

“Prevalence of nonfermenting Gram negative bacilli infection in tertiary care Hospital in Ahmedabad, Gujarat”

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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* accounting for 1845(76.97%), followed by *A. baumannii* 512(21.36%) etc.

Conclusion: *P.aeruginosa* and *A.baumannii* are most common NFGNB isolated in our study from patient of surgical site infection, urinary tract infection, bacteremia etc. *P.aeruginosa* showed good sensitivity to imipenem and pip.tazobactam. *A.baumannii* also showed good sensitivity to imipenem.

Key words: Nonfermenter, Gram negative bacilli, Imipenem.

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 Non-fermenters 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 non-fermenters 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.³In recent year due to liberal and empirical use of antibiotics, non-fermentative 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 extended spectrum β -lactmases and metallo β -lactmases.⁴

With this background in mind, the present study was undertaken to identify various nonfermenter from patient admitted to our hospital. Study was also done to assess their clinical significance, antimicrobial susceptibility

pattern and to identify various healthcare related problem.

Material and Methods:

A total 20721 clinical specimen were received in bacteriology laboratory Department of Microbiology, for culture and sensitivity during July 2010 to June 2011, which included 4926 urine, 5272 pus/pus swab, 1011 sputum, 4395 blood culture, 164 tracheal aspirate/secretion, 195 throat swab, 1056 Cerebrospinal fluid and 1117 body fluids and other samples at our institute. 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 non-fermenter 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-2010) guidelines.⁵ *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 was used as a control strain.

Observations & Results :

From received 20721 various clinical samples 10018(48.34%) samples were found to be positive for bacterial culture and 326(1.57%) for *Candida*, other were culture negative or normal flora. From total isolates 1862(18.58%) were belongs to Gram positive and 8156(81.4%) Gram negative organisms, and from them 2397(23.93%) of various clinical samples were found to be positive for nonfermenter Gram negative bacilli. All isolates were analyzed by specimens; nonfermenter Gram negative bacilli were isolated from pus, urine, sputum, blood, tracheal secretion or aspirate, body fluids, CSF and other samples. In which 3413 (31.78%) specimens showed polymicrobial infection with nonfermenter (other organism was also nonfermenter) as well as other than nonfermenter organisms while three specimens with *Candida*. In the present study, the majority of the non-fermenters were isolated from pus (58.65%), followed by urine (10.8%), sputum (6.96%), body fluids (6.59%), blood (6.54%), tracheal aspirate/secretion(4.21%), CSF(0.87%), Throat swab(0.29%) and 5.03% from other specimens. The majority of the nonfermenters were isolated from pus and urine samples and accounting for 69.45% of total isolates. Specimenwise distribution of nonfermenter Gram negative bacilli shown in table 1. In our study, most common isolates were *P.aeruginosa*

(76.97%) followed by *A.baumannii* (21.36%), *Pseudomonas sp.* (0.54%), *Acinetobacter sp.*(0.50%), *S.maltophilia* (0.020%), *Moraxella sp.* (0.12%) , *C. meningosepticum* (0.08%), *A.lwoffii* (0.08%), *P.putida*(0.08%) and *P.stutzeri* (0.04%). Out of 268 samples from respiratory tract 101 tracheal aspirate/secretion and 167 sputum samples yielded nonfermenter Gram negative bacilli in which *P.aeruginosa* was most common pathogen followed by *A.baumannii* and the three isolates of *Moraxella sp.* one from sputum.

A total 259 urine samples yielded nonfermenter Gram negative bacilli in which *P.aeruginosa* was major pathogen accounting for 87% of total isolates followed by *A.baumannii* , *Pseudomonas sp.* and *A. lwoffii*. Among the 157 isolates from blood 87(55.41%) were *P.aeruginosa*, 64(40.76%) *A. baumannii*, 3 *Pseudomonas sp.*, 2 *Chryseobacterium meningosepticum* and 1 *Acinetobacter sp.*. From the 21 samples of CSF 17(80%) *A.baumannii* and 4(20%) were *P.aeruginosa* and from the body fluids and other samples *P.aeruginosa* was most common isolates followed by *A.baumannii*.

The patient having infection in throat also yielded same for *P.aeruginosa* and *A. baumannii* other than it two isolates of *Moraxella sp.* were found. It was observed that almost in all clinical specimens *P.aeruginosa* was most common followed by *A.baumannii*. All the isolated were sensitive to polymyxin B except *C.meningosepticum*.

From the isolates of *P.aeruginosa*, 94% were sensitive to imipenem, pip.tazobactam(75.9%), amikacin(39.6%), aztreonam(39.8%), cefepime (28.7%) ceftazidime (24.6%) and ciprofloxacin(16.53%). While isolated *P.putida* showed 100% sensitivity to imipenem, amikacin and cefepime, 50% sensitivity to ceftazidime, pip.tazobactam, ciprofloxacin and aztreonam. *A.baumannii* were sensitive to imipenem in 72.9% followed by amikacin(38.8%), doxycycline(22.3%),

cotrimoxazole(10%) and for cephalosporins sensitivity is less than 10%. Isolated all strain of *A.lwoffii* were sensitive to imipenem and doxycycline and all were resistant to cephalosporins, and 50% sensitive to amikacin and cotrimoxazole. *Acinetobacter sp.* showed totally resistant to cephalosporins and cotrimoxazole and 77.8% sensitive to imipenem and amikacin. *S.maltophilia* were 100% sensitive to cotrimoxazole and 100% resistant to imipenem, aminoglycosides and cephalosporins. Other rare isolates of *P.putida*, *Moraxella sp.* and *C.meningosepticum* were almost highly sensitive to tested antibiotics. While *Pseudomonas sp.* showed only 10.1% resistance to imipenem, 16.7 % sensitive to ciprofloxacin and mixed response to other tested antibiotics. Isolated all *S. maltophilia* were sensitive to cotrimoxazole. Sensitivity pattern of nonfermenter Gram negative bacilli shown in table 2.

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 nonfermenters were isolated *P.aeruginosa* predominated followed by genus *Acinetobacter* resembling to study done by A Malini *et al* and Vijaya D *et al.*^{4,6} Highest number of Non-fermenting Gram negative bacilli isolates were from pus samples and this is similar to observation made by A Malini *et al.*⁴ Resistant patterns among nosocomial bacterial pathogens may vary from country to country and also within the same country, over time.⁷ Sensitivity pattern of *P. aeruginosa* for imipenem and cefepime is similar with isolate from Karnataka but differ for amikacin.⁴ 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 6%

only.⁸ Similarly *Acinetobacter baumannii* showed higher rate of resistance to amikacin, and ceftazidime, compare to study at Bangalore.⁹ *S.maltophilia* is inherently resistant to aminoglycosides and β -lactam agents including carbapenems but consistently sensitive to cotrimoxazole.¹⁰

P. aeruginosa and *A. baumannii* are the most common NFGNB isolated in our study. Their role as healthcare-associated pathogens is well established and they have caused UTI, septicemia, SSI and respiratory tract infection. *P. aeruginosa* and *A. baumannii* has shown good

sensitivity to imipenem. 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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Fig. 1 Scheme use in the study of identification of nonfermenting Gram negative Bacilli(up to genus level)

- OF = Oxidative Fermentative test for glucose, maltose, mannitol, sucrose, lactose
- BCC= Burkholderia Ceapcia Complex
- PPA = Phenyl alanine deaminase

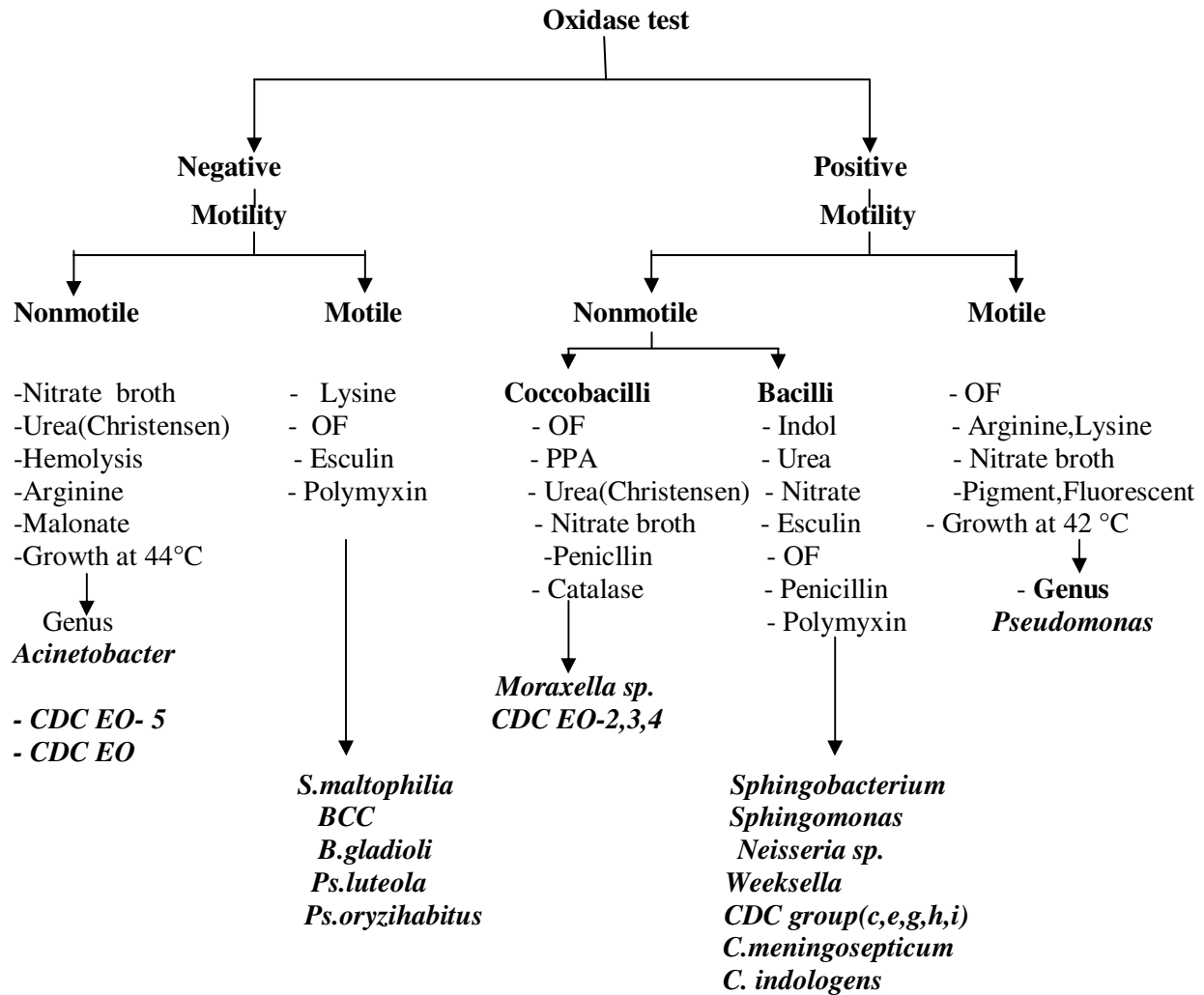


Table 1 : Specimenswise distribution of nonfermeter Gram negative bacilli

Species	Pus/Pus swab	Urine	Sputum	Blood	Tracheal aspirate/ secretion	CSF	Throat swab	Body fluid	Other	Total & %
<i>Pseudomonas Aeruginosa</i>	1142	227	127	87	51	04	04	118	85	1845(76.9%)
<i>Pseudomonas sp.</i>	06	03	01	03	00	00	00	01	00	14(0.54%)
<i>Pseudomonas putida</i>	02	00	00	00	00	00	00	00	00	02(0.08%)
<i>Acinetobacter baumannii</i>	242	27	37	64	50	17	1	39	35	512(21.36%)
<i>Acinetobacter sp.</i>	09	00	00	01	00	00	00	01	01	12(0.5%)
<i>Acinetobacter lwoffii</i>	00	02	00	00	00	00	00	00	00	02(0.08%)
<i>S.maltophilia</i>	05	00	00	00	00	00	00	00	00	05(0.2%)
<i>Cryobacterium meningosepticum</i>	00	00	00	02	00	00	00	00	00	02(0.08%)
<i>Moraxella sp.</i>	00	00	01	00	00	00	02	00	00	02(0.12%)
<i>P.stutzeri</i>	00	00	01	00	00	00	00	00	00	01(0.04%)
Total	1406 (58.65%)	259 (10.80 %)	167 (6.96%)	157 (6.54%)	101 (4.21%)	21 (0.87%)	07 (0.29%)	158 (6.59%)	121 (5.03%)	2397

Table 2: Sensitivity pattern of the nonfermenting Gram-negative bacilli Isolated in Present study

Antimicrobial agent	P.aerugino sa	P.puti da	Pseud o. sp.	Psedo . stutze ri	A.bau m- manii	A. lwoff ii	Acinet o. sp.	S.mal to philia	Moraxe lla sp.	C.meningo- septicum
Imipenem	94	100	90.9	100	72.9	100	77.8	0	100	100
Amikacin	39.6	100	61.5	100	38.8	50	77.8	0	100	100
Ceftazidime	24.6	50	15.4	100	5.8	0	0	0	100	100
Cefepime	28.7	100	33.3	100	7.6	0	0	0	100	100
Pip.tazobact am	75.9	50	53.8	100	-	-	-	-	-	-
Aztreonam	39.8	50	30.8	100	-	-	-	-	-	-
Cotrimoxaz ole	-	-	-	-	10	50	0	100	50	100
Doxycyclin e	-	-	-	-	22.3	100	66.7	00	66.7	100

(Pip.tazobactam=Piperacillin tazobactam) The sensitivity pattern shown in the table is the percentage.

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