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Original Research Article

An Observational Assessment of the in-Vitro Antibiogram of Non-Fermenting Gram Negative Bacilli in a Tertiary Care Hospital

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Abstract

Aim: To identify non-fermenting Gram negative bacilli isolated from various clinical samples up to genus and species level along with study of their antimicrobial sensitivity/ resistance pattern.

Methodology: This study was carried out in the department of Microbiology, over 200 samples of clinical specimens collected from in- patients admitted to various departments of ANMMC, Gaya during a period of 1 year study. A detailed history of the patients was recorded including the current underlying disease like Diabetes or malignancy. The samples for the study were collected from patients with NFGNB infection, admitted during the period of study. NFGNB was isolated from blood, CSF and other body fluids. Patients with colonization of NFGNB with no apparent clinical infection and isolates from improperly collected samples were excluded from the study. The samples were collected from respiratory tract (RT), pus, wound, urine and blood. A preliminary (gram stain) examination was carried out for RT, pus, wound and urine samples, following which they were incubated in appropriate media.

Results: Pus samples constituted majority of specimens accounting for 45.5%. Urine and Sputum samples accounted for 14% & 13% of specimens respectively. Stool, Blood, Pleural fluid, Ascitic fluid and CSF samples accounted for remaining 27.5%. In the decreasing order of frequency, the NFGNB showed 54.41% sensitivity to Carbenicillin, 45.58% to Piperacillin and Tazobactam, 29.42% to Piperacillin, 26.48% sensitivity to Ticarcillin, 23.53% sensitivity to Amoxyclav, 16.18% sensitivity to Netilmicin and 7.36% sensitivity to Penicillin. NFGNB showed a sensitivity of 52.95% to Ceftazidime, 48.53% to Cefaperazonea and 42.65% to Ceftriaxone. Maximum resistance was observed for Cefuroxime and minimum for Ceftazidime. Ps. aeruginosa showed a sensitivity of 54.16% for Cefoperazone, 60.42% sensitivity for Ceftazidime, 49.84% sensitivity for Cefepime.

Conclusion: This study gives an alarming sign towards high prevalence of multi drug resistant NFGNB. It may be concluded that growth of NFGNB cannot be overlooked and should be confronted with high index of suspicion. Precise identification of these bacteria up to genus and species level, imperative clinicmicrobiological correlation and careful antibiotic prescription shall go a long way in improving clinical outcomes of patients.

Keywords: Penicillin, cephalosporin, bacteria, resistance.

Introduction

Non-fermenting Gram-negative bacilli (NFGNB) are taxonomically a group of aerobic non-spore-forming bacilli that either do not utilize carbohydrates as the source of energy or degrade them through metabolic pathways other than by fermentation [1]. They are widely distributed in nature as saprophytes (organisms obtaining nutrients directly from dead organic matter or wastes) found in soil, water, and sewage or as commensals on human skin or in the human gut. Several are found in the hospital environment [2–4].

These organisms were previously thought to exist either as commensals or contaminants. However, recent findings have established a clinical significance and pathological association with blood stream infections, especially in immunocompromised hosts and patients with haematological malignancy [5]. NFGNB are known to account for 15% of all bacterial isolates from clinical microbiology laboratories [6]. Data from the Surveillance and Control of Pathogens of Epidemiological importance study revealed that approximately a quarter of Gram-negative bacteremia was attributed to NFGNB [7].

These bacteria can be frequently isolated from samples of patients suffering from septicemia, meningitis, pneumonia, urinary tract infection and surgical wound infection. Some of the risk factors that can contribute to NFGNB infections include 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. Currently Pseudomonas aeruginosa and Acinetobacter baumannii are the most commonly isolated non-fermenters pathogens for humans. Other species that can be isolated include opportunistic pathogens like P. fluorescence, P. stutzeri, Stenotrophomonas maltophilia, P. putida and P. cepacia [8, 9].

Development of resistance in non-fermenters is multifactorial. Factors involved are- mutations in genes encoding porins, efflux pump mechanisms, penicillin binding proteins, chromosomal beta lactamases [10]. Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent; therefore, this study was conducted with an objective to identify non-fermenting Gram negative bacilli isolated from various clinical samples upto genus and species level along with study of their antimicrobial sensitivity/ resistance pattern.

Materials and Methods:

This study was carried out in the department of Microbiology on 200 samples of clinical specimens collected from inpatients, admitted to various departments of ANMMC, Gaya during a period of 1 year study. A detailed history of the patients was recorded including the current underlying disease like Diabetes or malignancy. The samples for the study were collected from patients with NFGNB infection, admitted during the period of study. NFGNB was isolated from blood, CSF and other body fluids. Patients with colonisation of NFGNB with no apparent clinical infection and isolates from improperly collected samples were

excluded from the study. The samples were collected from respiratory tract (RT), pus, wound, urine and blood.

Methodology

A preliminary (gram stain) examination was carried out for RT, pus, wound and urine samples, following which they were incubated in appropriate media. The RT samples were inoculated in 5% sheep blood agar (BA), Macconkey agar (MA), and chocolate agar (CA) which were incubated overnight at 37◦ c and were observed for growth for 48 hrs. The pus and wound swab sample were plated on 5% sheep BA, MA, Thioglycollate which were incubated overnight at 37◦ c and were observed for growth for 48 hrs. For the urine samples, gram stain smear was made by placing a loopful urine sample on a clean slide and allowed to air dry. These samples were then plated by a 4mm loop onto 5% sheep BA and MA for semi-quantitative analysis. Isolates which were significant in semi quantitative culture of urine were included in the study. A Brain Heart Infusion broth was used for blood culture. The bottle was examined duly for turbidity and subculture was made at regular intervals on to BA, MA and any growth was processed further for identification. Cultures that showed growth in the first three days were included in the study.

For identification of species, biochemical tests were performed which include OF medium (Hugh and Leifson), Nitrate reducing broth, Citrate utilization test, Growth at room temperature 25◦ c-30◦ c, 37◦ c, 44◦ c, Hemolysis on a 5% sheep BA, Gelatin liquefaction and Hanging drop preparation for motility testing. Antibiogram was done by KIRBY-BAUER disc diffusion method. Only those NFGNB which grew either in pure culture or as predominant growth were identified in the study. All the tests were performed with positive and negative control.

Results:

In the present study, 200 samples were collected from clinical specimens with local infections, Septicemia, Respiratory tract infections, Urinary tract infections, Ear infections, meningitis and cervicitis samples from patients admitted to ANMMC, Gaya.

Pus samples constituted majority of specimens accounting for 45.5%. Urine and Sputum samples accounted for 14% & 13% of specimens respectively. Stool, Blood, Pleural fluid, Ascitic fluid and CSF samples accounted for remaining 27.5%.

Table 1: Various samples from which NFGNB were isolated

Sample	Number	%
Pus	91	45.5
Sputum	26	13
Ascetic fluid	7	3.5
Blood	15	7.5
Urine	28	14
Stool	12	6
Cervical discharge	5	2.5
Pleural fluid	8	4
CSF fluid	8	4

Table 2: Bacterial species isolated under each clinical diagnosis

Species	Local	RTI	UTI	GIT	Post op	Post traumatic	Septicemia	Total
Ps. aeruginosa	23	14	7	9	11	6	4	74
Ps. fluorescens	0	6	0	0	7	0	0	13

Ac. baumanii	9	7	4	5	5	2	2	34
Mixed Group	23	12	19	9	7	4	5	79

Out of all the cases, Pseudomonas aeruginosa was isolated in 74 cases which included 23 cases of local infection, 14 cases of respiratory tract infection (RTI), 9 cases of Gastrointestinal tract (GIT), 6 cases of post traumatic, 7 cases of urinary tract infection (UTI), 4 cases of septicaemia and 11 cases of postoperative (post OP) infection. Pseudomonas fluorescens was isolated from 13 cases including 6 cases of RTI and 7 of post OP infection. Acinetobacter baumanii was isolated from 34 cases which included 9 cases of local infection, 7 cases of RTI, 5 cases each of GIT and post OP and 4 cases of UTI and 2 cases each of post traumatic and septicaemia. Mixed growth (Proteus mirabilis, Proteus vulgaris, Citrobacter, MRSA, E coli, Klebsiella, Enterococci, Enterobacter species, Group A beta haemolytic streptococci, Salmonella spp, Shigellaspp) were seen a total of 79 cases which included 23 cases of local infection, 19 cases of UTI, 12 cases of RTI, 9 cases of GIT, 5 case of septicaemia, 7 cases of post OP infection and 4 cases of post traumatic infection.

Table 3: Antibiotic susceptibility pattern of NFGNB for penicillin group of drugs

Antibiotic	Sensitive	Resistant
Penicillin	10	126
Amoxyclav	32	104
Carbenicillin	74	62
Piperacillin+Tazobactam	62	74
Netilmicin	22	114
Ticarcillin	36	100
Piperacillin	40	96

Table 4: Antibiotic susceptibility pattern of NFGNB for cephalosporin group of drugs

Antibiotic	Sensitive	Resistant
Cefuroxime	16	120
Cefotaxime	34	102
Ceftriaxone	58	78
Cefaperazone	66	70
Ceftazidime	72	64
Cefipime	58	78

The antibiotic susceptibility pattern for various NFGNBs for penicillin group of drugs is shown in Table 3 and 4. In the decreasing order of frequency, the NFGNB showed 54.41% sensitivity to Carbenicillin, 45.58% to Piperacillin and Tazobactam, 29.42% to Piperacillin, 26.48% sensitivity to Ticarcillin, 23.53% sensitivity to Amoxyclav, 16.18% sensitivity to Netilmicin and 7.36% sensitivity to Penicillin. Maximum resistance was exhibited against penicillin (92.64% cases) and minimum against carbenicillin (45.58% cases).

Ps. aeruginosa showed a sensitivity of 68.75% to Carbenicillin followed by 41.66% sensitivity to Piperacillin+ Tazobactam. Ps. fluroscens showed a unit form sensitivity of 60% to Carbenicillin and Piperacillin+Tazobactam. Acinetobact erbaumanii showed a sensitivity of 40% to Piperacillin+Tazobactam, 26.66% sensitivity to Carbenicillin and Amoxyclav.

The antibiotic susceptibility pattern of NFGNB for cephalosporin group of drugs is shown in Table 4. NFGNB showed a sensitivity of 52.95% to Ceftazidime, 48.53% to Cefaperazonea and 42.65% to Ceftriaxone. Maximum resistance was observed for Cefuroxime and minimum for Ceftazidime. Ps. aeruginosa showed a sensitivity of 54.16% for Cefoperazone, 60.42% sensitivity for Ceftazidime, 49.84% sensitivity for Cefepime. Ps. Fluorescens showed uniform sensitivity of 60% to Ceftriaxone, Ceftazidime, and Cefaperazone. Ac. baumanii showed a susceptibility of 46.66% to Ceftazidime, 53.34% to Ceftriaxone and 33.33% to Cefepime and Cefaperazone.

Discussion:

NFGNB are ubiquitous in the environment. They are now recognised as important healthcare-associated and opportunistic pathogens [14]. Bloodstream infections by NFGNB pose a challenge for clinicians as well as microbiologists where there are limited facilities in the laboratories for their identification and also because of their emerging antimicrobial resistance. The two most frequently encountered organisms, Pseudomonas aeruginosa and Acinetobacter baumanii complex, were also found in other studies [15,16].

In the present study, maximum NFGNB isolates were obtained from pus samples (45.5%) but variable isolation rates of NFGNB from pus samples have reported by other studies: Malini et al- 62.2%, Patel et al- 58.6%, Gokale and Metgud 58.4% [17-19]. Our results are much similar to the results of and Benanchinmardi et al, and Kalidas et al, who have reported it to be 22% and 27.8% respectively [20,21].

In this study, 14% NFGNB were obtained from urine samples. A study by Rajendra et al shows similar results with the isolation rate of NFGNB from urine to be 25.4% [22]. Jayapriya et al reported the NFGNB isolates obtained from urine to be 30.8% [23]. Many authors have reported very less isolation rates of NFGNB from urine samples. Benanchinmardi et al, Malini et al and Patel et al have reported NFGNB isolates obtained from urine as 11%, 11.9% and 11.8% respectively [17,18,20]. A study by Gokale and Metgud showed that only 8.2% NFGNB isolates were obtained from urine samples [19].

In the present study, 13% NFGNB were isolated from sputum samples. In studies by Kalidas et al, Malini et al, Gokale and Metgud the NFGNB reported from endotracheal secretions were 18.4%, 16.4%, 6.8% and 7.8% respectively [17,19,21]. In a study by Malini et al, and Patel et al, NFGNB obtained from sputum samples were 6.7% and 7% respectively which also correlates with results of this study [17, 18].

In this study, NFGNB isolated from blood samples were 7.5%. Benanchinmardi et al, and Aamal et al, have reported NFGNB isolates obtained from blood as 6% and 8% respectively thus showing similarity with the results of this study [20, 24]. On the other hand, Sidhu et al, and Rajendra et al, have reported higher isolation rate of NFGNB from blood samples i.e.-36.3% and 24.5% respectively [22, 25].

NFGNBs showed resistance of 47.05 % to Ceftazidime, 51.47 % to Cefaperazone, 57.35% to Cefepime which are commonly used by the clinicians in our hospital. Ps. aeruginosa showed 27.08% resistance to Ciprofloxacin in our study. In various other studies by Taneja et al., AlgunU et al., Prakash KS et al., Wong fu et al. and Smitha S et al., it ranged from 12.5% to 83% [26-31].

Conclusion:

This study gives an alarming sign towards high prevalence of multi drug resistant NFGNB. It may be concluded that growth of NFGNB cannot be overlooked and should be confronted with high index of suspicion. Precise identification of these bacteria up to genus and species level,

imperative clinic microbiological correlation and careful antibiotic prescription shall go a long way in improving clinical outcomes of patients.

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