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Evaluate the Distribution of Gram Negative Non Fermenting Bacteria and their Resistant Pattern in Clinical Isolates among the Rural Population in South India

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A B S T R A C T

Non-fermenting gram negative bacilli (NFGNB) are a group of aerobic, non spore forming bacilli. They either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. They are ubiquitous in nature. Although they are commonly considered to be environmental contaminants, they have emerged as important nosocomial pathogens. Aim of this study was to characterize the prevalence of NFGNB distribution from various clinical isolates and to evaluate their antibiotic sensitivity patterns. Material and methods: A total 11,040 various clinical specimen were received in bacteriology laboratory, Department of Microbiology at Kamineni Institute of Medical Sciences. Non fermenters are identified and further analysed as per the guidelines. Antimicrobial susceptibility testing was performed by Kirby beaur disc diffusion method. Results: Among 11,040 clinical samples 354 yields NFGNB. Pseudomonas species (63.55%) and Acinetobacter species (32.20%) were the most commonly isolated NFGNB. A high level of antibiotic resistance was recorded. Ciprofloxacin (71.2) and Gentamicin (54.33) were the drugs with maximum activity. Conclusion: Identification of NFGNB and monitoring their antimicrobial susceptibility pattern helps in proper management of the treatment.

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 (Koneman *et al*., 2006). Aerobic Non Fermenting Gram Negative Bacilli (NFGNB) usually considered as contaminants, are emerging as important nosocomial pathogens as they have a tendency to colonize various surfaces and have pivotal role in their emergence as important nosocomial pathogens. Nonfermenters can cause vast variety of infections and account for approximately 15% of all Gram negative bacilli cultured from clinical specimens (Murray *et al*., 2003). They may differ in their pathogenic potential and transmissibility and many are multidrug resistant (Siou *et al*., 2009), Depending on several factors such as use of immunosuppressant substance, abusive use of wide spectrum antimicrobial agents, prolong surgical procedure and inadequate instrumentation, they are endogenous or exogenous in origin (Patel *et al*., 2013). In

recent years due to liberal and empirical use of antibiotics, NFGNB emerges as an important health care associated pathogen. They have been incriminated in infections such as septicemia, pneumonia, Urinary tract infection and surgical site infection. NFGNB are innately resistant to many antibiotics.

Antimicrobial treatment of the infections caused by these agents is difficult due to its multidrug resistance (MDR).For this reason, accurate identification of non-fermenters is important for appropriate patient management

The main objective of this study includes to isolate and identify the Non Fermenting Gram Negative Bacilli from clinical samples. And to evaluate the antibiotic sensitivity pattern of the isolates.

Materials and Methods

This study was conducted for a period of 2 years (July 2012 to June 2014) at Kamineni Institute of Medical Sciences Narketpally, District Nalgonda, Hyderabad (A.P), India.

A total of 11,040 clinical specimens were received in bacteriology laboratory, Department of Microbiology, which includes urine (1884), pus/pus swab (2921), sputum (1368), blood culture (1780), other respiratory secretions (983), Cerebrospinal fluid (531) and indwelling devices (641) and other samples. All the samples received were further plated on Blood agar, MacConkey agar, Nutrient agar, and incubated at 37°C for 18-48 hours. Growth was recorded, and lactose non fermenting colonies were further analysed and processed as per the standard guidelines All the Gram-negative bacilli that grew on Mac Conkey agar or blood agar, whether oxidase positive or negative were inoculated on Triple sugar iron agar medium (TSI). Organisms that grew on Triple Sugar Iron agar producing an alkaline reaction were provisionally considered to be non fermentative gram negative bacilli, and were further inoculated into Hugh and Leifson's medium for glucose, lactose, sucrose and maltose fermentation to find out whether a particular organism was oxidizer or non-oxidizer.

Samples were plated on blood agar (BA) and Mac Conkey's agar (MA) and incubated at 37°C for 48 hours before being reported as sterile. The isolates that showed non lactose fermenting (NLF) colonies on MA and failed to acidify the butts of triple sugar iron (TSI) agar were provisionally considered as NFGNB and they were further identified by using a standard protocol for identification.

The characters assessed were gram staining morphology, motility (by hanging drop), catalase test, oxidase test, citrate utilization, urea hydrolysis, hemolysis on 5% sheep blood agar, growth on 6.5% NaCl, nitrate reduction, pigment production, indole production, lysine and ornithine decarboxylation, arginine dihydrolase test, growth at 40°C and 42°C, oxidation of 1% glucose, lactose, sucrose, maltose, mannitol, xylose (Hugh and Leifson's medium), growth on 10% lactose agar and gelatin liquefaction test.

Further Antimicrobial sensitivity was determined by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). Results were interpreted in accordance with central laboratory standards institute (CLSI) guidelines (Clinical Laboratory Standards Institutes. Performance Standards for antimicrobial susceptibility tests, 2009). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Results and Discussion

Among 11,040 clinical samples, total of 354 NFGNB were isolated from 348 samples (due to polymicriobial growth) which accounted

for an isolation rate of NFGNB to be 3.20%. Monomicrobial growth was seen in 266 (76.43%) specimens, whereas 82 specimens showed polymicrobial growth. Out of 82 specimens, 76 were both fermenters and non fermenters but 6 samples yielded both as non fermenters. Out of the fermenters, *Klebsiella* spp. and *E.coli* were most commonly isolated. Non fermenters were isolated from variety of clinical specimens. Majority of isolated were from surgical site infections SSI (21.26%) followed by ET Tube 20.40% urine 19.25% and respiratory secretions (18.39%). *P.aeruginosa* was the most common isolate, accounting for 225 (63.56%) followed by *Acinetobacter spp* (32.20%) and *Moraxella spp* 3.67%. *Burkholderia spp* and *Stenotrophomonas spp* were only 1 (0.28%). Accounting for sensitivity pattern *Pseudomonas spp* showed maximum resistance to ciprofloxacin, Piperacillin followed by Gentamicin and Ciprofloxacin, whereas *Acinetobacter* showed high level of resistance to Ceftazidime, Co-trimoxazole and Piperacillin followed by Ciprofloxacin and Gentamicin and all the organisms showed sensitivity towards polymyxin B. Whereas all the isolates of *Acinetobacter* species were found maximally sensitive to polymyxin B, all the isolates of *Burkholderia spp* and *Stenotrophomonas spp* showed maximum sensitivity to fluroquinolones, cephalosporins and co-trimoxazole.

The Age group in our study is between 21 to 70 years were (77.3%). And this observation correlated to the study conducted by Sachdev and Deb (1980).

There was a preponderance of the infection in males in our study. Similar observation was made in other studies by Rajan *et al.,* (2001) and Wisplinghoff *et al.,* (1999). This finding can be explained on the basis that males are more active in outdoor activities so they are more prone to infections and trauma. The total NFGNB isolated from surgical site infections were (21.26%), which is similar to other studies by Malini *et al.,* (2012) and Gokale *et al.,* (2012) where pus is the commonest sample from which majority of the NFGNB were isolated.

Comparison of isolation rate of NFGNB in various studies

Study series	Year	% of NFGNB Isolated
Malini A *et al*	2009	4.5
Jayanthi S study	2012	5.2
Juyal D *et al*	2013	9.32
our study	**2014**	**3.20**

Comparison of commonest isolates in various studies

Study series	Year	*Pseudomonas* spp (%)	*Acinetobacter* spp (%)
Malini A *et al*	2009	64.6	25.3
Upgade A *et al*	2012	43	21
Patel PH *et al*	2013	76.97	21.36
Nautiyal S *et al*	2014	62.92	21.05
Present study	2014	63.55	32.20

In comparison with the studies done by Malini *et al.,* (2012) and Nautiyal *et al.,* (2014) *Pseudomonas* spp isolation rates of 64.6% and 62.92% respectively, were similar to our study, and *Acinetobacter* spp were isolated at 25.2% and 21.05%, which is slightly less as compared to our study. Upgade *et al.,* (2012) and Patel *et al.,* (2013) isolated *Pseudomonas* spp 43% and 76.97% respectively, whereas *Acinetobacter* spp isolation rate was 21% in both the studies.

Pseudomonas spp was found to be commonest non fermenter in all of the studies followed by *Acinetobacter*. This is in concordance to the findings of our study. In our study the most common Gram Negative Non Fermenting organisms isolated was *Pseudomonas* spp 225 (63.55%) followed by *Acinetobacter* spp 114 (32.20%).

The NFGNB are known to be responsible for wide range of nosocomial infections. Resistance pattern among nosocomial bacterial pathogens may vary widely from country to country at any given time and within the same country over time (Prashanth *et al*., 2004). Because of these variations a surveillance of the nosocomial pathogens for resistograms in a given set up is needed in order to guide appropriate selection of empiric therapy. Various international authorities emphasize that every hospital should have its individual antibiotic sensitivity pattern since the standard antibiotic sensitivity pattern may not hold true for every area. Most of our patients were from surgical wards and not from ICU settings. Furthermore our patients came from rural areas without much exposure to antibiotics. In the present study, from the antibiotic sensitivity pattern it is clear that most of the isolates showed high degree of resistance suggesting that majority of the first and second line drugs were ineffective and this further confirms the multi drug resistant (MDR) attribute of NFGNB.

Antibiotic susceptibility

In present study, amongst the *Pseudomonas* spp, high level of resistance was recorded for Ciprofloxacin (71.20%), followed by Gentamicin (54.33%) and to both Ceftazidime and Piperacillin (52.88%)

A study done by Juyal *et al.,* (2013) reported high level of resistance to Ciprofloxacin 73.77% followed by 51.64% resistance to Gentamicin. Patel *et al.,* (2013) had also reported 83.3% Ciprofloxacin resistance in their study.

Amongst the Aminoglycosides, Gentamicin (54.33%) demonstrated higher resistance than Amikacin (36.44%). Similar results were also demonstrated in Jayanthi *et al.,* (2012) study where Gentamicin (30.3%) showed higher resistance than Amikacin (15.5%).

In the present study, amongst the *Acinetobacter* spp higher rate of resistance was reported in Ceftazidime (82.30%) followed by Co-trimoxazole (79.51%). Similarly higher rate of resistance was reported in Ceftazidime, Piperacillin and Ciprofloxacin in a study done by Sinha *et al.,* (2007).

Only one strain of *Stenotrophomonas* spp was isolated in our study, which was sensitive to Co-trimoxazole and Ciprofloxacin, but resistant to Aminoglycosides and Imipenem. Similar results of Cotrimoxazole sensitivity were also reported by Malini *et al.,* (2012) and Steinberg *et al*., (2010).

Screening for MDR isolates in the present study, 48.5% isolates were multidrug resistant, showing acquired non susceptibility to at least one drug in three or more antimicrobial categories. This was in concordance to the Amutha *et al*., (2009) study showing 45.2% of MDR isolates, but

Mathai *et al.*, (2012) study showed higher MDR isolates of 70%. This can be explained on the basis as their study was done on the ICU patients who were mostly on ventilators and had more chances of hospital acquired infection with multidrug resistant strains. In our study a overall Imipenem resistance among NFGNB was 9.60%. This collaborates well with the study by Gladstone *et al.*, (2005) and Nautiyal *et al.*, (2014).

Comparison of the isolation rate of MDR NFGNB in various studies

Study series	Year	% of MDR NFGNB
Amutha R	2009	45.2
Mathai AS *et al*	2012	70
Jayanthi S study	2012	39.4
Present study	**2014**	**48.5**

Comparison of total Imipenem resistance in NFGNB in various studies

Study series	Year	% of Imipenem resistance
Gladstone P *et al*	2005	12.2
Patel PH *et al*	2013	6
Nautiyal S *et al*	2014	11.6
Present study	**2014**	**9.60**

Table.3 Sample-wise Distribution (n=348)

Sample	Number of cases	Percentage
SSI	74	21.26
ET Tube	71	20.40
Urine	67	19.25
Sputum, throat swab	64	18.39
Wound Swab	29	8.34
Blood	26	7.47
Body fluids	9	2.59
Indwelling devices	7	2.01
CSF	1	0.29
TOTAL	348	100

Table.5 Distribution of isolated NFGNBs (n = 354)

Organisms isolated	Number	Percentage
Pseudomonas spp	225	63.56
Acinetobacter spp	114	32.20
Moraxella spp	13	3.67
Burkholderia spp	1	0.28
Stenotrophomonas spp	1	0.28
Total	354	100

Table.8 Antibiotic resistance pattern of NFGNB (n=354)

Antibiotic	*Pseudomonas* n (%)	*Acinetobacter* n (%)	*Moraxella* n (%)	*Burkholderia* n (%)	*Stenotrophomonas* n (%)
Piperacillin	119(52.88)	85(75.22)	1(7.69)	1(100)	1(100)
Amikacin	82(36.44)	73(64.40)	1(7.69)	1(100)	1(100)
Gentamicin	122(54.33)	77(68.14)	2(15.38)	1(100)	1(100)
Tobramicn	66(29.33)	65(57.50)	1(7.69)	1(100)	1(100)
Netilmicin	58(25.77)	61(53.50)	1(7.69)	1(100)	1(100)
Ciprofloxacin	160(71.2)	81(71.38)	5(38.46)	0	0
Ofloxacin	70(31.11)	73(64.40)	1(7.69)	0	0
Norfloxacin	38(16.88)	15(13.15)	-	-	-
Cotrimoxazole	-	90 (79.51)	0	0	0
Ceftazidime	119(52.88)	93(82.30)	3(23.02)	0	0
Ceftazidime-Clavulanic Acid	63(28)	63(55.75)	0	0	0
Piperacillin/ Tazobactum	58(25.77)	74(65.48)	0	1(100)	1(100)
Imipenem	6(2.66)	26(23.00)	0	1(100)	1(100)
Polymyxin-B	0	0	0	1(100)	0
Total isolates	225	114	13	1	1

Total of 11,040 samples were received which yielded 354 NFGNB, resulting in an isolation rate of 3.20%.These NFGNBs were identified and screened for antibiotic sensitivity patterns. The most common isolate was *Pseudomonas* spp - 225(63.55%), followed by *Acinetobacter*spp-114(32.20%). Other isolates were *Moraxella* spp - 13(3.67%), *Burkholderia* spp - 1(0.28%) and *Stenotrophomona s*spp - 1(0.28%). *Pseudomonas* spp showed maximum resistance to Ciprofloxacin (71.20%), Gentamicin (54.32%) followed by Ceftazidime and Piperacillin both accounting for 52.88% resistance. *Pseudomona s*spp showed maximum sensitivity to Polymyxin-B (100%) and Imipenem (97.34%). *Acinetobacter* spp showed maximum resistance to Ceftazidime (82.30%), followed by 79.50% resistance to Cotrimoxazole and 75.22% resistance to Piperacillin.MDR NFGNB accounted for 48.5%. Total Imipenem resistance was reported to be 9.03%. NFGNB though regarded as contaminants are important bacteria causing wide range of nosocomial infections. Variability in sensitivity pattern emphasizes the need for identification of NFGNB and to monitor their susceptibility patterns as it will help in proper management of the infections caused by them.

Prevalence of pathogens often varies dramatically between communities, hospitals in the same community and among different patient populations in the same hospital. Thus it is important for clinicians to remain updated with prevalence and antimicrobial susceptibility pattern of the circulating pathogens in their practice setting and the antimicrobials to be used for empiric therapy should be selected accordingly.

More importantly these organisms have great potential to survive in hospital environment. Thus improved antibiotic stewardship and

infection control measures like maintaining good housekeeping, equipment decontamination, strict attention to hand washing and isolation procedures especially in high risk areas should be implemented to prevent the emergence and spread of multidrug resistant NFGNB in the healthcare setting.

De-escalation of antibiotics should be done depending upon the antibiotic sensitivity reports.

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