero, Rapoport, & Corso, 2009).

Over the past 10-15 years an alarming rate of dissemination of carbapenemases, especially MBLs to members of Enterobacteriaceaefamily, with few reported epidemics (Nordmann et al., 2011, Singh, H., et al2018). Various studies from India have also reported a prevalence of carbapenem resistance in members of Enterobacteriaceae and non-fermenters.(Mala, A. A. et

al 2019) The majority of those are imipenem hydrolysing enzyme (IMP), Verona integron encoded metallo-β-lactamase (VIM), *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase-1 (NDM-1)(Amudhan, Uma, Arunagiri, & Sekar, 2012; Kumarasamy et al., 2010; Nagaraj, Chandran, Shamanna, & Macaden, 2012, Singh, J. et al 2018).

Carbapenemases can be detected by phenotypic or genotypic methods in the laboratory. Critical zone diameters and MICs are used as screening assays for identification of carbapenemases(CLSI, 2016). Several assays for confirmation of presence of carbapenemases have been described, including modified Hodge test (MHT) (Amjad et al., 2011); carba NP test (Nordmann, Poirel, & Dortet, 2012), modified carbapenem inactivation method (mCIM) (van der Zwaluw et al., 2015), imipenem/imipenem-EDTA disk potentiation test (I/IE test) (Franklin, Liolios, & Peleg, 2006) and PCR/qPCR based molecular tests (Bialvaei, Kafil, Asgharzadeh, Yousef Memar, & Yousefi, 2016). The current study was conducted to analyse the carbapenemase activity in GNB isolates using different phenotypic methods from clinical specimens. Samples have been separated on a daily basis by collection of all carbapenems resistant and subcultured for the performance of three phenotypic tests, namely MHT, MBL and mCIM tests.

2. METHODS AND MATERIALS

The study was conducted over a period of three months (January – March 2017) in a tertiary care hospital of Delhi, India. The clinical specimens were received from various departments of the hospital. Two hundred Gram negative isolates from different clinical specimens like pus, exudates, wound swabs, respiratory samples etc. were processed as per the standard protocols.

Inclusion Criteria

All Gram-negative bacilli belonging to Enterobacteriaceae*, Pseudomonas* spp.*, Acinetobacter* spp.which were:

1. Non-susceptible to any one of the carbapenems, viz. meropenem, imipenem and ertapenem isolated from pus
2. Isolated from pus, wound swabs, sputum, tracheal aspirates, tissues, high vaginal swabs and other miscellaneous samples.

Exclusion criteria

Gram negative isolates sensitive to all carbapenems were excluded.

Culture and Identification

Clinical specimens were inoculated on Blood agar and MacConkey agar and incubated at 37 °C overnight. Gram staining and biochemical tests were performed to identify GNBwith standard methods.

Antimicrobial susceptibility testing (AST)

AST was performed by disc diffusion method to various drugs as per CLSI guidelines(CLSI, 2016)*viz.* eftazidime (30 μg), cefotaxime (30 μg), amikacin (30 μg), netilmicin (30 μg), piperacilin/tazobactam (100/10 μg), cefoperazone/sulbactam (75/30 μg), colistin (10 μg),

ertapenem (10 μg) and meropenem (10 μg), imipenem (10 μg). The following zone diameter (in mm) breakpoints for carbapenem drugs according to CSLI were used.

Acinetobacter spp:

Drugs used	Sensitive	Intermediate	Resistant
Imipenem	>22	19-21	<18
Meropenem	>18	15-17	<14

Pseudomonas aeruginosa:

Drugs used	Sensitive	Intermediate	Resistant
Doripenem	>19	16-18	<15
Imipenem	>19	16-18	<15
Meropenem	>19	16-18	<15

Enterobacteriaceae:

Drugs used	Sensitive	Intermediate	Resistant
Ertapenem	>22	19-22	<18
Imipenem	>23	20-22	<18
Meropenem	>23	20-22	<18

Detection of carbapenemases

All isolates showing intermediate susceptibility or resistance to meropenem/imipenem/ertapenem by the disc diffusion method were further evaluated by modified Hodge test, disk potentiation test for metallo-β-lactamases and modified carbapenem inactivation method.

The sensitivity and specificity were calculated for MHT and mCIM tests as follows:

$$Sensitivity = \frac{True\ positive}{True\ positive + False\ positive}\ x\ 100$$

$$Specificity = \frac{True\ negative}{True\ negative\ +\ False\ negative}\ x\ 100$$

3. RESULT

The study was performed from January to March, 2017. Two hundred isolates were screened during this period in which at least one of the carbapenems was resistant. The isolates were recovered from varied types of specimens including wound swabs (75), pus (68), tracheal aspirates (26), tissue (10), sputum (6), HVS (3) and other miscellaneous specimens (12). *Acinetobacter* species was the most common organism isolated (n=74), followed by *Klebsiella* (n=58), *Pseudomonas* (n=35)*, E.coli* (n=28), *Citrobacter* (n=3) and *Enterobacter* (n=2) (Table 1).

Table 1: The distribution of isolates collected for different organisms

Organism	Number of isolates
Pseudomonas	35
Klebsiella	58
E. coli	28
Acinetobacter	74
Citrobacter	3
Enterobacter	2

According to the antibiogram calculated, third generation cephalosporins (3GC) showed 100% resistance in the case of *Acinetobacter.* High resistance against cephalosporins was also seen in *Klebsiella* (93%) and *Pseudomonas* species (94%). (Figure 3). Other drugs like Amikacin, Piperacillin/Tazobactam, Ciprofloxacin, Netilmicin, Cefoparazone/Sulbactam, Imipenem, Meropenem show mild to higher percentage of resistance ranging from 47% to 97%in different genera. No isolate demonstrated resistance against colistin (Table 2).

Table 2: An antibiogram showing percentage resistance to various drugs in carbapenem resistant isolates**.** Abbreviations: N, Number of isolates; 3GC, cephalosporins, Ak, Amikacin; Cf, Ciprofloxacin; Nt, Netilmicin; Cs, Cefoparazone/Sulbactam; Pt, Piperacillin/Tazobactam; Ert, Ertapenem; Imp, Imipenem; Mer, Meropenem; Coli, Colistin.

Organisms	N	3GC	Ak	Cf	Nt	Cs	Pt	Ert	Imp	Mer	Coli
Acinetobacter	74	100%	95%	96%	54%	47%	49%		93%	93%	0%
E. Coli	28	86%	82%	75%	72%	68%	83%	83%	72%	83%	0%
Pseudomonas	35	94%	89%	83%	74%	80%	83%		80%	83%	0%
Klebsiella	58	93%	92%	97%	92%	97%	98%	95%	95%	97%	0%

Out of 200 isolates tested for carbapenemase enzyme by three different methods (MHT, MBL (synergy test) and mCIM tests) 113,173 and 116 isolates were positive to carbapenemase by MHT, MBL, mCIM tests, respectively (Table 4). While four isolates were indeterminate for carbapenemase by MHT, 38 isolates tested indeterminate for mCIM whereas none for MBL (Figure 4).mCIM test displayed highest positivity rate with 58% positive isolates in comparison to MHT and MBL which showed 56.5% and 86.5% positivity, respectively.

Table 3: Comparison of MHT, MBL, mCIM tests

	Positive	Negative	Indeterminate
MHT	113	83	4
I/IE disk potentiation test	173	27	0

| mCIM | 116 | 46 | 38 |

The sensitivity of mCIM for detection of carbapenemase production as compared to MHT was 65.7% and its specificity was 64.6%. In our study, 173 MBL producing isolates were detected. The sensitivity of MHT to detect MBL producers was 50.3%. In comparison, mCIM test had a sensitivity of 57.2% for the detection of MBL production.

4. DISCUSSION

The members of family Enterobacteriaceae give rise to a variety of clinical infections and are an important reason of infections of urinary tract, blood stream, surgical site, intra-abdominal site etc. *Acinetobacter* species were found to be the major organism isolated in our study. Maximum (41.1%) isolates were obtained from respiratory secretions. Similar studies have also reported respiratory tract infection as a predominant infection caused by *A. baumanii*(Jaggi, Sissodia, & Sharma, 2012; Villers et al., 1998). Kirby Baur disk diffusion method confirmed that all carbapenem resistant isolates were sensitive to colistin. Similar trends were observed by a stud in 2009 (Lascols et al., 2011).

CRE isolation has been associated with nosocomial infection mortality ranging from 29% to 52% (Hyle, Ferraro, Silver, Lee, & Hooper, 2015). Despite therapy, the mortality due to nosocomial pseudomonas pneumonia is approximately 70% (Chastre & Trouillet, 2000). Various studies report that carbapenem resistant *P. aeruginosa* demonstrate resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment (Obritsch, Fish, MacLaren, & Jung, 2004). This is corroborated in our study, with all *Acinetobacter* isolates showing resistance to third generation cephalosporins, 95% showing resistance to amikacin and ciprofloxacin and over half of the isolates were resistant to piperacillin-tazobactam and netilmicin. Similarly, *Pseudomonas* isolates demonstrated 80 to 95% resistance to all classes of drugs. Majority of CRE were also resistant to all drugs except colistin. None of our isolates were resistant to colistin which is the last resort drug available for treatment of CRE and CR-AB. Recent data from CDC has also reported the greatest proportion of antibiotic resistancein*Klebsiella* spp. (Centers for Disease & Prevention, 2013).Three different methods MHT, MBL (synergy test) and mCIM tests were performed during the study period in which 113, 89 and 116 isolates were found to be positive to carbapenemase, respectively. Likewise, 83, 111 and 46 isolates were found to be negative for carbapenemase in MHT, MBL (synergy test) and mCIM tests respectively. While 4 and 38 isolates were indeterminate for carbapenem by MHT and mCIM respectively whereas none for MBL. mCIM test contribute to be the highest test with 58% positive in comparison to MHT. While MBL show 86.5% positive that is the highest prevalence in India. As discussed above, in between MHT and mCIM test has shown sensitivity test of 65.7% and a specificity of 64.6%.

One study evaluated that the sensitivity and specificity of mCIM in carbapenem-resistant Enterobacteriaceae (93% and 100% respectively) and carbapenem-resistant*Pseudomonas* spp. (100% each). The overall sensitivity and specificity of CIM (95.8% and 100%, respectively) and MHT (76.8% and 94.3% respectively) was also determined. The Carba NP test is a rapid and accurate phenotypic method that is recommended by CLSI for carbapenemase detection (Dortet, Poirel, & Nordmann, 2012; Tijet, Boyd, Patel, Mulvey, & Melano, 2013).

5. CONCLUSION

We suggest that carbapenemase production occurs more commonly in GNB of Enterobacteriaceae,*Pseudomonas* spp. and *Acinetobacter* spp. In this particular study we have found that MBL producers are more common in India. mCIM test performed better as compared to MHT for detection of MBL producers. The phenotypic investigation has been performed in our study and it is advisable to use genotypic test as well to know the different types of KPC, NDM and OXA classes in detail. In conclusion, carbapenem resistant Gram-negative bacilli are rampant in our setting and are almost always multidrug resistant. Newer phenotypic tests can be used to detect this resistance for infection control and epidemiological investigations.

6. REFERANCE

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