Original Research Paper

Volume - 11 | Issue - 09 | September - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

EMERGENCE OF MULTIDRUG RESISTANT NFGNB AS AN IMPORTANT CAUSE OF INFECTIONS

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ABSTRACT **Background:** The non fermenter gram negative bacilli (NFGNB) are primarily opportunistic pathogens and have emerged as an important cause of health care associated infections. **Methods:** The non fermenter organisms were isolated from various clinical specimens in a microbiology laboratory of a tertiary care hospital and were identified by the conventional bacteriological identification methods. Susceptibility testing was performed by methods as recommended by Clinical Laboratory Standard Institute (CLSI). **Results:** A total of 10,456 clinical specimens were processed, of which 442 (4.23%) non fermenter spp. were isolated. Most common infection caused by non fermenters was abscess. Maximum numbers of isolate (52.94%) were of Pseudomonas aeruginosa, followed by Acinetobacter baumannii (42.76%). Other species isolated were Acinetobacter calcoaceticus (1.81%), Pseudomonas putida (1.13%), Acinetobacter lwoffii (0.68%), Pseudomonas stutzeri (0.45%), Stenotrophomonas maltophilia (0.23%). The isolation rate of non fermenters from ICU was much higher compared to general ward. High antibiotic resistance was noted for commonly used antibiotics like cephalosporins, quinolones, aminoglycosides. A total 58.60% of multi drug resistant (MDR) non fermenter isolates were obtained. The isolation rate of MDR isolates of non fermenters was more from ICU compared to general ward. **Interpretation and Conclusion:** Nosocomial infections resistant to most antimicrobials caused by non fermenter organisms have emerged, especially in ICU. Early identification and continued surveillance will help to prevent their spread in hospital environment.

KEYWORDS : Non fermenter, antimicrobial resistance, nosocomial infections

INTRODUCTION

An increasing incidence was seen during the 1970s of resistant members of the family Enterobacteriaceae involved in nosocomial infections. It lead to therapeutic introduction of new broad spectrum antibiotics in hospitals and a subsequent increase in the importance of non fermenter gram negative bacilli (NFGNB) including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter spp.[1] Though primarily regarded as contaminants, they are becoming increasingly important as opportunistic pathogens.[2] A high degree of multi drug resistance is seen in NFGNB.[3] This study was done to know the prevalence of non fermenters isolated from different infections and to study their antimicrobial susceptibility profile.

MATERIAL AND METHODS

The study was carried out in the department of Microbiology from October 2014 to October 2016. Relevant clinical specimens from patients with bacteraemia, septicemia, skin and soft tissue infection, lower respiratory tract infection, meningitis, urinary tract infection, ascitis, keratitis were collected from inpatient departments by standard collection procedures.[4]

Microscopy of all the clinical specimens was done. Smears of specimens were stained using Gram method and examined for pus cells and different morphological forms of organisms.[5] All specimens were inoculated on 10% sheep blood agar and MacConkey agar and incubated at 37°C for 18-24 h. The non fermenter organisms were identified by reaction on triple sugar iron agar and by oxidative – fermentative test (Hugh and Leifson). They were further subjected to various biochemical reactions and the non fermenter species was identified by P.C. Schreckenberger scheme.[6]

The significance of the isolated non fermenter species was assessed by the presence of pus cells along with the gram negative bacilli in the stained smear from the sample, monomicrobial infection, isolation of the same organism from repeat sample.[7] The organisms isolated were also clinically co-related with the clinical condition of the patient and presence of risk factors like instrumentation and surgery like catheterizations, tracheostomy, lumbar puncture, dialysis, lavages and placement of shunts and prostheses, prolonged corticosteroid, antibiotic, antimetabolic, anticancer therapy, underlying metabolic or chronic infectious diseases, burns, open wounds, prolonged hospital stay.[6,8] Antimicrobial susceptibility testing was performed as per the CLSI guidelines.[9]

RESULTS

A total of 10,456 clinical specimens were processed, of which 442 (4.23%) non fermenter spp. were isolated. Most common infection caused by non fermenters was abscess (34.62%) followed by pneumonia (23.98%) as shown in Table 1. Major risk factors associated with non fermenter infections were prolonged antibiotic use (34.61%), mechanical ventilation (21.70%), trauma (18.63%), extended hospital stay (17.20%), followed by I.V. catheterization (9.76%), diabetes mellitus (8.82%), burn wound (7.35%), post-surgical (4.90%).

Table 1: Different Infections Caused By Non Fermenter Species

Sr. no	Non fermenter infections	Specimen type	Total no. of isolates (%)	
1	Abscess	Pus	153 (34.62)	
2	Pneumonia	Sputum	69	106
		Tracheal aspirate	20	(23.98)
		Bronchoalveolar lavage	9	
		Endotracheal tube secretions	8	
3	Urinary tract infection	Urine	52 (11.76)	
4	Wound infection	Wound swab	51 (11.54)	
5	Bacteraemia	Blood	41(9.28)	
6	Pleural effusion	Pleural fluid	26 (5.88)	
7	Ascitis	Ascitic fluid	10 (2.27)	
8	Meningitis	CSF	2 (0.45)	
9	Keratitis	Corneal scraping	1 (0.23)	
		Total	442 (100)	

Out of the total, maximum number 234 (52.94%) isolates were of *Pseudomonas aeruginosa*, followed by 189 (42.76%) *Acinetobacter baumannii*. Other species isolated were *Acinetobacter calcoaceticus* (1.81%), *Pseudomonas putida* (1.13%), *Acinetobacter lwoffii* (0.68%), *Pseudomonas stutzeri* (0.45%), *Stenotrophomonas maltophilia* (0.23%) as shown in Table 2. Male to female ratio of 1.23% was obtained. The maximum 99 (22.40%) isolates were observed in the age group of 41-50 years.(Table 3) The isolation rate of non fermenter species from ICU (16.09%) was more compared to the general wards of (3.68%).

Table 2: Different Non Fermenter Species Isolated

Non fermenter spp. isolated	Number of isolates (%)
Pseudomonas aeruginosa	234 (52.94)
Acinetobacter baumannii	189 (42.76)
Acinetobacter calcoaceticus	8 (1.81)
Pseudomonas putida	5 (1.13)

Acinetobacter lwoffii	3 (0.68)
Pseudomonas stutzeri	2 (0.45)
Stenotrophomonas maltophilia	1 (0.23)
Total	442 (100)

Table 3: Distribution Of Non Fermenter Isolates According To The Age And Sex Of Patients Infected With Non Fermenter Organisms

Age group	Male	Female	Total (%)
0 – 10 yr	28	18	46 (10.40)
11 -20 yr	16	11	27 (6.11)
21 -30 yr	39	28	67 (15.16)
31 – 40 yr	37	39	76 (17.19)
41 -50 yr	51	48	99 (22.40)
51 -60 yr	41	28	69 (15.61)
>60 yr	32	26	58 (13.13)
Total	244 (55.20%)	198 (44.80%)	442 (100)

The most sensitive antibiotic against *P. aeruginosa* was polymyxin B (100%) followed by imipenem (86.32%), piperacillin/tazobactam combination (80.77%) and amikacin (64.96%). In case of Acinetobacter species most sensitive drug was also polymyxin B (100%). Sensitivity of *Acinetobacter baumannii* for imipenem was 69.84%. High resistance was noted for commonly used antibiotics like cephalosporins, quinolones, aminoglycoside, β-lactam/β-lactamase inhibitor combination. An isolate of *Stenotrophomonas maltophilia* obtained was found to be susceptible to cotrimoxazole, levofloxacin, ticarcillin - clavulanate. A total 58.60% of multi drug resistant non fermenter isolates were obtained. Out of the total Pseudomonas spp. isolated, 55.60% were MDR while out of the total Acinetobacter spp. isolated, 62.5% were MDR. Highest number of MDR isolates was obtained from ICU (93.24%).

DISCUSSION

In the present study, isolation rate of non fermenters obtained was 4.23%. *Pseudomonas aeruginosa* has long been recognized as the most common non fermenter organism pathogenic for humans but *Stenotrophomonas maltophilia* and *Acinetobacter baumannii* also are now recognized as important pathogens.[10] In the present study, *Pseudomonas aeruginosa* was the most common species isolated accounting for 52.94%. It was followed by *Acinetobacter baumannii* (42.76%), *Acinetobacter calcoaceticus* (1.81%), *Pseudomonas putida* (1.13%), *Acinetobacter lwoffii* (0.68%), *Pseudomonas stutzeri* (0.45%), *Stenotrophomonas maltophilia* (0.23%).

In the present study, the non fermenter spp. were isolated from various infections like bacteraemia, septicemia, skin and soft tissue infection, lower respiratory tract infection, meningitis, urinary tract infection, ascitis, keratitis with maximum isolates obtained from abscess. NFGNB causes serious infections in immunocompromised and hospitalized patients especially those admitted to intensive care units (ICU).[11] Out of the total 442 non fermenters isolated, a total of 74 (16.74%) isolates were obtained from ICU in the present study.

Among the isolated non fermenter species high resistance was noted for commonly used antibiotics like cephalosporins, quinolones, aminoglycosides. Resistance for β-lactam/β-lactamase inhibitor combination was less in *Pseudomonas aeruginosa*. Resistance to imipenem was observed in 30.16% of *Acinetobacter baumannii* and 13.68% of *Pseudomonas aeruginosa* isolates. All the isolates were sensitive to polymyxin B. A total 58.60% of MDR non fermenter isolates were obtained in which maximum isolates were obtained from ICU. Out of the total Pseudomonas spp. isolated, 55.60% were MDR while out of the total Acinetobacter spp. isolated, 62.5% were MDR. In a study by Biswal I et al in 2014, 36.2% of the isolated *P. aeruginosa* were MDR.[12] According to Golia S et al in 2016, multi-drug resistant isolates of *P. aeruginosa* were 8.92%.[13] Khanal S et al in 2012 studied tracheal aspirates isolates in which MDR acinetobacter isolated were 85.4%.[14] According to study by Sinha N et al in 2013, 87% of Acinetobacter spp. was MDR.[15] NFGNB pose a particular difficulty for health care community because they represent problem of multidrug resistance to maximum, so there is an urgent need to study the antibiotic sensitivity pattern of commonly isolated NFGNB.[16]

CONCLUSION

Non fermenting gram negative bacilli (NFGNB) have emerged as important healthcare associated pathogens. NFGNBs are primarily opportunistic pathogens causing infection in seriously ill hospitalised patients, immunocompromised patients. Judicious use of antimicrobial agents is essential to prevent the emergence of multi drug resistant bacteria especially in the ICU.

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