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RESEARCH ARTICLE

PREVALENCEOF EXTENDED SPECTRUM β LACTAMASES (ESBL) IN NON-FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL ISOLATES.

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ABSTRACT

Background and Objectives: Non fermenting gram negative bacilli (NFGNB) producing Extended spectrum β lactamases (ESBL) are 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 ESBL producing non fermenting gram negative bacilli from clinical isolates and their antibiotic resistance pattern. **Methods:** A total of 389 non fermenting gram negative bacilli 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 ESBL production detection by phenotypic confirmatory double disk diffusion test using ceftazidime with and without clavulanic acid. **Results:** A total of 199(51.15%) isolates were found to be Extended spectrum β-lactamase producers. Majority of *Acinetobactercalcoaciticus-baumaniicomplex* 80 (80.8%) were ESBL producer where as 114(41.6%) of *Pseudomonas aeruginosa*isolates were found to be ESBLproducers. ESBL producing organisms were more drug resistant compared to ESBL non producers; **Conclusion:** The prevalence of ESBL was 51.15% among NFGNB. Significantly higher resistance rate was observed by these isolates to almost all the drugs routinely used.

Key words: NFGNB,Extended spectrum β-lactamase, double disk diffusion test. Pseudomonas, Acinetobacter

INTRODUCTION:

NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum beta- lactamases (ESBLs)[1].

Over the last 4 years, various extended spectrum Beta-lactamases have been found in *P aeruginosa*. These enzymes are penicillinase derivatives (class A β-lactamses), metallo-enzymes (class B enzymes) or oxacillinases (class D enzymes). Most of them confer a high degree of resistance to third generation cephalosporins such as the widely prescribed ceftazidime[2].

Even though plasmid mediated beta lactamases were found nearly 30 years ago in this bacterial species, extended spectrum derivatives have only been reported recently, probably because of detection difficulties and/or the presence of a naturally-occurring AmpC (cephalosporinase) system which once derepressed, may confer ceftazidime resistance[2].

AIMS AND OBJECTIVES:

1. Detection of Extended spectrum β-lactamase producing NFGNB.
2. Study of antibiotic resistance pattern of NFGNB.
3. Risk factors associated with the Extended spectrum β-lactamase producing NFGNB infection.

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 fluidsetc. 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 received during study period.

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Sample processing:

All the samples were processed for routine bacterial culture as per standard protocol[3]. 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 37^0 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[3,4].

Antimicrobial susceptibility test:[5,6]

Antimicrobial susceptibility test was carried out with modified Kirby-Bauer disk diffusion method using current CLSI[5] 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[5].

The isolates were further subjected to following tests:

1. ESBL production detected by phenotypic confirmatory double disk diffusion test using ceftazidime with and without clavulanic acid.[14,18,24].

1. Detection of ESBL:[5]

- Phenotypic confirmatory Double disk diffusion test (Cephalosporin/Clavulanate combination disk):

A suspension of the test isolate equivalent to 0.5 McFarland turbidity was swabbed on Muller Hinton agar plates and disks of ceftazidime (30µg) were placed adjacent to ceftazidime/clavulanic acid (30µg/10µg) disks at a distance of 20mm from each other. After overnight incubation at 37^0 C, the inhibition zones were measured.

Interpretation: If the isolate showed a zone diameter of 5mm or more with ceftazidime/clavulanic acid when compared to ceftazidime disk alone was considered as ESBL producer

Statistical analysis:

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

Ethical consideration:

The protocol for this study was approved by the institutional Ethical Committee. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for Human Experimentation in Biomedical Research[7]. Approval was obtained from the subjects by taking informed consent.

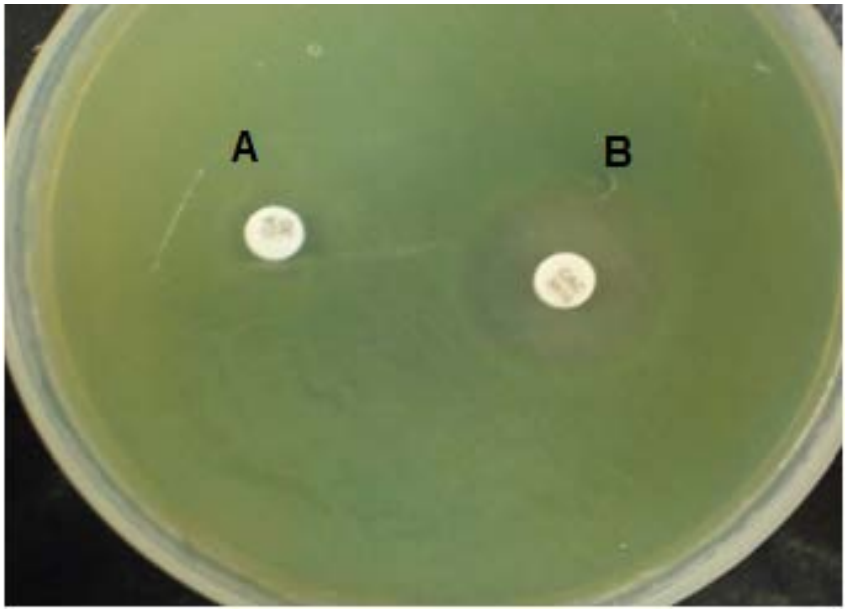

Figure 1: ESBL-Phenotypic confirmatory disk diffussion test:

a) Ceftazidime
b) Ceftazidime with clavulanic acid positive for ESBL production

RESULTS:

Of2758 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 abcess (2), corneal scraping (1) and tracheal secretion (1).

Table 1: Extended spectrum β-lactamase producing nonfermenting gram negative clinical isolates.

Organism	Number of isolates	Extended spectrum β-lactamaseproducing organisms no (%)
Pseudomonas aeruginosa	274	114(41.6)
Acinetobactercalcoaciticus-baumaniicomplex	99	80(80.8)
Acinetobacter lwoffii	10	3(30)
Acinetobacterhemolyticus	6	2(33.33)
TOTAL	389	199(51.15)

- A total of 199(51.15%) isolates were found to be Extended spectrum β-lactamase producers.
- Majority of *Acinetobactercalcoaciticus-baumaniicomplex* 80 (80.8%) were Extended spectrum β-lactamase producer.

Table 2: Prevalence ofExtended spectrum β-lactamase among different non-fermenting gram negative organisms

Organism	Extended spectrum β-lactamase positive no (%)
Pseudomonas aeruginosa (274)	114 (41.6)
Acinetobactercalcoaciticus-baumaniicomplex (99)	80 (80.8)
Acinetobacter lwoffii (10)	3 (30)
Acinetobacter hemolyticus (6)	2 (33.33)
TOTAL (389)	199 (51.15)

Table 4: Distribution ofExtended spectrumβ-lactamasepositive non-fermenting gram negative bacilli isolate in the hospital ward

Wards	Extended spectrum β-lactamase positive no (%)
Surgery	60(30.15)
Medicine	51(25.62)
Orthopedics	32(16.08)
Burns	19(9.54)
ENT	15(7.53)
OBG	13(6.53)
Pediatric	8(4.02)
Ophthalmology	1(0.5)
NICU	0
Total(389)	199(51.15)

Maximum number of the Extended spectrum β-lactamaseharbouring non-fermenting gram negative bacilli isolates were obtained from the Surgery, Medicine andOrthopedics wards.

Table 5: Comparison of Antibiotic resistance pattern of Extended spectrum β-lactamase positive and Extended spectrum β-lactamase negative Non-fermenting gram negative bacilli.

Antibiotics	Extended spectrum β-lactamase negative NFGNB n=190		Extended spectrum β-lactamase positive NFGNB n=199		p value
	Resistant	%	Resistant	%	
Gentamicin	37	19.47	78	39.19	0.05
Amikacin	7	3.68	37	18.59	0.01
Gatifloxacin	30	15.78	59	29.64	0.05
Levofloxacin	44	23.15	79	39.69	0.05
Cefipime	15	7.89	42	21.1	0.05
Ceftazidime	17	8.94	199	100	0.0001
Cefoxitin	0	0	39	19.59	0.0001
Piperacillin-tazobactam	0	0	39	19.59	0.0001

ESBL producing organisms were more drug resistant compared to ESBL non producers; difference was statistically significant towards all the antibiotics used in the present study.

Table 6: Comparison of Antibiotic sensitivity pattern of Extended spectrum β-lactamase positive positive Non-fermenting gram negative bacilli.

Antibiotics	Extended spectrum β-lactamase positive NFGNB n=199	
	Sensitive	%
Gentamicin	121	60.8
Amikacin	162	81.4
Gatifloxacin	140	70.35
Levofloxacin	120	60.3
Cefipime	130	65.32
Piperacillin-tazobactam	160	80.4
Imipinem	162	81.4
Polymyxin B (300µg)	199	100

Table 7: Analysis of the risk factors for non-fermenting gram negative bacilli infection by ESBL positive isolates

Risk factors	ESBL positive No (n=199) (%)
Burns (30)	18(9.04)
Carcinomas (18)	14(7.03)
Catheterization(136)	119(59.79)
Chronic ailment(116)	74(37.18)
Diabetis mellitus. (18)	11(5.52)
HIV Positive(9)	7(3.51)
Hospitalization of 5 days or more (200)	151(75.87)
ICUs (Intensive care units) (6)	4(2.01)
Neurological Disorders(6)	4(2.01)
Sepsis (9)	5(2.51)
Surgical Intervention(173)	139 (69.84)

The major risk factors for infection with Extended spectrum β-lactamases producing non-fermenting gram negative bacilliwerehospitalization 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 *Acinetobacterbaumanii*are the most common nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent.[9]

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 β-lactamase and metallo β-lactamase (MBL) enzymes. With the increase in occurrence and types of these multiple β-lactamase enzymes, early detection is crucial.[8]

Antimicrobial treatment of the nosocomial infections caused by these agents may be compromised by multiple drug resistance to β-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 β-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*[9].

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[9].

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*Acinetobactercalcoaciticus-baumanii complex*, 10(2.57%) were *Acinetobacterlwoffiiand* 6(1.54%) were *Acinetobacterhemolyticus.* Study conducted by Malini A, Deepa E K, et al. reported nonfermenting gram negative bacilli isolation rate as 4.5%. *Pseudomonasaeruginosa* as the most common isolate (53.8%)[4].

Maximum number of non fermenting gram negative bacilli were isolated from pus (53.21%) followed by sputum (15.68%) and urine (14.13%).NoyalMariya Joseph, SujathaSistla et al.[10] 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%). Baheraet al.[11] isolated 37.36 % *P.aeruginosa* from bronchoalveolar lavage, 23.07 %from blood, 15.38%from tracheal aspirate.

Extended spectrum β-lactamases:

Pseudomonads are more versatile than *Enterobacteriaceae* in acquiring drug resistance by various mechanisms[12,13]. Clinical Laboratory Standards Institute (CLSI) guidelines do not describe any method to detect ESBL production in *P.* aeruginosa.[14]

ESBL was detected by phenotypic confirmatory disk diffusion method using ceftazidime with and without clavulanic acid as described by Singhal S et al.[16] Similar method was adopted by various other studies[14,15,19].

The prevalence of ESBL in our study is 199 (51.15%) which is similar to the report 53.6% by Chatterjee S S, Karmacharya R, et al.[17]ESBLs are major problem in hospitalized patients worldwide and have been involved in epidemic outbreak in many institutions in India, Europe and USA[17].

The prevalence of ESBL production among different non-fermenting gram negative bacilli in our study is as follows: *Pseudomonas aeruginosa*114 (41.6%), *Acinetobactercalcoaciticus-baumanii complex* 80 (80.8%),*Acinetobacterlwoffii*3 (30.00%) and *Acinetobacterhemolyticus*2 (33.33%).

Various studies from India have reported prevalence of ESBL producers among *Pseudomonas aeruginosa* ranging from 3.3% to 77.3%[14,18,19,17,20,21,22,23].and among Acinetobacter species ranging from 14.2% to 54.6%.[16,23,25,27] ESBL production in Acinetobacter has been found to be 46% in Turkey[28]and 54.6% in Korea[29].

Distribution among different samples:

Maximum number of ESBL producing nonfermenters were isolated from pus 96 (48.24%) and urine 29 (14.57%) samples[4].

Aggarwal R, Chaudhary U et al.[14] reported maximum ESBL producing isolates from sputum and tracheostomy wound swabs (28.57%). Different studies have reported range of ESBL producing NFGNB isolates in pus (24.13-43.3%), sputum (32.8-28.57%) and urine (19.04-29.4%).[14,31]

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 β-lactamases especially in high pressure units like Surgery, Medicine and Orthopedics where newer β-lactams are being routinely prescribed[30].

In the study by Sinha M et al.significant number of ESBL producing isolates were obtained from patients admitted in ICU 38%, followed by surgical 22%, medical 18% and burns ward 13%[6].

Male to female ratio was 1.74: 1.

Maximum number of 44 (22.11%) of ESBL isolates were in 31-40 years age group,

Mean age in the study group is 38.1 ± 18.48 years.

There was no statistically significant difference observed between male and female gender regarding ESBL producers. Least ESBL production was observed among samples taken from age above 70 years. S K Meharwal**,** NeelamTaneja, et al.[32]reported male to female ratio as 1.09 : 1 and maximum number of patients belonged to the age groups 16-45 years.

Antibiotic sensitivity pattern of ESBL producing organisms:ESBL producing isolates showed statistically significant higher resistance to levofloxacin (39.69%), Cefipime (21.1%), Gatifloxacin (29.64%), Gentamicin (39.19%), Amikacin (18.59%) and cefoxitin (19.59%) compared to ESBL negative isolates.

ESBL-mediated resistance to 3rd Generation Cephalosporin in *P. aeruginosa* as reported by Uma et al.[14] 77.30% and Mathur P et al.[22]64%. Aggarwal R et al.[14] reported that among the combination group, 96.66% of resistance was seen to Ticarcillin/Clavulanate and 63.33% of resistance rate was observed to Ampicillin/Sulbactam. This shows that these combination drugs are unreliable for therapeutic purposes.

ESBL-producing bacteria are frequently resistant to many other classes of antibiotics, including aminoglycosides and fluoroquinolones. This is due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL[34].This fact has also been observed in our study.

Different studies have reported ESBL isolates range of resistance to Gentamicin (65-66.6%), Amikacin (11.1-78.6%), third generation Cephalosporin (64-77.3%), fluoroquinolones (11.1%-27%) and cotrimoxazole (65-70%).[14,22,31,26,33].

Therapeutic options:

Among the 199 ESBL producing organisms lower resistance was observed with amikacin 37(18.6%) gatifloxacin 59 (29.65%) and to cefipime 69 (34.67%). These can be considered potent agents in the treatment of infections caused by these isolates.

Similar pattern was observed by many others.[9,26]. Taneja N et al.[9]quoted that a combination of β-lactam agent and an aminoglycoside has been most commonly used.

Risk factors for different β-lactamase producing non-fermenting gram negative bacilli infection.

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

ESBL production in septicaemic cases of our centre was 2.51%. A much higher prevalence was reported by Jain et al.[24] 86.6% and 32% by Battacharjee A et al.[27]
Zavaskiet al.[35] observed that ICU stay increased the risk for acquisition of MBL producing *P.aeruginosa*. Varaiyaet al.[36] reported 20.8% MBL producers among *P.aeruginosa* isolates in ICU. M Shanthi, Uma Sekar, et al.[37]found more than half of the patients hospitalized in ICU acquire a nosocomial infection.

CONCLUSION:

A prospective study conducted to know the prevalence of different β-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*Acinetobactercalcoaciticus-baumanii complex*, 10(2.57%) were *Acinetobacterlwoffiiand* 6(1.54%) were *Acinetobacterhemolyticus.*
- The prevalence of ESBL producing organisms was 199(51.15%), AmpC β-lactamases was 66(16.96%) and metallo β-lactamases was 49 (12.6%).
- Coexistence of ESBL and AmpC producers observed among 39 (10.02%) isolates and AmpC and MBL producers among 11(2.82%) isolates .

➢ Majority of ESBL producers were sensitive to Imipenem, Piperacillin-tazobactam and Amikacin. 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 ESBL producers. Maintenance of strict antibiotic policyin the hospital is a must to fight against antibiotic resistance.

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