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Research Paper

BACTERIOLOGICAL PROFILE AND DRUG RESISTANCE PATTERN OF NONFERMENTING GRAM NEGATIVE BACILLI INFECTION IN TERTIARY CARE HOSPITAL IN AHMEDABAD, GUJARAT

Microbiology

KEYWORDS : Nonfermenter, Gram negative bacilli, Imipenem, Polymyxin , colistin

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ABSTRACT

Introduction: The present study was planned to identify the nonfermenters isolated from various clinical samples, to assess their clinical significance, to know the type of healthcare-associated infections they caused, and to know their anti-microbial sensitivity pattern.

Material and Methods: The nonfermenters were identified using a standard conventional method including motility, oxidase production; oxidation-fermentation etc. and antibiotic susceptibility testing was performed with the help of the Kirby-Bauer disc diffusion method.

Observations and Results: The most common isolates were Pseudomonas aeruginosa (65.32%) followed by Acinetobacter baumanii (32.61%) etc.

Conclusion: P. aeruginosa and A. baumannii are the most common NFGNB isolates causing UTI, septicemia, SSI and respiratory tract infection. Both has shown emerging resistance to imipenem. Only 100% sensitive antibiotics were Polymyxin and Colistin to them.

Introduction:

The term nonfermentative Gram-negative bacilli means all aerobic gram- negative rods that show abundant growth within 24 hrs on the surface of Kligler iron agar(KIA) or Triple sugar iron(TSI) medium, but neither grow in nor acidify the butt of this media. [1]

Nonfermenters can cause a vast variety of infections and account for approximately 15%of all Gram negative bacilli cultured from clinical specimens.[2] Non-fermenters may differ in their pathogenic potential and transmissibility, and many are multidrug resistant. For this reason, accurate identification of nonfermenters to species level is important for appropriate patient management.[2]

Their infection are endogenous or exogenous origin, depending on several factor such as:

use of immunosuppressant substance, abusive use of wide spectrum antimicrobial agents, prolong surgical wide spectrum antimicrobial agents, prolong surgical procedure and inadequate instrumentation.[3]In recent year due to liberal and empirical use of antibiotics, nonfermentative

Gram-negative bacilli are emerge as a important health care associate pathogen. They have been

incriminated in infections such as a septicemia, pneumonia, Urinary tract infection and surgical site infection. Non-fermenting Gram-negative bacilli are innately resistant to many antibiotics and are known to produced extrended spectrum ß-lactmases and metallo ßlactmases.[4]

The present study was undertaken to identify various nonfermenters from patient admitted to our hospital and to assess their antimicrobial susceptibility pattern.

Material and Methods:

A total 21367 clinical specimen were received in Department of Microbiology, for culture and sensitivity during Novemeber 2013 to October 2014. All the samples were plated on blood agar, MacConkey agar and nutrient agar, and incubated at 37°C for 18-48 hours and growth recorded, and lactose nonfermenting colonies were followed. Morphology and motility of the organisms were determined by Gram staining and hanging drop method respectively and oxidase test was done. All the Gram-negative bacilli grew on Mac Conkey agar or blood agar, whether oxidase positive or negative were inoculated on Triple sugar iron agar medium (TSI). Organisms grew on Triple Sugar Iron and produced an alkaline reaction were provisionally considered to be nonfermenter gram negative bacilli, and were inoculated into Hugh and Leifson's medium for glucose, lactose, sucrose and maltose to find out whether a particular organism was oxidizer or non-oxidizer and identified particular organism its biochemical reactions characteristics. Initial clues that an unknown isolate is a nonfermenter are:[1]

a) Lack of evidence of glucose fermentation.
b) Positive cytochrome oxidase reaction.
c) Failure to grow on MacConkey agar.

Scheme for identification (up to genus level)of nonfermenting Gram negative bacilli shown in figure 1.The antimicrobial susceptibility testing was performed with the help of the Kirby-Bauer disc diffusion method using commercially available discs on Muller- Hinton (MH)agar. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI-2013) guidelines.[5] *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains.

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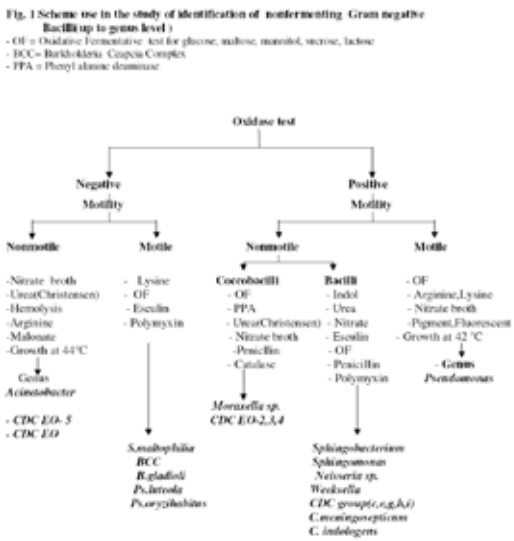

Observations & Results :

From received 21367 various clinical samples 2183 (10.21%) samples were nonfermenter Gram negative bacilli. In the present study, the majority of the nonfermenters were isolated from swab (61.30%) , Urine (7.96%) , Sputum (5.95%) , Tracheal aspirates (5.17%) and Blood (4.89%). Specimen wise distribution of nonfermenter Gram negative bacilli shown in table 1. 374 (17.10%) specimens showed polymicrobial infection with nonfermenter organisms. In our study, most common isolates were *P.aeruginosa* (65.32%) followed by *A.baumanii* (32.61%), *Acinetobacter sp.* (1.01%). Organism wise distribution of nonferementer gram negative bacilli is shown in table 2.

Table 1 : Sample distribution showing growth of nonfermenter gram negative bacilli

Specimen	Number	%
Swab	1339	61.30
Urine	174	7.96
Sputum	130	5.95
Tracheal aspirate	113	5.17
Blood	107	4.89
Tissue	57	2.60
Pus	50	2.28
Fluids	46	2.10
CSF	39	1.78
Others	128	5.86

Table 2 : Organismwise distribution of nonfermenter gram negative bacilli

Organism	Number of isolates	%
Pseudomonas aeruginosa	1426	65.32
Acinetobacter baumannii	712	32.61
Acinetobacter sp.	22	1.01
Stenotrophomonas maltophilia	6	0.27
Acinetobacter lwoffii	5	0.23
Pseudomonas sp.	5	0.23
Pseudomonas stutzeri	4	0.18
Acinetobacter calcoaceticus	1	0.05
Non-fermenting gram negative rods	1	0.05
Stenotrophomonas sp.	1	0.05

From the isolates of *P.aeruginosa 100%* were sensitive to Polymyxin B and colistin followed by Imipenem (64.4%) , Piperacillin – Tazobactam (40.2%) , Cefepime-Tazobactam (38.7%) and Cefoperazone-sulbactam (34.6%). *Acinetobacter baumanii* were sensitive to Polymyxin and colsitin in 100% followed by Cefoperazone-sulbactam (47.5%) and Ampicillin sulbactam (21.6%). While *Pseudomonas* showed only 31.8 % resistance to imipenem , A.baumanii showed 76.8% rsistance. Sensitivity pattern of two most common nonfermenter Gram negative bacilli were shown in table 3 & 4.

Table 3 : Antibiotic susceptbility pattern of Pseudomonas aeruginosa

Pseudomonas aeruginosa	%R	%I	%S
Polymixin B	0	0	100
Colistin	0	0	100
Imipenem	31.8	3.8	64.4
Meropenem	31.8	3.8	64.4
Ertapenem	52.1	2.1	45.8
Piperacillin/Tazobactam	59.5	0.3	40.2
Cefepime-Tazobactam	61	0.3	38.7
Cefoperazone/Sulbactam	64.2	1.2	34.6
Ticarcillin/Clavulanic acid	65.4	0	34.6
Aztreonam	71.6	0.7	27.7
Cefepime	76.4	0.1	23.5
Netilmicin	76.6	0.1	23.3
Amikacin	76.7	0.1	23.2
Ofloxacin	77.1	0	22.9
Chloramphenicol	77.8	0	22.2
Ceftazidime	78.3	0	21.7
Levofloxacin	78.3	0	21.7
Moxifloxacin	78.4	0	21.6
Gatifloxacin	78.4	0	21.6

Piperacillin	79.3	0.1	20.6
Cefpirome	80.4	0	19.6
Tobramycin	80.4	0	19.6
Gentamicin	81.3	0	18.7
Cefotaxime	87.7	0	12.3
Ceftriaxone	87.7	0	12.3
Ampicillin/Sulbactam	88.9	0	11.1
Doxycycline	91.6	0	8.4
Minocycline	91.6	0	8.4
Trimethoprim/ Sulfamethoxazole	92.7	0	7.3
Tetracycline	93.5	0	6.5

Table 4 : Antibiotic susceptbility pattern of Acinetobacter baumanii

Acinetobacter baumanii	%R	%I	%S
Polymixin B	0	0	100
Colistin	0	0	100
Cefoperazone/Sulbactam	48.9	3.5	47.5
Ampicillin/Sulbactam	77.7	0.7	21.6
Imipenem	76.8	6.4	16.8
Meropenem	76.8	6.4	16.8
Ertapenem	78	5.2	16.8
Tetracycline	86.3	0	13.7
Doxycycline	86.3	0	13.7
Minocycline	86.3	0	13.7
Levofloxacin	79.5	7.1	13.4
Moxifloxacin	79.5	7.1	13.4
Gatifloxacin	79.5	7.1	13.4
Amikacin	94.5	0.3	5.3
Netilmicin	94.6	0.2	5.2
Tobramycin	95.1	0	4.9
Chloramphenicol	95.1	0	4.9
Trimethoprim/Sulfamethoxazole	95.6	0	4.4
Gentamicin	95.7	0.1	4.1
Cefpirome	96.9	0	3.1
Ofloxacin	97.6	0	2.4
Cefepime	97.7	0	2.3
Ciprofloxacin	97	0.8	2.3
Cefotaxime	98.7	0	1.3
Ceftriaxone	98.9	0	1.1
Cefuroxime	99	0	1
Ceftazidime	100	0	0
Aztreonam	100	0	0
Piperacillin/Tazobactam	100	0	0
Ticarcillin/Clavulanic acid	100	0	0

Discussion:

Nonfermenter Gram negative bacilli were considered to be a contaminant in past have now emerge as an important health care pathogen. *P. aeruginosa* and *Acinetobacter* species are known to be nosocomial pathogens.[4] In the present study non-fermenters were isolated *P.aeruginosa* predominated followed by genus *Acinetobacter* resemling to study done by A Malini *et al* and Vijaya D *et al.*[4,6]

Resistant patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time. [7] In a study of Taneja N. *et al* from Chandigarh 42% of *P. aeruginosa* isolates were found to be resistant to imipenem while in our it is 31.8% only.[8]Similarly *Acinetobacter* baumanii showed higher rate of resistance to amikacin, and ceftazidime, compare to study at Bangalore.[9]

P. aeruginosa and *A. baumannii* are the most common NFGNB isolated in our study. Their role as health care associated pathogens is well established and they have caused UTI, septicemia, SSI and respiratory tract infection. *P. aeruginosa* and A. baumanii has shown good sensitivity to imipenem but in present study we noted resistance to these drugs too. The different species of NFGNB have shown a varied sensitivity pattern in our study. Therefore, identification of NFGNB, and monitoring their susceptibility patterns, are important for the proper management of the infections caused by them.

Conclusion: Our study highlights the fact that it is essential to establish the clinical relevance of the isolated NFGNB, before they are considered as pathogens. This would avoid unnecessary usage of antibiotics and emergence of drug-resistant strains.

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