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Prevalence of Non-Fermenting Gram Negative Bacilli and their Antibiotic Sensitivity Pattern at a Tertiary Care Hospital in Tamilnadu, India

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A B S T R A C T

Non-fermenting gram negative bacilli (NFGNB) are known to account for nearly 12-16% all bacterial isolates from a clinical microbiology laboratory. The most common infections caused by these organisms were septicemia, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis, etc. As multidrug resistances being very common and increasing among NFGNB and Pseudomonas and Acinetobacter being the most predominantly isolated NFGNB and its resistance towards colistin and imepenam type of antimicrobials is of major concern. To isolate, identify and characterize the prevalence of NFGNB along with their antimicrobial sensitivity pattern among the patients attending a tertiary care centre in Tamilnadu. A prospective study was conducted in our hospital for a period of two years from Jan2012 to Dec 2013. A total of 5052 clinical specimens were received during the above said period. Out of this 1699 were urine specimens, 315 were pus, 988 blood, 1470 respiratory samples which includes sputum and tracheal secretions, and 580 were other than the above mentioned samples (body fluids, stool, tissue biopsy, vaginal swabs etc). The isolates that showed non lactose fermenting (NLF) colonies on Mac conkey agar and failed to acidify the butts of triple sugar iron (TSI) agar were provisionally considered as NFGNB. Antimicrobial sensitivity was determined by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). Antibiotic discs were placed and plates were incubated at 37°C for 18‑24 hrs. Results were interpreted in accordance with central laboratory standards institute (CLSI) guidelines. In our study out of 5052 clinical samples 517 samples had shown positive for non-fermenting gram negative bacilli with a prevalence of 10.2%. Pseudomonas aeruginosa (53.9%) was found to be the most common organism isolated from the clinical samples followed by *Acinetobacter baumanni* (36.7%). The antibiotic sensitivity pattern varies for different clinical samples but colisitin and imipenam had shown the maximum sensitivity pattern for all the clinical samples. The sensitivity pattern for gentamicin, ceftazidime and ciprofloxacin was in the range of 30 - 70% which means highest resistance was seen with these antimicrobials. It is important to establish the clinical relevance of the isolated NFGB, before are considered as pathogens to avoid unnecessary usage of antibiotics and emergence of drug-resistant strains.

Introduction

The non-fermentative gram-negative bacilli (NFGB) are group organisms with characteristics of aerobic, non-spore forming, gram negative bacilli which either utilize carbohydrates as a source of energy nor degrade them through metabolic pathways other than fermentation. (Winn *et al.,* 2006) These organisms are most commonly recovered from hospital environment, which would cause device related infections and they are often resistant to disinfectants and are considered to be more hazardous as it has the potential to spread from patient-to-patient either via fomites or through the hands of the medical personnel. (Steinberg and Rio, 2005; Quinn, 1998) Now recently these non-fermenting bacteria which are associated with different nosocomial infections are becoming increasingly resistant to the commonly used antibiotics and are also known to produce extended spectrum β- lactamases and metallo β- lactamases. (McGowan, 2006) NFGNB are known to account for nearly 12-16% all bacterial isolates from a clinical microbiology laboratory. The most common infections caused by these organisms were septicemia, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis, etc. (Bergogne-Berezin and Towner, 1996; Mehta *et al.,* 2001) These heterogeneous group includes organisms like *Pseudomonas* spp, *Acinetobacter* spp, *Alkaligenes* spp, *Stenotrophomonas maltophilia, Burkholderia cepaciacomplex* (BCC). Currently *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most commonly isolated non-fermenters pathogenic for humans. Infections caused by other species are relatively infrequent (Fass *et al.,* 1996).

Few studies were done to identify the risk factors for NFGNB infections and it identified immunosuppression (oncology patients on cytotoxic therapy/radiotherapy, organ transplant patients and even patients with AIDS), neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostic and therapeutic procedures were the common risk factors for acquiring NFGNB infections and prolonged hospital stay, use of broad spectrum antibiotic and underlying host factors were found to be the best predictors of the outcome of this infection (Quinn, 1998).

Multidrug resistance is very common and increasing among NFGNB. Carbapenem resistance among *Pseudomonas* and *Acinetobacter* is of major concern. Carbapenemase activity in *A. baumannii* is mainly due to carbapenem-hydrolyzing class D b-lactamases (CHDLs) which is very much specific for this species. These enzymes belong to 3 unrelated groups of clavulanic acid resistant b-lactamases represented by OXA-23, OXA-24, and OXA-58 that can be either plasmid or chromosomally encoded (Poirel and Nordmann, 2006).

In case of *P. aeruginosa* the dominant mechanism of carbapenem resistance is loss of carbapenem specific porin OprD2 (Quinn *et al.,* 1988). There are number of strains which had been identified and proved showing resistance to essentially most of the commonly used antibiotics. Recently few studies conducted in India had identified the antimicrobial susceptibility pattern for NFGNB (Samanta *et al.,* 2011; Gautam *et al.,* 2009; Malini *et al.,* 2009).

Aim

To isolate, identify and characterize the prevalence of NFGNB along with their antimicrobial sensitivity pattern among the patients attending a tertiary care centre in Tamilnadu.

Materials and Methods

A Prospective study was conducted in our hospital for a period of two years from Jan 2012-December 2013. The pathogens data were extracted from Culture and sensitivity registers which was audited for correctness by Head of Microbiology department on weekly basis. The bed strength of our hospital is 550 with 30 beds in the intensive care unit. A total of 5052 clinical specimens were received during the above said period. Out of this 1699 were urine specimens, 315 were pus, 988 blood, 1470 respiratory samples which includes sputum and tracheal secretions, and 580 were other than the above mentioned samples (body fluids, stool, tissue biopsy, vaginal swabs etc).

All the samples were plated on blood agar (BA) and MacConkey's agar (MA) and incubated at 37°C for 48 hours before being reported as sterile. The isolates that showed non lactose fermenting (NLF) colonies on MacConkey agar and failed to acidify the butts of triple sugar iron (TSI) agar were provisionally considered as NFGNB and they were further identified by using a standard protocol for identification. The characters assessed were gram staining morphology, motility (by hanging drop), catalase test, oxidase test, citrate utilization, urea hydrolysis, hemolysis on 5% sheep blood agar, growth on 6.5% NaCl, nitrate reduction, pigment production, indole production, lysine and ornithine decarboxylation, arginine dihydrolase test, growth at 40°C and 42°C, oxidation of 1% glucose, lactose, sucrose, maltose, mannitol, xylose (Hugh and Leifson's medium), growth on 10% lactose agar and gelatin liquefaction test.

Antimicrobial sensitivity was determined by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). Briefly a suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland standards and then plated as a lawn culture onto MHA. Antibiotic discs were placed and plates were incubated at 37°C for 18-24 hrs. Results were interpreted in accordance with central laboratory standards institute (CLSI) guidelines. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. Antibiotics which were used for testing the susceptibility are Amikacin, Gentamycin, Ciprofloxacin, Ofloxacin, piperacillin, PIT, aztreonam, tobramycin, ceftazidime, colistin, Imepenam, meropenam and norfloxacin.

Results and Discussion

In our study out of 5052 clinical samples 517 samples had shown positive for non-fermenting gram negative bacilli with a prevalence of 10.2%. Table 1 shows the total number of non-fermenting gram negative bacilli isolated from different clinical samples. It is seen from the table that *Pseudomonas* and *Acinetobacter* are the two prevalent Non fermenting gram negative bacilli which were isolated and among the *Pseudomonas* genus *P. aeruginosa* and *P. fluorescens* are the two common species which were isolated and among the *Acinetobacter* it was *A.baumannii* and *A. Iwoffii* were the two common species which were isolated. Among all the four organism *Pseudomonas aeruginosa* (53.9%) was found to be the most common organism isolated from the clinical samples followed by *Acinetobacter baumanni* (36.7%). Whereas the prevalence of *Pseudomonas fluorescens* and *Acinetobacter iwoffii* was 6% and 3% respectively. *Pseudomonas aeroginosa* was found to be more common among the pus and urine samples and less common in blood samples, whereas *Acinetobacter baumannii* was found to be more common in blood samples and respiratory tract specimens and least common among the samples taken from pus. Table 2 shows the antibiotic sensitivity pattern among the *Pseudomonas* species from the different clinical samples collected. It is observed from the table that the antibiotic sensitivity pattern varies for different clinical samples but colisitin and Imipenam had shown

the maximum sensitivity pattern for all the clinical samples. Apart from colistin and imipenam, piperacillin and tazobactum combination had shown the next highest sensitivity pattern among the various clinical samples. Piperacillin when used alone had shown the sensitivity pattern only between 50 – 65%. Aztreonam is the next antimicrobial with a maximum sensitivity pattern ranging between 65% - 80%. The sensitivity pattern for gentamicin, ceftazidime and ciprofloxacin was in the range of 30 - 70% which means highest resistance was seen with these antimicrobials. Norfloxacin was sensitive only for urine sample with a sensitivity of only 37%. The antibiotic sensitivity pattern among the *Acinetobacter* species from the different clinical samples collected were shown in Table 3. It is inferred from the table that the sensitivity pattern for *Acinetobacter* was almost similar to that of the *Pseudomonas* organism except for norfloxacin antimicrobial which had shown a sensitivity pattern of 70% in the urine samples.

Non-fermenters are ubiquitous in environment. Although frequently they are considered as commensals or contaminants, the pathogenic potential of NFGNB has been established beyond doubt by their frequent isolation from clinical materials and their association with certain dreadful diseases (Winn *et al.,* 2006; Prashanth and Badrinath, 2004). The available data suggests that NFGNB are remarkable microorganisms because of their epidemiological complexity, propensity to cause outbreaks of infection and antimicrobial resistance (Rahbar and Hajia, 2006; Boroumand *et al.*, 2007; Taherikalani *et al.,* 2008). They are now considered as the most important nosocomial pathogens especially among the immune compromised hosts for causing variety of infections. Resistance to antimicrobials has become more common among NFGNB and the resistance has now extended to all commonly used antibiotics. Multi drug resistance among these organisms makes the treatment more difficult and expensive (Kharangate *et al.*, 2001).

Studies carried out by different researchers have reported varied isolation rates. In the present study the prevalence of NFGNB among the clinical samples was 10.2% and this was almost similar to the results of a study from Chandigarh (Taneja *et al.,* 2003) where NFGNB were isolated in 10% of clinical samples. Contradicting to our study a study from Amritsar (Sidhu *et al.,* 2010) reported a very high isolation rate of 45.9% and another study from Bangalore reported it to be 21.80% (Kumari *et al.,* 2007). A study from Saudi Arabia (Eltahawy and Khalaf, 2001) had shown the prevalence as 16% and a study from Kolar, Karnataka (Malini *et al.,* 2009). reported NFGNB to be isolated only in 4.5% of clinical samples and similarly a study from Brazil had also reported a very low isolation rate of only about 2.18% (Bruno *et al.,* 2011). *Pseudomonas* was found to be commonest non fermenter in all of these studies followed by *Acinetobacter* and this is in concordance to our finding and also the most common species among *Pseudomonas* was *aeroginosa* and among *Acinetobacter* it is *baumannii.*

In our study, highest number of the NFGNB isolates were from respiratory samples, similar to the observations made by others (Taneja *et al.,* 2003; Mishra *et al.,* 1986). NFGNB were commonly involved in wound infections resulting from road traffic accidents and chronic non-healing ulcers. The clinical conditions associated with NFGNB infection in our study included surgical site infection (SSI), ventilator-associated pneumonia (VAP), urinary tract infection (UTI), septicemia and chronic nonhealing ulcer because of endocrinopathy like diabetes mellitus. *P. aeruginosa* and *A. baumannii* were more commonly isolated from respiratory samples, followed by pus and urine.

Table.1 Total number of non-fermenting gram negative bacilli isolated from Different clinical samples

Name of the organism	Pus	Blood	Urine	Respiratory tract	Others	Total
P.aeruginosa	60 (75%)	18 (42.8%)	42 (57.5%)	142 (48.6%)	17 (56.6%)	279 (53.9%)
A.*baumannii*	14 (17.5%)	21 (50%)	24 (32.8%)	121 (41.4%)	10 (33.3%)	190 (36.7%)
P.fluorescens	4 (5%)	1 (2.3%)	4 (5.4%)	21 (7.1%)	2 (6.6%)	32 (6.1%)
A.Iwoffii	2 (2.5%)	2 (4.7%)	3 (4.1%)	8 (2.7%)	1 (3.3%)	16 (3%)
Total	80 (100%)	42 (100%)	73 (100%)	292 (100%)	30 (100%)	517 (100%)

Table.2 Antibiotic sensitivity pattern among the *Pseudomonas* species from the different clinical samples collected

Antibiotics	Pus (n=64)	Blood (n=19)	Urine (n=46)	Respiratory tract (n=163)	Others (n=19)
Amikacin	44 (68.7%)	15 (78.9%)	23 (50%)	93 (57%)	14 (73.6%)
Gentamicin	34 (53.1%)	14 (73.6%)	12 (26%)	93 (57%)	12 (63.1%)
Ceftazidime	34 (53.1%)	12 (63.1%)	29 (63%)	106 (65%)	13 (68.4%)
Ciprofloxacin	46 (71.8%)	12 (63.1%)	17 (36.9%)	120 (73.6%)	12 (63.1%)
Pipericillin	43 (67.1%)	9 (47.3%)	23 (50%)	108 (66.2%)	10 (52.6%)
PIT	53 (82.8%)	18 (94.7%)	41 (89.1%)	132 (80.9%)	16 (84.2%)
Aztreonam	41 (64%)	15 (78.9%)	34 (73.9%)	128 (78.5%)	15 (78.9%)
Tobramycin	40 (62.5%)	16 (84.2%)	18 (39.1%)	93 (57%)	14 (73.6%)
Colistin	58 (90.6%)	19 (100%)	46 (100%)	149 (91.4%)	18 (94.7%)
Imepenam	58 (90.6%)	19 (100%)	46 (100%)	163 (100%)	18 (94.7%)
Norfloxacin	0	0	17 (36.9%)	0	0

Table.3 Antibiotic sensitivity pattern among the *Acinetobacter* species from the different clinical samples collected

Antibiotics	Pus (n=16)	Blood (n=23)	Urine (n=27)	Respiratory tract (n=129)	Others (n=11)
Amikacin	10 (62.5%)	14 (60.8%)	14 (51.8%)	58 (44.9%)	8 (72.7%)
Gentamicin	7 (43.7%)	10 (43.4%)	12 (44.4%)	36 (27.9%)	10 (90.9%)
Ceftazidime	6 (37.5%)	8 (34.7%)	14 (51.8%)	36 (27.9%)	6 (54.5%)
Ciprofloxacin	7 (43.7%)	15 (65.2%)	13 (48.1%)	36 (27.9%)	9 (81.8%)
Pipericillin	7 (43.7%)	13 (56.5%)	14 (51.8%)	58 (44.9%)	5 (45.4%)
PIT	15 (93.7%)	22 (95.6%)	25 (92.5%)	82 (63.5%)	11 (100%)
Aztreonam	13 (81.2%)	20 (86.9%)	18 (66.6%)	53 (41%)	11 (100%)
Tobramycin	13 (81.2%)	15 (65.2%)	17 (62.9%)	64 (49.6%)	11 (100%)
Colistin	16 (100%)	21 (91.3%)	27 (100%)	112 (86.8%)	11 (100%)
Imepenam	16 (100%)	22 (95.6%)	26 (96.2%)	129 (100%)	11 (100%)
Norfloxacin	0	0	19(70.3%)	0	0

Our study is in concordance with reports of other authors for multi-drug resistance among the *P. aeruginosa* (Takeyama *et al.,* 2002; Jombo *et al.,* 2008). High degree of resistance to almost all the routinely used antibiotics was seen and this finding is in line with the study from Chandigarh (Taneja *et al.,* 2003). Though imipenem showed good activity to all the NFGNB, but emerging resistance to this group of drug is of major concern. Previous studies by other authors also have reported carbapenem resistance among NFGNB. (Taneja *et al.,* 2003; Gladstone *et al.,* 2005) In the present study only 5% of *Acinetobacter* species and 8% of *Pseudomonas* species were imipenem resistant and this was in contrast to the findings of Gladstone *et al.,* from Tamil Nadu and Joseph *et al.,* from Pondicherry who have reported the same to be 12.2% and 50% respectively (Taneja *et al.,* 2003; Gladstone *et al.,* 2005). In our study, *Acinetobacter* strains percentage sensitivity for Colistin and Imipenem was 95.6% and 98.3% respectively. Imipenem monotherapy have also been proved effective in many studies (Sidhu *et al.,* 2010).

The strains of *Acinetobacter* species showed higher rate of resistance to ciprofloxacin, amikacin, ceftazidime and piperacillin in a study in Bangalore which is almost in par with the present study (Sinha *et al.,* 2007). Resistance to 3rd generation Cephalosporin, Ceftazidime showed 38% in *Pseudomonas* and 58% among Acenitobacter and it was found to be higher than the studies done by Kumarietal and Mishra *et al.,* which had reported the resistance in the range of 35 – 40% for both *Pseudomonas* and *Acinetobacter* (Mishra *et al.,* 1986; Kumari *et al.,* 2007).

Pseudomonas showed (45%) resistance to Gentamicin which is concordant with (Murugan *et al.,* 2010) who also reported (42.8%) resistance to Gentamicin. In the present study Ciprofloxacin resistance to *Pseudomonas* is (39%) which is very much lower than (Deepak Juyal *et al.,* 2013) who had reported the prevalence of resistance as 73.7%.

The antibiotic susceptibility patterns may change with time and may vary from hospital to hospital. Susceptibility patterns may be altered due to resistance transfer and mutant selection from indiscriminate and excessive use of antibiotics. Furthermore, most of our patients came from rural areas without much exposure to antibiotics. Differences in susceptibility could be attributed to these above mentioned factors.

Non-fermenter gram-negative bacilli though regarded as contaminants are important bacteria causing both hospital- and community-acquired infection. *P. aeruginosa* and *A. baumannii* were the most common NFGB isolated in our study. They have been associated with UTI, septicemia, SSI, VAP and other chronic wound infection and most of them were multidrug-resistant. *P. aeruginosa* has shown good sensitivity to colistin, imipenem, amikacin and cefoperazone/sulbactum combination. *A. baumannii* shows good sensitivity to colistin, imipenem and amikacin. Prompt identification of NFGB upto the species level along with monitoring of their susceptibility patterns are important for proper management of the infection caused by them.

It is also important to establish the clinical relevance of the isolated NFGB, before they can be considered as pathogens to avoid unnecessary usage of antibiotics and emergence of drug-resistant strains. Continued awareness of the need to maintain good housekeeping, equipment decontamination, strict attention to hand washing and isolation procedures are the measures necessary to control the previously unabated spread of these organisms

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