Characterization and antimicrobial susceptibility pattern of non-fermenting gram negative bacilli from various clinical samples in a tertiary care hospital

Gomathi Maniyan[1,*], Dillirani Vedachalam[2], Nithya Chinnusamy[3]

[1]Assistant Professor, [2]Professor, [3]Assistant Professor, Dept. of Microbiology,

***Corresponding Author:**
Email: gomathym2013@gmail.com

Abstract

Background: Non fermenting Gram Negative Bacilli (NFGNB) once considered as contaminants have now emerged as a major cause of life threatening nosocomial infections and as multidrug resistant pathogens. Aim: To isolate and identify the NFGNB and their antimicrobial susceptibility pattern and to detect the Extended Spectrum Betalactamases (ESBL) among the isolated non-fermenters.

Materials and Methods: This Cross sectional study conducted in Medical College and Hospital for one year and samples collected like pus, urine, endotracheal aspirates, blood, sputum and body fluids were identified using standard protocol, which includes Grams staining, test for motility, catalase test, oxidase test, OF test and various biochemical reactions.

Results: Out of 110 clinically significant isolates of non-fermenters, 54(49%) were Pseudomonas aeruginosa, 36(32.7%) Acinetobacter baumannii, 8(7.3%) Acinetobacter lwoffi, 6(5.4%) S.maltophilia and Pseudomonas stutzeri and Burkholderia cepecia 3(2.8%). The antimicrobial susceptibility pattern revealed maximum resistance to Gentamycin (61.8%), Cotrimoxazole (60%), followed by Ciprofloxacin(50.9%) and Cefotaxime(47.3%). Sensitivity to Polymyxin B (100%) followed by Imipenem and Meropenem (75.5%). ESBL production was 18.18%.

Conclusion: Pseudomonas aeruginosa and Acinetobacter baumannii were the most common NFGNB isolated in this study. Difference in antimicrobial susceptibility by nonfermenters pose a great problem in treating these infections. ESBL production by these organisms lead to high morbidity and mortality.

Keywords: Non-fermenter, Antimicrobial susceptibility, ESBL, Pseudomonas, Acinetobacter

Introduction

Non fermenting Gram Negative Bacilli (NFGNB) are aerobic, non-spore forming organisms that either do not use carbohydrates as a source of energy (or) degrade them through metabolic pathways other than fermentation[1,2,3]. 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. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory[3]. Non fermenting Gram Negative Bacilli cause various infections including wound infections, urinary tract infections, meningitis, pneumonia, septicemia, osteomyelitis, etc.[4] Risk factors includes immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, invasive diagnostics and therapeutic procedures. They are recovered with increasing frequency from clinical specimens. Prolonged hospital stay, broad spectrum antibiotic use and underlying host factors are the best predictors of outcome[5]. This group includes organisms from diverse genera like *Pseudomonas, Acinetobacter, Stenotrophomonas, Burkholderia, Alcaligenes, Weeksella,* etc., currently; *Pseudomonas aeruginosa and Acinetobacter baumanii* are the most commonly isolated non-fermenters pathogenic for humans whereas infections caused by other species are relatively infrequent.[3] It is the most common cause of wound infection among Gram negative bacteria with an isolation rate of up to 62%[6]. *Ps. aeruginosa* is the leading cause of pneumonia in the ICU patients with a mortality of 80 -100% [7]Members of non-fermenting gram negative bacteria show resistance to a wide range of commonly used antibiotics by several mechanisms like antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change targets or cellular functions[8]. The antimicrobial inactivating enzymes are beta lactamases including Extended Spectrum Betalactamases (ESBL), AmpC, Non-metallo beta lactamases and Metallo betalactamases (MBL)[9]. Multidrug resistance (MDR) exhibited by non-fermenters pose a major clinical problem in treating infections caused by them. Therefore, early identification and institution of appropriate treatment is necessary to reduce the morbidity and mortality due to these organisms in hospitalized patients[10]. The present study was therefore taken to identify the non-fermenters from various clinical specimens and to determine their antimicrobial susceptibility pattern and to detect the production of Extended Spectrum Betalactamases in non-fermenters (ESBL) and also by different phenotypic methods. This may provide the necessary information to formulate a hospital antibiotic policy and also to prevent the spread of multidrug resistant strains in the community.

Materials and Methods

This Cross sectional study was conducted in the Department of Microbiology in a tertiary care hospital

over a period of one year from July 2014 to June 2015. Samples were collected from various patients who attended the OPD and wards in whom satisfied the inclusion criteria. The inclusion criteria included were hospitalized patients undergoing treatment in ICU medical, surgical and pediatric ward, Patients affected with burn wounds, Patients with non-healing ulcer, Diabetic patients with ulcers, Patients with provisional diagnosis of Septicemia and Pneumonia, Patients with indwelling urinary catheter and on ventilators. Exclusion criteria included patients on prior antibiotic therapy. Isolates of repeated samples from the same patient were not included in the study and patient who did not give consent.

Isolation and Identification is mainly based on the Gram staining, Motility, colony morphology on Nutrient Agar, MacConkey Agar and Blood Agar. All the catalase positive, oxidase positive and negative, non-lactose fermenting colonies on Mac Conkey agar were provisionally 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 in dole production, citrate utilization, urease production, nitrate reduction, growth at 42˚C , Sensitivity to Polymyxin B and following special biochemical tests and grouped according to P.C. Schreckenberger scheme.[1]

Antimicrobial Susceptibility Testing:

Disc diffusion method: Antimicrobial susceptibility was performed for all the isolates by modified Kirby - Bauer disc diffusion method. Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines (January 2014, M100-S24- Volume 34 No.1, Table 2B-2, Page 62/63).

Detection of ESBL: All the isolates which were included in this study were subjected to ESBL screening test using Cefotaxime and Ceftazidime discs. Cefotaxime disk (30mg), cefotaxime – clavulanic acid (30mg/10mg) ceftazidime (30mg) & ceftazidime clavulanic acid (30mg/10mg) were placed on surface of agar. The plates were incubated at 35^0C for 16-18 hours and diameter of zone of inhibition produced was recorded. A 5mm increase in zone diameter for combination disc than that when tested alone confirmed the presence of ESBL production. ATCC *Escherichia coli* 25922 & *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control respectively.

Results

All the isolates were characterized to the species level and results were analyzed.

Age distribution of non-fermenters was analyzed which showed, majority of the patients were from the age group of less than 10 years of age 23(20.91%), followed by 21-30 years 21(19.09%) and elderly patients 21(19.09), >60 years of age. Of the 110 isolates 72(65.4%) were from males and 38 (34.5%) from females. Among the non-fermenters, 43 (39%) were isolated from pus, 20 (18.1%) 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. Majority of isolates of non-fermenters were from Surgical ward (40%) followed by ICU (20%). Wound swab following surgery/trauma (40%) was the major risk factor contributes to the infection with non-fermenters. The findings of antimicrobial susceptibility testing and ESBL detection is depicted in tables.

Table 1: Distribution of clinical isolates (n=110)

Speciality	Clinical isolates	Percentage (%)
Surgery	32	29.09
Intensive care unit	22	20.01
Medicine	16	14.55
OG	11	10
Urology	9	8.18
Burns	6	5.45
Ortho	5	4.55
Paediatrics	4	3.64
Otorhinolaryngology	3	2.73
TB ward	1	0.91
Dermatology	1	0.91
Total	110	100

Table 2: Speciation of non-fermenters (n = 110)

Clinical isolates	Number	Percentage (%)
Pseudomonas aeruginosa	54	49
Acinetobacter baumanii	36	32.7
Acinetobacter lwoffi	8	7.3
Stenotrophomonas maltophilia	6	5.4
Pseudomonas stutzeri	3	2.8
Burkholderia cepacia	3	2.8
Total	110	100

Table 3: Sample wise organism isolation (n=110)

Specimen	P.aeruginosa. (n=54)		P.stutzeri (n=3)		B.cepacia (n=3)		A.baumannii (n=36)		A.lwoffi (n=8)		S.maltophilia (n=6)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pus	19	35.2	2	66.7	0	0	19	52.8	0	0	3	50
sputum	2	3.7	0	0	3	100	2	5.6	2	25	0	0
urine	14	25.9	0	0	0	0	2	5.6	4	50	0	0
WS	13	24.1	0	0	0	0	4	11.1	1	12.5	1	16.7
Blood	4	7.4	1	33.3	0	0	4	11.1	1	12.5	1	16.7
As.fluid	1	1.85	0	0	0	0	0	0	0	0	1	16.7
ET swab	1	1.85	0	0	0	0	4	11.1	0	0	0	0
Pl.fluid	0	0	0	0	0	0	1	2.8	0	0	0	0
Total	54		3		3		36		8		6	

Table 4: Antimicrobial susceptibility pattern of nonfermenters (n=110)

Drugs	Ps.aeruginosa (n=54)		A.baumaniii (n=36)		A.lwoffii (n=8		P.stutzeri (n=3)		S.maltophilia (n=6)		B.cepaciaa (n=3)	
	S	%	S	%	S	%	S	%	S	%	S	%
Gentamycin	22	40.7	18	50	4	50	1	33.3	-	-	-	-
Amikacin	32	59.3	26	72.2	6	80	2	66.7	2	33.3	-	-
Ciprofloxacin	23	42.6	18	50	4	50	2	66.7	6	100	1	33.3
Ofloxacin	23	42.6	18	50	4	50	2	66.7	6	100	1	33.3
Ceftazidime	30	56	20	55.6	6	80	2	66.7	-	-	1	33.3
Cefotaxime	-	-	20	55.6	6	80	-	-	-	-	1	33.3
Pip - Taz	39	72	26	72.2	8	100	3	100	1	16.7	1	33.3
Cotrimoxazole	-	-	20	55.6	4	50	-	-	6	100	3	100
Imipenem	43	79.6	27	75	8	100	3	100	-	-	2	66.7
Meropenem	43	79.6	27	75	8	100	3	100	-	-	2	66.7
Polymyxin - B	54	100	36	100	8	100	3	100	6	100	-	-

Table 5: ESBL production in nonfermenters (n=110)

Organism	Total No.	ESBL producers	Percentage (%)	One proportion Z-Test
Ps.aeruginosa	54	9	16.7	0.0001
A.baumanii	36	6	16.7	0.0009
A.lwoffi	8	2	25.0	0..7516
Ps.stutzeri	3	1	33.3	0.9885
S.maltophilia	6	1	16.7	0.2420
B.cepacia	3	1	33.3	0.9885
Total	110	20	18.18	

Discussion

Non fermenting Gram Negative bacilli (NFGNB) are being isolated with increasing frequency from clinical specimens and treatment failure due to their multidrug resistance in recent years. 110 clinical isolates of non-fermenting Gram negative bacilli isolated from various clinical samples like pus, urine, endotracheal aspirates, blood, sputum, body fluids and were evaluated for their role in infections in hospitalized patients including the characteristics of their drug resistance. In this study maximum number of isolates were from Surgical wards (28.18%) followed by Intensive care unit (20.01%) and Medicine (14.55%) ward [**Table 1]**. Outbreaks of Burkholderia cepacia complex septicemia have been documented worldwide in intensive care units (ICUs), oncology units and renal failure patients[11].

On analyzing the risk factors, it was present in 95% of the infections caused by Non-fermenters and the commonest risk factor associated was Surgery/Trauma (40%) followed by ICU stay (20%), prolonged antibiotic therapy(9.9%), Catheter and Instrumentation (8.18%) followed by Diabetes mellitus (6.36%), Burns (9.09%), Malignancy and Ventillator associated pneumonia (0.91%), cardiac failure, end-stage renal disease, cancer, hepatitis and human immunodeficiency virus[12]. A longer hospital stay in a high-risk unit, use of mechanical ventilation, admission as inpatient into the ICUs, and underlying co-morbid conditions have been identified as the risk factors.

In the present study, the commonest isolates were *Pseudomonas aeruginosa* 54 (49%) followed by *Acinetobacter baumanii* 36(32.7%), *Acinetobacter lwoffi* 8(7.3), *S.maltophilia* 6(5.4%), *Pseudomonas stutzeri* and *Burkholderia cepecia* 3(2.8%) among the NFGNB **(Table 2**). Among the 54 *Pseudomonas aeruginosa* isolated, 19(35.2%) were from pus, 2(3.7%) from sputum, 14(25.9%) from Urine, 13 (24.1%) from Wound swab, 4(7.4%) from blood, 1(1.85%) from Ascitic fluid and ET swab **(Table 3).** Because of high intrinsic resistance of different NFGNB to different antimicrobial agents, the value of proper identification and resistance testing is foremost important in a given setup to guide appropriate selection of empiric therapy. The antimicrobial susceptibility pattern of *Ps.aeruginosa* showed 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%) and Polymyxin B 54(100%)**(Table 4).** *Acinetobacter spp.,* were the second most common isolate among the nonfermenters contributes to 40% *A.baumannii* (n=36) showed 27(75%) sensitivityto Meropenem and Imipenem followed by Amikacin and Piperacillin tazobactum 26(72.2%), Ceftazidime and Cotrimoxazole 20(55.6%), Gentamycin and Ciprofloxacin and Ofloxacin 18(50%) each respectively. 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%).**(Table 4)** In the present study, the antimicrobial susceptibility 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 tazobactum 1(16.7%).**(Table 4)** Multidrug resistance is a major problem with non-fermenting gram negative bacilli and so the infections caused by them are very difficult to be treated. Polymyxins are the remaining antimicrobial drug class with fairly consistent activity against multidrug resistant strains of non-fermenters.

ESBL continued to be a major challenge in healthcare institutions, hence knowledge about their prevalence is very essential to initiate appropriate antimicrobial therapy. In the present study, all the 110 isolates were screened for ESBL production and confirmed by CLSI phenotypic confirmatory method. 20(18.18%) isolates were found to be ESBL producers.(p value – 0.0001 as per one proportion Z-Test) which is statistically significant. In the present study, 9(16.7%) *Pseudomonas aeruginosa*, followed by *A.baumannii* 6 (16.7%), *A.lwoffi* 2(25%), *P.stutzeri* 1(33.3%) and *B.cepacia* and *S.maltophilia* 1(16.7%) were ESBL producers.**(Table 5)** While *S.maltophilia* and *B.cepacia* show intrinsic resistance to ß-lactams, ESBL production by *P.aeruginosa* and *A.baumannii* is significant. (P value of *P.aeruginosa* – 0.0001 and *A.baumannii* – 0.0009) which is statistically significant. In the present study, maximum sensitivity among ESBL producers was seen with Piperacillin-tazobactum (100%) followed by Imipenem and Meropenem (90.5%).Due to difference in antimicrobial susceptibility pattern in different hospitals, frequent studies are valuable in deciding most adequate therapy. The prevalence and sensitivity of non-fermenters often varies between communities, in the same community and hospitals, among different patient populations in the same hospital. Faced these variations, the physician in clinical practice has the responsibility of making clinical judgments and should access to recent data on the prevalence and antimicrobial resistance pattern of commonly encountered pathogens.

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The present study observed highest resistance of NFGNB against Gentamycin & Cefotaxime antibiotics which are commonly used drugs. This necessitates the judicious use of these antibiotics in empirical therapy. Maximum sensitivity was observed with newer agents like carbapenams and pipercillin-tazobactum and polymyxin. Moderately sensitive to Aminoglycosides and Fluroquinolones. Major risk of using monotherapy is the emergence of antibiotic resistance as observed in the present study which showed high rate of multidrug resistance and ESBL producers.

Antibiotic therapy either empirical or documented is based upon antibiotic combination supplemented by the knowledge of local epidemiology of susceptibility pattern in choosing a suitable combination. Therefore combination therapy such as piperacillin-tazobactum, quinolones amikacin, imipenam-amikacin would be an ideal choice of therapy on the basis of antimicrobial susceptibility testing as observed in this study along with an adequate infection control measures especially in the surgical and ICU units.[13,14]

Conclusion

Observations from present study showed that the aerobic NFGNB which are usually considered as contaminants are now emerging as important nosocomial pathogens. The various clinical samples from which they were isolated proved their existence in all the sites leading to a range of diseases. Different

antimicrobial susceptibility pattern and multidrug resistance exhibited by non-fermenters pose great problems in treating these infections. ESBL and MBL production by these organisms lead to high morbidity and mortality and we were left with the only option of treating them by potentially toxic drugs like Colistin and Polymyxin B. Care in detection, evaluation of effective antibiotic option, judicious use of antibiotics by instituting antibiotic policy and infection control measures will help to fight against these multidrug resistant non-fermenters in the effective management of patients. The present need is that all the health care institutions should have a coordinated effort to curtail inappropriate use of antibiotics, their own antimicrobial stewardship program, and vigilant detection of resistant non-fermenters, regular surveillance and infection control protocols to control the increasing incidence of highly resistant non-fermenters.

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How to cite this article: Maniyan G, Vedachalam D, Chinnusamy N. Characterization and antimicrobial susceptibility of pattern of non-fermenting gram negative bacilli from various clinical samples in a tertiary care hospital. Indian J Microbiol Res 2016;3(4):387-391.