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Characterization and Antibiotic Sensitivity Pattern of Nonfermenting Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital

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A B S T R A C T

Non-fermenting Gram negative bacilli (NFGNB) are a group of bacteria which are aerobic, non sporing and most commonly they are saprophytes. They are also found as commensals in both man and animals. NFGNB are previously considered as a contaminant but now it is emerged as a important cause for life threatening nosocomial infections and also multidrug resistant organisms. Materials and methods: One year prospective study was conducted and our aim is to characterise the non fermenters from various clinical samples and their antibiogram. 110 isolates from various clinical samples with different age groups were included. The clinical samples like pus, urine, endotracheal aspirates, blood, sputum and body fluids were collected under aseptic precaution and identified by using standard protocol. It includes Gram's staining, Motility Testing, Catalase test, Oxidase test, OF test and various biochemical reactions. The susceptibility testing was done by Kirby bauer disc diffusion method. A total of 110 NFGNB were isolated and among that nonfermenters, *Pseudomonas aeruginosa* (49%) was the predominant, followed by *Acinetobacter baumanii* (35%), *Acinetobacter lwoffi* (7.3%), *Stenotrophomonas maltophilia* (5.4%), *and Burkholderia cepacia* (2.8%). *Pseudomonas aeruginosa* showed good sensitivity to Polymyxin B (100%), Meropenem and Imipenam (79.6%) followed by Piperacillin tazobactam (72%) and Amikacin (59.3%). *Acinetobacter baumanii* showed 92% sensitivity to Polymyxin B followed by 69% sensitivity to Meropenem and Imipenam. *P.aeruginosa* and *A.baumanii* were the commonest nonfermenters that are isolated in our study. They are associated with various infections like urinary tract infection, blood stream infections, surgical site infections and ventilator associated pneumonia. *P.aeruginosa* showed better sensitivity to following antibiotics: Polymyxin B, Meropenem, Imipenem and Amikacin. Therefore it is essential to identify the nonfermenters and to know their antibiotic sensitivity pattern.

Introduction

Non fermenting Gram Negative Bacilli (NFGNB) are a group of organisms which are aerobic and non-sporing that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. [1]

Most commonly these bacteria occur as

saprophytes in the environment and also found as commensals in the human gut. [2] These are ubiquitous in nature particularly in soil and water. Although frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections in immuno-compromised hosts. NFGNB accounts for about 15% of all bacterial isolates from a clinical microbiology laboratory. [3,4]

Non fermenting Gram Negative Bacilli can cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicaemia, osteomyelitis, etc., Associated with risk factors like immuno-suppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics and therapeutic procedures. Outcome of the disease mainly depends on prolonged hospital stay, broad spectrum antibiotic use and underlying host factors. [5,6,8]

In the National Nosocomial Infection Surveillance (NNIS) survey from the Centre for Disease Control and Prevention (CDC), Infections caused by non fermenters is the fourth most common cause of hospital acquired infections. [7]

This group of non fermenters includes *Pseudomonas, Acinetobacter, Stenotro-phomonas, Burkholderia, Alcaligenes* and *Weeksella spp*, etc. Among these non fermenters, *Pseudomonas aeruginosa* is the commonly isolated non fermenter followed by *Acinetobacter baumanii*. Infection caused by these two nonfermenters is pathogenic for humans whereas infections caused by other species are less frequent. [3]

NFGNB show resistance to antibiotics due to production of extended spectrum β lactamases and metallo β lactamases. [4, 9, 10] Nonfermenters can cause opportunistic as well as nosocomial infections and this nosocomial infections caused by nonfermenters are most commonly observed in debilitated and immune compromised patients. [1,5,10]

This study was undertaken to identify the nonfermenters isolated from various clinical samples and their antimicrobial susceptibility pattern.

Materials and Methods

The present study was undertaken at the Department of Microbiology, Vijayanagara Institute of Medical Sciences, Ballari for a period of one year. A total of 110 non fermenters were isolated from various clinical samples such as pus, sputum, urine, blood, ET tube and body fluids were included.

Identification is mainly based on the Gram staining, Motility testing, and growth on Nutrient Agar, Mac Conkey Agar and Blood Agar. The isolates which are catalase positive, oxidase positive or negative, non lactose fermenting colonies on Mac Conkey agar were identified by colony morphology and pigment production. They were inoculated in Triple sugar iron (TSI) agar slope. The colonies which failed to acidify the TSI agar were considered as non fermenters and subjected to the following tests such as Indole, Citrate, Urease, Nitrate reduction, growth at 42^0C and sensitivity to Polymyxin B. [1]

The sensitivity test was performed by Kirby-bauer disc diffusion method using commercially available discs (Himedia). The results were interpreted as per the CLSI guidelines. [11] *Pseudomonas aeruginosa* ATCC 27853 was used as control strain.

Results and Discussion

A total of 110 non fermenters were isolated from various clinical samples. Among the 110

non fermenters, 43 (39%) were isolated from pus, 20 (18.1%) were from urine, 19 (17.2%) from wound swab, 11 (10%) from blood, 9 (8.1%) from sputum,, 5 (4.5%) from endotracheal aspirate and 3 (2.7%) from body fluids. (Table: 1)

Majority of isolates of non fermenters were from Surgical ward (40%) followed by ICU (20%), Medicine (14.6%), OBG (10%), Urology (8%), Burns (5.5%), Ortho (4.6%), Paediatrics (3.6%), Otorhinolaryngology (2.7%), TB ward (0.9%), Dermatology (0.9%). (Table: 2)

Among the nonfermenters, *Pseudomonas aeruginosa* (49%) was the predominant isolate followed by *Acinetobacter baumanii* (35%), *Acinetobacter lwoffi* (7.3%), *Stenotrophomonas maltophilia* (5.4%), and *Burkholderia cepecia* (2.8%). (Table: 3)

Pseudomonas aeruginosa showed good sensitivity to Polymyxin B (100%), Meropenem and Imipenam (79.6%) followed by Piperacillin tazobactam (72%) and Amikacin (59.3%). Acinetobacter baumanii showed 92% sensitivity to Polymyxin B followed by 69% sensitivity to Meropenem and Imipenam.

Among all the isolates maximum resistance was recorded for Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin (50.9%) and Cefotaxime (47.3%). (Table: 4)

Non fermenting Gram Negative bacilli (NFGNB) are being isolated with increasing frequency from various clinical samples. In recent years, the failure to treatment due to their multidrug resistance has led to the interest to carry out this study. In the present study out of 110 non fermenters, 43(39%) were isolated from pus, 20 (18.1%) from urine, 19(17.2%) wound swab, 11(10%) blood, 9(8.1%) sputum, 5 (4.5%) from endotracheal aspirate and 3(2.7%) from body fluids. In a study conducted by Gokale etal they reported that 58.4% non fermenters were isolated from pus/wound discharge followed by 23% from blood, 8.2% from urine, 4.5% from sputum and 2.3% from pleural fluid. [(2)]

A study conducted by Kirtilaxmi etal also stated that the isolation rate of pus was 21%, 11% from urine, 7% from blood and 17% from tracheal aspirate. [(3)]

In another study conducted by Kalidas etal they observed that the isolation rate was 27.9% from pus sample, 18.4% from tracheal aspirate, 16.4% from sputum and16.4% from blood and15.9% from urine. [(12)]

In the present study maximum number of non fermenters were isolated from Surgical wards (28.2%) followed by Intensive care unit (20%) and Medicine ward (14.6%). Similar findings were also reported by Anupurba et al., that higher prevalence rate of non fermenters was observed in surgery wards (29.9%).

The second highest prevalence observed in this study was intensive care units (20%) followed by Medical ward (14.6%).

In another study conducted by Keertilaxmi B etal they also reported that the isolation of NFGNB from intensive care units was 37%. [(3)] Among the non fermenters in recent years there are outbreaks of *Burkholderia cepacia* complex septicaemia have been documented worldwide in intensive care units (ICUs), oncology units and renal failure patients. [(13)]

In this present study, the commonest NFGNB isolated were *Pseudomonas aeruginosa* 54(49%) followed by *Acinetobacter baumanii* 39(35%), *Acinetobacter lwoffi* 8(7.3%), *S.maltophilia* 6 (5.4%), and *Burkholderia cepecia* 3(2.8%).

Table.1 Nonfermenting gram negative bacilli from various clinical samples

(n=110)

Clinical Samples	No. of Isolates	% of Isolates
Pus	43	39%
Urine	20	18%
Wound swab	19	17%
Blood	11	10%
Sputum	9	8.5%
ET tube	5	4.6%
Body fluids	3	2.8%
Total	110	100%

Table.2 Distribution of clinical isolates

(n=110)

Speciality	Clinical Isolates	Percentage (%)
Surgery	32	29.1
Intensive care unit	22	20
Medicine	16	14.6
OBG	11	10
Urology	9	8.2
Burns	6	5.4
Ortho	5	4.6
Paediatrics	4	3.6
Otorhinolaryngology	3	2.7
TB ward	1	0.9
Dermatology	1	0.9
Total	110	100

Table.3 Speciation of nonfermenting gram negative bacilli

(n=110)

Organisms	No. of Isolates	Percentage (%)
Pseudomonas aeurginosa	54	49
Acinetobacter baumanii	39	35
Acinetobacter lwoffi	8	7.3
Stenotrophomonas maltophilia	6	5.4
Burkholderia cepacia	3	2.8
Total	110	100

Table.4 Antimicrobial susceptibility pattern of nonfermenting gram negative bacilli

(n=110)

Antibiotics	*P.aeruginosa* (n=54)		*B.cepecia* (n=3)		*A.baumanii* (n=39)		*A.lwoffi* (n=8)		*S.maltophilia* (n=6)	
	S	%	S	%	S	%	S	%	S	%
Gentamicin	22	40.7	-	-	18	46	4	50	-	-
Amikacin	32	59.3	-	-	26	66	6	80	2	33.3
Ciprofloxacin	23	42.6	1	33.3	18	46	4	50	6	100
Ofloxacin	23	42.6	1	33.3	18	46	4	50	6	100
Ceftazidime	30	56	1	33.3	20	51	6	80	-	-
Cefotaxime	-	-	1	33.3	20	51	6	80	-	-
Piperacillin-tazobactam	39	72	1	33.3	26	66	8	100	1	16.7
Cotrimoxazole	-	-	3	100	20	51	4	50	6	100
Imipenam	43	79.6	2	66.7	27	69	8	100	-	-
Meropenem	43	79.6	2	66.7	27	69	8	100	-	-
Polymyxin B	54	100	-	-	36	92	8	100	6	100

*S-Sensitive and *- Not tested

A study conducted by Kalidas et al., also reported that *Pseudomonas* aeruginosa (50.2%) was the predominant isolate followed by *A.baumannii* (24.9%), *A.lwoffi* (5.5%), *S.maltophilia* (3%) and *Burkholderia cepacia*(7%). [12] In the present study *Pseudomonas spp* and *Acinetobacter spp* were the commonest NFGNB isolated which correlates with other studies. [3,16,17]

Because of the prevalence of high intrinsic resistance of different NFGNB to different antimicrobial agents in recent years, the absolute identification of non fermenters and their resistance pattern should be performed at microbiology laboratory. As this can be an important guide for the clinicians for appropriate selection of empiric therapy. In this study, the antimicrobial susceptibility pattern of *P.aeruginosa* showed 54 (100%) sensitivity to Polymyxin B, 43 (79.6%) sensitivity to Imipenem and Meropenem followed by Piperacillin tazobactum 39 (72%), Amikacin 32(59.3%), Ceftazidime 30 (56%), Ciprofloxacin and Ofloxacin 23(42.6%) and Gentamycin 22 (40.7%) which correlates with the study conducted by Grewal *et al.,* [18] and Kaur et al.,[19].

A Study from Gokale *et al.,* also reported that most of the isolates of *Pseudomonas aeruginosa* were sensitive to Meropenem (96.2%), followed by Ciprofloxacin (50.4%) and Amikacin (49.5%). [2]

A study conducted by Nautiyal etal reported that all the isolated *Pseudomonas aeruginosa* were sensitive to Polymyxin B [14] which correlates with our study as well. Kirtilaxmi etal reported the sensitivity of *Pseudomonas aeruginosa* were ceftazidime (60%), Gentamycin (65%), Piperacillin-tazobactum (73.3%), Imipenem (80%), Amikacin (83.3%), Ciprofloxacin (58.3%) [3] It is similar to our study.

In the present study, the isolates of *A.baumannii* showed 27 (69%) sensitivity to Meropenem and Imipenem followed by Amikacin and Piperacillin tazobactum 26 (66%), Ceftazidime and Cotrimoxazole 20 (51%), Gentamycin and Ciprofloxacin and

Ofloxacin 18 (46%) each respectively. Most of the isolates were sensitive to Polymyxin B 36 (92%). These results are similar to the study conducted by Nautiyal et al., [14]

In the present study, all the isolates of *A.lwoffi* were sensitive to Imipenem, Meropenem and Piperacillin tazobactum 8(100%) followed by Cefotaxime, Ceftazidime and Amikacin 6 (80%), Gentamycin, Cotrimoxazole and Ciprofloxacin and Ofloxacin 4 (50%), Polymyxin B (100%). Similar to the present study, another study conducted by Nautiyal etal reported that all the isolated *A.lwoffi* were 100% sensitive to Polymyxin B. [14]

In our study, among the isolated *S.maltophilia* majority were sensitive to Cotrimoxazole, Ciprofloxacin and Ofloxacin and Polymyxin B 6 (100%), followed by Amikacin 2(33.3%) and Piperacillin tazobactum1 (16.7%). These results are similar to another study conducted by Nautiyal etal They reported that all the isolated *S.maltophilia* were 100% sensitive to polymyxin B. [14] Similar results were also shown in another study conducted by Deepak etal They reported that all the isolated *S.maltophilia* showed 100% sensitivity to Ciprofloxacin and Cotrimoxazole and 33.3% to piperacillin tazobactum and 16.67% to Gentamycin [15] *S. maltophilia* is intrinsically resistant to most β lactams, including carbapenems. [13] In our study, among the isolates of *Burkholderia cepacia* (3), all were sensitive to Cotrimoxazole 3(100%), followed by Imipenem and Meropenem 2(66.7%), Ciprofloxacin, Ofloxacin, Cefotaxime and Ceftazidime 1(33.3%). In contrast to the present study, Kalidas etal showed the 92.8% sensitivity to Imipenem and Cotrimoxazole, 85% to Ceftazidime and Ciprofloxacin and 57% to Piperacillin tazobactum. [12]

Burkholderia cepacia show intrinsic resistance to aminoglycosides and polymyxins due to the presence of inducible chromosomal β-lactamases and altered penicillin-binding proteins leads to resistance to β-lactams.

They also mediate resistance to chloramphenicol, trimethoprim and fluoroquinolones through its antibiotic efflux pumps. [13]

Observations from the present study showed that aerobic NFGNB which are usually considered as contaminants are now emerging as important nosocomial pathogens. Different antimicrobial susceptibility pattern and multidrug resistance by nonfermenters cause difficulty in treating the infections. ESBL and MBL production by these organisms lead to high morbidity and mortality and we have only option of treating them by potentially toxic drugs like Colistin and Polymyxin B. All the health care institutions should have their own antimicrobial policy, regular surveillance and infection control protocols to avoid high incidence of resistant non fermenters.

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