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Epidemiology and Antibiogram of Non fermentative Gram negative bacilli isolated from various clinical samples in a tertiary care teaching hospital in South India

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Abstract

Non-fermentative gram-negative bacilli (NFGNB) are group of aerobic, non-spore bearing bacilli. According to recent studies, these organisms have been increasingly isolated from patients admitted in hospital. This is a prospective study conducted in department of Microbiology from January to March 2018, at SVIMS, a tertiary care teaching hospital, tirupathi. NFGNB were isolated and characterized by various biochemical tests. Antimicrobial sensitivity testing was performed by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines 2018. In our study, 220 clinical samples had shown growth of NFGNB. Peak isolation of NFGNB was observed in pus samples (32%) followed by urine (23%). NFGNB isolated were Pseudomonas spp (64%), Acinetobacter baumanii (18%), Acinetobacter lwoffii (14%), Burkholderia cepacia complex (BCC) (2%), Moraxella spp (1%) and Stenotrophomonas spp (0.4%). Pseudomonas spp showed a good sensitivity for aminoglycosides, penicillins, and cephalosporins. Imipenem resistance was 22%. The most effective antimicrobial against Acinetobacter spp was cefaperazone plus sulbactum. All the NFGNB were 100% sensitive to Polymyxin B. P. aeruginosa and A. baumannii are the most prevalent NFGNB isolated in our study. Their part as healthcare-associated pathogens is well recognized and implicated in causing clinical conditions like urinary tract infections (UTI), septicemia, Surgical site infections (SSI's), and Ventilator associated pneumonia (VAP). Therefore, speciation of NFGNB, and keeping track of their susceptibility patterns, are essential for proper management of the healthcare associated infections resulted by them.

Keywords: Acinetobacter baumannii, Acinetobacter lwoffii, non-fermenting Gram-negative bacilli, Pseudomonas aeruginosa

Introduction

Non-fermentative gram-negative bacilli (NFGNB) are group of aerobic, non-spore bearing bacilli that either do not use carbohydrate as source of energy or degrade them through metabolic pathways other than fermentation. They exhibit resistance to beta-lactams including carbapenems and also to other group of antibiotics**.[**1]

These organisms have been implemented as a cause of clinical diseases including meningitis, septicemia, wound infections and urinary tract infections.[2,3] Recent studies say that, these organisms have been increasingly isolated from hospitalized patients**.[**2]

Non-fermentative gram-negative bacilli (NFGNB), which are saprophytic in nature, now appeared as important health care associated pathogens which were earlier considered to be commensals or contaminants. NFGNB have common characteristics of clinical significance that justify their inclusion and study in a single group. Frequently NFGNB are implicated in causing device related infections. Many a time, NFGNB are resistant to disinfectants and have the capacity to spread from one patient to another through fomites or the hands of the health care workers. [4-7]

One fourth of gram negative bacteremia is contributed by NFGNB as per data from the Surveillance and Control of pathogens of Epidemiological importance (SCOPE) study. [8]

Species level identification of NFGNB in conjunction with monitoring their susceptibility patterns are essential for genuine management of these NFGNB infections. Antibiogram patterns may alter with time and also differs from one hospital to another.[9]

In the laboratory, these organisms are frequently difficult to identify, since they are usually non-reactive in the conventional systems routinely used for the identification of facultative pathogens. The methods used for the identification of these organisms are in large part of cumbersome, requiring a variety of media, reagents and test conditions, time consuming and are susceptible to interpretation error. A variety of commercial systems have been developed for the identification of these organisms.[10-13]

The NFGNB are misdiagnosed as Pseudomonas and Acinetobacter, based on biochemical properties. But, there are many species are present apart from pseudomonas and Acinetobacter.

With this literature in view, the study and speciation were conducted to characterize the non-fermentative gram-negative bacilli (NFGNB) and to study their antibiogram with the available facilities and information.

Materials and Methods

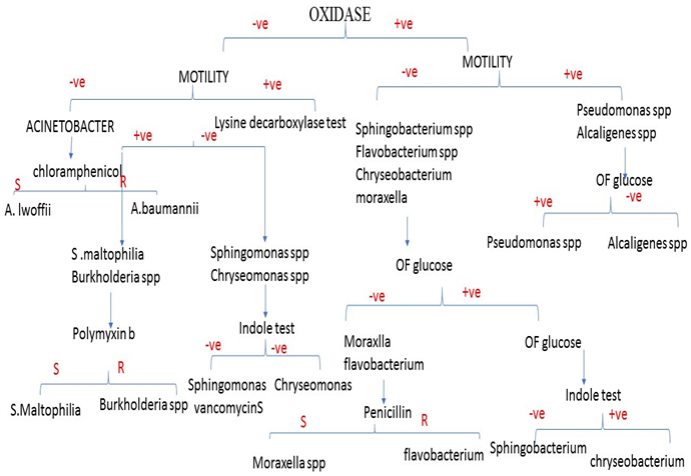
This is a prospective study conducted in our hospital for a period of 3 months from January to March 2018 conducted in department of Microbiology, SVIMS, a tertiary care teaching hospital, Tirupathi.

Microbiological diagnosis was performed on various clinical samples that were submitted to the department of Microbiology as per standard protocols. All the samples were processed on nutrient agar, blood agar, MacConkey agar and chocolate agar. 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 (Figure 1). The characteristics assessed for genus/species identification were morphology, motility (by hanging drop), Gram staining, catalase test, oxidase test, citrate utilization, urea hydrolysis, indole production,

lysine decarboxylation and ornithine decarboxylase test, arginine dihydrolase test, Oxidative/Fermentative (O/F) test (Hugh and Leifson's medium).[14]

Sensitivity testing to antimicrobials was performed on Muller Hinton agar (MHA) by Kirby-Bauer disk-diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines 2018.[15] Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains. Antibiotics which were used for testing the susceptibility are Amikacin (30 µg), Gentamicin (10µg), Cefotaxime (30µg), Cefaperazone+sulbactam (75/10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Cotrimaxazole (1.25/23.75µg), piperacillin-tazobactam (100/10 µg), Aztreonam (30µg), ceftazidime (30µg), Polymyxin (300U), Imipenem (10µg), and meropenem (10µg) and Tigecycline (15µg) on Mueller–Hinton agar were studied. For Polymyxin B, no CLSI guidelines are available for interpretation of disc diffusion technique as regards to NFGNB. For these isolates, the recommendation of Galani et al [16] was adopted. For each isolate data regarding, patient hospital identification number, admission department, type of sample, clinical diagnosis and demographic data were documented. In our study for analyzing the data, we considered intermediate susceptible isolates as resistant by deploying CLSI 2018 breakpoints. [15].

Figure 1: showing the schematic of differentiation of NFGNB [1,17].



Statistical analysis: Data was entered and analyzed using Microsoft Excel. Categorical variables like proportion of bacterial infections across different wards & ICU's, age groups, gender were expressed as percentage. Pattern of microbes, sites of infection and resistance rates were analyzed and expressed as percentage.

Results

In our study, out of 16985 samples, 220 clinical samples had shown growth of NFGNB. NFGNB contributed 1.29% of culture positivity. In our study 60% NFGNB are isolated from male patients and 40% from female patients (Figure 2). NFGNB isolated were Pseudomonas spp (64%), Acinetobacter baumanii (18%), Acinetobacter lwoffii (14%), Burkholderia cepacia complex (BCC) (2%), Moraxella spp (1%) and Stenotrophomonas spp (0.4%) (Figure 3)

Among all the organisms, Pseudomonas aeruginosa (64%) was found to be the most common organism isolated from the clinical samples followed by Acinetobacter baumannii (18%). Among the Acinetobacter spp, two common species isolated were A. baumannii and A. lwoffii.

Among the clinical samples, NFGNB were isolated from pus (32%), urine (23%), blood (19%), body fluids (14%), respiratory tract (10%), and reproductive tract (2%) (Figure 3). Peak isolation of NFGNB was observed in pus samples (32%) followed by urine (23%).

Pseudomonas aeruginosa 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. Pseudomonas spp showed a good sensitivity for aminoglycosides, penicillins, and cephalosporins. Imipenem resistance was 22%.

The antimicrobial susceptibility pattern among the Acinetobacter species isolated from the different clinical samples collected has shown high resistant to ceftazidime, ciprofloxacin, levofloxacin and sensitive to Polymyxin, Imipenem and cefaperazone-sulbactam, ranging from 60-100% (Table-1). The most effective antimicrobial against Acinetobacter spp was cefaperazone plus sulbactum. Aminoglycoside resistance noted in Acinetobacter baumanii and Acinetobacter lwoffii were 41% and 58% respectively. Imipenem resistance observed in Acinetobacter baumanii and Acinetobacter lwoffii were 66% and 74% respectively. Twenty percent of Burkholderia cepacia complex (BCC) was resistant to piperacillin plus tazobactam and ciprofloxacin. Strikingly, one being resistant to imipenem among 3 isolated Moraxella spp. Stenotrophomonas spp were sensitive to all antimicrobials tested. All the NFGNB were 100% sensitive to Polymyxin B.

Figure 2: Gender distribution of NFGNB

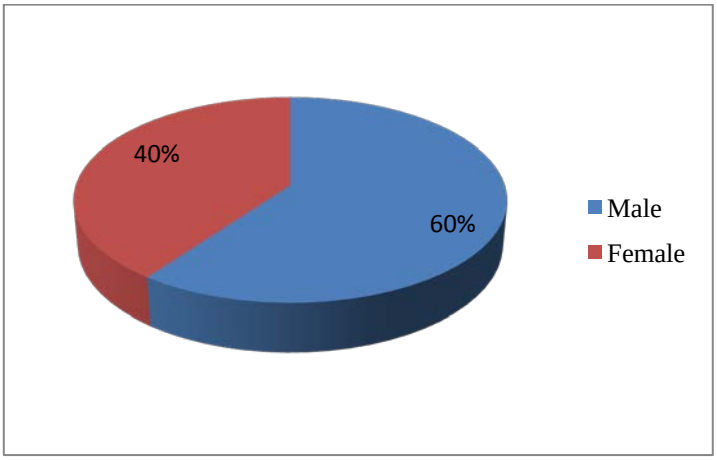


Figure 3: Percentage of individual organisms among isolated NFGNB

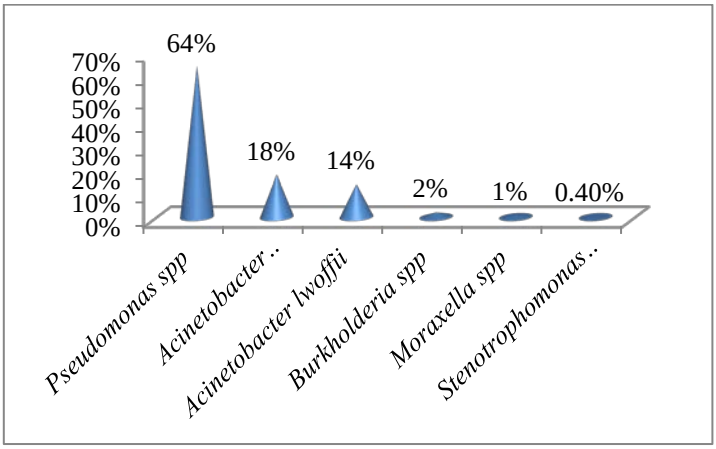


Figure 4: Sample wise distribution of isolated NFGNB

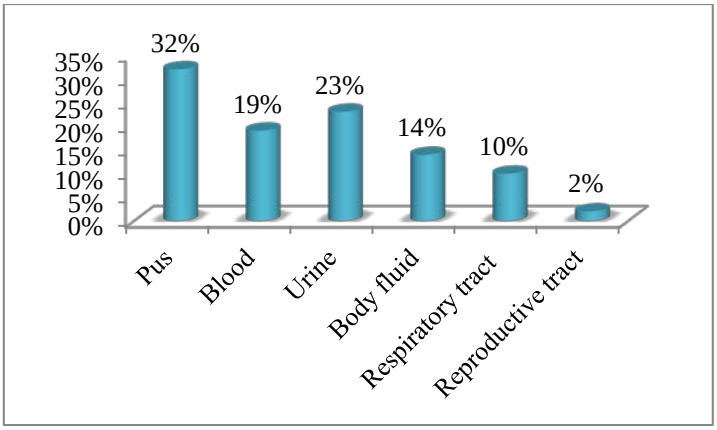


Table1: Antibiotic resistance pattern of isolated NFGNB

S.N.	Organism	Percentage of Resistance pattern								
		AK	CFS	PTZ	I	GEN	LE	CF	CTZ	Pb
1	Pseudomonas spp(141)	7%	7%	12%	22%	11%	-	20%	31%	Nil
2	Acinetobacter baumannii (39)	41%	20%	46%	66%	36%	65%	-	74%	Nil
3	Acinetobacter lwoffii (31)	58%	19%	55%	74%	52%	74%	-	77%	Nil
4	Burkholderia cepaciacomplex (5)	Nil	Nil	20%	Nil	Nil	Nil	20%	Nil	100%
5	Moraxella spp(3)	Nil	Nil	33%	33%	Nil	Nil	-	Nil	Nil
6	Stenotrophomonas spp(1)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Discussion

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.[18,19] The available data suggests that NFGNB are remarkable microorganisms because of their epidemiological complexity, propensity to cause outbreaks of infection and antimicrobial resistance.[20,21,22] 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.[23] 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 aeruginosa and among Acinetobacter was A.baumannii.

In our study, clinical spectrum caused by NFGNB infection comprises surgical site infection (SSI), ventilator-associated pneumonia (VAP), urinary tract infection (UTI), and septicemia. NFGNB were frequently encountered in wound infections following road traffic accidents and chronic non-healing ulcers. P.aeruginosa and A. baumannii were more often isolated from pus samples followed by urine and blood.

Our study is in concordance with reports of other authors for multi-drug resistance among the P. aeruginosa.[24,25] Greater level of resistance to almost all the commonly administered antimicrobials was seen and this finding is in line with the study from Chandigarh.[26] Though imipenem showed good activity to all the NFGNB, but emerging resistance to this group of drugs is of major concern. Previous studies by other authors also have reported carbapenem resistance among NFGNB.[26, 27] In the present study 70% of Acinetobacter species and 22% 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.[26,27] In our study, Acinetobacter strains percentage sensitivity for polymyxin was 100%.

Maximum level of resistance to ciprofloxacin, amikacin, ceftazidime, levofloxacin and piperacillin-tazobactam was shown by strains of Acinetobacter species in a study in Bangalore which is almost in par with the present study.[28] Resistance to 3rd generation cephalosporin, Ceftazidime showed 31% resistance in Pseudomonas and 74% among Acinetobacter. Compared to studies done by Kumari et al.[29], which had reported the resistance in the range of 35 – 40% for both Pseudomonas and Acinetobacter. Kumari et al [29] in these studies they reported similar results for Acinetobacter and lesser resistance for Pseudomonas.

Pseudomonas showed (11.3%) resistance to Gentamicin which is different compared to Murugan et al [30] who reported (42.8%) resistance to Gentamicin. In the present study Ciprofloxacin resistance to Pseudomonas is (20%) which is very much lower than (Deepak Juyalet al., 2013) [31] who had reported the prevalence of resistance as 73.7%.

Antibiogram patterns may alter with time and also differs from one hospital to another. Alterations in susceptibility patterns may be due to mutational adaptations and resistance transfer from indiscriminate and excessive use of antibiotics. Resistance transfer may be due to alterations in gene expression or by acquisition of genetic material. Moreover, majority of our patients hail from rural areas without substantial exposure to antimicrobials. Dissimilarity in susceptibility patterns could be accreditated to these above mentioned factors. Nevertheless, NFGNB considered as contaminants are major bacteria resulting in both health care associated and community-acquired infections. Most common isolates in our study were P. aeruginosa and A. baumannii. UTI, septicemia, SSI, VAP and other chronic wound infection are the clinical conditions caused by NFGNB in our study. Majority of them were multidrug-resistant. P. aeruginosa has shown good susceptibility to polymyxin, imipenem, amikacin and cefaperazone /sulbactam. A. baumannii shows good sensitivity to polymixin, Imipenem and Amikacin. Species level identification of NFGNB in conjunction with monitoring their susceptibility patterns is essential for genuine management of these NFGNB infections. It is also essential to establish the clinical significance of the isolated NFGNB and their association with infection. It is better to avoid unnecessary usage of antimicrobials before contemplating NFGNB as pathogens, finally preventing the emergence of drug-resistant strains. Continued awareness of the need to maintain good housekeeping, equipment decontamination, strict adherence to hand hygiene and isolation precautions are the mandate measures necessary to control the previously unabated spread of these organisms.

Conclusion

NFGNB are emerging as an important opportunistic pathogen and are resistant to commonly used antimicrobials. The interaction between these multidrug resistant pathogens and the increasing number of immune compromised patients poses a challenge for the microbiologists and clinicians. To conclude, the most common NFGNB isolated in our study were P. aeruginosa and A. baumannii. Their part as healthcare-associated pathogens is well recognized and implicated in causing clinical conditions like urinary tract infections (UTI), septicemia, Surgical site infections (SSI's), and Ventilator associated pneumonia (VAP). .P. aeruginosa has shown good sensitivity to imipenem, amikacin, and cefaperazone-sulbactam. A. baumannii shows good susceptibility to Imipenem and cefaperazone-sulbactam. The different species of NFGNB have shown a varied sensitivity pattern in our study.

Therefore, Therefore, speciation of NFGNB and keeping track of their susceptibility patterns, are essential for appropriate management of the healthcare associated infections resulted by them. Our study highlights the fact that it is essential to establish the clinical significance of the isolated NFGNB and their association with infection to avoid unnecessary usage of antimicrobials and emergence of drug resistance.

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