Original Research Article

A clinical and microbiological study of non-fermenting gram negative bacilli in a tertiary care hospital

Ami Patel[1], Suman Singh[2*]

[1]Resident Doctor, [2]Professor & HOD, Department of Microbiology, Pramukhswami Medical College, Karamsad, Gujarat, INDIA.
Email: dramicpatel@gmail.com

Abstract

Background: Aerobic non-fermenting Gram-negative bacilli (NFGNB) once considered as contaminants are now commonly associated with life-threatening infections and have emerged as multidrug resistant nosocomial pathogens. In spite of being pathogens of great clinical significance, their identification and antimicrobial sensitivity, being tedious and time consuming, is not routinely reported by conventional methods where automated systems are not available. Aims: To study the prevalence of NFGNB in our hospital along with the comparison between conventional method and Vitek 2 automated system for identification and antimicrobial susceptibility to find the percentage agreement between the two methods. Settings and Design: This prospective observational study was conducted in the Department of Microbiology at a tertiary care teaching hospital over a period of one year. Methods and Material: Isolation, identification and antibiotic sensitivity test of these NFGNB was performed by conventional method and Vitek 2 automated system. Statistical analysis used: The statistical analysis was performed by SPSS 14.0 with application of two samples T-test and ANOVA. Agreement between two methods was analysed using Kappa. Results: Prevalence of NFGNB was 26.52% predominantly from pus (22.2%) followed by Sputum (20.6%). Male (70.9%) patients were more compared to female (29.1%).*P.aeruginosa* (38.62%) and *A.baumannii* (34.92%) were the main NFGNB followed by *S.paucimobilis* (6.87%) and *A.lwoffii* (5.29%). Antimicrobial sensitivity in *P. aeruginosa* ranged from 56.2-94.5% to various drugs while *A. baumannii* was found to be multidrug resistance (87.9%).Faster reporting, identification and Antimicrobial susceptibility within 6.42 and 12.89 hours respectively, was the major advantage with moderate agreement (p value 0.476) between Vitek 2 and Conventional method, which means both the method can be used for the Identification. Conclusions: NFGNB are now emerging as organisms of nosocomial infections, making the identification and antimicrobial sensitivity necessary for patient management. If possible, automated systems must be used for faster and reliable result for better care and management of patients.

Key words: Non fermenting Gram-negative bacilli, *Pseudomonas aeruginosa, Acinetobacter baumannii,* Vitek 2.

[*]**Address for Correspondence:**
Dr Suman Singh, Professor & HOD, Department of Microbiology, Pramukhswami Medical College, Karamsad, Gujarat, INDIA.
Email: dramicpatel@gmail.com

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INTRODUCTION

Non fermenters are a group of aerobic, non-spore forming gram negative bacilli that are either incapable of utilizing carbohydrates as a source of energy, or degrade them via oxidative rather than a fermentative pathway.[1] Non-fermenters can cause a vast variety of infections and accounts for approximately 15% of all Gram negative bacilli cultured from clinical specimen.[2] Less than 1/5th of all Gram negative bacilli isolated from clinical specimens received in the routine laboratories are likely to be non-fermentative bacilli. Although non-fermenters are commonly considered as commensals or contaminants; they have emerged as important nosocomial pathogens with frequent outbreaks. [3-9] Spectrum of disease by *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* the most common NFGNB are well established as nosocomial pathogens. Other NFGNBs like *Burkholderia cepacia, Stenotrophomonas maltophilia, Sphingomonas paucimobilis, Ralstonia pickettii*or, *Achromobacter spp*. have been increasing since the early 1970s.[10-12] These pathogens primarily affect patients with co-morbidities such as cystic fibrosis (CF), immunosuppression, organ transplantation, and malignancy. Higher rate of

hospitalized patients with serious underlying diseases, large environmental distribution as potential reservoirs for human infections and intrinsic high-level of antibiotic and biocide resistance in NFGNB are contributing factors for this emergence. [13-15] In-spite of being important as human pathogens, very few clinical microbiology laboratories are able to identify these organisms as a routine because of their complicated taxonomy, slow growth, need for use of special culture media and large spectrum of complex biochemical test required for their identification by conventional tecniques.[16] To overcome this problem a number of semi or fully automated systems like Phoenix, Microscan, Vitek 2 etc. have been introduced which are expected to give faster and better results that can be very critical in patient care but they are not available in a routine microbiology laboratory for use. Prevalence and antibiogram of non-fermenting Gram negative bacilli has not yet been reported using conventional method and automated system VITEK2 from this part of India. The present work is designed to study the prevalence of nonfermenting gram negative bacilli with their clinical and microbiological profile in our hospital. We would also compare VITEK 2, a fully automated identification / antibiotic susceptibility testing (AST) system for its efficacy in rapid and accurate identification and AST with conventional manual methods for better patient management.

MATERIALS AND METHODOLOGY

The present study was conducted at the Shree Krishna Hospital located in Karamsad, a tertiary care hospital with a capacity of 550 beds. This is a prospective observational study. The duration of the study was one year from April 2014 to April 2015. In the duration of one year, 189 consecutive non-repetitive isolates of Non fermenters obtained from various clinical samples such as pus, sputum, urine, blood, endotracheal secretion, cerebrospinal fluid, pleural fluid, peritoneal fluid, tracheostomy secretions, CVP tip, Catheter tip and other relevant clinical material submitted to Microbiology Laboratory for Culture and Sensitivity testing from outdoor as well as indoor patients of Shree Krishna Hospital, Karamsad, were included in the study. All the samples received in bacteriology section of laboratory were inoculated on blood agar, Nutrient Agar, Chocolate agar, MacConkey agar and incubated at 37°C for 48 h before being reported as sterile. The isolates which were non-lactose fermenting and showed alkaline/no change (K/NC) reaction on triple sugar iron agar were provisionally considered as NFGNB. Isolates were identified using Conventional method and automated system VITEK 2 system (bioMérieux) for identification and antibiotic sensitivity testing. The non-fermenting gram negative bacilli (NFGNB) were identified up to genus or species level based on Motility test, Oxidase test, Catalase test, Indole production, Nitrate reduction, Citrate utilization, Urease test, Phenylalanine deaminase test, Triple Sugar Iron agar (TSI agar) test, Arginine dehydrolase test, Lysine decarboxylase production, Ornithine decarboxylase production, Gelatin liquefaction, Malonate utilization, Acetamide, Esculin hydrolysis, DNAse hydrolysis. The susceptibility testing was performed using Kirby-Bauer disc diffusion method using commercially available discs according to Clinical Laboratory Standards Institute guidelines.

RESULTS

In the present study total numbers of clinical specimens processed in the Microbiology Laboratory of Shree Krishna Hospital from April 2014 to April 2015 were 7743, out of which 2613 were culture positive. Out of all culture positive samples, 693 (26.52%) were positive for NFGNB, but only 189 NFGNB have been included in present study. Out of 189 samples highest NFGNB were isolated from pus sample 22.2%,followed by Sputum 20.6%, ET 19%, and lowest among Catheter tip, Femoral tip, Splenic swab, TT (0.5% each). [Table 1 and Figure 1]

Table 1: Specimen wise distribution of NFGNB in the present study (n=189)

Specimens	No of Isolates	%
BAL	2	1.1
Blood	6	3.2
Catheter Tip	1	0.5
CVP Tip	2	1.1
Drain	2	1.1
ET	36	19.0
Femoral Tip	1	0.5
Pus	42	22.2
Splenic Swab	1	0.5
Sputum	39	20.6
Swab	1	0.5
TT	28	14.8
Urine	28	14.8
Total	**189**	**100**

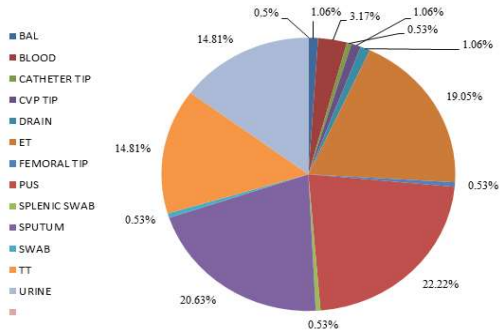

Figure 1: Specimen wise distribution of NFGNB in the present study

Isolation rate of NFGNB was highest among male (70.9%) as compared to females (29.1%). [Table-2]

Table 2: Gender distribution

Organism	Female (%)	Male (%)	Total (%)
Ac.baumanii	12(18.2)	54(81.8)	66(100)
Ac.lwoffi	5(50)	5(50)	10(100)
Ac.haemolyticus	0(0)	1(100)	1(100)
Acinetobacter spp.	2(100)	0(0)	2(100)
Burkholderia cepacia	1(12.5)	7(87.5)	8(100)
Burkholderia pseudomallei	0(0)	1(100)	1(100)
Burkholderia spp	0(0)	1(100)	1(100)
Myroides spp	0(0)	3(100)	3(100)
Ps.aeruginosa	22(30.1)	51(69.9)	73(100)
Ps.luteola	2(100)	0(0)	2(100)
Ps.stutzeri	3(75)	1(25)	4(100)
Sphingomonas paucimobilis	5(38.5)	8(61.5)	13(100)
Stenotrophomonas paucimobilis	3(60)	2(40)	5(100)
Total	**55(29.1)**	**134(70.9)**	**189(100)**

Highest nonfermenters were isolated from Pus (22.2%). *P.aeruginosa* was highest *(38.62%)*from pus samples (11.1%) followed by *A.baumannii (34.92%)* from ET (11.1), lowest for *Acinetobacter hemolyticus* and *Burkhoderia pseudomallei (0.5%)* [Table-3]

Table 3: Species distribution of NFGNB in different clinical specimens (n=189)

Organism	BAL(%)	Blood(%)	Catheter Tip (%)	CVP Tip (%)	Drain (%)	ET (%)	Femoral Tip (%)	Pus (%)	Splenic swab (%)	Sputum (%)	Swab(%)	TT (%)	Urine (%)	Total
A.baumannii	0.5	1.1	0	0	0.5	11.1	0	5.29	0	5.28	0	7.93	2.64	34.92
A.lwoffi	0	0	0	0.5	0	0	0	2.64	0	1.1	0	0	1.1	5.29
A.haemolyticus	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5
Acinetobacter spp.	0	0.5	0	0	0	0	0	0	0	0	0	0	0.5	1
B.cepacia	0	0	0	0	0	0.5	0	1.58	0	0.5	0	0.5	1.1	4.23
B. pseudomallei	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0.5
Burkhoderia spp	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0.5
Myroides spp.	0	0	0	0	0	0	0	0	0	0	0	0	1.58	1.58
P.aeruginosa	0.5	1.1	0	0	0.5	5.82	0	11.1	0.5	6.87	0.5	5.29	6.34	38.62
P.luteola	0	0	0.5	0	0	0	0	0	0	0	0	0	0.5	1.05
P.stutzeri	0	0.5	0	0.5	0	0.5	0	0	0	0	0	0	0.5	2.11
S. paucimobilis	0	0	0	0	0	0	0	0.5	0	5.82	0	0	0.5	6.87
S. maltophilia	0	0	0	0	0	1.1	0.5	0	0	0	0	1.1	0	2.64
Total	**1.1**	**3.2**	**0.5**	**1.1**	**1.1**	**19**	**0.5**	**22.2**	**0.5**	**20.6**	**0.5**	**14.8**	**14.8**	**100**

P.aeruginosa and *A. baumannii* showed highest sensitivity to Colistin. *P. aeruginosa* was sensitive to most of the drugs where as *Myroide spp.* showed highest resistant pattern to all the drugs.[Table-4]

Table 4: Susceptibilities of nonfermentative bacilli to antimicrobial agents (n=189)

Antibiotic	*A.baumannii(%)*	*A.lwoffii(%)*	*A.haemolyticus (%)*	*Acinetobacter spp(%)*	*B.cepacia(%)*	*B.pseudomallei (%)*	*Burkholderia Spp(%)*	*Myroides Spp(%)*	*P.aeruginosa (%)*	*P.luteola(%)*	*P.stutzeri(%)*	*S.paucimobilis (%)*	*S.maltophilia (%)*
AK	10.6	80	100	100	-	-	-	0	60.3	50	75	92.3	-
AS	10.6	70	100	100	-	-	-	-	-	-	-	84.6	-
AZ	-	-	-	-	-	-	-	0	54.8	50	75	92.3	-
CPM	10.6	80	100	100	-	-	-	0	54.8	50	100	76.9	-
CIP	10.6	60	100	100	-	-	-	0	52.1	50	75	53.8	-
COT	12.1	60	100	100	50	0	100	0	-	-	-	53.8	100
CTX	10.6	70	100	100	-	-	-	0	-	-	-	76.9	-
CAZ	10.6	60	100	100	50	0	0	0	56.2	50	75	84.6	-
CTR	6.1	80	100	100	-	-	-	-	-	-	-	76.9	-
GEN	10.6	90	0	0	-	-	-	0	56.2	50	75	84.6	-
IMP	12.1	90	100	100	-	-	-	0	56.2	50	75	100	-
MRP	12.1	90	100	100	37.5	0	100	0	57.5	50	75	92.3	-
PI	9.1	70	100	100	-	-	-	0	52.1	50	75	76.9	-
PIT	10.6	70	100	100	-	-	-	0	58.9	50	100	76.9	-
LE	10.6	70	100	100	37.5	0	0	0	53.4	50	50	53.8	53.8
TCC	12.1	70	100	100	25	0	100	53.4	53.4	50	75	76.9	-
TI	-	-	-	-	-	-	-	0	52.1	50	75	23.1	-
TOB	10.6	80	100	100	-	-	-	0	58.9	50	75	84.6	-
CO	95.5	100	100	100	0	0	0	0	94.5	50	100	100	-

AS- Ampicillin-sulbactam, AK- Amikacin, AZ- aztreaonam, CPM- Cefepime, CIP- Ciprofloxacin, COT- Co-trimoxazole, CTR- Ceftriaxone, CAZ- Ceftazidime, CTX- Cefotaxime, GEN- Gentamicin, IMP- Imipenem, MRP- Meropenem, PI- Piperacillin, PIT- Pieracillin- tazobactam, LE- Levofloxacin, TCC- Ticarcillin-clavulonic acid, TI- Ticarcillin, TOB- Tobramycin, CO- Colisitin.

Time taken for the identification of Nonfermenters by Vitek 2 was 6.42 hours, whereas it was 103.74 hours by the Conventional methods. Rapid identification of an organism helps clinician to start appropriate antibiotic according to the organism which was identified. This way it improves patient management and treatment outcome. [Table-5]

Table 5: Time taken for identification of nonfermenters by Vitek 2 and Conventional methods: (n=189)

Average time taken for identification	Time in hours
VITEK 2	6.42
Conventional method	103.74

Mean time taken for antimicrobial susceptibility of NFGNB by Vitek 2 was 12.89 hours whereas time taken by conventional method was 18.4 hours.[Table-6]

Table 6: Time taken for AST of Nonfermenters by Vitek 2 and Conventional methods. (n=189)

Average time taken for AST	Time in hours
VITEK 2	12.89
Conventional method	18.4

Major errors were observed for Imipenem (2.66%) and Meropenem(2.43%), Minor errors seen for Cefepime (12.32%), Ciprofloxacin (9.23%), Amikacin (2.57%), Ceftazidime(2.63%) and Piperacillin-tazobactam (1.33%). [Table-8]

Table 8: Discordant results obtained for isolates by Kirby-Bauer disk diffusion test to VITEK 2 Compact (n=189)

Antibiotic (n)	Disc diffusion	Vitek 2	%	Interpretation
Amikacin(2)	R	I	2.57	Minor error
Cefipime(9)	R	I	12.32	Minor error
Imipenem(2)	S	R	2.66	Major error
Meropenem(2)	S	R	2.43	Major error
Piperacillin-tazobactam(1)	R	I	1.33	Minor error
Ciprofloxacin(6)	R	I	9.23	Minor error
Ceftazidime(2)	R	I	2.63	Minor error

R- Resistant, S-Sensitive, I-Intermediate sensitive

Highest isolation of NFGNB were from patients with indwelling devices (52.9%), followed by 49.7% from the patients who had undergone various operative procedures, 16.9% from patients with DM.[Table-7]

Table 7: Distribution of NFGNB with different clinical conditions (n=189)

Organism	DM	HIV	TB	Malignancy	Pregnancy	COPD	Others	Operative procedures	I/W devices
A.baumannii	4.76	0	0	0.52	0	5.82	0.52	17.46	22.75
A.lwoffii	0	0	0	0	0	0.52	0	2.64	1.58
A.haemolyticus	0	0	0.52	0	0	0	0.52	0	0.52
Acinetobacter spp	0.52	0	0	0	0	0	0	0.52	0.52
B.cepacia	1.05	0	0	0.52	0	0	0	2.11	3.70
B.pseudomallei	0	0	0.52	0	0	0	0	0.52	0
Burkholderia spp	0	0	0.52	0	0	0	0	0.52	0
Myroides spp	0.52	0	0	0	0	0	0	0.52	1.58
P.aeruginosa	7.4	0	0.52	2.11	0.52	2.11	2.11	22.22	17.98
P.luteola	0	0	0	0	0	0	0	1.05	1.05
P.stutzeri	0	0	0	0	0	0	0	0	0.52
S.paucimobilis	2.64	0.52	0.52	0	1.05	2.64	0	0	1.05
S.maltophilia	0	0	0	0	0	0	0	2.11	1.58
Total	**16.93**	**0.52**	**2.64**	**3.17**	**1.58**	**11.11**	**3.17**	**49.73**	**52.91**

DM-Diabetes mellitus, HIV- Human Immunodeficiency Virus, COPD- Chronic Obstructive pulmonary disease, TB- Tuberculosis

P value of T test is 0.476 which means fair agreement observed between Vitek 2 and Conventional methods for identification.[Table-9]

Table 9: Overall agreements between Conventional method and Vitek 2 for Identification of NFGNB

VITEK vs Conventional	0.476*

*p value of T test excluding *A.juni* and *Burkhoderia spp.*

P value of T test is <0.0001 which means Vitek gave rapid identification and antimicrobial susceptibility results as compared to conventional methods

Table 10: Overall time taken for identification and AST by VITEK and conventional method

VITEK VS CONVENTIONAL	<0.0001*

*P value of T test

DISCUSSION

Infections caused by Gram negative non-fermenting aerobic bacteria are increasing day by day. These Nonfermenting Gram negative bacteria have complex physicochemical properties which require a battery of tests for their precise identification.[17] In addition identification of these nonfermenters has often being neglected and there is still much confusion regarding the taxonomic status of many of these nonfermenters.[18] Therefore we intended to identify commonly encountered, clinically significant gram negative nonfermenting bacteria from clinical specimen along with their antimicrobial susceptibility pattern. We also compared Vitek 2 and Conventional methods used for identification and antimicrobial sensitivity of NFGNB, along with correlation between the two methods used. In our study prevalence of NFGNB was 26.52% . Different researchers have reported variable prevalence rates of Nonfermenters ranging from 23% to 67% in studies conducted from 1993 to 2015from different parts of the world like Nepal[19], China[20] and different parts of India like Ahmedabad[21], Kolkata[22]. This prevalence of 26.52% in our study is similar to finding by different

researchers from Nepal[19] (29.62%), China[20] (31.62%) and Ahmedabad[21] (23.93%), but study conducted in Kolkata got a prevalence value (12.18%) which is significantly lower than ours[22]. Rao and Shivananda[23], (1993) reported higher (66.88%) prevalence rate of non-fermenters. In the present study distribution of NFGNB was maximum from pus (22.2%), followed by sputum (20.6%) and ET (19%) (Table.1). Pus was commonest among all the specimens by different researchers as well as in our study. Isolation rate of Nonfermenters from pus varied geographically, ranging from 27.86%-58.4% in studies observed from different areas like Nepal[19], Kolkata[22], Ahmedabad[21] and Karnataka[24]. In our study we have encountered *P.aeruginosa* as most common Nonfermenter (38.62%) followed by *A.baumannii (34.92%).* Other significant NFGNB isolated were: *Sphingomonas paucimobilis (6.87%), A.lwoffii (5.29%), Burkholderia cepacia (4.23%), Stenotrophomonas maltophilia (2.64%), Pseudomonas stutzeri (2.11%), Myroides spp. (1.58%), Pseudomonas luteola (1.05%), Burkholderia pseudomallei (0.5%).* (Table. 3) Age and sex are the most important host factors which influence the level of innate immunity and susceptibility to all type of infections. In general, incidence and death rate of infectious disease is greater in males than in females which may be due to the difference in hormonal factors between the two sexes that influences the innate immunity.[27] Distribution of the NFGNB is highest among males 70.9% as compared to females 29.1% (Table. 2). Same results were obtained by Kalidas *et al.*[22], in which 55% were male, and 45% were female. Predominance for male gender was also seen in Benachinmardi *et al.*[26], 68% were males and 32% were female. In our study majority of NFGNB were from age group of 21-30 years (23.2%), and in this age group most commonly encountered organism was *A.baumannii* 40.9%, second most common age group in our study was 61-70 year (19%). In the present study, 52.9% organism were isolated from patients with indwelling devices, followed by 49.7% from the patients who had undergone various operative procedures both major and minor, followed by 16.9% from patients with diabetes mellitus. Study done by, Malini *et al.*, in which maximum isolation of NFGNB were from patients with RTA and from nonhealing ulcers (60.62%).[107] (Table. 7)

P.aeruginosa the most common NFGNB was maximum sensitive to Colistin (94.5%) followed by Amikacin (60.3%), Tobramycin and Piperacillin-tazobactam (58.9% each), Meropenem (57.5%), Imipenem, Gentamicin (56.2% each), with least sensitive for Ciprofloxacin and Piperacillin (52.1% each). (Table. 4) Second most frequently isolated organism in our study, *A.baumannii* showed were maximum sensitivity to Colistin 95.5% followed by Imipenem, Meropenem, Trimethoprim-sulfamethoxazole and Ticarcillin-clavulanate (12.1% each), Amikacin, Cefepime, Ciprofloxacin, Cefotaxime, Gentamicin, Levofloxacin, Piperacillin-tazobactam and Tobramycin for (10.6% each) with least sensitivity to Ceftriaxone (6.1%). (Table.4) *Myroides* species produce a chromosomally mediated noninducible metallo-β-lactamase which is capable of hydrolyzing Cephamycins, Penicillins, Cephalosporins, Aztreonam, Imipenem, and Meropenem.Despite their low levels of virulence, these bacteria are resistant to many antimicrobial agents, this may favor nosocomial infections or infections in immunocompromised individuals. In our study, *Myroides spp* were 100% resistant to Amikacin, Aztreonam, Cefepime, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Ceftazidime, Gentamicin, Imipenem,Meropenem, Piperacillin, Piperacillin-tazobactam, Levofloxacin, Ticarcillin-clavulanate, Tobramycin.All multidrug resistant *Myroides spp* isolated in our study were from catheterized patients, after culture reports for *Myroides spp* catheter were removed from all patients. After 48 hours of catheter removal repeat urine culture showed no growth of *Myroides spp.*This suggests that catheterized patients were most likely colonized with *Myroides spp.* The development of automated systems with in-built expert systems has allowed an increase in both the reproducibility and reliability of the results and consequently the quality of the reporting. These systems have also modified daily working practice, providing the ability to communicate early provisional results to the clinician. Conventional methods are tedious, time-consuming, and sensitive to transcription errors. So this draw backs of Conventional methods and increasing work load has led us to promote the use of an automated system for ID and AST. In the present study, Time taken for the identification of non-fermenters using Vitek 2 was 6.42 hours, while by conventional method it was 103.7 hours. Mean time taken by VITEK 2 for antimicrobial susceptibility was 12.89 hours, while in the conventional method mean time taken was 18.40 hours (Table. 5, Table. 6). when comparing Vitek 2 and Conventional method with respect of time, P value of T test was <0.0001, which means VITEK 2 gave rapid identification and antimicrobial susceptibility results as compared to conventional methods. Comparing Vitek 2 and Conventional method for identification of nonfermenter no disagreement was found for most commonly encountered organisms i.e, *P.aeruginosa* and *A.baumannii.* p value for overall agreement between Vitek 2 and Conventional method for identification of NFGNB was 0.476 (Table. 9). This moderate agreement between Vitek 2 and Conventional method means either method can be used for the identification of the Nonfermenters. The advantage of using Vitek 2 over Conventional method is that it is easy to perform and gives faster results. Variable results were

seen for *A.lwoffii, A.juni, Burkholderia pseudomallei,* and *Acinetobacter spp.* AST reported by disc diffusion was in agreement with VITEK 2 except for Amikacin, Cefepime, Ciprofloxacin, Ceftazidime, Piperacillin-tazobactam. Categorial agreement for these antibiotics was not calculated because antibiotic susceptibility results given by VITEK 2 as intermediate were reported by Conventional methods as Resistant. So the statistical analysis was not performed for these antibiotics. To overcome this problem, we determined errors reported in AST. Susceptibility testing for following drugs Ampicillin-sulbactam, Piperacillin, Ticarcillin, Ticarcillin-clavulanate, Tobramycin could not be established by VITEK 2 because these antibiotics are not included in the database of VITEK 2 AST cards. Comparable results between Vitek 2 and Conventional methods were found for Cefotaxime (p value 0.945), ceftriaxone (p value 0.919), Trimethoprim-sulfamethoxazole (0.916), Colistin(0.901), Gentamicin (0.902), Levofloxacin(0.399)and Meropenem(0.399). (Table. 7). There was Major errors observed for Imipenem (2.66%) and Meropenem(2.43%), Minor errors seen for Cefepime (12.32%), Ciprofloxacin(9.23%), Amikacin (2.57%), Ceftazidime(2.63%) and Piperacillin-tazobactam (1.33%) (Table. 8). For AST, the errors were within the range specified by FDA which is, major error rate must be less than 3% of all the susceptible organisms tested and very major error rate 1.5% or less. Antimicrobial susceptibility comparison for nonfermenters using Vitek 2 and disc diffusion method has not yet been performed by any other researcher. So comparison of Vitek 2 and Conventional method for Antimicrobial susceptibility testing was not possible.

CONCLUSION

Total samples received in the Microbiology laboratory of Shree Krishna Hospital were 7743, with a culture positivity rate of 33.7%. The prevalence rate of NFGNB was 26.52%. Majority of NFGNB isolates from clinical samples were in age group of 21-30 years (23.2%). The numbers of male patients (70.9%) were more as compared to female patients (29.1%). *P.aeruginosa* was the commonest Nonfermenter (38.62%) that was isolated from pus samples (28.8%). Pus was the most common specimen (22.2%) for isolation of Nonfermenters followed by Sputum (20.6%) and ET (19%) . *Myroides spp* showed highest resistant pattern (98%) for antibiotics tested which was followed by *Acinetobacter baumannii* which showed MDR (87.9%) and PDR (4.5%). Majority of NFGNB were isolated from patients with indwelling devices (52.9%) followed by patients who had undergone minor or major surgeries(49.7%) and from patients who are suffering from diabetes (16.9%). Time was the major advantage of VITEK 2 over Conventional method in identification (6.42 vs103.7 hours) and AST (12.89 vs 18.40 hours) with good agreement. Overall moderate agreement (p value- 0.476) was found between Vitek 2 and Conventional method for identification of NFGNB. Both Vitek 2 and Conventional method are useful in Identification and Antimicrobial susceptibility testing. But Vitek 2 is superior because it is easy to use and takes less time for reporting. Disagreement between Vitek 2 and Conventional method was found for Acinetobacter lwoffii, Acinetobacter juni and Burkholderia pseudomallei. For antimicrobial susceptibility testing performed using VITEK 2 compact, Major errors were observed for Imipenem (2.66%) and Meropenem(2.43%), while Minor errors seen for Cefepime (12.32%), Ciprofloxacin(9.23%), Amikacin (2.57%), Ceftazidime(2.63%) and Piperacillin-tazobactam (1.33%)

REFERENCES

1. Veenu, Sikka R, Arora DR: Isolation and susceptibility pattern of Non fermenting Gram negative bacilli from clinical samples. IndianJ Med Microbiol. 1998;17(1):14-18
2. Siou Cing Su,Mario vaeechotte,Lenie Dijkshoorn,Yu Fang Wei,Ya Lei Chen and Tsung Chain Chang.Identification of non-fermenting Gram-negative bacteria of clinical importance by an oligonucleotide array. J Med Microbiol 2009;58: 596-605.
3. In: Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, *et al.*., editors. Nonfermenting Gram negative bacilli. In: Koneman's color Atlas and textbook of Diagnostic Microbiology. 6 th ed. USA: Lippincott Williams and Wilkins Company 2006;305-391.
4. Prashanth K, Badrinath S. *In vitro* susceptibility pattern of *acinetobacter* species to commonly used cephalosporins, quinolones, and aminoglycosides. Indian J Med Microbiol 2004;22:97-103.
5. Rahbar M, Hajia M. Detection and quantitation of the etiologic agents of ventilator-associated pneumonia in endotracheal tube aspirates from patients in Iran. Infect Control Hosp Epidemiol 2006;27:884-885.
6. Boroumand MA, Esfahanifard P, Saadat S, Sheihkvatan M, Hekmatyazdi S, Saremi M, *et al.*. A report of *Pseudomonas aeruginosa* antibiotic resistance from a multicenter study in Iran. Indian J Med Microbiol 2007;25:435-436.
7. Japoni A, Alborzi A, Kalani M, Nasiri J, Hayati M, Farshad S. Susceptibility patterns and cross-resistance of antibiotics against *Pseudomonas aeruginosa* isolated from burn patients in the South of Iran. Burns 2006;32:343-347.
8. Taherikalani M, Etemadi G, Geliani KN, Fatollahzadeh B, Soroush S, Feizabadi MM. Emergence of multi and pan-drug resistance*Acinetobacter baumannii* carrying blaOXA-type -carbapenemase genes among burn patients in Tehran, Iran. Saudi Med J 2008;29:623-624.
9. Kharangate NV, Pinto MJ, Rodrigues S, Verenkar MP. Characterization of nonfermenters from clinical samples. J Assoc Physicians India 2001;49:324-326.

10. Davies JC, Rubin BK. Emerging and unusual gram-negative infections in cystic fibrosis. Semin Respir Crit Care Med 2007; 28(3):312-321.
11. Enoch DA, Birkett CI, Ludlam HA. Non-fermentative Gramnegative bacteria. Int J Antimicrob Agents 2007;29(3): 33-41.
12. Rolston KV, Kontoyiannis DP, Yadegarynia D, Raad II . Nonfermentative Gram-negative bacilli in cancer patients: increasing frequency of infection and antimicrobial susceptibility of clinical isolates to fluoroquinolones. Diagn Microbiol Infect Dis 2005;51(3):215-218.
13. Cervia JS, Ortolano GA, Canonica FP. Hospital tap water as a source of Stenotrophomonas maltophilia infection. Clin Infect Dis 2008;46(9):1485-1487.
14. Bezanson GS, MacInnis R, Potter G, Hughes T. Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. Int J Food Microbiol 2008;127(2):37-42.
15. Higgins CS, Murtough SM, Williamson E, Hiom SJ, Payne DJ, Russell AD, *et al.*. Resistance to antibiotics and biocides among non-fermenting Gram-negative bacteria. Clin Microbiol Infect 2001;7(6):308-315.
16. Yashodara P, Shyamala S. Identification and characterization of nonfermenters from clinical specimens. Indian J Med Microbiol 1997;15:195-197.
17. Mehta M, Punia JN, Joshi RM. Antibiotic resistance in Pseudomonas aeruginosa strains isolated from various clinical specimens- a retrospective study. Indian J Med Microbiol 2001; 19(4):232.
18. Nagoba BS, Deshmukh SR, Ulka G Gude *et al.*. Invitro susceptibility of Pseudomonas aeruginosa to different antibiotics. Indian J Med Microbiol 1997,15: 185-186.
19. Dipak B, Sanjay K, Mukesh S. Prevalence of Non-Fermentative Gram Negative Bacilli Infection in Tertiary Care Hospital in Birgunj, Nepal. *Int.J.Curr.Microbiol.App.Sci* 2015; 4(7): 301-307
20. Chang T.C., Huang A.Y.H. Rapid differentiation of fermentative from nonfermentative gram negative bacilli in positive blood cultures by an impendance method. *J. Clin Microbiol*2000*;*38: 3589 - 3594.
21. Parimal HP, Jayshree DP, Sanjay DR, Bimal C, Parul S. Prevalence of nonfermenting Gram negative bacilli infection in tertiary care Hospital in Ahmedabad, Gujarat.*Indian J Basic Appl Med Res* 2013; 6(2): 608 – 613.
22. Kalidas RIT, Falguni NAG, Hirak J R, PK Maity.Prevalence and Susceptibility Profiles of Nonfermentative Gram-negative Bacilli Infection in a Tertiary Care Hospital of Eastern India.*Indian J Clin Pract*2013;24(5):451-455
23. Rao, P.S., Shivananda, P.G. Bacteraemia due to non fermenting gram negative bacilli in immunocompromised patients. *Indian J Med Microbiol,* 1993;11: 95- 99.
24. Gokhale S, Metgud SC. Characterization and antibiotic sensitivity pattern of nonfermenting Gram negative bacilli from various clinical samples in a tertiary care hospital. Belgaum J Pharm Biomed Sci 2012;17:1-5.
25. Malini A., Deepa E.K., Gokul B.N, Prasad S.R.Nonfermenting Gram-Negative Bacilli Infection in a tertiary care Hospital in Kolar, Karnataka. *J. Lab Physicians* 2009;1(2): 62 -66.
26. Benachinmardi KK, Padmavathy M, Malini J, Naveneeth BV. Prevalence of non-fermenting Gram-negative bacilli and their in vitro susceptibility pattern at a tertiary care teaching hospital. J Sci Soc 2014;41:162-166.
27. Pandya Y, PhD Thesis, "Microbiology and Epidemiology studies of Pseudomonas aeruginosa with special emphasis on Nosocomial Infections." Pramukhswami Medical College, Gujarat, June 2011:125-126.

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