Isolation And Identification Of Non-Fermenting Gram Negative Bacilli From Various Clinical Samples At A Tertairy Care Hospital In North Karnataka

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Abstract:

Background and Objectives: Non fermenting gram negative bacilli (NFGNB) are a group of heterogenous, aerobic, non sporing bacteria. They are saprophytes in nature and are also found as commensals in man and other animals. This study aims at isolation, identification and antibiotic susceptibility of non fermenting gram negative bacilli from various clinical specimens and to find out their clinical significance among the inpatients admitted at Basaveshwar Teaching and General Hospital, Gulbarga.

Materials & Methods: 150 isolates from various age groups of both male and female patients were included in the study. A detailed history was elicited and the clinical specimens were collected under aseptic precautions and subjected to preliminary biochemical test and further speciation was done.

Results: In the present study Pseudomonas aeruginosa, Acinetobacter baumanii, Pseudomonas fluorescens were isolated of which Pseudomonas aeruginosa was the most common. The non fermenting gram negative bacilli were isolated 29.68% from local wound infection, 21.8% from post operative wound infection, 20.3% from respiratory tract infections, 9.37% from gastrointestinal tract infections, 6.25% from urinary tract infections and 4.68% from septicaemia cases. Non fermenting gram negative bacilli showed variability in their antibiotic susceptibility results. Most of them were resistant to various groups of drugs.

Interpretation and conclusion: The non fermenting gram negative bacilli infection is mainly seen in patients with serious underlying risk factors like prolonged stay in hospital, catheterization, underlying diseases like diabetes, malignancies and chronic pulmonary disease. Ciprofloxacin, Ofloxacin, Amikacin, Imipenem appeared to be effective drugs in treating non fermenting gram negative bacilli infections.

Keywords: Non fermenting gram negative bacilli (NFGNB), antibiogram, Ps. aeruginosa, Ac. baumanii, ciprofloxacin, Ofloxacin, Imipenem.

Introduction:

Non fermenting gram negative bacilli (NFGNB) are heterogenous group of aerobic, non-sporing bacteria, which do not utilize glucose as source of energy or utilize it oxidatively. They comprise about 1/5th of all gram negative bacilli (GNB)[1,2].

Non-fermenters (NF) are emerging with increasing frequency as agents of opportunistic and often serious infection as well as nosocomial infection[2]. They are most commonly isolated from patients with serious underlying disease who had abusive use of wide spectrum antimicrobials agents, prolonged surgical procedures, prolonged hospital stay, inadequate mechanical instrumentation or tracheostomy, genitourinary instrumentation, in burns patients, low birth weight babies. Their infections are observed in extreme age groups like neonates, young children and geriatric age[3]. They are frequently isolated from cases such as septicemia, meningitis, pneumonia, urinary tract infection and surgical wound infection[4,5].

Among the species that are opportunist pathogens in immunologically compromised host either by disease or treatment Pseudomonas aeruginosa (P. aeruginosa) is eminent followed by Acinetobacter baumanii (Ac. baumanii), Ps. fluorescence, Ps.stutzeri , Stenotrophomonas maltophilia, Ps.putida, Ps.cepacia[5].

Antimicrobial treatment of the infection caused by these agents is difficult due to its multi drug resistance (MDR)and rapid selection of high level MDR to various groups of antibiotics like Beta-lactam , Aminoglycosides and fluroquinolones posing problem for both treatment and infection control[6].

The isolation rate of NFGNB was increasing in our lab, hence this study was undertaken to identify, speciate and study the sensitivity pattern of NFGNB and also to know the clinical significance of these infected organisms.

Meropenem.
Ps. *fluorescens* showed a sensitivity of 5(83.3%) to *Imipenem* and 4(66.66%)sensitivity were seen with *Meropenem*.
Ac. *baumanii* showed a sensitivity of 9 (75%) to *imipenem* and 5 (41.66%)sensitivity were seen with *Meropenem*.

Discussion:
During the study period from January 2009 to December 2009 at Basaveshwar Teaching and General Hospital, Gulbarga, 150 specimens from various clinical conditions like local infection, post operative infection, post traumatic infection, respiratory tract infections, urinary tract infection, septicaemia, gastrointestinal tract infection and genital tract infection were collected and subjected for further processing. NFGNB were isolated from 64 samples which included infections caused by Ps.*aeruginosa*, Ps.fluorescens, Ac.baumanii were isolated similar to other studies by *Yashodhara P* et al[7].

There was a preponderance of the infection in Males in our study. Similar observation was made in other studies by Rajan R et al[8] and Wisplinghoff H et al[9].

The mean duration of stay in hospital was 25.3 days in our study.
In our study 70% were from ICU where as in Algur et al study it was 47.2%[10]. Other patients were from surgery wards orthopaedics wards, ENT wards, OBG wards Medical wards and paediatric wards.

In our study NFGNB's were most commonly isolated from pus sample. This is similar to earlier studies done by Mishra E et al[11] and Yashodhara P et al[7].

Ps.aeruginosa, Ac.baumanii were the most common isolates from local infection like cellulitis, diabetic foot, ear discharge and burns in our study which was similar to other studies by Rajan R et al[8].

Ps.aeruginosa was the main etiological agent responsible for 52.7% local infections in our study. However it was higher in studies by Yashodhara P et al[7] 66.95% , in Mishra et al study 66%, in Resmi Rajan et al 89.9% and in Cristine et al study 72.5%[12,13]. The differences in the percentages of various parameters may be due to the variation in the sample size.

In our study Pseudomonas aeruginosa caused 58% of Post operative wound infection and Pseudomonas fluorescens caused 23.57% . In a study by Resmi Rajan et al Pseudomonas aeruginosa caused 34.09% of post operative wound infection[8]. In a study by Yashodhara et al Pseudomonas fluorescens caused 5.8% of post operative infection[7].

Infections related to abdomen included peritonitis cases in our study Ac .baumanii was the most common NFGNB isolate. In our study patients who had been catheterised for >72 hours, urinary tract infection was common with Pseudomonas aeruginosa and Acinetobacter baumanii. Not much inference could be obtained as the number isolated was very small 2%. Ps.aeruginosa and Ac. baumanii both are known to cause recurrent and chronic urinary tract infection and often multi drug resistant. The most common organism causing Respiratory tract infection was Ps.aeruginosa (20.45%) followed by Ac.baumaanii (17.8%). In study by Mark et al Ps.aeruginosa isolated was 6%(12). Most patients had underlying pathology like COPD, Tuberculosis, Pneumonic consolidation and those who were exposed to repeated nebulisation.

NFGNB displays a wide and variable spectrum of antibiotic sensitivity. There is no antibiotic to which all strains are susceptible[15].

NFGNB are uniformly resistant to Penicillin group of drugs[12]. NFGNB showed sensitivity of 28.12% to Piperacillin in our study and the sensitivity ranged from 70% to 85% in other studies by Rajan R et al[8], Prakash K S et al[14] Troillet N et al [15]. Piperacillin+Tazobactam is a preferred drug for treating NFGNB infections and showed a sensitivity of only 43.51% in our study.

Ps.aeruginosa showed a resistance of 56.02 % to Piperacillin+Tazobactam in our study as compared to 2% resistance in a study by Prakash K S et al [14]. The low sensitivity in our study could be due to excessive use of Piperacillin+Tazobactam combination in our hospital. Netilmicin resistance in Ps.aeruginosa in our study was 86.96%. In other studies by Taneja N et al[16] and Prakash K S et al it ranged from 60 - 88%[14]. Ac .baumanii showed a sensitivity of 25 % to Amoxyclav in our study. In studies conducted by Jawad et al it showed a sensitivity of 57% and in Wong fu et al 25%[14,17].

Ac.baumanii showed a sensitivity of 25% to Piperacillin in our study. In studies conducted by Wong fu et al it showed a sensitivity of 20% and in a study by Taneja et al it showed 40% sensitivity[17,6]. Ac. baumanii showed a resistance of 58.4 % to Piperacillin+Tazobactam in our study, similar to study by Jawad et al[16].

Table No.1: Various specimens included in the study

Si no	Sample	No of cases	Percentage
1	Pus	68	45.33
2	Sputum	21	14
3	Ascitic fluid	5	3.33
4	Blood	7	4.66
5	Urine	23	15.33
6	Stool	11	7.33
7	Cervical Discharge	4	2.66
8	Pleural Fluid	6	4
9	CSF Fluid	5	3.33

Table No.2: Various antibiotics used with their concentration

Piperacillin(Pc)-100mcg	Ciprofloxacin(Cf)-5mcg
Ticarcillin(Ti)-75mcg	Ofloxacin(Of)-5mcg
Carbenicillin(Cb)-100mcg	Gentamycin(G)-10mcg
Cefaperazone sulbactam(Cs)-75mcg	Amikacin(AK)-30mcg
Cefepime(Cpm)-30mcg	Netilimicin(Nt)-30mcg
Cefotaxime(Ce)-30mcg	Meropenem (M)-10mcg
Ceftriaxone(Ci)-30mcg	Imepenem(I)-10mcg
Ceftazidime(Ca)-30mcg	

Table No.3: Bacterial species isolated under each clinical infections

Species	Local infection	RTI	UTI	GIT	Post op infection	Post traumatic infection	Septicemia	Total
Ps.aeruginosa	15	9	3	4	5	4	2	46
Ps.fluorescens	-	2	-	-	4	-	-	6
Acinetobacter	4	2	1	2	1	1	1	12
Mixed Group	13	14	25	19	14	15	4	84

NFGNBs showed resistance of 46.88 % to Ceftazidime, 52.57 % to Cefaperazone, 57.82 % to Cefepime which are commonly used by the clinicians in our hospital.

Ps. aeruginosa showed a sensitivity of 43 % in our study to third generation Cephalosporins. In a study by Krishna Prakash et al it was 67% and in study by Resmi Rajan et al study it was 98.2%[9,13]. NFGNBs showed 43.75 % resistance to Ofloxacin and 37.5% to Ciprofloxacin in our study.

Ps.aeruginosa showed 28.66% resistance to Ciprofloxacin in our study. In various other studies by Taneja et al, Algu ?i et al, Krishna Prakash et al, Troillet N et al, Smitha S et al, Wong fu et al it ranged from 12.5% to 83%[10,14,15,17,18].

NFGNBs showed a good sensitivity to Amikacin 70.31% in our study which is similar to other studies by Prakash K S et al, Wong Fu et al and Taneja et al [6,14,17]. Gentamicin showed a sensitivity of 58.7 % in our study. In a study by Taneja et al it showed 61.2% and 73.5% in Resmi Rajan et al[6,9].

However Ps.fluorescens showed least sensitivity of 33.3 % to Gentamicin which was comparable to study by Yashodhara et al 25%[7]. NFGNB's showed an overall 15.66 % resistance to Imipenem in our study. In a study by Taneja et al it showed 36%[06]. Pseudomonas aeruginosa showed 13.04% resistance to Imipenem in our study, in other studies done by Taneja et al, Rajan R et al, Gupta E et al, Troillet N et al, Smitha S et al, Wong fu et al showed a range of 11.8% - 81.5%[6,8,9,13,15,16,17].

NFGNB's showed a resistance of 40.6 % to Meropenem which was higher, compared to Imipenem in our study. It is known that Meropenem develops resistance earlier than Imipenem. In study by Gupta E et al resistance to Meropenem was 22.16 %[09]. Ps.aeruginosa showed 36.96% resistance to Meropenem and it ranged from 4.2 % to37.3 % in different studies. Carbapenem resistance of

Table No.4: Antibiotic sensitivity patern of NFGNB isolated from various specimen

-Antibiotics	Ps aeruginosa (n=46)		Ps fluorescence (n=6)		Ac baumanii (n=12)	
	S	R	S	R	S	R
Carbenicillin	31 (67.3%)	15 (32.6%)	04 (66.6%)	02 (33.3%)	03 (25%)	09 (75%)
Ticarcillin	12 (26.1%)	34 (73.9%)	01 (16.6%)	05 (83.3%)	02 (16.6%)	10 (83.3%)
Piperacillin	14 (30.4%)	32 (69.6%)	01 (16.6%)	05 (83.3%)	03 (25%)	09 (75%)
Piperacillin+ Tazobactam	20 (43.5%)	26 (56.5%)	04 (66.6%)	02 (33.3%)	05 (41.6%)	07 (58.3%)
Netilmicin	06 (13.1%)	40 (86.9%)	01 (16.6%)	05 (83.3%)	02 (16.6%)	10 (83.3%)
Cefotaxime	13 (28.3%)	33 (71.7%)	00 (0%)	06 (100%)	02 (16.6%)	10 (83.3%)
Ceftriaxone	19 (41.3%)	27 (58.7%)	02 (33.3%)	04 (66.6%)	06 (50%)	06 (50%)
Cefaperazone + Sulbactam	24 (52.2%)	22 (47.8%)	03 (50%)	03 (50%)	04 (33.3%)	08 (66.6%)
Ceftazidime	28 (60.8%)	18 (39.2%)	02 (33.3%)	04 (66.6%)	06 (50%)	06 (50%)
Cefepime	22 (47.8%)	24 (52.2%)	02 (33.3%)	04 (66.6%)	04 (33.3%)	08 (66.6%)
Amikacin	35 (76.1%)	11 (23.9%)	03 (50%)	03 (50%)	07 (58.3%)	05 (41.6%)
Gentamicin	27 (58.7%)	19 (41.3%)	02 (33.3%)	04 (66.6%)	07 (58.3%)	05 (41.6%)
Ciprofloxacin	33 (71.7%)	13 (28.3%)	02 (33.3%)	04 (66.6%)	05 (41.6%)	07 (58.3%)
Ofloxacin	29 (63%)	17 (37%)	03 (50%)	03 (50%)	04 (33.3%)	08 (66.6%)
Imipenem	40 (86.9%)	06 (13.1%)	05 (83.3%)	01 (16.6%)	09 (75%)	03 (25%)
Meropenem	29 (63.1%)	17 (36.9%)	04 (66.6%)	02 (33.3%)	03 (25%)	09 (75%)

Ac. baumanii was similar to Ps. aeruginosa.

Conclusion:

Large number of NF isolated from different patients has an etiological role to play in infections and is reflected by the fact that, in repeated cultures same organisms were re-isolated. Most of the patients had high risk factors like prolonged stay in hospital especially in ICUs, catheterisation (both urinary and intravenous), diabetes, burns and malignancy. The most common isolates were Ps. aeruginosa 30.66% followed by Ac. baumanii 8 % Ps. fluorescens 4%. Most common clinical conditions were ulcers, post operative wounds, COPD, peritonitis and burns cases.

The most effective antibiotics are Amikacin, Imipenem, Ciprofloxacin, Ofloxacin, and Carbenicillin. Most of the NFs isolated were resistant to Penicillin group of drugs. Repeated exposure of organisms to antimicrobial agents is thought to enhance the development and maintenance of resistance. Also presence of antimicrobial agent in sub lethal concentration makes an environment suitable for development of resistance.

Organisms are resistant to drugs commonly employed in therapy emphasizes that NFs need to be taken more seriously and should not be discarded as mere contaminants or non pathogens. Identification of these organisms can throw more light on their prevalence and pathogenic role.

The sensitivity pattern changes from hospital to hospital and population to population. Treating NFGNB systemic infection is usually by broad spectrum intensive treatment

antimicrobial, regular antimicrobial susceptibility surveillance and strict infection control measures are required to contain this emerging antibiotic resistance among NFGNBs.

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