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PROFILE OF NONFERMENTING GRAM NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL LABORATORY

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Abstract

Introduction: Non-Fermentative or Non Fermenting Gram Negative Bacilli (NFGNB) are widely distributed in nature as saprophytes, found in soil, water, sewage or as commensals on human skin or in the human gut and some in hospital environment. These organisms are commonly isolated from patients with serious underlying diseases such as patients with prolonged antibiotic therapy, endotracheal intubation, catheterization, burn patients and in extremes of age like neonates, children and geriatrics age group. They are responsible for septicemia, meningitis, brain abscess, endocarditis, pneumonia, urinary tract infection and surgical site infections.

Materials and Methods: This a prospective study, conducted in the Department of Microbiology, VSS Medical College & Hospital, Burla over a period of two years from October 2014 to September 2016. A total of 9204 non repetitive clinical specimens like sputum, throat swabs, endotracheal tube secretions, pus, urine, blood, and body fluids were collected using strict aseptic precautions from various clinical departments and immediately processed.

Observation & Results: In this study 9204 clinical samples were processed in Microbiology Laboratory, out of which 3955 specimens showed growth; 571 of these positive cases showed mixed cultures of two organisms. Hence total number of isolates summed upto 4526, including 1350 isolates of gram-negative bacilli. Out of these, 505 cases are found to be nonfermenters. *Pseudomonas aeruginosa* is the most common isolate accounting for 349 (69.30%) followed by *Acinetobacter baumanii* 84(16.63%) and next to it is *Pseudomonas fluorescens* 42(7.92%). The maximum number of cases are from Surgery ward accounting for 26.53% followed by Medicine ward 20.39% and 17.82% from Obstetrics and Gynaecology ward. The most effective drugs against *Pseudomonas aeruginosa* isolates showed sensitivity to Meropenem (78%) followed by Imipenem (73.56%) and Amikacin (51.23%). *A.baumannii* isolates showed highest sensitivity to meropenem (76%) followed by imipenem(74%) and chloramphenicol(62%)., *S.maltophila* showed 100% resistance to ceftazidime and cefotaxime, but 100% sensitive to amikacin, imipenem, meropenem, aztreonam, and ciprofloxacin.

Conclusion: Most of the NFGNB are resistant to commonly used antibiotics, hence antibiogram should be followed before successfully treating these cases. Above all, the Hospital Infection Control Committee should be active enough to avoid infections due to NFGNB as well as other organisms and the Hospital Antibiotic Policy should be followed strictly.

Introduction

Bacteria are single celled prokaryotic microorganisms without any phototrophic activity. They vary widely in their nutritional demands and sources of metabolic energy. The major mechanism for generating metabolic energy in bacteria is by the process of fermentation of carbohydrates.[1] There are certain bacteria which do not ferment carbohydrates, primarily glucose as source of their energy. There are 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 fermentation and taxonomically are called Non-Fermentative or Non fermenting gram negative bacilli (NFGNB).[2] During 1950s, the nosocomial infections were predominantly caused by *Staphylococcus aureus.* Recently the pattern of Hospital acquired infections (HAI) has shifted to Gram negative bacilli (GNB). The common organisms causing HAI, amongst the Gram-negative bacilli are Enterobacteriaceae and nonfermenters.[3]

NFGNB are widely distributed in nature as saprophytes, found in soil, water, sewage or as commensals on human skin or in the human gut and some in hospital environment.[1, 3, 4] Recently there has been tremendous interest in these group of organisms as non-fermenters are considered to be important pathogens of many infectious disease and recovered with increasing frequency from clinical specimen.[5] They are resistant to physical and chemical compounds like povidone-iodine, chlorhexidine and quaternary ammonium compounds.[6] NFGNB are responsible for 15% of all bacterial isolates from clinical microbiological laboratory in the recent years. These organisms are commonly isolated from patients with serious underlying diseases such as, patients with prolonged antibiotic therapy, endotracheal intubation, catheterization, burn patients and in extremes of age like neonates, children and geriatrics age group. They are isolated from a variety of clinical specimens such as urine, pus, blood, sputum, pleural fluid, Broncho Alveolar Lavage (BAL), ascitic fluid, CSF. [4] They are responsible for septicemia, meningitis, brain abscess, endocarditis, pneumonia, urinary tract infection and surgical site infections.[4] These organisms are also found in malignant disorders, after bone marrow transplantation and stem cell transfusion, and in ventilator associated pneumonia cases. [5, 6, 7]

The multi drug resistant nonfermenting gram negative bacteria especially *Pseudomonas aeruginosa* and *Acinetobacter* species are on the rise. *Pseudomonas* species are resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, narrow spectrum and extended-spectrum cephalosporins, cefotaxime and ceftriaxone.[8,9,10] A variety of resistance mechanisms have been identified in *P.aeruginosa, A. baumanii & Burkholderia* species, such as enzyme production, over expression of efflux pumps, porin loss and target-site alterations. Multiple resistance genes frequently coexist in the same organism.[11] Carbapenems are often used for treating infections due to multi drug resistant isolates. However, resistance to these antibiotics is increasing, leading to an ever-restricted therapeutic choice. In *Pseudomonas aeruginosa,* resistance to β-lactam agents can be due to the overproduction of chromosome-encoded cephalosporinase, alteration of the outer membrane protein OprD, overexpression of the efflux system, and acquisition of exogenous β-lactamases. Efforts are underway to address these varied clinical challenges and have concentrated on enhanced infection control practices, better screening methods, determination of optimal usage of existing antibiotics, and development of novel antimicrobials.[12] This present study in our hospital will help to make an attempt to isolate and identify the NFGNB by using some simple protocol and to find out the incidence of NFGNB with regard to the nature of organisms and its sensitivity pattern. This study has been proposed after considering the above mentioned problems. The aim of the study is to

characterize nonfermenting gram negative bacilli isolated from different clinical samples and to detect the antibiotic sensitivity pattern of these isolates.

Materials and Methods

This a prospective study, conducted in the Department of Microbiology, VSS Medical College & Hospital, Burla over a period of two years from October 2014 to September 2016. A total of 9204 non repetitive clinical specimens like sputum, throat swabs, endotracheal tube secretions, pus, urine, blood, and body fluids were collected using strict aseptic precautions from various clinical departments and immediately processed. Clinical history of the patient, co-morbidities like diabetes mellitus (DM), prolonged hospitalization, indwelling intravascular catheters, patients on ventilators and in ICU, history of surgical intervention, history of use of antibiotics especially the Carbapenem group were noted. The clearance from the Ethical Committee of the institute was obtained before start of the study, bearing IEC No. 2014/P-I-RP/14M-O-MIC-028/026.

Inclusion Criteria

Clinical specimens received in the Microbiology laboratory from patients attending to OPD and admitted to different wards in VIMSAR, Burla.

Exclusion criteria

1. Specimens received from the peripheral centres.
2. Non clinical specimens.
3. Samples which on primary examination seem to be contaminated.

Collection & processing of samples

1. Respiratory tract samples, sputum and throat swabs -Expectorated sputum samples were collected in a sterile wide mouthed container. Gram stains of sputum samples were microscopically examined to assess contamination with upper respiratory tract secretions. The sample was considered as excellent if more than 25 pus cells and less than 10 epithelial cells per low power field were found. Throat swabs were collected from the posterior pharynx and tonsils and subjected to smear and processed. In patients on ventilator, lower respiratory tract secretions were sent in a Luken's trap. The tracheal samples were placed in a sterile tube and washed down with 1ml of sterile normal saline (NS). The resulting suspension was vortexed and used to inoculate the plates for culture. The endotracheal tube tips were received in sterile tubes. The tips including the bore were washed with 1ml sterile normal saline solution was vortexed thoroughly and the resulting suspension was used to inoculate the plates for culture. All these samples were inoculated on 5% sheep BA, MCA and incubated overnight at 37˚C and observed for growth for 48 hrs.

2. Pus, wound swabs- A preliminary Gram's stain was done. Then these samples were inoculated on 5% sheep BA, MCA, and a tube of thioglycollate broth which were incubated overnight at 37^{o}C aerobically and were observed for growth for 72 hrs. The samples showing no growth on solid agar plates but showing growth in the thioglycollate broth, subculture was done from the broth onto BA and MCA to see the enrichment of aerobic and facultative organisms.

3. Urine- Clean catch mid- stream urine was collected in sterile plastic universal containers & catheterized urine aspirated in a sterile syringe in 5-10 ml amounts. Samples were then inoculated by a 4mm bacteriological culture loop onto 5% sheep BA and CLED agar plates for semi-quantitative analysis. Only those isolates, which were found significant in semi quantitative culture of urine were further processed. If the patient was on antibiotics or having frequency of micturition, or showing few colonies of *Staphylococcus aureus,* was taken as significant and processed.

4. Blood- BHI broth was used for blood culture. 5 ml of blood collected aseptically in a bottle containing 50ml of broth and incubated at 37^{o} C under aerobic conditions. Blood samples collected in BHI bottles were incubated at 37ºC and routinely inspected twice a day (at least for first 3 days) for signs of microbial growth or turbidity. If visible growth appears, bottle was opened aseptically, small amount removed with a sterile loop and Gram stain of smear examined for presence of

microorganisms. Subcultures were performed by streaking a loopful on MacConkey & Blood agar and processed biochemically. Bottles incubated for 7-10 days before giving negative culture report. **5**. Different body fluids pleural fluid, peritoneal fluid, fluid from drainage catheters, CSF, ascitic fluid, were collected in sterile vials. These were inoculated into to 5% sheep blood agar, MacConkey agar plates and processed.

Microscopic examination of collected samples

Routinely Gram stain was done for each sample. It differentiated gram positive from gram negative bacteria and gave insight about their morphology and arrangement, presence or absence of spores, yeast like cells, pus cells, epithelial cells. Any gram-negative rod growing on blood agar but poorly or not at all on MacConkey agar were suspected to be a nonfermenter. These isolates were inoculated onto TSI agar. All isolates showing alkaline/alkaline (k/k) reaction on TSI were subjected for oxidation fermentation of glucose, lactose, mannitol and maltose tests, motility by hanging drop and semisolid motility medium, growth at 44^{o}C and a set of biochemical reactions like cytochrome oxidase test, catalase production, urea hydrolysis, citrate utilization, nitrate reduction test, decarboxylation of arginine, lysine, ornithine tests, gelatin liquefaction, esculin hydrolysis, for identification upto species level. Antibiotic susceptibility test of isolates was performed by using Kirby Bauer disc diffusion and double disc synergy methods as per CLSI guidelines (2016).

Observation & Results

In this study 9204 clinical samples were processed in Microbiology Laboratory, received from patients admitted into different wards of the institution during October 2014 to September 2016, out of which 3955 specimens showed positive growth; 571 of these positive cases showed mixed cultures of two organisms. Hence total number of isolates summed up to 4526, including 1350 isolates of gram negative bacilli. Out of these 505 cases are found to be nonfermenters. Of these 202 were obtained in pure cultures and 303 were isolated in mixture with some other bacteria or yeast.

The NFGNB comprised 5.5% of total clinical specimens, 11.15% of the total isolates and 37.4% of the total gram negative isolates. 214 (5.4%) were from urine, 211 (5.6%) were from pus and wound discharges, 41 (4.5%) were from sputum, 17 (3.8%) were from throat swab and ETT secretions, 17 (2%) were from blood and 5 (5%) were from body fluids and Gender distribution of isolated NFGNB from samples. (Figure 1)

In this study there is male preponderance of infection accounting for 56.6% (286) and females 43.4% and distribution of NFGNB from samples according to age.

The highest number of patients belong to the age group of 31-40 years comprising 55.5% of males and 44.4% females, followed by the age group 41-50 years in which the males accounted for 53% and females 46.5% (Figure 2)

Pure culture isolates from urine was 41.6%, pus 45.5%, sputum 14.6%, throat swab 17.6% , blood 35.2%, body fluids 40%. Mixed culture isolates were urine was 58.4%, pus 54.5%, sputum 85.4%, throat swab 82.35%, blood 64.7%, body fluids 60%. *Pseudomonas aeruginosa* is the most common isolate accounting for 349 (69.3%) followed by *Acinetobacter baumanii* 84(16.6%) and next to it is *Pseudomonas fluorescens* 42 (7.9%) (Figure 3)

Maximum number of cases are from surgery ward accounting for 26.5% followed by medicine ward 20.4% and 17.8% from obstetrics and gynaecology ward.

DM posed as the major risk factor followed by TB, indwelling catheters. *Pseudomonas aeruginosa* were most commonly associated with DM patients accounting for 64.1% followed by *Acinetobacter baumanii* 29%.

The most effective drugs against *Pseudomonas aeruginosa* isolates showed sensitivity to meropenem (78%) followed by imipenem (73.6%) and amikacin (51.2%). *A.baumannii* isolates showed highest sensitivity to meropenem (76%) followed by imipenem (74%) and chloramphenicol(62%)., *S.maltophila* showed 100% resistance to ceftazidime and cefotaxime, but 100% sensitive to amikacin, imipenem, meropenem, aztreonam, and ciprofloxacin.(Figure 4)

Fig- 1: **Gender wise distribution of NFGNB from different clinical samples**

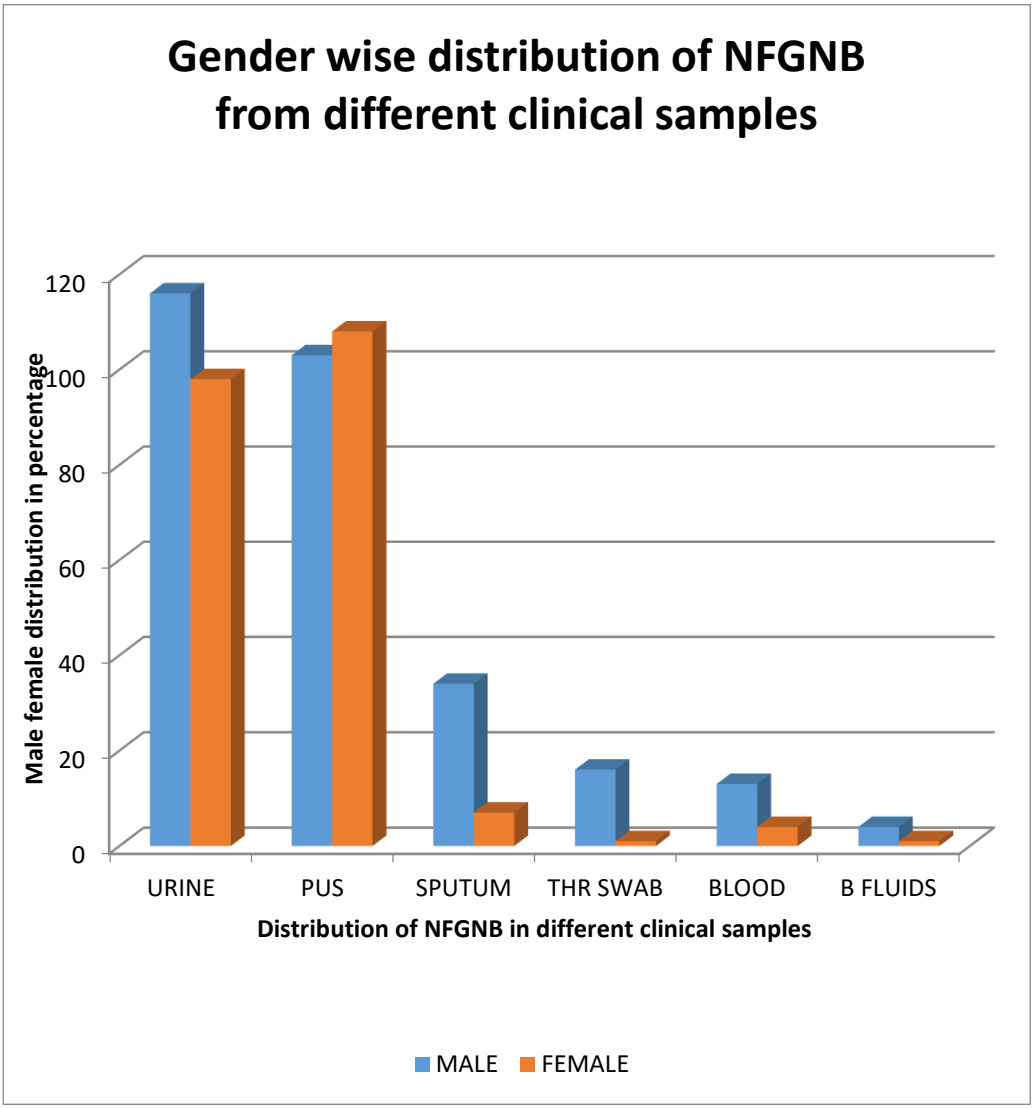
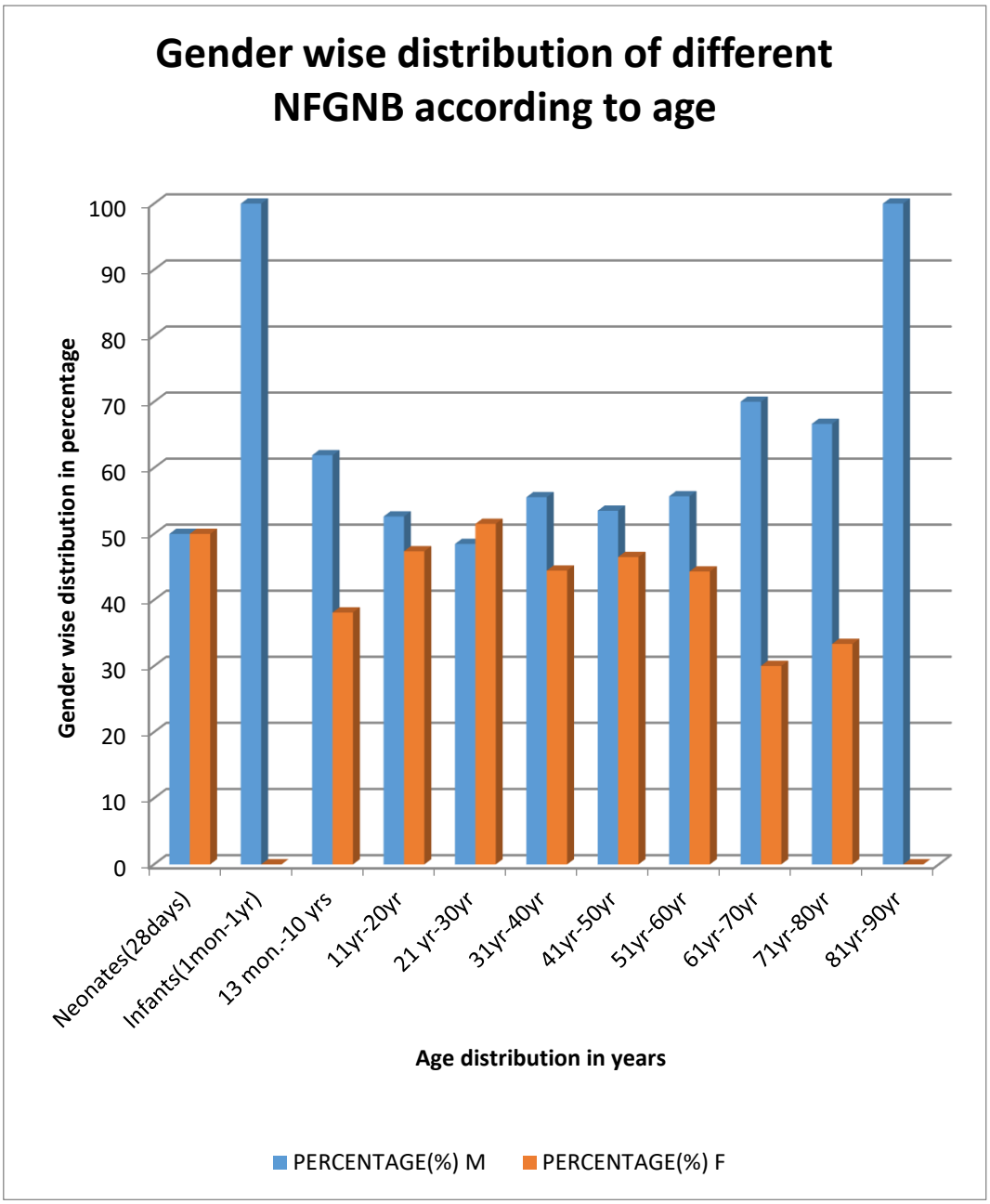


Fig- 2: **Gender wise distribution of different NFGNB according to age**



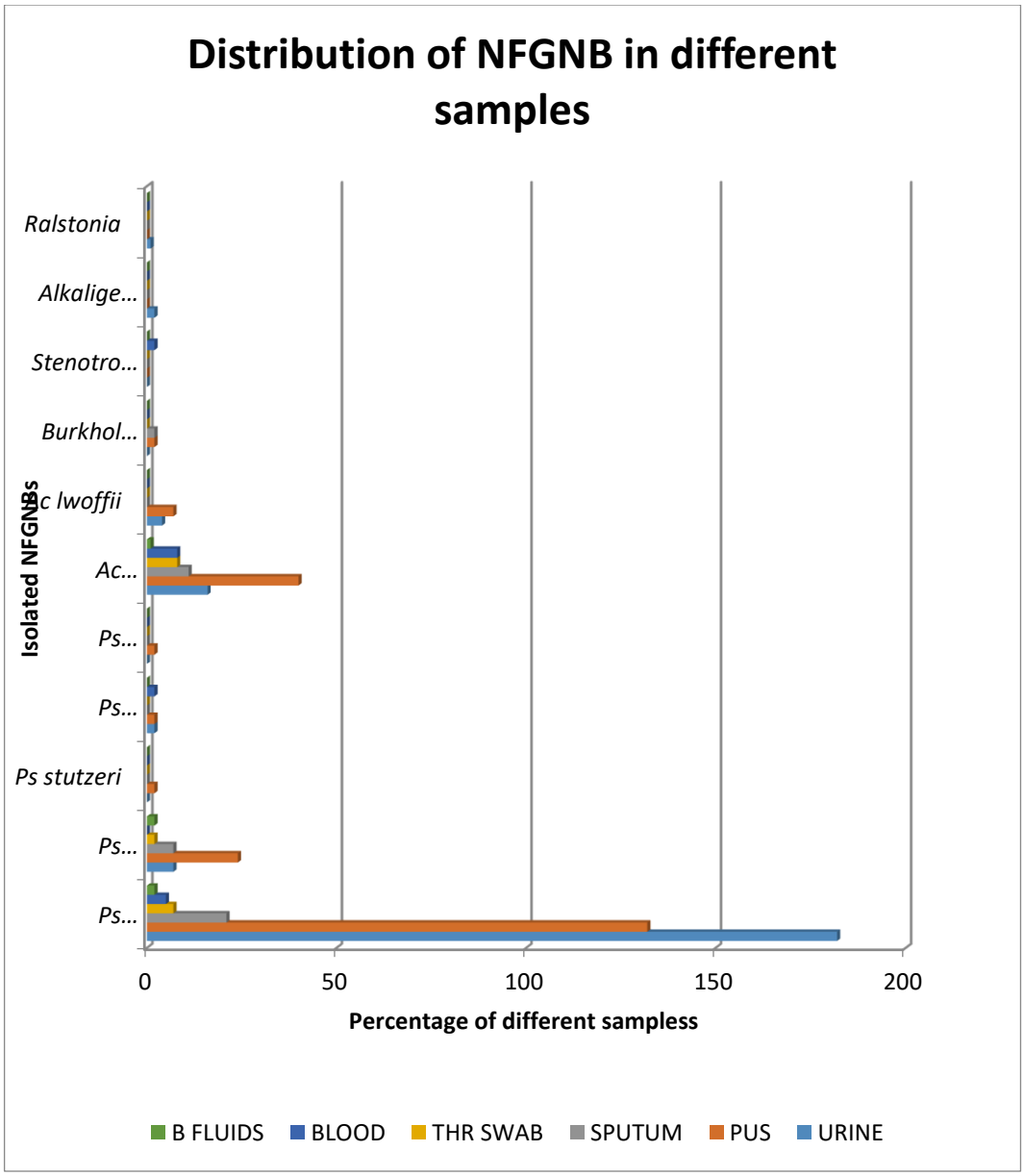

Distribution of NFGNB in different samples
Ralstonia
Alkalige…
Stenotro…
Burkhol…
Ac lwoffii
Ac…
Ps…
Ps…
Ps stutzeri
Ps…
Ps…
Isolated NFGNBs
0
50
100
150
200
Percentage of different sampless
B FLUIDS
BLOOD
THR SWAB
SPUTUM
PUS
URINE

FIGURE 3

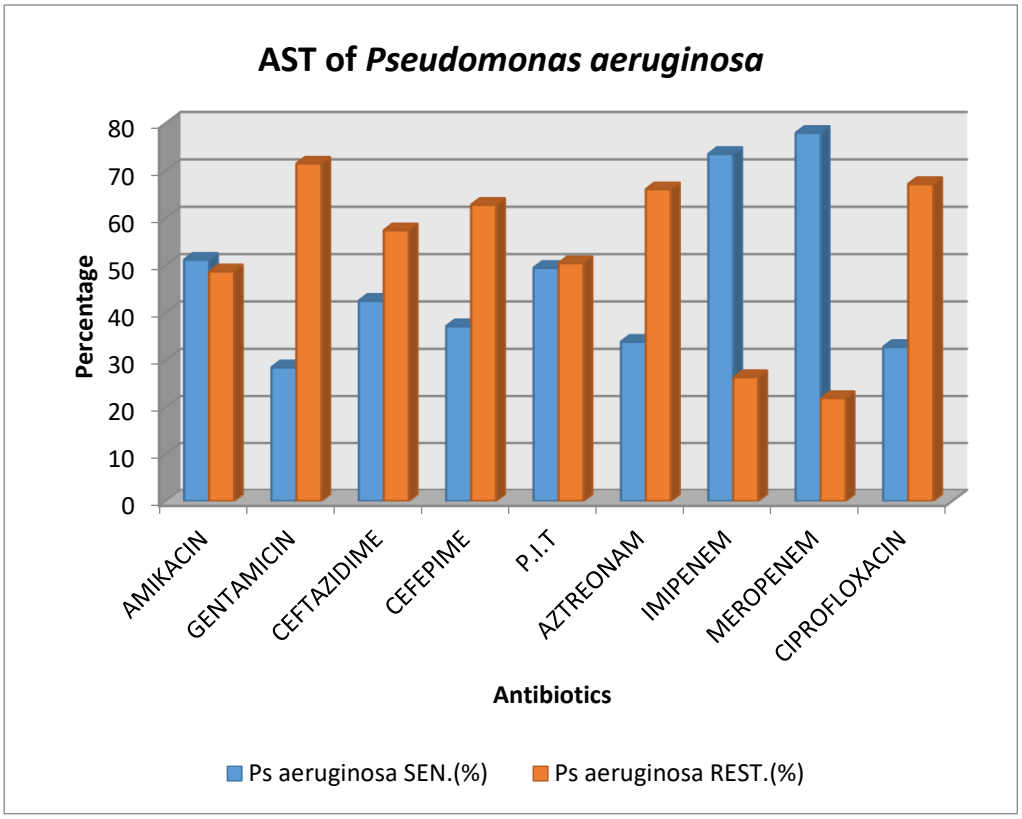


FIGURE 4

Discussion

The NFGNBs are widely distributed in nature as saprophytes or as commensals.[13,14] NFGNB that were considered to be contaminants in the past but in the recent years, they have emerged as important pathogens causing nosocomial as well as community acquired infection. [15,16] They have been reported to cause infections at almost all sites of body or to colonize almost any site subjected to injury[17]. This prospective study was conducted in the department of Microbiology of VIMSAR Burla from October 2014 to September 2016 and detailed history of the patients regarding age-sex distribution, duration of illness, antibiotic therapy were noted. In this study a total of 9204 clinical samples were collected from patients admitted in various wards at VIMSAR. The clinical samples were urine, pus, blood, body fluid, sputum, throat swab, endotracheal secretions and bronchoalveolar lavage. The NFGNB comprised 5.5% of total clinical specimens, 11.15% of the total isolates and 37.4% of the total gram negative isolates. As per the observations made from different studies NFGNB comprised 15% of the total isolates in study by Gales in US, Canada and Europe in 1997-99, 4.5% in 2009 by Malini at Kolar[13], 11.6% in 2013 by Patel in Gujarat[14], 12.8% in the study by Rit & Nag in 2013 in Eastern India.[15]

In this study the highest number of patients belong to the age group of 31-40 years comprising 55.5% of males and 44.4% females, followed by the age group 41-50 years in which the males accounted for 53% and females 46.5%. Yashodhara and Shyamala also revealed in their study that these organisms were more commonly found in the older age group with a male predilection.[18,19]

There is a male preponderance of infection in this study accounting for 56.6% (286) and females accounted 43.36%. A study by Rit K et al reported a similar result where males accounted for 55% of the cases and females 45%. Gardener et al also reported increased incidence of NFGNB in males. In this study out of total 505 NFGNB isolates, 214 (5.4%) were from urine, 211 (5.6%) were from pus and wound discharges, 41 (4.5%) were from sputum, 17 (3.8%) were from throat swab and ETT secretions, 17 (2%) were from blood and 5 (5%) were from body fluids. Malini A et al. in 2009 isolated non fermenters from 189 clinical specimens as 61.9% from pus, 12.16% from urine,12.7% from sputum, 11.6% from blood and 1.6% from body fluids. Patel in 2013 isolated from clinical samples 58.6% from pus ,6.6% from body fluids ,6.5% from blood, 7% from sputum, 0.8% from urine, 0.3% from throat swab and 4.2% from tracheal aspirate. In the same year a study in Eastern India reported isolation of nonfermenters from tracheal aspirate (18.4%), sputum (16.4%), blood (16.4%), pus (27.9%), urine (15.9%) and rest 4.9%. In this study out of 505 NFGNB isolates, 202 were isolated in pure culture and 303 from mixed cultures. Pure culture isolates from urine was 41.6%, pus 45.5%, sputum 14.6%, throat swab 17.65%, blood 35.2%, body fluids 40%. Mixed culture isolates were urine was 58.4%, pus 54.5%, sputum 85.36%, throat swab 82.35%, blood 64.7 %, body fluids 60%. Similar results were also shown by Gardener P et al 1970, Parimal HP et al 2013. In this study the highest number of patients were from surgery ward accounting for 26.5% followed by medicine ward 20.4% and 17.8% from obstetrics and gynaecology ward. Among all the clinical samples processed, *Pseudomonas aeruginosa* is the most common isolate accounting for 349 (69.3%) followed by *Acinetobacter baumanii* 84 (16.6%) and next to it is *Pseudomonas fluorescens* 42(7.9%). Patel PH et al in 2013 reported 76.97% *Pseudomonas aeruginosa* which was the commonest one followed by *Acinetobacter baumanii* 21.36% from the clinical isolates. In 2009 Malini A et al reported *Pseudomonas aeruginosa* 53.8%, *Acinetobacter baumanii* 22.2%, *Pseudomonas flourescens* 10.8% from the clinical specimens. Similar type of study by Memish ZA et al [20] in 2012 reported 72.9% *Pseudomonas aeruginosa* followed by 25.3% *Acinetobacter baumanii* and 1.8% *Stenotrophomonas maltophila* from the clinical specimens. Pathi et al [21] isolated 8.4% (319) *Pseudomonas aeruginosa* from 3783 positive isolates. Parimal HP et al 2013 who reported *Pseudomonas aeruginosa* 76.97% and *Acinetobacter baumanii* 1.36%. Fastidious NFGNB could not be cultured in our laboratory, so not included in this study. In this study we have observed that NFGNB were commonly isolated from cases of UTI, SSI, burn cases, RTI, sepsis, cases of CSOM and different body fluids. DM posed as the major risk factor followed by TB, indwelling catheters. *Pseudomonas aeruginosa* were most commonly associated with DM patients accounting for 64.15% followed by *Acinetobacter baumanii* 29%. Similar results were observed by Quinn JP et al [22] 1998 and Gardener P et al 1970.46,47 Meropenem and imipenem are the most effective antibiotics against NFGNB with overall sensitivity 94% and 93% respectively followed by amikacin (87%), ciprofloxacin (81%), aztreonam (77%), cefepime (76%) and gentamicin (67%). The most effective drugs against *Pseudomonas aeruginosa* isolates showed sensitivity to meropenem (78%) followed by imipenem (73.56%) and amikacin (51.23%). The sensitivity results are in concordence with the study by Agrawal G et al [23] 2008 and Nagoba et al [24] 1997. In the present study 27% of *P.aeruginosa* isolates showed imipenem resistance, similar to the study by Taneja N et al [25] 2008(42%) and Sarkar et al [26] 2006 (36.36%). *A.baumannii* isolates showed highest sensitivity to meropenem (76%) followed by imipenem(74%) and chloramphenicol(62%). This result is well correlated with the results of Dheepa M et al[27] 2012 and Parimal HP et al 2013. In our study 24% of *A.baumannii* were meropenem resistant , which is similar to study by Lautenbach E -et al[28] 2009. In present study, *S.maltophila* showed 100% resistance to ceftazidime and cefotaxime, but 100% sensitive to amikacin, imipenem, meropenem, aztreonam, and ciprofloxacin. This is comparable to study by Alonso A et al [29] 1997 and Parimal HP et al 2013. Sensitivity of *A.lwoffii* isolates was highest to imipenem(100%), amikacin (100%) and aztreonam (89%) which is similar to the study by Patil JR et al [30] 2001 and Parimal HP et al 2013. Similar observations were made by Forbes BA et al [31] 1998 and Daniel et al 1997[32]. The higher percentage of either sensitivity or resistance to many drugs could be due to very low number of isolates of *S.maltophila, A.lwoffii & Burkholderia cepacia* complex.

Conclusion:

We processed 9204 clinical samples. The NFGNB isolated from them is 505. Out of 505 NFGNB isolates, 60% (303) were co-isolated along with some other bacteria, and 40% (202) isolates were isolated in pure growths. There is a higher preponderance of infection in males (56.63%) as compared to females (43.36%) in this study. Most NFGNB's were isolated from pus samples, followed by urine, sputum and tracheal secretions. Higher isolation of organisms were reported in the age group of 31 to 40 years followed by 41-50 years. Diabetes mellitus, intravascular catheterization, burns, prolonged hospitalization, and immunocompromised state are the common risk factors found to be associated. *Pseudomonas aeruginosa* (69.3%) was the commonest bacteria isolated in this study followed by *Acinetobacter baumanii* (16.6%).

The most effective antibiotics were meropenem with sensitivity of 94%, imipenem 93.2%, amikacin 87.56%, ciprofloxacin 80.88%, aztreonam 77.5%, cefepime 75.8%, gentamycin 67.3%, piperacillin – tazobactam 58.6%, and ceftazidime 55.3%. *Pseudomonas aeruginosa* isolates were more sensitive to meropenem (78%) followed by imipenem (73.56%) and amikacin (51.23%). *Acinetobacter baumanii* isolates were more sensitive to meropenem (76%) followed by imipenem (74%), chloramphenicol (62%) and amikacin (57%). Most of the NFGNB are resistant to commonly used antibiotics, hence antibiogram should be followed before successfully treating these cases. Above all, the Hospital Infection Control Committee should be active enough to avoid infections due to NFGNB as well as other organisms and the Hospital Antibiotic Policy should be followed strictly.

References

1. Jawetz , Melnick And Adelbergs Medical Microbiology
2. Koneman Ew, Alen Sd, Janda Wm, Schreckenbeiger Pc,Winn Wc. The Non Fermenting Gramnegative Bacilli. Color Atlas And Text Book Of Diagnostic Microbiology.5th Edition, Philadelphia: J.B.Lippincott, 1997;253-309.
3. Steinberg Jp , Rio Dc. Gram Negative and Gram Variable Bacilli. Principles And Practice Of Infectious Diseases 2005;2:2751-68.
4. Gales Ac,Jones Rn,Forward Kr,Linaes J,Sader Hs,Verhoef J.Emerging Importance Of Multidrug Resistant Acinetobacter Species And S maltophila As Pathogens In Seriously Ill Patients: Genetic Patterns, Epidemiological Features And Trends In The Sentry Antimicrobial Surveillance Program. Clinical Infectious Disease 2001;32:104-13.
5. Fujita J, Negayama K, Fujita T Et Al. Activity Of Antibiotics Against Various Stains Of Clinically Isolated Glucose Nonfermenting Gram Negative Bacteria J Intern Med 1991;6:553-555.
6. Noberto Jp. Pseudomonas. In:Borriello Sp,Murray Pr, Funke G. Microbiology And Microbial Infections. 10th Edition Washingtondc: American Society For Microbiology;2005.P.16-1606.
7. Rubi Sj,Granto Pa,Wasilauskas Bl. Glucose Nonfermenting Gram Negative Bacteria. In: Lenna,Hausler Wj Jr, Shadomy Hj. Manual Of Clinical Microbiology 4th Edition.Washington Dc : American Society For Microbiology; 1985.P.330- 49.
8. Edith Bh, Deborah Ah ,Speert Dp. *Pseudomonas* . In: Baron Ej, Jorgensen Jh ,Landry Ml,Pfaller Ml. Manual Of Clinical Microbiology . 9th Ed. Washington Dc: American Society For Microbiology ; 2007.P.734-48.
9. Livermore Dm. Beta-Lactamases In Laboratory And Clinical Resistance. Clinical Microbiology Rev8;1995:557-84.
10. Livermore Dm. Multiple Mechanisms Of Antimicrobial Resistance In *Pseudomonas aeruginosa.* Clinical Inf Disease2002; 34:634-40.
11. Krisztina M. Papp-Wallace,1,2 Andrea Endimiani,1,2,3 Magdalena A. Taracila,2 And Robert A. Bonomo1,2,4,5, Carbapenems: Past, Present, And Future. Antimicrobial Agents Chemotherapy. 2011 November; 55(11): 4943–4960.

12. Ryan S. Arnold, Md, Kerri A. Thom, Md, Ms, Saarika Sharma, Md, Ma, Michael Phillips, Md, J. Kristie Johnson, Phd, Daniel J. Morgan, Md, Emergence Of *Klebsiella pneumoniae* Carbapenemase-Producing Bacteria. South Med J. 2011;104(1):40-45.
13. Malini A, Deepak, Gokul Bn, And Prasad Sr Nonfermenting Gram-Negative Bacilli Infections In A Tertiary Care Hospital In Kolar, Karnataka,J Lab Physicians. 2009 Jul-Dec; 1(2): 62–66.
14. Parimal H Patel, Dr.Jayshree D Pethani , Dr.Sanjay D Rathod , Dr.Bimal Chauhan , Dr.Parul D Shah , Prevalence Of Nonfermenting Gram Negative Bacilli Infection In Tertiary Care Hospital Indian Journal Of Basic & Applied Medical Research; March 2013: Issue-6, Vol.-2, P. 608 – 613.
15. Rit K,Nag F, Rar Hj, Maity Pk. Prevalence And Susceptibility Profiles Of Nonfermentative GNB In A Tertiary Care Hospital Of Eastern India. Indian Journal Of Clinical Practice 2013 ;24(5):451-5.
16. Gardener P, Griffin Wb, Swartz Mn, Kunz Lj. Nonfermenting Gram- Negative Bacilli Of Nosocomial Interest. Amer J Med 1970; 48:735-749.
17. Pickett MJ, Pedersen MM. Nonfermentative Bacilli Associated with man:II. Detection and Identification. Amer J Clin Path 1970; 54:164-177.
18. Yashodhara P. Shyamala S. Identification and characterization of Nonfermenters from Clinical Specimens. IJMM 1997; 15: 195-197.
19. Dheepa Muthusamy, Appalaraju Boppe, Phenotypic Methods for the detection of various Betalactamases in carbapenem resistant isolates Of *Acinetobacter baumanii* at a Tertiary Care Hospital In South India, Journal Of Clinical And Diagnostic Research, 2012, Aug Vol 6, Issue 6 ,Page 970-973.
20. Mehmish Za, Shibl Am, Kambal Am, Ohlay Ya, Ishaq Am, Livermore Dm, Antimicrobial Resistance Among NFGNB In Saudi Arabia. Jour Of Antimicrobial Chemotherapy 2012;67(7):1701-5.
21. Pathi B, Mishra Sn, Panigrahi K, Poddar N, Lenka Pr, Mallick B, Et Al. Prevalence And antibiogram pattern of *Pseudomonas aeruginosa*. In A Tertiary Care Hospital From Odisha, India. Transworld Medical Journal2014; (3):77-80.
22. Quinn Jp. Clinical Problems Posed By Multiresistant Nonfermentating Gram Negative Pathogens. Clinical Infectious Diseases 1998; 27:117-124.
23. Agrawal G , Lodhi Rb , Kamalakar Up, Khadse Rk , Jalgaonkar Sv, Study Of Metallo-B-Lactamase production in Clinical Isolates Of *Pseudomonas aeruginosa* Year : 2008 | Volume : 26 | Issue : 4 | Page : 349-351
24. Nagoba Bs, Deshmukh Sr,Wadher Bj, Gude Ug, Gomashe Av, Tumane Pm. Induction Of Resistance To ciprofloxacin And Other fluoroquinolones In clinical And Environmental Isolates Of *Pseudomonas aeruginosa*. Ind J Med Microbiology 1998; 16:29-30
25. Taneja N, Maharwal S,Sharma M. Imipenem Resistant In Nonfermenters Causing Nosocomial Urinary Tract Infection. Ind J Med Sci 2003; 57:294-299.
26. Sarkar B, Biswas D, Prasad R, Sharma Jp. A Clinico-microbiological Study On The Importance Of *Pseudomonas* In Nosocomially Infected ICU patients, With Special Reference To Metallo-β-Lactamase Production. Ind J Pathol Microbiol 2006; 49:44-48.
27. Dheepa Muthusamy, Appalaraju Boppe, Phenotypic methods for detection of various betalactamases in carbapenem resistant isolates of *Acinetobacter baumanii* at a Tertiary Care Hospital in South India, Journal of Clinical and diagnostic Research,2012,Aug Vol 6, Issue 6 Page 970-973.
28. Lautenbach E, Synnestvedt M, Weiner Mg, Bilker Wb, Vo L, Schein J, Kim M Epidemiology and impact Of imipenem resistance in *Acinetobacter baumannii*. Infect Control Hosp Epidemiol. 2009 Dec;30(12):1186-92.
29. Alonso A And Martínez Jl, Multiple Antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrobial. Agents Chemotherapy. May 1997 Vol. 41 No. 5 1140- 1142

30. Patil Jr, Jog Nr, Chopade Ba. Isloation And Characterization Of *Acinetobacter spp*. from Upper Respiraory Tract Of Healthy Humans And Demonstration Of Lectin Activity. Ind J Med Microbiol 2001;19:30-35.
31. Forbes Ba, Sahm Df, Weissfeld As. *Pseudomonas, Burkholderia* And Similar Organism in Diagnostic Microbiology. 10th Edition, Baltimore:Mosby, 1998;448- 461
32. Daniel Ct, Chang Sc, Chen Yc Et Al , In Vitro Activities Of Antimicrobial Agents , alone and in combinations against *Burkholderia cepacia* isolated from blood, Diagnostic Microbial Diseases ,1997;28:187-191