

A Study of Isolation, Identification & Antibiotic Susceptibility Pattern of Non-Fermenting Gram Negative Bacilli Isolated From Various Clinical Samples at Tertiary Care Hospital Rajkot, Gujarat, Western India

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Abstract

Introduction: Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively. They occur as saprophytes in the environment and some are also found as commensals in the human gut. In recent year NFGNB have emerged as important health care associated pathogens. They have been incriminated in infections such as bacteremia, meningitis, pneumonia, urinary tract infections, surgical site infections, wound infections, osteomyelitis etc. **Materials and method:** During Nine Month study period, various samples were collected aseptically and transported immediately to the bacteriology laboratory. The pathogens were identified by standard laboratory procedures including Gram's staining, motility, culture, colony characters and biochemical reactions. Antimicrobial susceptibility testing was performed by modified Kirby Bauer method as per the CLSI guidelines using Muller-Hinton agar and available antibiotic disks. **Result:** A total number of 312 NFGNB were isolated from 4112 clinical samples. Most frequently isolates NFGNB were *Pseudomonas aeruginosa* (42.62%) and *Acinetobacter* spp. (25.32%). NFGNB isolates were more common in males (58.33%) as compared to females (41.67%). Maximum sensitivity was seen to Polymyxin B (100%), Meropenem (83.45) in *Pseudomonas aeruginosa* and Meropenem (78.48%), Pipracillin-tazobactam (62.02%) in *Acinetobacter* spp. **Conclusion:** NFGNB are emerging as important pathogens and shows resistance to commonly used antibiotics. Minimized use of available antimicrobial, regular use antimicrobial susceptibility surveillance and strict infection control measures are required to control this emerging antimicrobial resistance among NFGNB.

Keywords: Non fermenting gram negative bacilli, emerging pathogens, Antimicrobial susceptibility, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*.

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INTRODUCTION

Nonfermenting gram-negative bacilli (NFGNB), which are saprophytic in nature, have emerged as important healthcare-associated pathogens. Nonfermenting gram-negative bacilli (NFGNB) are a taxonomically diverse group of aerobic, nonsporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively [1]. They occur as saprophytes in the environment and some are also found as commensals in the human gut [2]. Inherent resistance of these bacterial agents & their tendency to colonize various surfaces have been pivotal in their emergence as important nosocomial pathogens. The outbreaks of nosocomial infections, emerging antimicrobial resistance and epidemiological

complexity have made NFGNB the remarkable organisms [3]. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory [4]. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections, such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), ventilator associated pneumonia, wound infection, osteomyelitis and surgical site infections (SSI) [5]. Risk factors 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. Prolonged hospital stay broad spectrum antibiotic use and underlying host factors are the best predictors of outcome [6]. The most commonly occurring non fermentative gram negative bacilli are *Pseudomonas* spp., *Acinetobacter* spp., *Alkaligenes* spp., *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex (BCC). Currently *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most commonly isolated nonfermenters pathogenic for humans. Infections caused by other species are relatively infrequent [7]. Multidrug resistances is common and increasing among NFGNB and they are known to produce extended spectrum beta lactamases (ESBL's) and metallo beta lactamases (MBL's) [8]. As *Pseudomonas* and *Acinetobacter* are the most predominantly isolated NFGNB so carbapenem resistance among them is of major concern. Carbapenemase activity in *A.baumannii* is mainly due to carbapenem-hydrolyzing class D b-lactamases (CHDLs) that is mostly 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 [9]. In case of *P.aeruginosa* the dominant mechanism of carbapenem resistance is loss of carbapenem specific porin OprD2 [10]. Due to rampant use of antibiotics most of these organisms are now resistant to many routinely used antibiotics causing prescription failure [11]. Hence, this study was undertaken to isolate and identify NFGNB and also to characterize the antibiotic susceptibility pattern at a tertiary care hospital.

OBJECTIVES

To Isolate and identify non fermenters gram negative bacilli and to study antimicrobial susceptibility pattern of non fermenters gram negative bacilli isolates from various clinical specimens.

MATERIAL AND METHODS

The Cross sectional study for isolation, identification and antibiotic susceptibility testing of Non-fermenting gram negative bacilli from various clinical specimen in tertiary care hospital was carried out in Department of Microbiology, P.D.U Medical college and Hospital, Rajkot, Gujarat, India for a period of nine month (April 2019 to Dec 2019). During this study, the study population comprised of all hospitalized patients, OPD & ICU patients. Various

clinical samples like blood, pus, wound swabs, urine, sputum, ear swab & endotracheal secretions were collected in sterile container, irrespective of their age and sex & sent to the Microbiology laboratory along with the requisition forms filled with relevant clinical details of patients & processed as per the standard guidelines. During this period 4,112 samples were received and processed at bacteriology laboratory. Samples were inoculated on Nutrient Agar, Blood Agar and MacConkey Agar plates. Samples for blood culture were collected in BHI broth (Adult & Paediatric). Urine specimens were inoculated by semi-quantitative method. All the inoculated plates were incubated aerobically at 37°C in incubator for overnight (16-18 hrs). At the same time a smear was made on clean glass slides for gram staining for provisional identification. They were examined for pus cells and different morphological forms of microorganisms. The isolates were identified by standard microbiological techniques by studying their colony characteristics (size, shape, elevation, margin, surface, opacity, consistency, pigment production), morphology & biochemical reaction like Catalase, Oxidase test, Indole, Methyl red, Voges Proskauer, Citrate utilization test, Urease test and Triple sugar iron Test, Oxidation/fermentation for glucose, lactose, xylose, mannitol and maltose (Hugh and Leifson's media), Lysine and Ornithine decarboxylase and Arginine dihydrolase activity, ONPG test, Esculin test. Antibiotic susceptibility testing was done by Modified Kirby Bauer's disc diffusion method as per CLSI guidelines 2019 using commercially available discs [12].

RESULT

Total number of 312 non fermentative gram negative bacilli (NFGNB) isolated from the processed 4112 clinical specimen with isolation rate of 7.59%. Out of 4112 samples processed, 43 (10.48%) were from (ICU), 242 (8.09%) isolates were from Inpatient department and 27 (3.79%) isolates were from Outpatient department. Higher percentage of NFGNB strains were found in ICU compared to IPD & OPD.

NFGNB isolates were slightly higher in male (58.33%) than in females (41.67%). Maximum No. of cases were observed in the age group 31-40 (24.36%), 41-50 (16.67%), 51-60 (14.10%) followed by 21-30 (12.18%) & then <1 age group (11.85%). Maximum No. of cases observed in the age group 31-50 (41.03%).

Table 1: Age & Gender wise Distribution (n=312)

| Age Groups (days, years) | Gender | | Total (%) |
|--------------------------|-----------|------------|-----------|
| | Male (%) | Female (%) | |
| <1 | 17(9.34) | 20(15.38) | 37(11.85) |
| 1-10 | 8(4.39) | 6(4.62) | 14(4.49) |
| 11-20 | 12(6.59) | 3(2.31) | 15(4.81) |
| 21-30 | 20(10.99) | 18(13.84) | 38(12.18) |
| 31-40 | 35(19.23) | 41(31.54) | 76(24.36) |

| Age Groups (days, years) | Gender | | Total (%) |
|--------------------------|------------|------------|-----------|
| | Male (%) | Female (%) | |
| 41-50 | 38(20.88) | 14(10.77) | 52(16.67) |
| 51-60 | 31(17.03) | 13(10.00) | 44(14.10) |
| 61-70 | 13(7.14) | 12(9.23) | 25(8.01) |
| >71 | 8(4.39) | 3(2.31) | 11(3.52) |
| Total | 182(58.33) | 130(41.67) | 312(100) |

Majority of NFGNB were isolates from Pus 211 (65.62%), followed by Blood 51(16.34%) & Sputum 20(6.41%). Significantly higher number of NFGNB was isolates from Pus. Because increasing

patient acuity level, chronically ill patient who harbour antibiotic resistant bacteria & frequent use of broad spectrum antibiotics.

Table 2: Distribution of NFGNB isolates in various clinical samples (n=312)

| Sr. No | Specimen | No. of Specimens | NFGNB isolates (%) |
|--------|---------------|------------------|--------------------|
| 1 | Pus | 2458 | 211(65.62) |
| 2 | Ascitic fluid | 92 | 02(0.64) |
| 3 | Pleural fluid | 298 | 10(3.20) |
| 4 | Sputum | 488 | 20(6.41) |
| 5 | Swab | 155 | 08(2.56) |
| 6 | Urine | 163 | 10(3.20) |
| 7 | Blood | 458 | 51(16.34) |
| TOTAL | | 4112 | 312 |

Out of 312 isolates, most frequently isolates NFGNB were *Pseudomonas aeruginosa* 133 (42.62%) followed by *Acinetobacter* spp.79 (25.32%). Others

NFGNB isolated were *Pseudomonas* spp.51 (16.34%) & *Acinetobacter baumannii* 49 (15.70%).

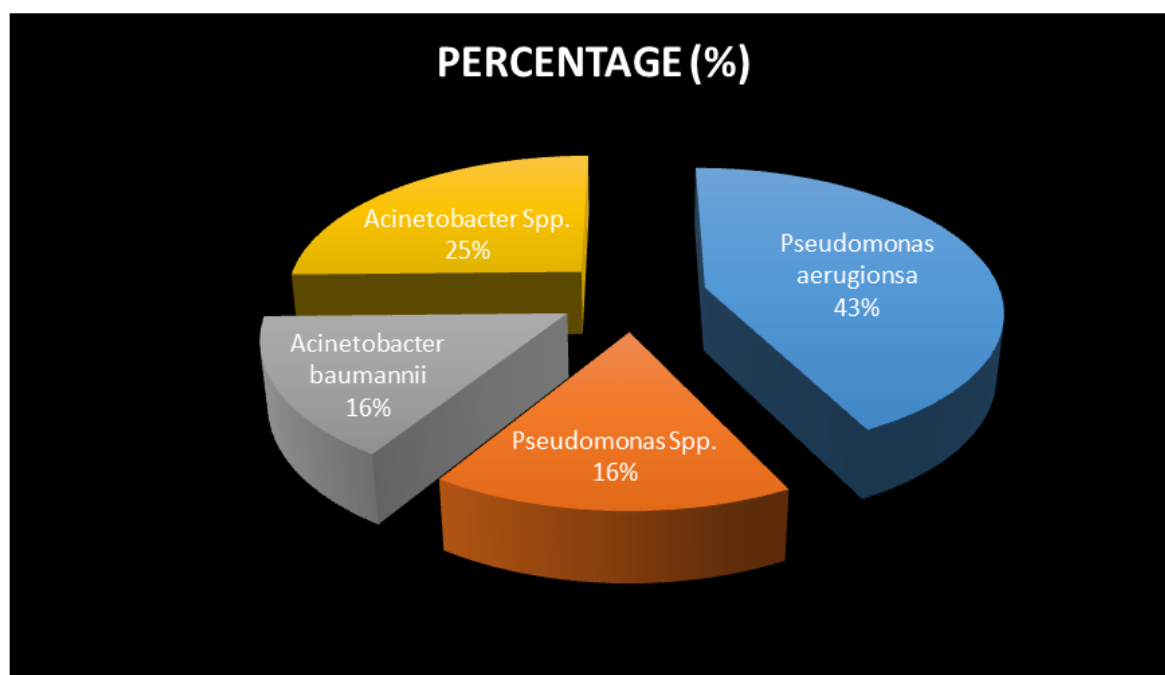


Figure 1: Non Fermentative Gram Negative Bacilli Isolates

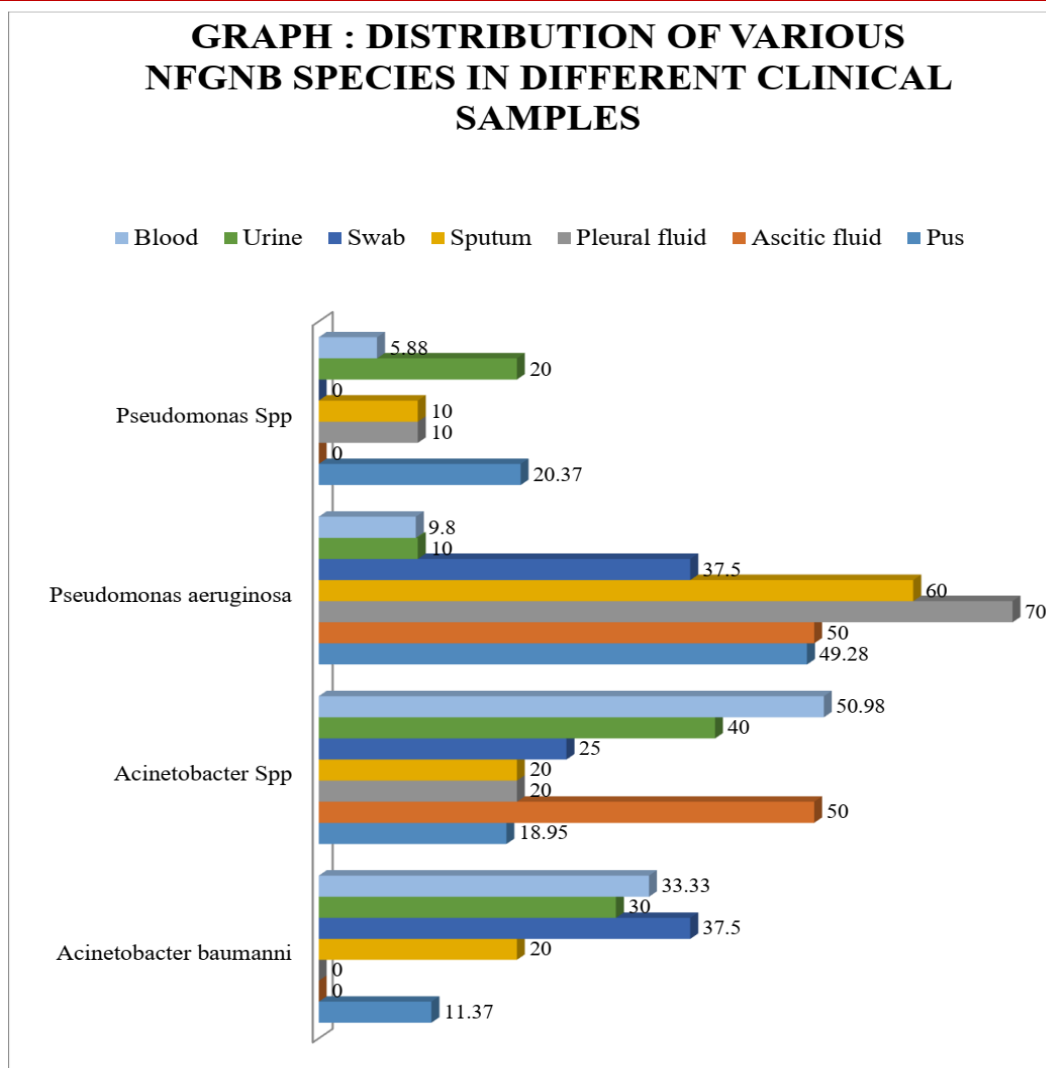


Figure 2: Distribution of Various NFGNB Species in Different Clinical Samples

Maximum sensitivity of *Acinetobacter baumannii* was seen to Meropenem (91.83%) followed by Piperacillin-tazobactam (73.46%), Tetracycline (59.18%), Levofloxacin (36.73%). Maximum Resistance to Cefotaxime (93.88%) followed by Ceftazidime (91.84%). NFGNB isolates from Urine showed 66.67 % resistance to Norfloxacin and 33.33% resistance to Nitrofurantoin.

Maximum sensitivity of *Acinetobacter* spp. was seen to Meropenem (78.48%), followed by Piperacillin-tazobactam (62.02%), Tetracycline (58.22%). Maximum resistance was observed to Cefotaxime (93.68%) followed by Ceftazidime (92.41%) & Cefepime (89.88%). NFGNB isolates from Urine showed 75% resistance to Nitrofurantoin and 25% resistance to Norfloxacin.

Maximum sensitivity of *Pseudomonas aeruginosa* to Polymyxin B (100%) followed by Meropenem (83.45), Aztreonam (67.66%), Piperacillin-tazobactam (61.65%). Maximum Resistance was observed to ciprofloxacin (71.43%) and Levofloxacin (69.93%). NFGNB isolates from Urine showed 100% resistance to Norfloxacin.

Pseudomonas spp. isolates showed 100% sensitivity to Polymyxin B followed by Meropenem (76.47%), Amikacin (58.82%), Piperacillin-tazobactam & Aztreonam (52.94%). Maximum resistance was observed to Ciprofloxacin (74.51%) followed by Levofloxacin (72.55%), Ceftazidime & Gentamicin (64.71%). NFGNB isolates from Urine showed 50 % resistance to Norfloxacin.

Table 3: Antimicrobial susceptibility pattern of *Acinetobacter baumannii* (n=49) & *Acinetobacter* spp. (n=79)

| Test/Report Group | Antibiotic | <i>Acinetobacter baumannii</i> | | <i>Acinetobacter</i> spp. | |
|-------------------|------------------------------|--------------------------------|---------------|---------------------------|---------------|
| | | Sensitive (%) | Resistant (%) | Sensitive (%) | Resistant (%) |
| Group A | Meropenem (MRP) | 45 (91.93) | 4 (8.17) | 62 (78.48) | 17 (21.52) |
| | Levofloxacin (LV) | 18 (36.73) | 31 (63.27) | 16 (20.25) | 63 (79.75) |
| | Ciprofloxacin (CIP) | 7 (14.28) | 42 (85.72) | 10 (12.65) | 69 (87.35) |
| | Ceftazidime (CAZ) | 4 (8.16) | 45 (91.84) | 6 (7.59) | 73 (92.41) |
| | Ampicillin/Salbactam (A/S) | 16 (32.65) | 33 (67.35) | 25 (31.64) | 54 (68.36) |
| | Gentamicin (GM) | 11 (22.44) | 38 (77.56) | 21 (26.58) | 58 (73.42) |
| Group B | Amikacin (AK) | 14 (28.57) | 35 (71.43) | 25 (31.64) | 54 (68.36) |
| | Pipracillin-Tazobactam (PIT) | 36 (73.46) | 13 (26.54) | 49 (62.02) | 30 (37.98) |
| | Cefotaxime (CTX) | 3 (6.12) | 46 (93.88) | 5 (6.32) | 74 (93.68) |
| | Cefepime (CPM) | 6 (12.24) | 43 (87.76) | 8 (10.12) | 71 (89.88) |
| | Cotrimoxazole (COT) | 12 (24.48) | 37 (75.72) | 23 (29.11) | 56 (70.89) |
| Group C | Tetracycline (TE) | 29 (59.18) | 20 (40.82) | 46 (58.22) | 33 (41.78) |
| Group U | Norfloxacin (NX) | 1 (33.33) | 2 (66.67) | 3 (75) | 1 (25) |
| | Nitrofurantoin (NIT) | 2 (66.67) | 1 (33.33) | 1 (25) | 3 (75) |

Table 4: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=133) & *Pseudomonas* spp. (n=51)

| Test/Report Group | Antibiotic | <i>Pseudomonas aeruginosa</i> | | <i>Pseudomonas</i> spp. | |
|-------------------|------------------------------|-------------------------------|---------------|-------------------------|---------------|
| | | Sensitive (%) | Resistant (%) | Sensitive (%) | Resistant (%) |
| Group A | Ceftazidime (CAZ) | 53 (39.84) | 80 (60.16) | 18 (35.29%) | 33 (64.71%) |
| | Gentamicin (GM) | 51 (38.34) | 82 (61.66) | 18 (35.29%) | 33 (64.71%) |
| Group B | Meropenem (MRP) | 111 (83.45) | 22 (16.55) | 39 (76.47) | 12 (23.53) |
| | Amikacin (AMK) | 61 (45.86) | 72 (54.14) | 30 (58.82) | 21 (41.18) |
| | Pipracillin-Tazobactam (PIT) | 82 (61.65) | 51 (38.35) | 27 (52.94) | 24 (47.06) |
| | Levofloxacin (LV) | 40 (30.07) | 93 (69.93) | 14 (27.45) | 37 (72.55) |
| | Ciprofloxacin (CIP) | 38 (28.57) | 95 (71.43) | 13 (25.49) | 38 (74.51) |
| | Cefepime (CPM) | 61 (45.86) | 72 (54.14) | 19 (37.25) | 32 (62.75) |
| | Aztreonam (AC) | 90 (67.66) | 43 (32.34) | 27 (52.94) | 24 (47.06) |
| Group O | Colistin/Polymyxin B | 133 (100) | 0 | 51 (100) | 0 |
| Group U | Norfloxacin (NX) | 0 | 1 (100) | 1 (50) | 1 (50) |

DISCUSSION

The non fermentative gram negative bacilli (NFGNB) are widely distributed in nature as a saprophyte or as commensals to man. They have now emerged as important healthcare-associated and opportunistic pathogens due to their frequent isolation from clinical materials and their association with various diseases.

In present study NFGNB isolation rate was 7.59%. Our study nearly correlates with study by, Madkey and Gajbhiye *et al.*, [13], which reported isolation rate of 5.19% and a study by Sudha Krishnan [14], which reported a total of 10.2% NFGNB isolates from all clinical specimens. In our study, NFGNB were isolated 43(10.48%) in ICU, 242(8.09%) in IPD and 27(3.79%) in OPD. Our observations correlate with the study by Madkey and Gajbhiye *et al.*, NFGNB strains were found in ICU (20.24%) compared to IPD (11.40%) and OPD (5.94%) [13]. A Malini *et al.*, they were isolated in ICU (8.47%), in IPD (6.92%) and in OPD (2.45%) [15].

In our study, NFGNB were slightly higher in Male 182(58.33%) than in females 130(41.67%). Male

preponderance was also seen in other studies. In a study by Jitendra *et al.*, they were isolated 56.92% in Male and 43.07% in Female [16]. A Malini *et al.*, they were isolated 68% in Male and 32% in Female [15].

Our study shows that Majority of NFGNB were isolates from Pus 211 (65.62%), followed by Blood 51(16.34%), Sputum (6.41%), Urine (3.20%). Our study are comparable with study by M. Nivitha *et al.*, which shows predominance of NFGNB in pus (52.3%) followed by sputum (20.9%), urine (9.9%), blood (4.7%) [17].

In the present study most frequently isolate NFGNB were *Pseudomonas aeruginosa* (42.62%) followed by *Acinetobacter* spp. (25.32%), *Pseudomonas* spp. (16.34%) *Acinetobacter baumannii* (15.70%). In Sudha Krishan *et al.*, *Pseudomonas aeruginosa* was the most common isolate (53.9%), followed by *Acinetobacter baumannii* (36.7%), and *Pseudomonas* spp.(6.0%), *Acinetobacter* spp.(3.0%) [14]. Jitendra *et al.*, *Pseudomonas aeruginosa* was the most common isolate (57.14%), followed by *Acinetobacter baumannii* (24.48%), and *Pseudomonas* spp. (14.28%), *Acinetobacter* spp. (4.08%) [16].

Maximum sensitivity of *Pseudomonas aeruginosa* to Colistin/Polymyxin B (100%) followed by Meropenem (83.45%), Aztreonam (67.66%), Piperacillin-tazobactam (61.65%). Maximum Resistance was observed to ciprofloxacin (71.43%). In the study by M Nivitha *et al.*, *Pseudomonas aeruginosa* highly sensitive to Colistin/Polymyxin B (100%), Meropenem (85%), Piperacillin-tazobactam (85%), Aztreonam (83%), Cefepime (74%). The highest resistance was recorded for Gentamicin (32%), Levofloxacin (31%) [17]. *Pseudomonas* spp. isolates showed 100% sensitivity to Colistin/Polymyxin B followed by Meropenem (76.47%), Amikacin (58.82%), Piperacillin-tazobactam & Aztreonam (52.94%). Juyal D *et al.*, [18], *Pseudomonas* spp. isolates showed 100% sensitivity to Colistin/Polymyxin B followed by Meropenem (78.57%), Amikacin (71.73%), Piperacillin-tazobactam & Aztreonam (53.57%).

Maximum sensitivity of *Acinetobacter baumannii* was seen to Meropenem (91.83%) followed by Piperacillin-tazobactam (73.46%), Tetracycline (59.18%), Levofloxacin (36.73%). In the study by, Parimal Patel *et al.*, *Acinetobacter baumannii* showed maximum sensitivity to Imipenem (72.9%) followed by Amikacin (38.8%), Doxycycline (22.3%) [19]. Maximum sensitivity of *Acinetobacter* spp. was seen to Meropenem (78.48%), followed by Piperacillin-tazobactam (62.02%). In the study by, parimal patel *et al.*, *Acinetobacter* spp. maximum sensitivity to Imipenem & Amikacin (77.8%) [19].

CONCLUSION

The present study highlighted the fact that non fermenter gram negative bacilli are emerging as important pathogens and shows resistance to commonly used antibiotics. More importantly these organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures like hand hygiene, using personal protective equipments, environmental cleaning, sterilization and disinfection of various instruments and devices used in Patient care and biomedical waste management should be implemented. Timely identification of nonfermenters and monitoring their susceptibility patterns will help in proper management of infections caused by them. Improved antibiotic stewardship and infection control measures should be implemented to prevent nosocomial infections and spread of drug resistant nonfermenters.

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