Original Research Article

Prevalence and Antibiotic Susceptibility of Gram Negative Nonfermenting bacilli Isolates Obtained from Different Clinical samples in a tertiary care hospital

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Abstract

Background: Non-fermenting gram-negative bacilli (NFGNB) have emerged as an important healthcare associated pathogens . Infections caused by these bacteria are almost always secondary to some predisposing factors in patients such as burns, prolonged antimicrobial therapy, immunosuppression etc. The aims and objective of the study was to isolate, identify and characterize the prevalence of NFGNB along with their antimicrobial sensitivity pattern among the patients attending a tertiary care centre in north India. **Materials and Methods** This Prospective study was conducted between October 2018 to December 2018 in the Department of Microbiology, Patna Medical College, Patna. A total of 1131 clinical samples were collected from patients admitted in ICU and various wards of the hospital. All the samples were collected and processed and identified as per standard microbiological guidelines.. Isolates which produced an alkaline/alkaline (K/K) reaction were provisionally identified as non-fermenters and were included in this study Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines using commercially available discs.**Results: A** Total of 143 NFGNB were isolated from 1151culture positive clinical samples accounting for an isolation rate of 12.42% . 92 (64.33%) isolates were obtained from male patients and 51(35.66%) were isolated from female patients. Maximum NFGNB 53 (37.06%) were obtained from age group of 41 to 60 years.. 58 (40.55%) NFGNB isolates were obtained from high-risk areas. Urine was the most common specimen (28.67%). *Acinetobacter baumannii* was the predominant isolate, 68 (47.55%) followed by *Pseudomonas aeruginosa* 63 44.05%) and *Burkholderia cepacia* complex 7 (4.89%). *P. aeruginosa*, *A. baumannii* and *A. lwoffii* isolates were 100% sensitive to polymyxin B and colistin, 74.6% sensitivity was reported towards imipenem and meropenem ,while less sensitivity was reported towards cephalosporins. *A. baumannii* isolates showed 43 (63.2%) to Imipenem and meropenem. *B. cepacia* showed very good sensitivity (100%) towards cefepime , imipenem (100%) , meropenem (100%), Cotrimoxazole (100%) and Piperacillin-tazobactum (71.4%). *S. maltophilia* were resistant towards ceftazidime, cefepime.**Conclusion:** It may be concluded that growth of NFGNB cannot be underestimated. NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections . Improved antibiotic stewardship and infection control measures should be implemented to prevent nosocomial infections and spread of drug resistant.

Keywords: Nonfermenters, Multidrug resistant, Nosocomial infection, Antibiotic stewardship

Introduction

The non-fermentative gram negative bacilli (NFGNB) consists of a diverse group of non-spore forming, aerobic bacilli that neither use carbohydrates as the source of energy nor degrade them through metabolic pathways other than fermentation. NFGNB are known to account for nearly 12-16% all bacterial isolates from a clinical microbiology laboratory[1].Certain conditions or diseases predispose the patients to infection with non-fermenters like malignancies particularly of reticuloendothelial system, instrumentation, surgery, catheterizations particularly of urinary tract, intravascular catheter-isation, lumbar puncture, tracheostomy, dialysis, lavages, placement of shunts, prosthesis and prolonged antibiotic usage and chronic infections. Burns, open wounds and exudative lesions are other predisposing factors[2].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[3]. 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[4]. The NFGNB are challenging to the microbiologists and the treating physician because of their intrinsically resistant nature, hardiness and mostly t they cause infection in immunosuppressed patients[5].Resistance to anti-microbials is common in these organisms and has increased over the years among NFGNB and number of strains are now resistant nearly to all commonly used antibiotics. 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[6]. 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

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isolated from various clinical samples upto genus and species level along with study of their antimicrobial sensitivity/ resistance pattern.

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

Materials and Methods

This Prospective study was conducted between October 2018 to December 2018 in the Department of Microbiology, Patna Medical College, Patna.A total of 1131 clinical samples were collected from patients admitted in ICU and various wards of the hospital of depending upon the clinical diagnosis of respective patients. These included: urine, pus, blood, ear swabs, high vaginal swabs, sputum, endotracheal secretions, tracheal aspirate and various body fluids. Out of total samples, 429 samples were collected from ICU patients and 702 samples were collected from patients admitted in various wards of the hospital. All the samples were collected and processed as per standard microbiological guidelines. Samples were inoculated on to Blood Agar (BA) and MacConkey Agar (MA) plates under strict aseptic conditions and plates were incubated at 37ºC for 24-48 hours under aerobic conditions. All isolates that showed non-lactose fermenting colonies on MA and those which grew only on BA and not on MA were subjected to Gram staining and all Gram-negative bacilli/cocci/coccobacilli obtained were then subjected to triple sugar iron test. Isolates which produced an alkaline/ alkaline (K/K) reaction were provisionally identified as non-fermenters and were included in this study and subjected to identification by various biochemical tests including Oxidative/ Fermentative (O/F) test for glucose, lactose, sucrose, mannitol and xylose, oxidase test, motility test, nitrate reduction test, lysine and ornithine decarboxylase test, arginine dihydrolase test, gelatin liquefaction test, urease test, indole production test, citrate utilization test, growth at 42ºC and 44ºC. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar as per CLSI guidelines using commercially available discs[7,8].Following antimicrobial discs were used: aztreonam(30µg), ceftazidime (30µg), cefepime (30µg), piperacillin-tazobactum (100µg/10 µg), imipenem (10µg), meropenem(10 µg), cotrimoxazole (25µg). gentamicin (10µg), amikacin (30µg) netilmicin (30 µg), ciprofloxacin (5µg), norfloxacin (30µg; for urinary isolates), polymyxin B (300 units) and colistin (10µg). Plates were incubated at 37º C for 18-24 hours and results were interpreted according to zone sizes mentioned in the CLSI guidelines[9]. *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains. All dehydrated media and antibiotic discs were used from HiMedia Labs, Mumbai, India. Statistical analysis was done by descriptive statistics using percentages and ratios method.

Results

Total 143 NFGNB were isolated from 1151culture positive clinical samples accounting for an isolation rate of 12.42% (Figure 1). 92 (64.33%) isolates were obtained from male patients and 51(35.66%) were isolated from female patients. Maximum NFGNB 53 (37.06%) were obtained from age group of 41 to 60 years followed by age-group of 21 to 40 years; 61-80 years; 0 to 20 years and minimum isolates were obtained from age group of more than 80 years. 58 (40.55%) NFGNB isolates were obtained from high-risk areas including intensive care units and dialysis units while 85 (59.44%) from other clinical area. Urine was the most common specimen (28.67%) followed by pus (26.57%), blood (15.38%), sputum (11.88%), tracheal aspirate (10.48%) and remaining 6.95% included other samples (Table 1).

Table 1: distribution of NFGNB isolates in different clinical sample.

Samples	No. of NFGNB (n=143)	Percentage
Urine	41	28.67
Pus/wound swab	38	26.57
Blood	22	15.38
Sputum	17	11.88
E.T. tube/tracheal aspirate	15	10.48
Catheter Tip	3	2.09
CVP tip	3	2.09
Drain tip	2	1.39
Throat swab	1	0.69
Other body fluids	1	0.69

Table 2: Prevalence of NFGNB isolates.

Isolates	Number (n=143)	Percentage
A. baumannii	68	47.55
P. aeruginosa	63	44.05
B. cepacia complex	7	4.89
Burkholderia pseudomallei	2	1.39
Acinetobacter lwoffii	2	1.39
Stenotrophomonas maltophilia	1	0.69

Table 3: Antibiotic susceptibility profile of commonly isolated NFGNB

Antibiotic Tested	*P. aeruginosa (n=63)*	*A. baumannii (n=68)*	*A. lwoffii (n=2)*	*B. cepacia (n=7)*	*B.Pseudomallei (N=2)*	*S. maltophilia (n=1)*
Aztreonam	26 (41.2%)	Not-tested	Not tested	Not tested	0(0%)	Not tested
Ceftazidime	21(33.3%)	4 (5.88%)	0 (00.0%)	4 (57.1%)	0(0%)	0 (0%)
Cefepime	28(44.4%)	6 (8.82%)	1(50%)	7 (100%)	0(0%)	0 (0%)
Ampicillin-sulbactum	Not tested	Not tested	1(50.0%)	Not tested	0(0%)	Not tested
Piperacillin-tazobactum	40 (63.4%)	34 (50.0%)	2 (100%)	5(71.4%)	0(0%)	0(0%)
Cotrimoxazole	Not tested	9(13.2%)	2(100%)	7 (100%)	2 (100%)	1 (100%)
Gentamicin	31(49.20%)	31 (45.5%)	2 (100%)	0(0%)	0(0%)	0(0%)
Amikacin	38 (60.3%)	42 (61.7%)	2 (100%)	0 (0%)	0(0%)	0 (0%)

Netilmicin	39(61.9%)	Not tested	Not tested	Not tested	0(0%)	Not tested
Ciprofloxacin	35(55.5%)	12 (17.6%)	1 (50%)	2 (28.5%)	0(0%)	0(0%)
Imipenem	47 (74.6%)	43 (63.2%)	2 (100%)	7 (100%)	2(100%)	0 (0%)
Meropenem	47(74.6%)	44 (64.7%)	2 (100%)	7 (100%)	2(100%)	0 (0%)
Polymyxin b	63 (100%)	68 (100%)	2 (100%)	0(0%)	Not tested	1(100%)
Colistin	63(100%)	68 (100%)	2 (100%)	0(0%)	Not tested	1 (100%)

Acinetobacter baumannii was the predominant isolate, 68 (47.55%) followed by Pseudomonas aeruginosa 63 44.05%) and Burkholderia cepacia complex 7(4.89%). Burkholderia pseudomallei, Acineto-bacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3.47% (Table 2). A. baumannii was more prevalent in high-risk areas (ICUs and Dialysis Units) in comparison to other clinical areas where P. aeruginosa is more prevalent. P. aeruginosa, A. baumannii and A. lwoffii isolates were 100% sensitive to polymyxin B and colistin, 74.6% sensitivity was reported towards imipenem and meropenem ,while less sensitivity was reported towards cephalosporins. A. baumannii isolates showed 43 (63.2%) to Imipenem and meropenem. B. cepacia showed very good sensitivity(100%) towards cefepime , imipenem (100%) , meropenem(100%),Cotrimoxazole(100%) and Piperacillin-tazobactum (71.4%). S. maltophilia was also found to be multidrug resistant pathogen showing resistance to various groups of antibiotics. All isolates of S. maltophilia were resistant towards ceftazidime, cefepime, Piperacillin-tazobactum, imipenem and meropenem. they were sensitive to cotrimoxazole, Polymyxin b and colistin (Table 3).

Discussion

Nonfermentative gram-negative bacilli are ubiquitous in nature. In the past, they used to be considered as contaminants or commensals. They have now emerged as important nosocomial and opportunistic pathogens due to their frequent isolation from clinical materials and their association with various diseases. These organisms are associated with life threatening infections such as- septicaemia, pneumonia, UTI, meningitis, surgical site infections, ventilator associated pneumonia, osteomyelitis etc. and resistance to antimicrobials have resulted in difficulty in treatment of infections caused by these bacteria[10].NFGNB are intrinsically resistant to various antimicrobials and are known to produce extended spectrum betalactamases (ESBL's) and metallobetalactamases (MBL's)[11]. In the present study, the isolation rate of NFGNB from clinical samples was 12.42% %. This was parallel to the results of a study from Kolkata by Kalidas et al and Rit K et al, where NFGNB were isolated in 12.18% and 12.2% respectively[12,13]. 92 (64.33%) isolates of NFGNB were obtained from male patients and 51(35.66%) from female patients. These results are similar to Ridhima et al who has reported NFGNB isolates from males as 69.7% and females as 30.3%[14]. In other study by Jayapriya et al who has reported NFGNB isolates from males as 71% and females as 29%[15]. In our study maximum NFGNB 53 (37.06%) were obtained from age group of 41 to 60 years followed by age- group of 21 to 40 years; 61-80 years; 0 to 20 years and minimum isolates were obtained from age group of more than 80 years. Ridhima et al reported that the age group which was maximum infected with NFGNB was 45-60 yrs which is similar to this study[14]. 58 (40.55%) NFGNB isolates were obtained from high-risk areas including intensive care units and dialysis units while 85 (59.44%) from other clinical area. In this study Urine was the most common specimen (28.67%) followed by pus (26.57%), blood (15.38%), sputum (11.88%), tracheal aspirate (10.48%) and remaining 6.95% included other samples. Simlar finding was observed by the study of Sarkar et al. where Urine was the most common specimen (29.44%) followed by pus (27.49%), blood (15.57%), sputum (12.90%) and tracheal aspirate (8.27%)[16] In many studies, NFGNB were most commonly isolated from pus[17]. According to a study by Shobha KL et al, nonfermenters were emerging as an important cause of urinary tract infections (9.44%)[18]. Frequent isolation of NFGNB from urine and pus samples in this study, could be attributed to the increase in number of critically ill, hospitalised patients requiring urinary tract catheterization and other instrumentations. Open wounds, surgical site infections, diabetes, malignancies and Prolonged hospital stay, bed sores, burns, and several underlying illnesses made these patients more vulnerable to NFGNB infections. In present study, Acinetobacter baumannii was the predominant isolate, 68 (47.55%) followed by Pseudomonas aeruginosa 63 (44.05%) and Burkholderia cepacia complex 7 (4.89%). Burkholderia pseudomallei, Acinetobacter lwoffii and Stenotrophomonas maltophilia altogether accounted for 3.47%. These results corroborated well with the studies of Goel V et al, where, A. baumannii (48.78%) was the most commonly isolated pathogen followed by P. aeruginosa (37.71%).[19] . However, in other studies, the most common isolate was P. aeruginosa, followed by A. baumannii[20].In this study A. baumannii was more prevalent in high-risk areas (ICUs and Dialysis Units) in comparison to other clinical areas where P. aeruginosa is more prevalent. This study corroborated well with the result of the study by Goel V et al[19], showing A. baumannii being the commonest isolate followed by P. aeruginosa from high risk areas.16 In our study, prevalence of A. baumannii was more in high risk areas, possibly due to increased colonisation of A. baumannii in hospital environment, including humidifiers, nebulizers, anaesthetic equipments, ventilators, healthcare workers etc. causing nosocomial opportunistic infections in patients with severe underlying illnesses[17].P. aeruginosa, A. baumannii and A. lwoffii isolates were 100% sensitive to polymyxin B and colistin, 74.6% sensitivity was reported towards imipenem and meropenem ,while less sensitivity was reported towards cephalosporins. A. baumannii isolates showed 43 (63.2%) to Imipenem and meropenem. B. cepacia showed very good sensitivity (100%) towards cefepime , imipenem (100%),meropenem (100%), Cotrimoxazole(100%) and Piperacillin -tazobactum (71.4%). S. maltophilia was also found to be multidrug resistant pathogen showing resistance to various groups of antibiotics. All isolates of S. maltophilia were resistant towards ceftazidime, cefepime,Piperacillin-tazobactum, imipenem and meropenem. they were sensitive to cotrimoxazole, Polymyxin b and colistin. Our study is in concordance with reports of other authors for multi-drug resistance among the P. aeruginosa . 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[21], . 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 [21,22]. In the present study only 36.8%of Acinetobacter species and 25.4% 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.In our study, Acinetobacter strains percentage sensitivity for Colistin and Polymyxin b is 100%. Imipenem and meropenem was 63.2% and 64.7% respectively. Imipenem and meropenem monotherapy have also been proved effective in many studies , Sidhu et al[23]. In the present study, B. cepacia showed very good sensitivity towards cefepime (100%), imipenem (100%), meropenem (100%) piperacillin-tazobactum(100%) and cotrimoxazole (100%). All

isolates were resistant to polymyxin B and colistin as B. cepacia shows intrinsic resistant towards these drugs [11]. Kalidas et al and Sidhu et al have also reported similar antibiogram of B. cepacia in their studies[12,23].

Conclusion

It may be concluded that growth of NFGNB cannot be under estimated. NFGNB are now emerging as important pathogens causing a wide range of nosocomial infections. Identification of NFGNB and monitoring of their susceptibility profiles are essential due to their variable sensitivity patterns and to help in proper management of the infections caused by this. It is noteworthy that as these bacteria also have a great potential to survive in hospital environment therefore, improved antibiotic stewardship, good housekeeping, equipment decontamination, strict protocols for hand washing, isolation procedures need to be implemented to prevent emergence and spread of multidrug resistant NFGNB in health care settings.

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