JMSCR Vol||04||Issue||11||Page 14057-14065||November 2016

www.jmscr.igmpublication.org
Impact Factor 5.244
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v4i11.87

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

A Study on Phenotypic Characterization of Carbapenemases among Gram Negative Bacilli in a Tertiary Care Hospital

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ABSTRACT

Introduction: *The emerging resistance to Carbapenems which are generally considered as life saving drugs to treat infections caused by ESBL and AmpC producing bacteria, has become a serious issue worldwide. It is therefore necessary to detect Carbapenemases to limit the spread of multidrug resistant organisms for effective Antibiotic surveillance and Infection Control in the Hospital.*

Aim & Objectives: *To detect the presence of MBL, KPC Carbapenemase and their Co-Existence among the Carbapenem resistant clinical isolates of Gram Negative Bacilli*

Materials & Methods: *The present study was carried out in a Tertiary care hospital; to detect Carbapenamases among Gram Negative Bacilli by Inhibitor based combined Disc tests in which Phenylboronic Acid and Dipicolinic acid are incorporated onto Meropenem discs.*

Results: *A Total of 718 strains of Gram Negative Bacilli comprising of 516 strains of Enterobacteriaceae and 202 Non-fermenters were included in the study. Out of these 718 strains, 89 strains were resistant to carbapenems, of which 2.5% (18 /718) were KPC (Klebsiella Pneumoniae Carbapenemase –class A) producers, 8.08%(58/718) were MBL (Metallo Beta Lactamases-class B) producers .Co-existence of MBL and KPC was observed in 1.25% (9 /718) of isolates and no mechanism was detected in 4 isolates.*

Conclusion: *Inhibitor based combined disc test is simple and cost effective phenotypic test for detecting Carbapenem resistance in the Laboratory. Antibiotic stewardship programme has to be implemented in Hospital to achieve good Infection control for better patient outcome and reduce the health care costs.*

Key words: *Carbapenemase, KPC, MBL, Enterobacteriaceae, Non-fermenters.*

Introduction

Owing to the inadvertent use of Antibiotics, which has resulted in the growing prevalence of Antimicrobial resistance, Hospital acquired infections caused by especially the Gram Negative Bacilli has become an emerging global threat to mankind. The most common Nosocomial isolates are primarily *Pseudomonas aeruginosa*, Escherichia *coli, Klebsiella spp.and Acinetobacter spp*[1].

The selective pressure exerted by the indiscriminate use of beta-lactam antibiotics have led to the selection of a variety of mutated forms of beta

lactamases such as the ESBLs, AmpC beta lactamases[2]. Introduced in 1980s, Carbapenems are widely considered as the drugs of choice for the treatment of severe infections caused by them.

The emerging resistance to Carbapenems poses a significant therapeutic threat as the treatment options are very limited leading to the use of parenteral Colistin, Polymyxin B and Tigecycline[3]. Carbapenem resistance has been attributed to various causes such as reduced expression of outer membrane proteins, increased efflux systems and production of carbapenemases which can inactivate carbapenems by causing their hydrolysis[4]. Another important cause for Carbapenem resistance is over production of ESBL, or Amp C enzyme in organism with porin loss[5].

The impetus for this study was the increasing problem of carbapenem resistance among Gram-negative bacilli .Therefore, with this background, the study is undertaken to detect the frequency of occurrence and detection of MBL & KPC carbapenemases by phenotypic methods, among Gram Negative Bacilli, which when failed to identify, may lead to inappropriate therapy, treatment failure which not only incur higher health care costs but also leads to increase in mortality rates.

Materials & Methods

This prospective study was conducted in a Tertiary care hospital from January 2016 to June 2016. A total of 718 Gram Negative Bacilli isolated from various clinical samples such as pus, urine, blood, sputum and other body fluids (Ascitic fluid, etc) were taken. The samples were processed and identified by standard bacteriological techniques[6] and Antimicrobial susceptibility testing was done by using commercially available disc (Himedia, Mumbai, India) in accordance with Kirby Bauer's disc diffusion method[7]. Piperacillin-Tazobactam 100/10µg (PT), Gentamicin 10µg (GEN), Amikacin 30µg (AK), Ciprofloxacin 5µg (CIP), Trimethoprim-Sulfamethoxazole 1.25/23.75µg (COT), Ceftazidime 30 µg (CAZ), Ceftriaxone 30 µg (CTR), Cefotaxime 30 µg (CTX), Imipenem 10µg (IPM), Meropenem 10µg (MR), Colistin 25 µg (CL), and Polymyxin B 300U were used in the antibiotic susceptibility tests . Zone sizes were interpreted according to CLSI guidelines[7]. Imipenem resistant isolates were subjected to Inhibitor based combined disc test for detection of Carbapenemases[8, 25].

Detection of Carbapenemases[8,25]:

Detection of MBL and KPC was done by Inhibitor based Combined disk test using Meropenem (MER) (10µg) and Meropenem with 400µg of Phenylboronic acid (MER/PBA) for KPC detection, Meropenem with 1000µg of Dipicolinic acid (DPA) for MBL, and Meropenem with both Dipicolinic acid (1000µg) and Phenylboronic Acid (400µg) for identifying co-existence of KPC and MBL in an isolate[8]. The interpretation of results is as follows:

A ≥ 5mm increase in zone diameter around MER/DPA, when compared to MER alone is seen with MBL production [25] as seen in fig.1.

A ≥ 4mm increase in zone diameter around MER/PBA, when compared to MER alone is seen with KPC production[25] as seen in fig.2.

In the case of the triple combination (MER+DPA+BOR) the zone was compared to MER+DPA and MER+BOR respectively. An isolate possessing both KPC and MBL, would produce a zone around the triple combination disc ≥5 mm than both around [MER+BOR]and [MER+DPA][8]. as seen in fig.3.

Fig.1: MBL Detection[25]

A ≥ 5mm increase in zone diameter around MER/DPA, when compared to MER alone is seen with MBL production[25].

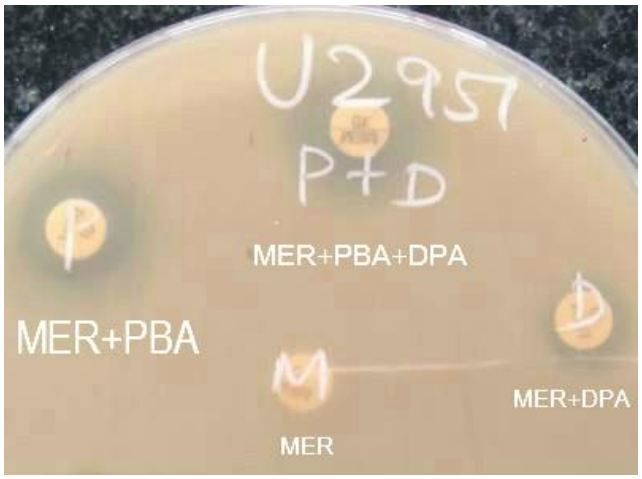


Fig.2: KPC Detection[25]

A ≥ 4mm increase in zone diameter around MER/PBA, when compared to MER alone as seen with KPC production[25].

Fig 3: Both KPC and MBL detection[8]

Isolate possessing both KPC and MBL, would produce a zone around the triple combination disc ≥5 mm than both around [MER+BOR] and [MER+DPA][8].

Results

The study was conducted in a Tertiary care Hospital, for a period of six months from January2016 to June 2016. A total of 718 Gram-negative bacilli isolated from various clinical samples (Urine, Blood. Pus, and Respiratory which includes Sputum, ET secretion) were taken in the study. Total *Enterobacteriaceae* isolates were 516 and total Non-Fermenters were 202. Total Carbapenem resistant isolates were 89 of which 32 isolates belong to *Enterobacteriaceae* family and 57 were Non-fermenters as shown in Table 1.

Prevalence of Carbapenem resistance among Gram negative bacterial isolates was 12.40% [89/718]. Prevalence of Carbapenem resistance among Enterobacteriaceae was 6.20% (32/516). Prevalence of Carbapenem resistance among the Non Fermenting Gram negative Bacilli was 28.22% (57/202).

Most of the Carbapenem resistant isolates are detected in Patients samples from Surgery (47.19%), followed by ICU (20.22%), Medicine (19.10%), Orthopaedics (6.74%), Urology (4.49%), as seen in figure;4. Majority of the Carbapenem resistant isolates were from Pus (38.20%), followed by Urine (30.34%) which is evident as shown in the figure; 5.

In our study, we found that 18/89 (20.22%) were KPC producers, 58/89 (65.17%) were MBL producers and co-existence of KPC and MBL was observed in 9 isolates (10.11%) as shown in the Table no.2.and no mechanism was detected in 4 isolates .

Similarly in our study, we found that 73.03% of our patients (65/89) who harbour Carbapenem resistant isolates were Diabetic as seen in Table 3 and were admitted for amputation of the affected foot , and some for wound debridement and Skin graft. Their duration of Hospital stay was more than 10 days as seen in Table 4. Significance of risk factors and co-morbid conditions for the increase in carbapenem resistant isolates is thus established.

Table: 1: Organism wise distribution of Carbapenem resistant Gram Negative Bacilli isolated:

Organisms	Total number isolated	Total Carbapenem resistant strains	Percentage of resistance
Pseudomonas spp.	64	16	25%
Pseudomonas aeruginosa	91	20	21.98%
Acinetobacter spp.	47	21	44.68%
Escherchia coli	276	6	2.17%
Klebsiella pneumoniae	91	17	18.68%
Klebsiella oxytoca	58	3	5.17%
Enterobacter spp.	19	5	26.32%
Proteus mirabilis	35	1	2.86%
Proteus vulgaris	19	0	0%
Salmonella typhi	1	0	0%
Citrobacter spp.	11	0	0%
Providencia spp.	2	0	0%
Morganella spp.	3	0	0%
Serratia marcescens	1	0	0%
TOTAL	718	89	12.40%

Table: 2: Distribution of Carbapenemase producers among the Gram Negative Bacilli isolated:

Organisms	Number of Carbapenem Resistant Strains Isolated	KPC only n (%)	MBL only n(%)	Both KPC and MBL n (%)
Pseudomonas spp.	16	2 (12.5%)	10 (62.5%)	2 (12.5%)
Pseudomonas aeruginosa	20	2 (10%)	16 (80%)	2 (10%)
Acinetobacter spp.	21	1 (4.76%)	18 (85.71%)	0 (0%)
Escherchia coli	6	4 (66.67%)	1 (16.67%)	1 (16.67%)
Klebsiella pneumoniae	17	7 (41.18%)	6 (35.29%)	4 (23.53%)
Klebsiella oxytoca	3	1 (33.33%)	2 (66.67%)	0 (0%)
Enterobacter spp.	5	1 (20%)	4 (80%)	0 (0%)
Proteus mirabilis	1	0 (0%)	1 (100%)	0 (0%)
TOTAL	89	18 (20.22%)	58 (65.17%)	9 (10.11%)

Table 3: Risk factors associated with Carbapenem resistant Isolates:

Risk factors	No. of isolates
Duration of Hospital stay ≥ 8 days	89/89 (100%)
Catheterisation	53/89 (59.55%)
Intravenous line	68/89 (76.40%)
Previous antibiotic use	55/89 (61.80%)
Mechanical ventilation	35/89 (39.33%)
Endotracheal Intubation	43/89 (48.31%)
Skin graft application	54/89 (60.67%)

Table 4: Co-Morbid conditions associated with Carbapenem resistant Isolates:

Co-morbid conditions	No. of isolates
Diabetes	65/89 (73.03%)
Chronic Renal Failure patients on Dialysis	30/89 (33.71%)
Cancer	2/89 (2.25%)
Tuberculosis	4/89 (4.49%)

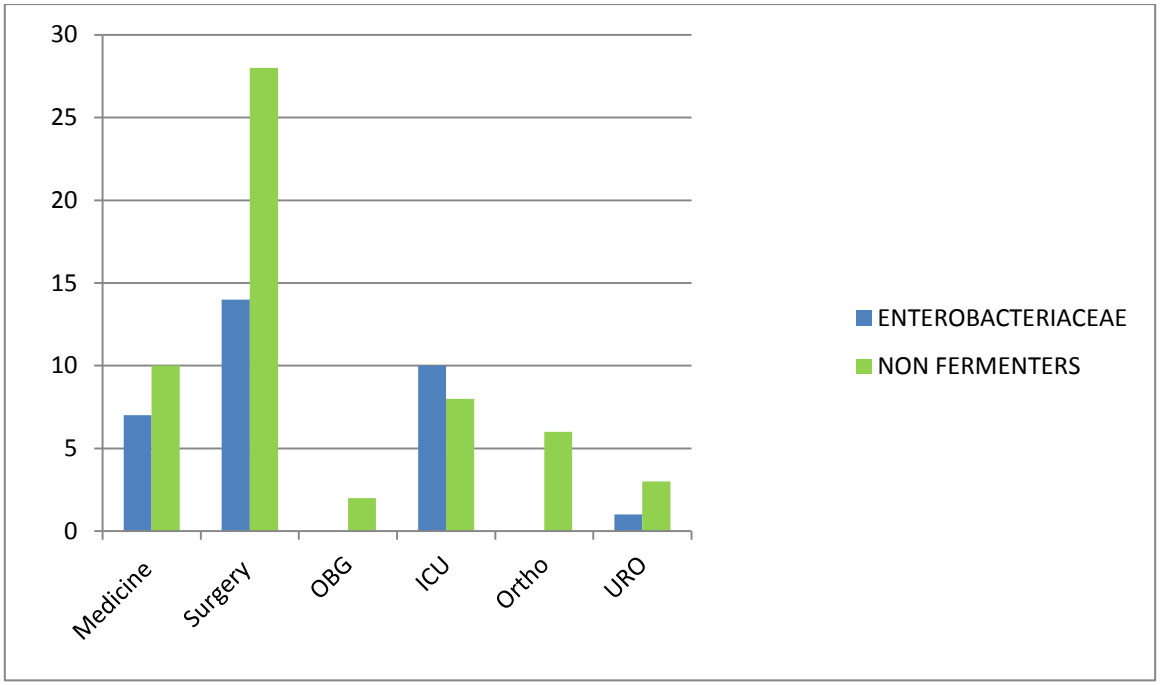


Fig: 4: Ward wise distribution of Carbapenem resistant Gram negative Bacilli in the hospital

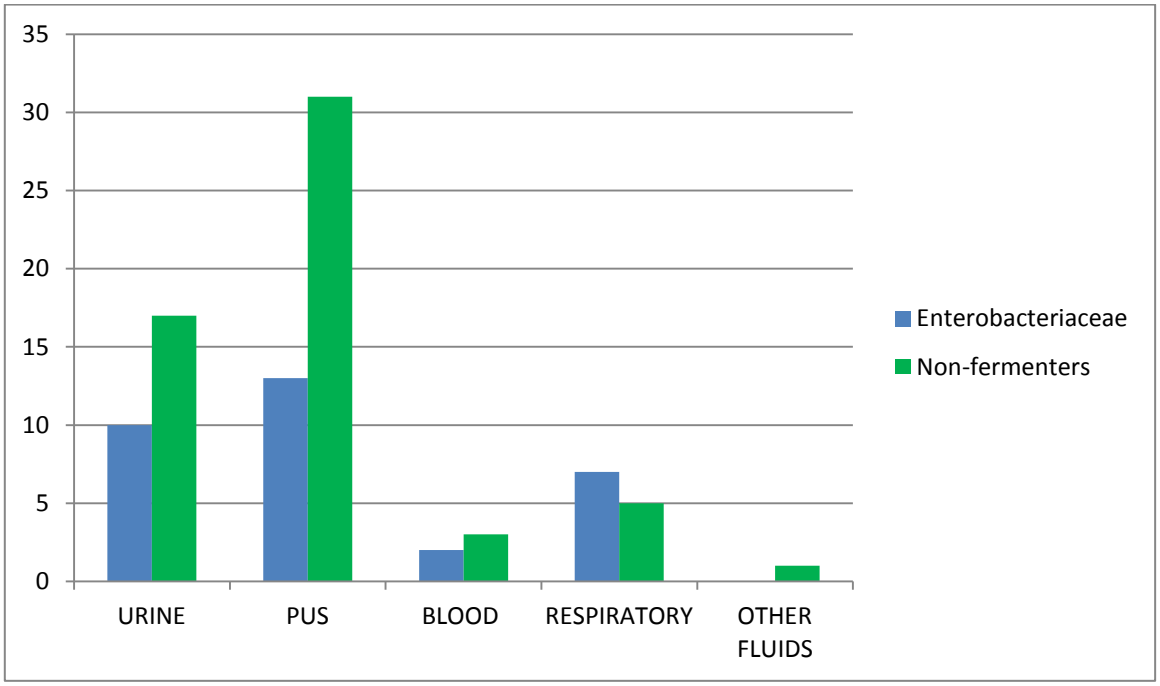
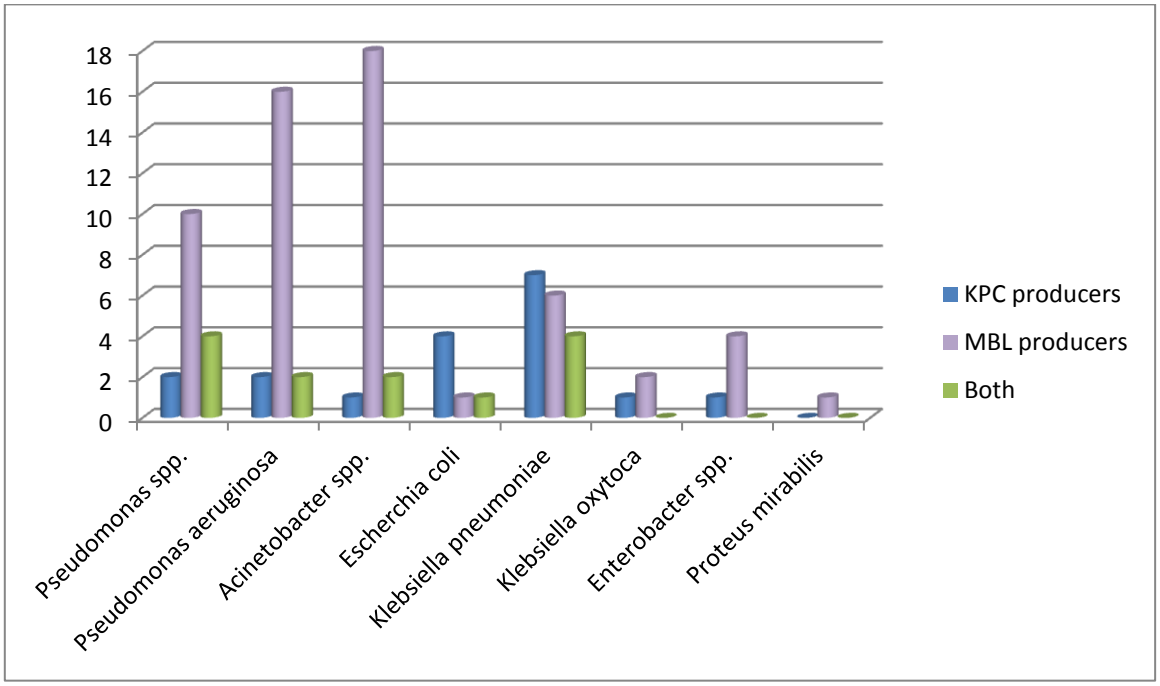


Fig: 5: Sample wise distribution of Carbapenem resistant Gram negative Bacilli isolates in the hospital



Fig; 6: Distribution of Carbapenemase producers among the Gram Negative Bacilli isolated

Discussion

The present study shows the prevalence of Carbapenem resistance among Gram Negative Bacilli is 12.40% which is comparable to the study done by Noyal et al [9], which was found to be 14.3% among Gram negative Bacilli, whereas Shivesh P et al [10] reported 15%, and Sasikala et al [11] reported 10.9%.

Prevalence of Carbapenem resistance among *Enterobacteriaceae* is found to be 6.2% which is comparable to the study done by Datta et al [12] where they found 7.87% carbapenem resistance.

In our study, of the total Gram negative Bacilli isolated, 8.08% of them are MBL producers (58/718) and MBL production is predominant in *Acinetobacter spp*. 31.03% (18/58).

MBL production among Enterobacteriaceae in our study was found to be 2.71% (14/516) while the study done by Datta et al [12] reported 5.75% MBL type Carbapenemase among Enterobacteriaceae strains and KPC production among Enterobacteri-aceae was found to be 2.51% (13/516).

The bacteria having MBL has the potential to spread rapidly (horizontal MBL gene transfer) within the hospital environment and also across continents posing both therapeutic and control management problem. In recent years, MBL genes have spread from *P. aeruginosa* to members of the Enterobacteriaceae (Peleg AY, 2005; Nordmann P, 2002)[13, 14]. Invasive infections with MBL producing isolates are also associated with a higher morbidity and mortality (Walsh TR et al, 2005)[15]. Several studies have reported global increase in the prevalence of MBL-producing nonfermenting bacilli and Enterobacteriaceae (Walsh TR et al. ,2005; Garza-Ramos U, 2008; Toleman MA, 2002; Moayednia R, 2014; Bhattacharya D, 2013; Saha R, 2010; D G Deshmukh, 2011; A. Varaiya, 2008) [15,16,17,18,19,20,21,22,23].

In our study, of the total Gram Negative Bacilli isolated 2.51% (18/718) of them are KPC producers.and Maximum KPC production is observed in *Klebsiella pneumoniae* 38.89% (7/18) and the Co-existence is observed in 9 isolates (1.25%) whereas no significant mechanism was detected in 4 isolates (2 in *Acinetobacter spp*.and 2 *in Pseudomonas spp*.).

Since there are no currently known specific inhibitors for class D carbapenemases, (OXA enzymes), OXA-48 production should be considered for isolates that cannot be confirmed to produce a class A (KPC) or class B (MBL) carbapenemase, and should be investigated by

molecular methods[25]. Thus the Inhibitor based method appears to be the most accurate method in detecting all carbapenemases from class A and class B [26].

Most of the Indian studies reported carbapenemase production in non-fermenters like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* where the incidence ranged from 7% to 65% [24, 27, 28, 29]. However, very few studies that showed carbapenemase production including MBL and KPC in Enterobacteriaceae have been conducted in India so far and according to those reports, the occurrence of these enzymes ranged from 1% to 18% [30,31,32].

Conclusion

Our study reflects an alarming increase in the prevalence of Carbapenem resistant Gram Negative Bacilli in our Hospital. Formulation of Antimicrobial policy and institution of strict Infection control will thereby keeps the prevalence rate under check thereby improving patient health and associated healthcare costs and quality of care.

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