Original Research Paper

Volume-7 | Issue-11 | November-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Microbiology

ANTIBIOGRAM PATTERN OF NON FERMENTING GRAM NEGATIVE BACILLI IN A TERTIARY CARE HOSPITAL, IN ODISHA

Dr Kundan Kumar Sahu	Associate Professor, Department of Microbiology IMS & SUM Hospital, Kalinga Nagar, Bhubaneswar, Odisha.751003
Dr Rajashree Panigrahy*	Associate Professor, Department of Microbiology IMS & SUM Hospital, Kalinga Nagar, Bhubaneswar, Odisha.751003 *Corresponding Author
Dr Sunita Kabi	Assistant Professor, Department of Microbiology IMS & SUM Hospital, Kalinga Nagar, Bhubaneswar, Odisha.751003
Dr Smita Padhi	Resident of Department of Microbiology IMS & SUM Hospital, Kalinga Nagar, Bhubaneswar, Odisha.751003

ABSTRACT Non-fermentative gram negative bacilli have emerged as an impending pathogenic entity with the ability to show resistance for commonly used antimicrobials. With this background the present study was undertaken to detect the clinical distribution and antibiogram profile of non-fermenting gram negative bacilli isolated from the clinical samples such as pus, urine, blood etc. Different non-fermentative gram negative bacilli were isolated from 140 clinical samples obtained from various clinical departments (indoor only) of our hospital during January 2017 to July 2017. The isolates were identified using the standard basic tests including motility, catalase, oxidase production, indole, triple sugar iron agar test, citrate utilization, urease production, oxidation fermentation and phenylalanine deaminase tests. Antibiotic sensitivity testing of isolated gram-negative bacilli was performed by the Kirby Bauer disc diffusion method using Mueller Hinton Agar plates following the CLSI guidelines. 1093 samples were found to be positive for bacterial culture, while 140 (12.8%) samples showed growth of non-fermentative gram negative bacilli. The most common isolates were *Pseudomonas aeruginosa* accounting for (48.5%) followed by *Acinetobacter baumanii*(33.5%). Antibiotic sensitivity profile of these isolated organisms indicates higher sensitivity towards Imipenem, Cefipime- Sulbactam and Ceftazidime-Sulbactam with certain degrees of resistance towards Amikacin, Piperacillin and Co-trimoxazole. The study findings could be useful for the clinicians towards promoting rational use of antibiotics and contributing to abate unnecessary development of resistance.

KEYWORDS : Non fermenter Gram negative bacilli, Antibiotic susceptibility testing.

INTRODUCTION:

Non-Fermenting Gram-Negative Bacilli (NFGNB) are a group of aerobic, non-sporing, bacilli/coccobacilli that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative, rather than fermentative pathway. This group includes numerous organisms but the ones which are known to cause nosocomial infections are *Pseudomonas aeruginosa, Acinetobacter baumanii, Burkholderia cepacia complex (BCC) and Stenotrophomonas maltophila.* The pathogenic potential of nonfermentative gram negative bacilli (NFGNB) has been established beyond doubt because of their repeated isolation from clinical specimens and association with the disease. These diverse groups of microorganisms have familiar traits of clinical importance to justify their inclusion in a single group. NFGNB can cause a wide variety of infections and account for approximately 15% of all gram negative bacilli cultured from clinical samples[1]. Non-fermenters vary in their pathogenic prospective, transmissibility and many have developed resistance to antibiotics. In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which are widely used against the non-fermenters, making them as an important healthcare associated pathogen[2]. Non-fermenting gram negative bacilli are intrinsically resistant to many antibiotics and are known to produce extended spectrum beta-lactamases and metallo beta-lactamases [3]. Nonfermenters have been incriminated in infections such as pneumonia, septicaemia, urinary tract infection, surgical site infection and have the potential to spread from patient to patient via fomites or the hands of medical personnel [4].

AIMS AND OBJECTIVES:

- To isolate, identify and characterize nonfermenting Gram-negative bacilli from clinical isolates received from indoor hospital patients .
- To analyze the antibiotic sensitivity patterns of the isolates..

MATERIALS AND METHODS:

A total of 2264 clinical specimens from various patients admitted, were received in Microbiology Laboratory of our Medical College, for culture and sensitivity from January 2017 to July 2017. All the received specimens were inoculated in blood agar, Mac Conkey agar, CLED agar (Cysteine lactose electrolyte deficient Agar) and nutrient agar. The plates were incubated under aerobic condition at 37°C for 24- 48 hours, followed by their cultural characteristics. NFGNB were identified by colony characteristics and biochemical reactions as described in text book of diagnostic microbiology[5]. Morphology and motility of the organisms were determined by Gram stain and Hanging drop preparation method respectively. Every single gram negative bacilli/coccobacilli, oxidase positive or negative were inoculated on Triple sugar iron agar medium (TSI). Organisms showing alkaline reactions were initially considered to be non-fermenter gram negative bacilli. The isolates were identified up to the species level based on motility, pigment production, enzyme production (e.g. catalase, urease, nitrate reductase) and various biochemical tests including Hugh and Leifson's medium to find out whether a particular organism was oxidizer or non-oxidizer, indole test, citrate utilization, production of hydrogen sulphide and nitrate or nitrite reduction[6]. Provisional identification of the unknown isolates of being a non-fermenter is lack of evidence of glucose fermenter and positive cytochrome oxidase reaction [7].

Antimicrobial sensitivity testing was carried out for all the isolates with the help of the Kirby-Bauer disc diffusion method using commercially available discs on Muller- Hinton agar (MHA). The results were interpreted as per the Clinical and

Laboratory Standard Institute (CLSI-2014) guidelines[8]. Those organisms which were difficult to identify we used the automated VITEK 2 system. All the isolates were tested for ESBL and MBL when required. For ESBL we used the Double disc synergy test using ceftazidime (30mg) and ceftazidime with clavulinic acid (30mg+10mg). EDTA disc synergy test was used to detect MBL production by the isolates[9,10].

OBSERVATIONS:

1.DISTRIBUTION OF CLINICAL SAMPLES :-

WARD	TOTAL NO. OF SAMPLES	PERCENTAGE
HAEMATOLOGY WARD	36	25.7%
SURGERY	28	20%
ICU	22	15.7%
BURN WARD	16	11.4%
OBS AND GYNAE	13	9.2%
UROLOGY	11	7.85%
ORTHOPAEDICS	8	5.71%
OTORHINOLOGY	4	2.85%
DERMATOLOGY	2	1.42%

2.ISOLATION OF NON FERMENTER FROM DIFFERENT SAMPLES:-

	P.aeruginosa		P.stutzeri		A.baumanii		A.lwofii		S.maltophilia		B.cepacia	
PUS	21	30.88%	1	25%	20	42.5%	1	9.09%	3	50%	--	-
SPUTUM	4	5.8%	-	-	2	4.25%	2	18.18%	-	-	2	50%
BLOOD	7	10.2%	2	50%	5	10.6%	1	9.09%	2	33.3%	1	25%
URINE	18	26.4%	-	-	4	8.5%	5	45.45%	-	-	-	-
WOUND SWAB	14	20.5%	1	25%	11	23.4%	2	18.18%	-	-	1	25%
ASCITIC FLUID	2	2.94%	-	-	-	-	-	-	1	16.6%	-	-
ET SWAB	2	2.94%	-	-	3	6.38%	-	-	-	-	-	-
PL.FLUID	0	0	-	-	2	4.2%	-	-	-	-	-	-

3. SPECIATION OF NON FERMENTERS :-

SPECIATION OF NON FERMENTERS	NUMBER OF N.F ISOLATED FROM SAMPLES	PERCENTAGE	ESBL PRODUCER	MBL PRODUCER
Pseudomonas aeruginosa	68	48.5%	12(17.6%)	4(5.88%)
Pseudomonas stutzeri	4	2.85%	1(25%)	0
Acinetobacter baumani	47	33.57 %	9(19.1%)	3(6.38%)
Acinetobacter iwoffii	11	7.85%	3(27.2%)	1(9.09%)
Stenotrophomonas maltophilia	6	4.28%	1(16.6%)	0
Burkholderia cepacia	4	2.85%	0(0%)	O
TOTAL	140		26(18.5%)	8(5.71%)

4.ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON FERMENTERS:-

	P.aeruginosa		P.stutzeri		A.baumanii		A.lwoffii		S.maltophilia		B.cepacia	
Gentamycin	24	39.7%	1	25%	23	48.9%	5	45.4%	-	-	-	-
Amikacin	36	56.25%	3	75%	33	70.2%	7	63.6%	4	66.6%	-	-
Ciprofloxacin	29	42.6%	3	75%	22	46.8%	6	54.5%	4	66.6%	2	50%
Ofloxacin	32	47.05%	4	100%	23	48.9%	5	45.4%	3	50%	1	25%
Ceftazidime	40	58.8%	3	75%	24	51%	7	63.6%	-	-	2	50%
Cefotaxime	-	-	-	-	29	61.7%	9	81.8%	-	-	1	25%
Piperacillin+Tazobactum	48	70.5%	4	100%	24	51%	9	81.8%	1	16.7%	1	25%
Cotrimoxazole	-	-	-	-	32	68%	4	36.3%	6	100%	4	100 %
Imipenem	49	72.0%	4	100%	34	72.3%	11	100%	-	-	3	75%
Polymyxin-B	68	100%	4	100%	47	100%	11	100%	6	100%	-	-
Meropenem	49	72%	4	100%	34	72.3%	11	100%	-	-	3	75%

RESULTS:

Out of 4872 number of total samples, 2264 number were collected from indoor patients admitted to different wards of our hospital. Total 140(12.8%) samples were shown growth of nonfermenters out of 1093 number of culture positive among the indoor samples. Maximum nonfermenters (25.7%)were isolated from our haematology department as this is the area where immunocompromised patients are staying. Least number (1.42%) of nonfermenters were isolated from our dermatology department. A total 140 different samples yielded nonfermenter Gram negative bacilli in which *P.aeruginosa* was major pathogen accounting for 48.5% of total isolates followed by *A.baumanii* 33.5%. We have isolated 6 (4.28%) cases of *S.maltophilia*.. From the isolates of *P.aeruginosa, 72%* were sensitive to imipenem, pip.tazobactam(70.5%),amikacin(56.25%), ceftazidime (58.8%) and ciprofloxacin(42.6%) and polymyxin B(100%).While isolated *P.stutzeri* showed 100% sensitivity to imipenem, meropenem and piperacillin tazobactum. *A.baumanii* were sensitive to polymyxin B(100%) followed by meropenem imipenem in 72.3%. In the present study, the antimicrobial susceptibility among the isolated *S.maltophilia*, majority were sensitive to Cotrimoxazole and Polymyxin B 6(100%), followed by Amikacin and ciprofloxacin (66-6%). Sensitivity pattern of nonfermenter Gram negative bacilli shown in table 4.

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 140 isolates were screened for ESBL production and confirmed by CLSI phenotypic confirmatory method. *A.baumanii* 9 (19.1%) were followed by 12(17.6%) *Pseudomonas aeruginosa*, 3(27.2%) of *A.lwoffii and P.stutzeri* 1(25%) were ESBL producers.(Table 3) While *S.maltophilia* and *B.cepacia* show intrinsic resistance to ß-lactam. 4(5.88%) of *Pseudomonas aeruginosa*, 3(6.38%) of *A.baumanii and A.lwoffii* 1(9.09%) were MBL producers. 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.

CONCLUSION:

The present study highlighted the fact that NFGNBs have emerged as an important Pathogen and shows resistance to commonly used antimicrobials. More significantly, the isolated NFGNBs have great ability to survive in hospital environment, so effective methods of sterilization and infection control measures should be implemented. Early diagnosis and institution of empirical therapy based on local antibiogram data of the institute would reduce mortality and improve patient management.

REFERENCES:

1. Malini A, Deepa EK, Gokul BN, Prasad SR; Nonfermenting Gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. Journal of lab physicians 2009;1(2):62-66.
2. Gautam V, Ray P, Vandamme P, Chatterjee SS, Das A, Sharma K, et al.; Identification of lysine positive non-fermenting Gram negative bacilli (Stenotrophomonas maltophilia and Burkholderia cepacia complex).Indian Journal of Medical Microbiology 2009;27(2):128-133.
3. Veenakumari HB, Nagarathna S, Chandramuki A; Antimicrobial resistance pattern among aerobic Gram negative bacilli of lower respiratory tract specimen of intensive care unit patients in a neurocentre. Indian J Chest Dis Allied Sci 2007; 49:19-2297.
4. Mohammad Rahbar, Hadi Mehragan, Negar Haji Ali Akbari; Prevalence of Drug Resistance in Nonfermenter Gram-Negative Bacilli. Iranian Journal of Pathology2010; 15(2).
5. Koneman EW, Allen SD. Colour atlas and Text book of diagnostic Microbiology, 6th edition, Philadelphia, Lippincott-Williams & Wilkins Publishers,2006:p .624-662.
6. Veenu, Rama S, Arora DR. 1999 Isolation and susceptibility pattern of non-fermenting Gram negative bacilli from clinical samples. Indian J Med Microbiol;17(1):14-7.
7. Yashodara P, Shyamala S; Identification and characterization of nonfermenters from clinical specimens. Indian J Med Microbiol 1997; 15: 195-7.
8. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement. CLSI document M100-S18. Clinical and Laboratory Standards Institute 2014.
9. Fellpe Fernandez, Luis Martinez, M Carman, Juan A. Relationship between Betalactamase production, Outer membrane proteins and Penicillin binding proteins profiles on the activity of Carbapenems against clinical isolates of A.baumannii. J. Antimicrob. Chemo. 2003;51(3):p.565-74.
10. Loveena Oberoi, Nachhatarjit Singh, Poonam Sharma, Aruna Agarwal. ESBL, AmpC and MBL beta lactamases producing superbugs. Havoc in the ICU of Punjab, India. J of Clinical Diagnostic Reseach.2013;7(1):p. 70-73.