



## RESEARCH ARTICLE

**PREVALENCE OF AmpC  $\beta$  LACTAMASES IN NON-FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL ISOLATES.**Thipperudraswamy .T<sup>1\*</sup>, Shobha Nadigar<sup>2</sup>, Sudhindra.K.S<sup>3</sup>, Mahesh Kumar<sup>4</sup><sup>1</sup>Assistant Professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India<sup>2</sup>Professor and Head, Department of Microbiology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, India<sup>3</sup>Associate Professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India<sup>4</sup>Associate Professor, Department of Microbiology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, India

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**ABSTRACT**

**Background and Objectives:** Non fermenting gram negative bacilli (NFGNB) producing AmpC  $\beta$  lactamases an increasing cause of concern in the hospitals as they produce a therapeutic dilemma for the treating physician. The present study was undertaken to know the prevalence of AmpC  $\beta$  lactamases producing NFGNB from clinical isolates and their antibiotic resistance pattern. **Methods:** A total of 389 NFGNB were recovered from various clinical specimens. All the samples were processed for routine bacterial culture and antimicrobial susceptibility test as per standard protocol. They were further subjected to AmpC  $\beta$ -lactamase detection by Cefoxitin disc test and AmpC disk test. **Results:** Cefoxitin resistance was observed in 92 (23.65%) isolates, of these 66 (16.96%) isolates were confirmed by AmpC disk test. Among *Pseudomonas aeruginosa* 26 (9.48%) were AmpC  $\beta$ -lactamase producing while among *Acinetobacter* species 40 (34.78%) are AmpC  $\beta$ -lactamase producing. Majority of AmpC  $\beta$ -lactamase producers were resistant to Gentamicin (80.3%), Levofloxacin (75.75%), and Gatifloxacin (63.63%), respectively. All isolates were sensitive to Polymyxin B. **Conclusion:** The prevalence of AmpC  $\beta$ -lactamase was 16.96% among NFGNB. Significantly higher resistance rate was observed by these isolates to almost all the drugs routinely used.

**Key words:** NFGNB, AmpC  $\beta$ -lactamase, Cefoxitin disc test, AmpC disk test.

**INTRODUCTION:**

NFGNB are known to account for about 15% of all bacterial isolates from a clinical Microbiology laboratory. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important health care associated pathogen. They have been incriminated in infection such as, septicemia, meningitis, pneumonia, urinary tract infection and surgical site infection.<sup>1</sup>

*Pseudomonas aeruginosa* together with Enterobacterial species, possess a naturally-occurring cephalosporinase. The enzyme when expressed at a low level confers resistance to aminopenicillins, first generation cephalosporins such as cephalothin and cephamycins such as cefoxitin. This Amp C  $\beta$ -lactamase is not inhibited by the currently available beta lactam inhibitors, clavulanic acid, sulbactam and tazobactam.<sup>2</sup>

Gram-negative bacteria have at their disposal a plethora of resistance mechanism that they can sequester and/or evade, eluding the actions of carbapenems and other beta-lactams. The common form of resistance is either

through lack of drug penetration (i.e outer membrane protein (OMP) mutations and efflux pumps), hyperproduction of an AmpC-type beta-lactamase, and carbapenem-hydrolyzing beta-lactamases.<sup>3</sup>

**MATERIALS AND METHODS:**

The present study was undertaken at the Department of Microbiology, Karnataka Institute of Medical Sciences (KIMS), Hubli from Dec 2010 to Nov 2011.

**Source of data:**

Clinical samples such as pus, urine, blood, body fluid etc. obtained from patients admitted in Karnataka Institute of Medical Sciences hospital and received at the department of Microbiology.

**Inclusion criteria:**

Non repetitive, consecutive non-fermenting gram negative bacilli isolated from clinical samples obtained from hospitalised patients (IPD) received during study period.

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### Sample processing:

All the samples were processed for routine bacterial culture as per standard protocol.<sup>4</sup> Smears were prepared on clean glass slides. Gram stain performed and observed for the presence of any gram negative bacilli or gram variable cocco-bacilli. Samples were inoculated into Thio-glycollate broth, chocolate agar, MacConkey's agar and Blood agar . They were incubated at 37<sup>0</sup> C in ambient air for 24 to 48hours. Isolates were identified based on colony morphology, motility and relevant biochemical reactions. All organisms that grew on triple sugar iron agar and produced an alkaline reaction were provisionally considered to be NFGNB and identified further by using a standard protocol for identification.<sup>4,5</sup>

### Antimicrobial susceptibility test:<sup>6,7</sup>

Antimicrobial susceptibility test was carried out with modified Kirby-Bauer disk diffusion method using current CLSI<sup>9</sup> recommendations. Commercially available antibiotic disks (Himedia, Mumbai) were used. The antibiotic susceptibility profile against Gentamicin, Amikacin, Gatifloxacin, Levofloxacin, Cephalosporins (Cefoxitin, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime), Piperacillin-Tazobactam, Imipenem and Polymyxin B were studied. *Pseudomonasaeruginosa* ATCC 27853 was used as control strain.<sup>6</sup>

The isolates were further subjected to following tests:

AmpC  $\beta$ -lactamase production detected by using Cefoxitin disk and AmpC disk test.

### AmpC $\beta$ -lactamases production:<sup>7</sup>

- Screening for AmpC  $\beta$ -lactamase production was done by using Cefoxitin disk test (30 $\mu$ g).<sup>9</sup>

### Interpretation:

1. Reduced susceptibility to cefoxitin (zone <18mm) were considered screen positive and selected for confirmation of AmpC  $\beta$ -lactamase production.
- Phenotypic confirmation of AmpC  $\beta$ -lactamase<sup>6</sup> was done by AmpC disk test: A lawn culture of *Escherichia coli* ATCC 25299 prepared on MHA plate. Sterile disks (6 mm) moistened with sterile saline (20  $\mu$ l) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates incubated overnight at 37<sup>0</sup>C.

### Interpretation:

- A positive test appears as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk.
- A negative test will have undistorted zone.<sup>6,7</sup>

### Statistical analysis:

Chi square test was used with appropriate correction to see the significance of difference between the sensitivity of various drugs in AmpC producing strains using SPSS software.  $p \leq 0.05$  was considered significant.



Figure 1: Amp C disc test:

a) and b) Amp C disc test positive showing clear zone of indentation.

c) Amp C disc test negative showing no zone of indentation.

**RESULTS:**

A prospective study was conducted to know the prevalence of different  $\beta$ -lactamases among non-fermenting gram negative bacilli isolated from various clinical specimens received at the Department of Microbiology, Kamataka Institute of Medical Sciences, Hubli, during the period Dec 2010 to Nov 2011.

2758 bacterial isolates 389 (14.1%) were Non-fermenting gram negative bacilli recovered from various clinical

specimens like pus (207), sputum(61), urine(55), ear discharge (31), blood (8), cerebrospinal fluid (8), pleural fluid (6), ascitic fluid (6), post operative drain (3), aspiration from liver abscess (2), corneal scraping (1) and tracheal secretion (1).

92 screen positive isolates, dear distortion of zone of inhibition of ceftazidime was observed in 66(16.96%) isolates by AmpC disk test.

**Table 1: Confirmation of AmpC  $\beta$ -lactamases by AmpC disk test:**

Organism	No. of AmpC screen positive isolates	AmpC disk test positive isolates no (%)
<i>Pseudomonas aeruginosa</i> (274)	40	26(9.48)
<i>Acinetobacter calcoaceticus-baumannii</i> complex(99)	49	37(37.37)
<i>Acinetobacter Lwoffii</i> (10)	1	1(10)
<i>Acinetobacter hemolyticus</i> (6)	2	2(33.33)
TOTAL (389)	92(23.65%)	66(16.96)

Among the 389 total isolates 92(23.65%) were screen positive and 66 (16.96%) were confirmed as AmpC producers by AmpC disk test.

- Of the 389 isolates, 88 showed reduced susceptibility to ceftazidime and ceftazidime. Four isolates i.e. 3 *Pseudomonas aeruginosa* and 1 *Acinetobacter calcoaceticus-baumannii* complex, also showed blunting of the inhibition zone of ceftazidime adjacent to ceftazidime disk. Therefore a total of 92 (23.65%) isolates were presumptively considered as AmpC producers.
- A total of 66(16.96%) isolates were confirmed as AmpC  $\beta$ -lactamase positives by Amp C disc test.
- Graph 1 shows the proportion of Amp C positive and Amp C negative organisms.
- Amp C production was seen in 37 (37.37%) of *Acinetobacter calcoaceticus-baumannii* complex followed by 26 (9.48%) of *Pseudomonas aeruginosa*.
- Graph 2 depicts the Amp C isolates from different clinical samples.

**Table 2: Prevalence of AmpC  $\beta$ -lactamase among different non-fermenting gram negative organisms.**

Organism	AmpC $\beta$ -lactamase positive no (%)
<i>Pseudomonas aeruginosa</i> (274)	26 (9.48)
<i>Acinetobacter calcoaceticus-baumannii</i> complex (99)	37 (37.37)
<i>Acinetobacter Lwoffii</i> (10)	1 (10)
<i>Acinetobacter hemolyticus</i> (6)	2 (33.33)
TOTAL (389)	66 (16.96)

**Table 3: Co-existence of Extended spectrum  $\beta$ -lactamases, AmpC  $\beta$ -lactamases among different organisms.**

Organism (no)	ESBL and AmpC positive no (%)
<i>Pseudomonas aeruginosa</i> (274)	13 (4.74)
<i>Acinetobacter calcoaceticus-baumannii</i> complex (99)	23 (23.23)
<i>Acinetobacter Lwoffii</i> (10)	1 (10)
<i>Acinetobacter hemolyticus</i> (6)	2 (33.3)
TOTAL (389)	39 (10.02)

39 (10%) isolates demonstrated the coexistence phenotype of both Extended spectrum  $\beta$ -lactamase and AmpC  $\beta$ -lactamases.

Table 4: Co-existence of AmpC $\beta$ -lactamases and Metallo $\beta$ -lactamases among different organisms.

Organism (no)	AmpC and Metallo $\beta$ -lactamase positive no (%)
<i>Pseudomonas aeruginosa</i> (274)	4 (1.45)
<i>Acinetobacter calcoaceticus-baumannii</i> complex (99)	7 (7.07)
<i>Acinetobacter Lwoffii</i> (10)	0
<i>Acinetobacter hemolyticus</i> (6)	0
TOTAL (389)	11 (2.82)

11 (2.8%) isolates demonstrated the coexistence phenotype of both Metallo $\beta$ -lactamase and AmpC  $\beta$ -lactamases.

Table 5: Distribution of AmpC $\beta$ -lactamases positive non-fermenting gram negative bacilli isolate in the hospital ward.

Wards	AmpC $\beta$ -lactamase positive no (%)
Surgery	25(37.87)
Medicine	20(30.3)
Orthopedics	4(6.06)
Burns	2(3.03)
ENT	3(4.54)
OBG	9(13.63)
Pediatric	3(4.54)
Ophthalmology	0
NICU	0
Total(389)	66(16.96)

(Graph 5) depict distribution of Amp C  $\beta$ -lactamase isolates in the hospital.

Maximum number of the Extended spectrum  $\beta$ -lactamase, AmpC and Metallo $\beta$ -lactamase harbouring non-fermenting gram negative bacilli isolates were obtained from the Surgery, Medicine and Orthopedics wards.

Table 6: Comparison of Antibiotic resistance pattern of AmpC positive and AmpC negative Non-fermenting gram negative bacilli.

Antibiotics	AmpCnegativeNFGNB n=323		AmpC positive NFGNB n=66		p value
	Resistant	%	Resistant	%	
Gentamicin	85	26.31	53	80.3	0.01
Amikacin	47	14.55	31	46.96	0.05
Gatifloxacin	74	22.91	42	63.63	0.05
Levofloxacin	96	29.72	50	75.75	0.05
Cefipime	50	15.47	30	45.45	0.05
Ceftazidime	150	46.43	66	100	0.05
Cefoxitin	51	15.78	66	100	0.0001
Piperacillin-tazobactam	27	8.35	26	39.39	>0.05

AmpC producing organisms are more drug resistant, difference was statistically significant towards all the antibiotics used in the present study.

Table 7: Comparison of Antibiotic sensitivity pattern of AmpC β-lactamase positive Non-fermenting gram negative bacilli.

Antibiotics	AmpC β-lactamase positive NFGNBn=66	
	Sensitive	%
Gentamicin	13	19.69
Amikacin	35	53.03
Gatifloxacin	24	36.36
Levofloxacin	16	24.24
Cefipime	36	54.54
Piperacillin-tazobactam	40	60.6
Imipenem	42	63.63
Polymyxin B (300µg)	66	100

Table 8: Analysis of the risk factors for non-fermenting gram negative bacilli infection by AmpC positive isolates.

Risk factors	AmpC positive No (n=66) (%)
Burns (30)	2(3.03)
Carcinomas (18)	8(12.12)
Catheterization(136)	40(60.6)
Chronic ailment(116)	24(36.36)
Diabetis mellitus. (18)	3(4.54)
HIV Positive(9)	3(4.54)
Hospitalization of 5 days or more (200)	52(78.78)
ICUs (Intensive care units) (6)	1(1.51)
Neurological Disorders(6)	2(3.03)
Sepsis (9)	1(1.51)
Surgical Intervention(173)	49 (74.24)

The major risk factors for infection with AmpCβ-lactamases producing non-fermenting gram negative bacilli were hospitalization of 5 days or more, surgical intervention and catheterization.

**DISCUSSION**

Nonfermentative gram-negative bacilli (non-fermenters) cause a significant number of infections, particularly in the hospitalised patients and immunocompromised hosts. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most common nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent.<sup>8</sup>

Infections caused by *Pseudomonas aeruginosa* are difficult to treat as the majority of isolates exhibit varying degrees of innate resistance. Acquired resistance is also reported by the production of plasmid mediated AmpC beta lactamase, extended spectrum  $\beta$ -lactamase and metallo  $\beta$ -lactamase (MBL) enzymes. With the increase in occurrence and types of these multiple  $\beta$ -lactamase enzymes, early detection is crucial.<sup>9</sup>

Antimicrobial treatment of the nosocomial infections caused by these agents may be compromised by multiple drug resistance to  $\beta$ -lactams, aminoglycosides and fluoroquinolones. Imipenem, a broad spectrum beta-lactam antibiotic and the first carbapenem to be used for clinical use, is an important drug for treatment of such infections. Imipenem offers the advantage of being more stable to most  $\beta$ -lactamases than the third generation cephalosporins. Unfortunately paralleling its increasing use in the west, resistance to imipenem has increased mainly among gram negative bacilli and particularly *P. aeruginosa*.<sup>8</sup>

Resistance rates vary from country to country. Overall, isolates from Latin American countries show the lowest susceptibility rates to all antimicrobial agents followed by Asian-Pacific isolates and European strains. Strains from Canada exhibit the best global susceptibility testing results.<sup>8</sup>

In the present study, 389 (14.1%) isolates were non-fermenting gram negative bacilli recovered from various clinical specimens at the department of Microbiology, Karnataka Institute of Medical Sciences, Hubli from Dec 2010 to Nov 2011. Out of which 274(70.43%) were *Pseudomonas aeruginosa*, 99 (25.44%) were *Acinetobacter calcoaceticus-baumannii* complex, 10 (2.57%) were *Acinetobacter lwoffii* and 6(1.54%) were *Acinetobacter hemolyticus*. Study conducted by Malini A, Deepa E K, et al. reported nonfermenting gram negative bacilli isolation rate as 4.5%. *Pseudomonas aeruginosa* as the most common isolate (53.8%).<sup>10</sup>

Maximum number of non fermenting gram negative bacilli were isolated from pus (53.21%) followed by sputum (15.68%) and urine (14.13%). Noyal Mariya Joseph, Sujatha Sistla et al.<sup>11</sup> reported, non-fermenters (77.8%) were the most predominant pathogens causing Ventilator-Associated Pneumonia in the Critical Care

Units and the Medicine Intensive Care Unit (48.3%). Bahera et al.<sup>12</sup> isolated 37.36 % *P. aeruginosa* from bronchoalveolar lavage, 23.07 % from blood, 15.38% from tracheal aspirate.

#### AmpC $\beta$ -lactamases:

We identified 92 (23.65%) isolates as possible AmpC screen positive based on resistance to cefoxitin.<sup>6,9,13,14</sup> Among the 92 screen positive isolates only 66 (16.96%) were confirmed as AmpC producers by AmpC disk test. Cefoxitin resistance in non AmpC producers may be due to lack of permeation of porin or porin deficient mutants.<sup>15,16,17</sup>

In our study 71.73% of screen positive isolates were confirmed to produce AmpC  $\beta$ -lactamase. Similarly in the study conducted by Coudron PE et al.<sup>18</sup> among cefoxitin resistant isolates 64% were found to be non AmpC producers. Only 36% of screen positive isolates were confirmed to produce AmpC  $\beta$ -lactamase in Singal S et al.<sup>19</sup> study. Chatterjee S S, Karmacharya R, et al.<sup>20</sup> observed, cefoxitin resistance was evident in 97% isolates while only 59.4% isolates were confirmed to be AmpC  $\beta$ -lactamase producers. Hernandez et al.<sup>21</sup> demonstrated that interruption of a porin gene by insertion sequences is a common type of mutation that causes the loss of porin expression and increased cefoxitin resistance. Ananthan et al.<sup>22</sup> in Chennai got similar results where resistance to cefoxitin was mediated by both AmpC  $\beta$ -lactamase production and loss of OMP.<sup>12</sup> Other studies have reported 58.5-80.9% of the screen positive isolates confirmed as AmpC producers by AmpC disc test.<sup>28,29,26,30,25</sup>

Jennifer A et al. stated that plasmid-mediated AmpC  $\beta$ -lactamases have been responsible for nosocomial outbreaks of infection and colonization.<sup>17</sup>

Four isolates i.e. 3 *Pseudomonas aeruginosa* and 1 *Acinetobacter calcoaceticus-baumannii* complex, also showed blunting of the inhibition zone of ceftazidime adjacent to cefoxitin disk which may be due to inducible AmpC  $\beta$ -lactamase (6.06%) i.e. chromosomal encoded AmpC<sup>6,23</sup> which correlates with 7% of inducible AmpC  $\beta$ -lactamase in study conducted by Supriya U et al.<sup>18</sup> and Rodrigues C et al.<sup>24</sup>

Presently, all plasmid mediated AmpC  $\beta$ -lactamases have similar substrate profile to chromosomal AmpC  $\beta$ -lactamases. Only difference is chromosomal AmpC  $\beta$ -lactamases are inducible where as plasmid mediated AmpC  $\beta$ -lactamases are uninducible.<sup>13</sup>

The AmpC disk test provided a simple, convenient, and accurate means of detection of plasmid-mediated AmpC  $\beta$ -lactamases in organisms lacking a chromosomally mediated AmpC  $\beta$ -lactamase.<sup>17</sup>

Different studies have reported the prevalence of AmpC $\beta$ -lactamases ranging from 6-26%.<sup>26,28,29,30</sup> NoyalMariya Joseph, SujathaSistla et al.<sup>11</sup> reported that AmpC  $\beta$ -lactamases were produced by 60.7% of the members of non-fermenters. Coudron PE et al.<sup>18</sup> quoted very low occurrence 1.2% of AmpC in their study at USA.

In our study AmpC  $\beta$ -lactamase was observed in 37 (37.37%) of *Acinetobactercalcoaceticus-baumannii* complex followed by 26 (9.48%) of *Pseudomonas aeruginosa*, *Acinetobacterhemolyticus*2 (33.33%) and *Acinetobacterlwoffii*1 (10%).

Other studies in India have reported prevalence of AmpC $\beta$ -lactamase among *Pseudomonas aeruginosa* ranging from 17.3%-22%.<sup>13,14,26,27</sup> and among *Acinetobacter* species ranging from 28.52%-42.8%.<sup>28,29</sup>. Our study correlates with the study of SinghalS et al.<sup>28</sup> with respect to *Acinetobacter* species (28.57%).

#### Distribution among different samples:

Majority of Amp C producing organisms were isolated from pus 35 (53%). Study by Basak et al. quoted AmpC  $\beta$ -lactamase producing *Pseudomonasaeruginosa* isolates 44.7% from urine, 25.5% from pus and 17% from sputum.<sup>13</sup> Other studies have observed range of AmpC  $\beta$ -lactamase producing NFGNB from pus (25.5-56.79%), urine (19.75-44.7%) and blood (3-18%).<sup>13,14</sup>

#### Distribution of the isolates in the hospital:

Significant number of the ESBL, AmpC, MBL positive strains were isolated from Surgery ward 60 (30.15%), 25 (37.87%), 15 (30.61%), followed by Medicine 51 (25.62%), 20 (30.3%), 14 (28.57%) and Orthopedics 32 (16.08%), 4 (6.06%), 6 (12.24%) respectively.

It is apparent that various mechanisms exist for the production of multiple  $\beta$ -lactamases especially in high pressure units like Surgery, Medicine and Orthopedics where newer  $\beta$ -lactams are being routinely prescribed.<sup>31</sup>

K PrabhatRanjan, NeelimaRanjan, et al.<sup>32</sup> reported that *P. aeruginosa* was the most prevalent (29.6%) among all the pathogens isolated from the surgical wound. Anupurba and colleagues<sup>33</sup> quoted 32%, where asHani and colleagues<sup>34</sup> found a prevalence rate of 27.78%.Iraida E. Robledoet al.<sup>35</sup> reported 60% of resistant strains *Acinetobacter* species were from ICU.

Male to female ratio was 1.74: 1.

Amp C isolates 14 (21.21%) were in 11-20 years age group Mean age in the study group is 38.1  $\pm$  18.48 years.

There was no statistically significant difference observed between male and female gender regarding AmpC  $\beta$ -lactamases producers.

#### Antibiotic sensitivity pattern of AmpC producing organisms:

Lower resistance among AmpC producers was observed to cefipime 30(45.45%), amikacin 31(46.96%), and Piperacillin/ Tazobactum26 (39.39%). Higher resistance was observed to gentamicin 53 (80.3%), levofloxacin 50 (75.75%) and gatifloxacin 42 (63.63%).

Bhattacharjeeet al.<sup>14</sup> has reported that 94% of *Pseudomonas aeruginosa* sensitive to Piperacillin/ Tazobactum. Basaket al.<sup>13</sup> observed only 71.7% strains sensitive to Piperacillin / Tazobactum which is comparable with our study.

The only  $\beta$ -lactam antibiotics active against co-AmpC and ESBL producers are carbapenems.<sup>9</sup>

In the study by Supriya U et al. maximum sensitivity (89.1%) was seen with imipenem, followed by moderate activity with piperacillin/tazobactam (51.5%), amikacin (47.5%), carbenicillin (43.5%) and co-trimoxazole (43.5%).<sup>9</sup> Other studies have reported range of Amp C isolates resistance to Gentamicin (30.8-60.9%), Amikacin (17.6-52.5%)<sup>40,42</sup>, Piperacillin/ Tazobactum(0-48.5%)<sup>40,17,41</sup> and Imipenem (0-14%).<sup>9,13,36</sup>

#### Therapeutic options:

AmpC producers are susceptible to fourth generation cephalosporins like cefepime. In our study 36 (54.54%) sensitivity was observed with cefepime. More than 50% Amp C producers were also susceptible to Amikacin, Piperacillin-tazobactam and Imipenem. Supriya U et al reported higher susceptibility rate (89.1%) to imipenem.<sup>18</sup> Few other studies have noted 100% sensitivity to Imipenem.<sup>13,28</sup>

Though piperacillin-tazobactam (Inhibitor combination) not advocated for infections by AmpC producers, we found 60.6% being sensitive to this drug. Study by Khan MKR et al.<sup>37</sup> reported all AmpC producers sensitive to piperacillin-tazobactam.

The cost of imipenem is the limiting factor for using it in a poor country like ours. Thus amikacin and newer fluoroquinolones like gatifloxacin are cost effective alternative for treating such patients.

Imipenem is a carbapenem antibiotic, which is active against *P. aeruginosa* and *Acinetobacter* sp. This drug is highly  $\beta$ -lactamase stable and has an unusual property of causing a post antibiotic effect on gram negative bacteria. It is a small molecule, which can overcome the poor outer membrane permeability of  $\beta$ -lactams for *Pseudomonas* by penetrating through the porinomp D.<sup>38,39</sup>

Piperacillin and imipenem either alone or in combination with amikacin were used for treating the patients not responding to treatment with fluoroquinolones, aminoglycosides and ceftazidime.<sup>8</sup>

### Risk factors for different $\beta$ -lactamase producing non-fermenting gram negative bacilli infection.

In our study the major risk factors for infection with  $\beta$ -lactamase producing non-fermenting gram negative bacilli were Hospitalization of 5 days or more, Surgical intervention and Catheterization.

### CONCLUSION:

- A prospective study conducted to know the prevalence of different  $\beta$ -lactamases among 389(14.1%) non-fermenting gram negative bacilli isolated from various clinical specimens.
- Of these 274(70.43%) were *Pseudomonas aeruginosa*, 99(25.44%) were *Acinetobacter calcoaceticus-baumannii* complex, 10(2.57%) were *Acinetobacter lwoffii* and 6(1.54%) were *Acinetobacter hemolyticus*.
- By AmpC disk test 66(71.73%) of Cefoxitin resistance isolates, were confirmed as AmpC producers.
- Coexistence of ESBL and AmpC producers observed among 39 (10.02%) isolates and AmpC and MBL producers among 11(2.82%) isolates .
- Majority of AmpC producers were susceptible to Imipenem, Amikacin and cefepime. All the isolates were susceptible to Polymyxin B.
- Monitoring and judicious usage of cephalosporins and Imipenem, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with AmpC  $\beta$ -lactamases producers. Maintenance of strict antibiotic policy in the hospital is a must to fight against antibiotic resistance.

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