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ISOLATION IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY TEST OF NON-FERMENTERS FROM DIFFERENT CLINICAL SPECIMENS

Apurba Kumar Sarkar*, M. Shaker, Rajesh D. Fasle,

P.G. Department of Microbiology Mrs. KSK College, Beed, Maharashtra-431122
Corresponding author apurbas@ymail.com

ABSTRACT: The present study was undertaking to identify the non-fermenters from different clinical specimens, to assess types of infection they caused and study of antimicrobial susceptibility against them. During the study period 93 numbers of different clinical specimens like Pus, Urine, Stool and Sputum were analyzed. Out of the total 93 specimens non-fermenters were found in 49 samples with an isolation rate of 52.68%. The most frequently isolated organisms were *Pseudomonas aeruginosa (41.93%), Acinetobacter baumanni* (5.37%), *Pseudomonas putida* (3.22%), *Pseudomonas alcaligenes* (1.07%), *B. vesicularis* (1%), *P.mendocina* (1.07%), *B.cepacia complex* (1.07%), *CDC N0-1* (1.07%), *CDC-E0-5*(1.07%). The majority of non-fermenters were isolated from pus (78%), followed by urine (35%), and stool (31.25%). *Pseudomonas aeruginosa, Acinetobacter baumanni, Pseudomonas alcaligenes, B.cepacia complex and CDC N0-1* show multidrug resistant property. Imepenum-EDTA was the highest susceptible antibiotic against all isolates.

Keywords- Non-fermenters, Multidrug resistant, Imepenum-EDTA

INTRODUCTION

Gram negative organisms that are unable to ferment sugar's to generate energy for their cell function are known as non-fermenters. Organisms of this group are aerobic, non-spore forming bacilli that either unable to utilize carbohydrates as a source of energy or degrade them via oxidative metabolic pathways other than fermentation. [1] Member of this group create serious challenge for health care management because they shows the problem of multidrug resistance. [2] Non-fermenters are being encountered as opportunistic or niche pathogens that cause infection not only in patients who are immuno-compromised but also those who are healthy. The rates of infection in healthy persons are rare as compare to patients who are critically ill. [3] Infections caused by non-fermenters are in extreme group in age like geriatric age to neonates. [4] Due to the liberal and empirical use of antibiotics non-fermenters have emerged as important healthcare associated pathogens in recent years. [5] [6]

Species that are very common as opportunistic pathogens either by diseases or treatment in immunologically compromised host are *Pseudomonas aeruginosa*, is eminent followed by *Acinetobacter baumannii*, *Pseudomonas fluorescence*, *Pseudomonas stotzeri*, *stenatrophomonas maltophilia*, *Pseudomonas putida*. [7]

Members of this group are frequently isolated from sample of different wound infection, urinary tract infection, stool, and septicemia. [8] They are also spread in nature like soil, water, and on the surface in contract with soil or water. It has been reported that, non-fermenters may isolated from different equipment's, machineries used in hospital and even from the skin of health care workers. [3][9]. Organisms belonging to this group use a variety of resistance mechanisms, including

the production of enzymes, target sites alterations, efflux pumps production and lose of outer membrane proteins. They may process intrinsic and rapidly acquired types of resistance. Intrinsic resistant is due to the relative impermeability of the outer membrane which may lead to show the non-susceptibility of this group against most cephalosporin, ampicillin and macrolides. [10], [11]

MATERIALS AND METHOD

Total 93 clinical specimens were received from local hospitals, diagnostic and clinic, for isolation, identification and sensitivity of non-fermenters. Which included 50 pus samples, 23 urine samples, 16 stool samples, and 7 others sample. All samples were inoculated on sterile nutrient agar, Mc Conkey agar, Cetrimide agar, Leeds Acinetobacter agar. All positive organisms were further subjected to Gram staining, Oxidase and motility test. Then they were inoculated on TSI (triple sugar iron agar (Deep stab). Organisms grow on TSI agar and produce an alkaline butt and alkaline slant were initially considered as non-fermenter and were inoculated into oxidative fermentative media containing different types of sugar like Dextrose, Lactose, Xylose, Mannitol, and Maltose to find out whether a organisms was oxidizer or not.

As per [1] unknown isolates will be non-fermenters if they show the following property.

1). Lack of evidence of glucose fermentation

2). Positive cytochrome oxidase reaction

3). Failure to grow on Mac Conkey agar.

Further identification was carried out according to the standard biochemical's test. All identified isolates were subjected to antibiotic sensitivity test by using Kirby Bauer method. The antimicrobial agents tested were Ceftazidime-30 mcg, Ceftizoxime-30 mcg, Imepenem-EDTA 10/75 mcg/disc, Chloramphenicol-30 mcg disk, Ciprofloxacin-5 mcg, Tetracyclin 30 mcg, Erythromycin 15 mcg/disc, Gentamycin 10 mcg/disc, Kanamycin 30 mcg/disc.

RESULT AND DISCUSSION

The most frequently encountered non-fermentative Gram negative bacilli were found to be *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Pseudomonas putida*, *Pseudomonas alcaligenes*, *B. vesicularis*, *Pseudomonas mendocina*, *B. cepacia* complex, CDC NO-1, and CDC-EO-5 the identification was done on the basis of Koneman et al [1] and Kalidas et al [9]. The majority of non-fermenters were isolated from pus (41.93%), followed by urine (8.60%), and stool (5.37%), this result correlates with A Malini et al [6].

Pseudomonas aeruginosa was the predominant bacterial strain in all specimens. It is similar to other study Kalidas et. al [9] . ciprofloxacin.

Antibiogram of obtained clinical isolates was studied by using Kirby Baur method. Percentage effect of antimicrobial activity of mentioned antibiotics against obtained isolates were analyzed and recorded. (Table-5). Imepenem-EDTA shows the higher susceptibility to all isolates.

In conclusion Pseudomonas aeruginosa, *Pseudomonas alcaligenes*, *Acinetobacter baumanni*, CDC N0-1, and *B.cepacia* complex shows multi drug resistance property except is Imepenem-EDTA. Ciprofloxacin is also effective against isolated non-fermenters but Acinetobacter baumanni show 80% resistant against it.

Table 1: Sample distribution

Sl. No	Sample Name	Number
1.	Pus	50
2.	Urine	20
3.	Stool	16
4.	Others	7

Table 2: Biochemical Characteristic of motile and oxidase positive nonfermenters

Test	*P. aeruginosa*	*P. Putida*	*P. alcaligenes*	*B. vesicularis*	*P.mendocina*	*B.cepacia complex*
Oxidase	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Pyoverdin	+	+	-	-	-	-
Yellow	-	-	-	+	-	+
Glucose	+	+	-	+	+	+
Maltose	-	+	-	+	-	+
Lactose	-	-	-	-	-	+
Mannitol	-	-	-	-	-	+
Xylose	+	+	-	-	-	-
Arginine	+	+	-	-	+	-
Lysine	-	-	-	-	-	+
N03-N02	-	-	-	-	+	-
No3-N2	+	-	-	-	-	-
Urea	+	+	+	-	+	+
ONPG	-	-	-	-	-	-
Dnase	-	-	-	-	-	-
Aceteamide	+	-	-	-	-	-
Esculin	-	-	NA	+	-	-
H2S in KIA	-	-	-	-	-	-
Polymyxin	+	+	+	+	+	Not done
Starch hydrolysis	-	NA	-	+	-	+

Table 3: Biochemical Characteristic of non-motile and oxidase negative nonfermenters

Test	*Acinetobacter baumanni*	CDC-E0-5	CDC N0-1
Oxidase	-	-	-
Motility	-	-	-
Yellow Pigment	-	+	-
Urease	-	+	-
Nitrate Reduced	-	-	+
Growth at 37°C	+	+	+
Growth at 44°C	-	n/a	n/a
Hemolysis sheep blood	+	n/a	-
Gelatin Hydrolysis	-	-	-
OF Dextrose	+	+	+
OF Maltose	+	-	n/a
Arginine	-	-	-
OF Lactose	+	-	-
Growth on MacConkey	+	-	+/ Slow
H2S in KIA/TSI	-	-	-
	-	-	NA

Table 4: Frequency distribution of isolated prominent pathogens from different clinical specimens

Sl. No.	Isolates	Number of organisms	Percentage of occurrence
1.	*Pseudomonas aeruginosa*	39	41.93%
2.	*Pseudomonas putida*	3	3.22%
3.	*Acinetobacter baumanni*	5	5.37%
4.	*Pseudomonas alcaligenes*	1	1.07%
5.	*B. vesicularis*	1	1.07%
6.	*P.mendocina*	1	1.07%
7.	*B.cepacia complex*	1	1.07%
8.	*CDC N0-1*	1	1.07%
9	*CDC-E0-5*	1	1.07%

Table 5: Distribution of predominant bacterial pathogen in various clinical specimens

Sl. No	Isolated organisms	Specimens							
		Pus	%	Urine	%	Stool	%	Other	%
1.	*Pseudomonas aeruginosa*	29	58	5	25	3	18.75	2	28.57
2.	*Pseudomonas putida*	3	6	0	0	0	0	0	0
3.	*Acinetobacter baumanni*	5	10	0	0	0	0	0	0
4.	*Pseudomonas alcaligenes*	1	2	0	0	0	0	0	0
5.	*B. vesicularis*	0	0	1	5	0	0	0	0
6.	*P.mendocina*	0	0	0	0	1	6.25	0	0
7.	*B.cepacia complex*	0	0	0	0	1	6.25	0	0
8.	*CDC N0-1*	1	2	0	0	0	0	0	0
9.	*CDC-E0-5*	0	0	1	5	0	0	0	0

Table 6: Antibacterial resistance pattern (%) exhibited by obtained isolates

Name of Antibiotics	***P. aeruginosa***	***P. putida***	***A. baumanni***	***P. alcaligenes***	***B. vesicularis***	***P. mendocina***	***B. cepacia complex***	***CDC N0-1***	***CDC-E0-5***
Ceftazidime	2.56%	0	100%	100%	0	0	100%	100%	0
Ceftizoxime	100 %	100%	100%	100%	100%	100%	100%	100%	0
Imepenem-EDTA	0	0%	0	0	0	0	0	0	0
Ciprofloxacin	33.33%	0	80%	0	0	0	0	0	0
Tetracyclin	100%	100%	100%	100%	0	100%	100%	100%	0
Erythromycin	100%	100%	100%	100%	0	0	100%	0	0
Gentamycin	25.64%	0	0		0	0	0	100%	0
Kanamycin	100%	100%	20%		0	0	100%	100%	0
Chloramphenicol	100%	100%	100%	100%	0	0	100%	100%	0

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