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## **PHENOTYPIC CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF NON-FERMENTATIVE GRAM NEGATIVE BACILLI FROM CLINICAL SAMPLES**

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### **ABSTRACT**

Non fermenting gram negative bacteria were considered to be nonpathogenic and commensal of little significance. Recently there has been a tremendous interest in these organisms as they are being isolated from clinical specimens with increasing frequency. So 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 tertiary care hospital at Bangalore.

This study comprises of 148-isolates of Non Fermenting Gram Negative Bacilli from total of 2540 clinical specimens, collected from in- patients admitted to various departments during a period of 1 year. All the isolates were phenotypically characterised. In the present study, most common isolates were *Ps.aeruginosa* 43.9% followed by *Ps.fluoroscence* 16.2% & *Ac. baumannii* 9.4 %. Most common clinical conditions were local pyogenic infections, post- operative wounds, post traumatic wounds, lower respiratory tract infections & chronic suppurative otitis media. The most effective antibiotics were Imipenem, Amikacin, Aztreonam, Ticarcillin-clavulanic acid and levofloxacin in our study. Most of the NFs isolated were resistant to Penicillin group of drugs. 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. The most effective antibiotics were Imipenem, Amikacin, Aztreonam, Ticarcillin-clavulanic acid and levofloxacin in our study early diagnosis and institution of empirical therapy based on recent Antibiogram of the institute would reduce mortality and improve patient management.

**Key Words:** *Pseudomonas, Acinetobacter, Alkaligenes, Burkholderia cepacia, Antibiogram*

### **INTRODUCTION**

Non fermentative gram negative bacilli (NFGNB), although frequently considered to be commensals or contaminants, but the pathogenic potential of these organisms has been established beyond doubt because of their frequent isolation from clinical specimens and their association with the disease (Koneman *et al.*, 2006). These apparently heterogeneous microorganisms have common traits of clinical importance that justify their inclusion and study in a single group. They can be recovered from hospital environment, device related infections, are often resistant to disinfectants and have the potential to spread from patient to patient via fomites or the hands of medical personnel (Joanna S Brooke, 2012). Most of the non-fermenters cause nosocomial blood stream infections particularly in debilitated and immunocompromised hosts and are usually multidrug resistant (Mc growan *et al.*, 2006). Serious infections due to this group of organisms are currently being reported with increasing frequency and make a significant contribution to in-hospital mortality (Vidal *et al.*, 2003). Non Fermenting Gram Negative Bacilli (NFGNB) is innately resistant to many antibiotics and is known to produce extended spectrum Beta lactamases and metallo Betalactamases (Bohera, 2008). Increase in concern to treat these infections due to NFGNBs as they possesses intrinsic mechanisms of resistance to various groups of drugs, especially to carbapenems, which are widely used in clinical practice in health care settings (Gibb, 2002). The present study was undertaken to isolate, identify, characterize and to find out the association of NFGNB with clinical condition of the patient. Antimicrobial resistance pattern of the isolates was also studied.

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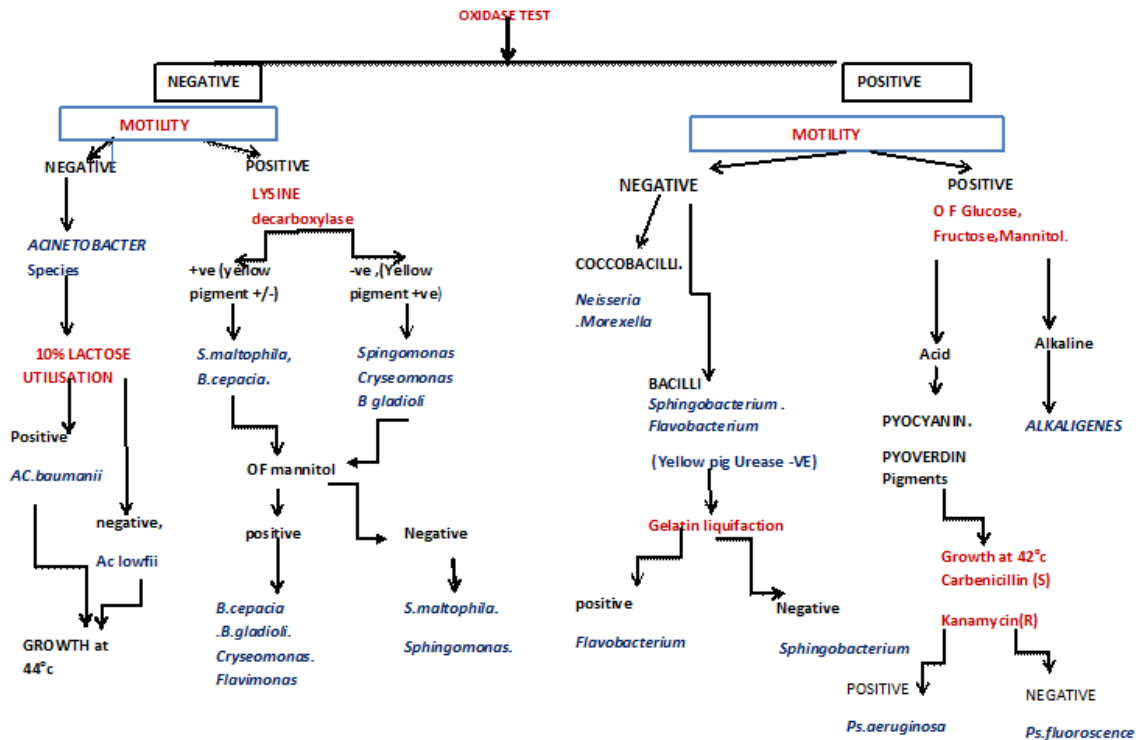
**MATERIALS AND METHODS**

This study comprises of 148-isolates of Non Fermenting Gram Negative Bacilli from total of 2540 clinical specimens, collected from in- patients admitted to various departments during a period of 1 year. The study was conducted as follows: Gram stain, Culture on Blood Agar & Mac Conkey Agar (Incubate aerobically at 37<sup>0</sup>c for 24 hrs) Isolates are inoculated on triple sugar iron agar (TSI) medium.

All organisms showing growth, alkaline slant no change in the butt in TSI are subjected to various biochemical tests, such as Gram stain, Oxidase test, Motility, Pigment production, Indole test, Urease test, Citrate test, Nitrate reduction test, Esculin hydrolysis, Fermentation of 10% lactose, Decarboxylation of Arginine(Ar),Ornithine(Or),Lysine(Lys). Oxidative fermentation (OF) of (Hugh-Leifson)-Glucose (G) Sucrose(S), Mannitol(Man), Maltose(Mal), Xylose(xyl), Lactose(L) and Gelatin liquefaction.

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-2012) guidelines (Gupta et al., 2006). *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 was used as a control strain.

Piperacillin(Pc)-100mcg, Imipenem(I)-10mcg, Levofloxacin(5mcg), Ticarcillin(Ti)-75mcg Meropenem(10mcg), Norfloxacin(Nx)-10mcg, Carbenicillin(Cb)-100mcg, Colistin(10mcg) Tetracyclin(T)-30mcg, Ticarcillin-clavulanic acid(75+10mcg), Polymyxin(Pb)-(300Iu) Cotrimoxazole(Co)-1.25mcg, Piperacillin-tazobactam(100+10mcg), Gentamycin(G)-10mcg Ceftazidime(Ca)-30 mcg, Amikacin(AK)-30mcg, Cefepime(Cpm)-30mcg,Netilmicin(Nt)-30mcg, Aztreonam(30mcg), Kanamycin(K)-30mcg are the drugs chosen for the anti-microbial testing.



**Chart 1: Scheme used for identification of non-fermenting gram negative bacilli**

**RESULTS**

In the present study 148 Non fermenters were isolated from 2540 clinical specimens of local infections, Septicemia, Respiratory tract infections, Urinary tract infections, Ear infections, local infections

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(cellulitis, diabetic foot, burns,) post-operative infections, post traumatic infection, stool satisfying both the inclusion and exclusion criteria, which were submitted to microbiology laboratory.

The isolation rate of NFGNB in this study was 5.8%.

There was a preponderance of the infection in Males in our study

Pus sample constituted majority of specimens accounting for 103(69.6%), followed by 26(17.56%) sputum samples

**Table 1: Bacterial species isolated under each clinical infections**

	septicemia	Respiratory tract infection	Local infections	Post OP infections	Uninary tract infections	CSOM	Post-traumatic infections	Gastro intestinal infections	Total
Ps.aeruginosa	6	10	29	10	2	5	2	1	65
Ps.fluorescence	-	3	11	7	-	1	2	-	24
Ac.baumannii	2	3	3	-	-	6	-	-	14
Ac.lwoffii	-	-	3	-	1	-	-	-	4
B.cepacia	-	1	1	-	-	-	1	-	3
Alkaligenes groups	-	-	-	-	1	-	-	-	1
Mixed isolates	3	12	7	6	-	3	6	-	37
Total	11	29	54	23	4	15	11	1	148

*Pseudomonas aeruginosa* was the most common isolate among all pathogen in all specimens *Pseudomonas fluorescence* was most isolated in local infections 11, followed by 7 cases of post-operative infections.

*Acinetobacter baumannii* was isolated from 6 cases of Chronic Suppurative Otitis Media (CSOM), 3 from local infections, and 2 each from blood & sputum.

*Acinetobacter lowfii* was isolated from 3 cases of local infection and 1 from urine.

3 cases of *Burkholderia cepacia* was isolated 1 each from respiratory, local infection & post traumatic infection.

Only one isolate of *Alkaligenes* in urine was isolated. Mixed growth (*proteus vulgaris*, *proteus mirabilis*, *MRSA*, *E coli*, *Enterococci* and *Klebsiella*) were mostly seen pyogenic infection (local infection, traumatic infection, post op infection & pus from CSOM). 10 cases were from respiratory infections, 3 from septicemia cases & 2 from endotracheal tube.

All the NFGNB together showed 98(66.2%) sensitivity to Ticarcillin +clavulanic acid, followed by 88(59.5%) sensitivity to piperacillin-tazobactam, and showed least sensitivity to piperacillin 33(22.3%).

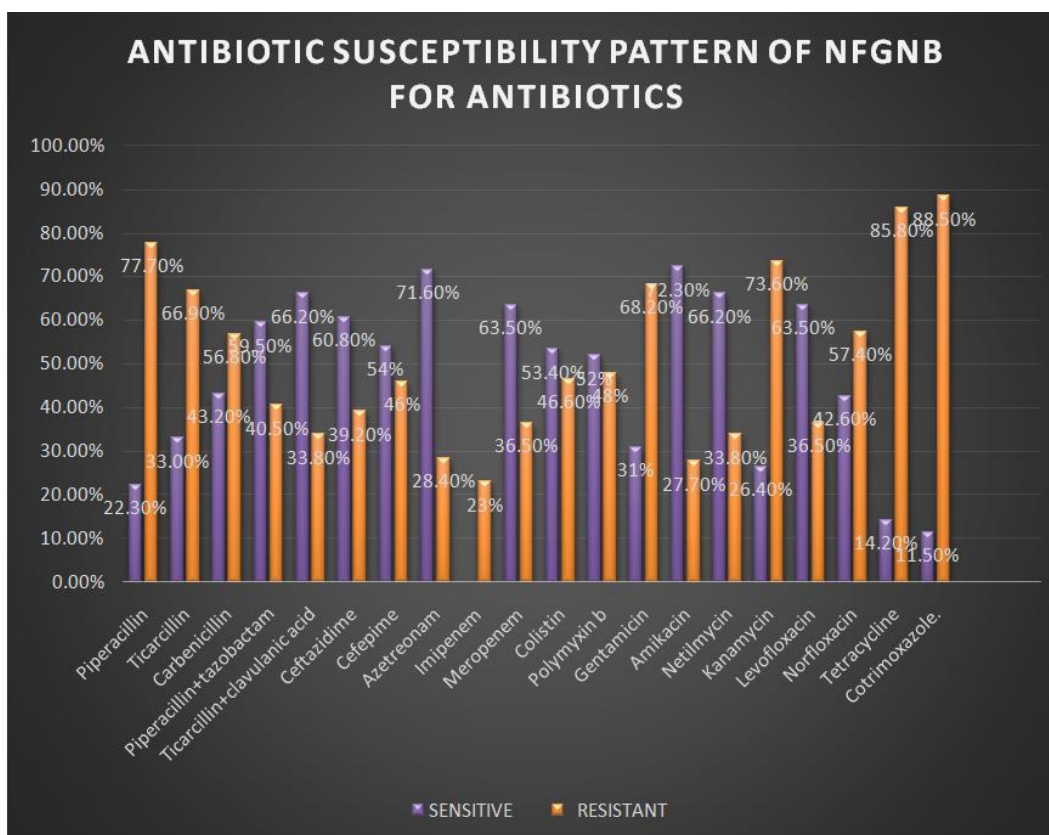
*Ps.aeruginosa* showed 73.8% sensitivity to Ticarcillin+clavulanic acid, 100% sensitivity to Carbenicillin & 100% resistance to kanamycin.

Both *Ac lowfii* & *B.cepacia* showed 75 to 100% resistance to penicillin groups.

*Ps fluorescence* showed 100% sensitivity to kanamycin, 54.2% sensitivity to piperacillin –tazobactam & 62.2 % to Ticarcillin-clavulanic acid. *Alkaligenes* showed resistance to Piperacillin, Ticarcillin, &

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Carbenicillin *Ps.aeruginosa* showed sensitivity of 42(64.6%) to ceftazidime, 36(55.4%) to cefepime, & 49(75.4%) to Aztreonam. *Acinetobacter species (baumanii & lowfii)* showed 66.6% to sensitivity ceftazidime, 55.55% to cefepime, and 72.2% to Aztreonam. *B. cepacia* shows 100% sensitive to ceftazidime, 66.7% to both cefepime & Aztreonam. Alkaligenes showed resistance Aztreonam all the NFGNB together showed 77% sensitivity to Imipenem, 63.5% sensitivity to Meropenem, 53.4% sensitivity to colistin, & sensitivity 52% polymyxin B. *Ps. aeruginosa* showed 78.5% sensitivity to Imipenem, followed by 64.6% sensitivity to Meropenem, colistin & polymyxin B showed 47.7% & 58.5% sensitivity respectively. *Ps fluorescence* showed 79.2% sensitivity to Imipenem, 62.5% to Meropenem, 58.3% to colistin and 41.7% sensitivity to Polymyxin B. *Acinetobacter spp (baumani & lowfii)* showed 77.8% sensitivity to Imipenem, 83.3% to Meropenem. 66.7% to Colistin, 14.5% sensitivity to Polymyxin B. *B cepacia* showed 66.7% sensitivity to Imipenem & Meropenem .100% resistance to colistin& 66.7% resistance to Polymyxin B. *Alkaligenes* showed 100% sensitivity to Imipenem & Meropenem & 100% resistance to colistin & Polymyxin. All the NFGNB together showed 47(31.8%) sensitivity to gentamicin, 107(72.3%) sensitivity to Amikacin, 98(66.2%) sensitivity to Netilmycin & only 39(26.4%) were sensitivity to Kanamycin



**Table 2: Antibiotic susceptibility pattern of nfgnb for antibiotics**

*Ps.aeruginosa* showed 35.4% (23) sensitivity to Gentamicin, 81.5% (53) to Amikacin, 70.8% (46) to Netilmycin & showed 100% resistance to Kanamycin. *Ps.fluorescence* showed only 33% (8) sensitivity to Gentamicin, 83.3% (20) to Amikacin, 70.8% (17) to Netilmycin & showed 100% sensitivity to Kanamycin.

*Acinetobacter species* showed 27.8% (5) sensitivity to both Gentamicin & Amikacin, 55.5% (10) sensitivity to Netilmycin, & showed 100% resistance to Kanamycin. *B cepacia* showed 100% resistance to

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Aminoglycosides. All the NFGNB together showed 63.5% sensitivity to levofloxacin, 42.6% sensitivity to Norfloxacin, & Tetracycline, Cotrimoxazole showed least sensitivity. *Ps.aeruginosa* showed 37(57%) sensitivity to levofloxacin, 19(29.3%) to Norfloxacin, 3(4%) to Tetracycline, and 4(6%) sensitivity to Cotrimoxazole. *Ps.fluorescence* showed 16(66.6%) sensitivity to Levofloxacin, 8(20.8%) to Norfloxacin, 1(4%) to Cotrimoxazole & 100% resistance to Tetracycline. *Acinetobacter spp* showed 16(89%) sensitivity to Levofloxacin, 7(29%) to Norfloxacin, 11(45.8%) to Cotrimoxazole & 11(45.8%) sensitivity to Tetracycline. *B.cepacia* showed 100% resistance to fluoroquinolones, 2(66.7%) sensitivity to both Tetracycline& Cotrimoxazole. *Alkaligenes spp* showed 100% sensitivity to both the fluoroquinolones, & 100% resistance both Tetracycline& Cotrimoxazole.

## DISCUSSION

Aerobic Non Fermenting Gram Negative Bacilli (NFGNB) usually considered as contaminants are emerging as important nosocomial pathogens.

During the study period 148 isolates of NFGNB out of 2540 clinical samples from various clinical conditions like septicaemia, local infection, post-operative infection, post trauma infections, respiratory tract infections (RTI), chronic suppurative otitis media (CSOM) were taken for the study. Isolation rate of non-fermenting gram negative bacilli in our hospital was 5.8%. Whereas it was 4.5% in study by Malini et al., *Pseudomonas species*, *Acinetobacter species*, *Burkholderia species*, *Alkaligenes* & Mixed growth organisms with *pseudomonas spp* were isolated during the study. There was a preponderance of the infection in Males in our study. The mean duration of stay in hospital was 8.62 days in our study.

In our study 20.3% were from ICU where as in Aljunied et al., (2004) study it was 47.2%. Other patients were from surgery wards, orthopedics wards, ENT wards, OBG wards Medical wards and pediatrics ward. In our study NFGNB's were most commonly isolated from pus sample. This is similar to earlier studies done by Meharwal (2002) that *Pseudomonas species* (45.5%) were the commonest followed by *Acinetobacter species* (39%).

*Ps.aeruginosa*, *Ac.baumannii* was the most common isolates from local infection like cellulitis, diabetic foot, ear discharge and burns. *Ps.aeruginosa* was the main etiological agent responsible for 46(70.7%) pyogenic infections in our study, in Resmi Rajan et al., (2001) 89.9%. The differences in the percentages of various parameters may be due to the variation in the sample size.

In our study *Pseudomonas aeruginosa* caused 15.4% and *Pseudomonas fluorescence* caused 29.2% of Post-operative wound infections. In a study by Resmi Rajan et al., (2001) *Pseudomonas aeruginosa* caused 34.09% of post-operative wound infection.

In our study only 2(1.3%) of *Pseudomonas aeruginosa* was isolated from urine of a patient who had been catheterized for >72 hours, One isolate was *Alkaligenes* from patient who was on long term care facility, another urine isolate was *Acinetobacter lowfii* from a patient also catheterized for more than 15 days. The non-fermenters are emerging as important cause of Urinary Tract Infections (Shoba et al., 2011; Joanna S Brooke, 2012). Non fermenters are known to cause chronic recurrent UTI & are often multi drug resistant.

Most common organism causing RTI was *Pseudomonas aeruginosa* 6.7%(10) followed by *Pseudomonas fluorescence* 0.2%(3) & *Ac.baumannii* 0.1%(2) from patients having underlying pathology like COPD, Tuberculosis, pneumonic consolidation & who were exposed to repeated nebulization.

*Ac.baumannii* was isolated from 14 clinical samples, 6 isolates were from case of CSOM, 3 from local infection like cellulitis & 2 from burn cases, 2 isolates were from patient with pneumonia with risk factor of antibiotic exposure & care in ICU, 1 isolate was from Endotracheal tube secretions.

*Burkholderia cepacia* was isolated from one patient having COPD with diabetes. One more was from local infection of a patient suffering from chronic diabetic ulcer and another one from elderly patient with fracture of femur.

Most common organisms associated with NFGNB were *E.coli*, *Proteus species*, *MSSA*, *MRSA*, *CONS* and *Citrobacter spp*.

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Piperacillin+tazobactam showed 59.5% sensitivity & resistance was 40.54%. Ticarcillin-clavulanic acid showed 69.6% sensitivity, and it was preferred drug for treating NFGNB infections in our study.

*Ac baumannii* showed only 14.3% sensitivity to Piperacillin in our study. In study conducted by Taneja et al., (2003) it showed 40% sensitivity.

*Ac baumannii* showed resistance of 70.8% to Piperacillin-Tazobactam in our study which was more than the study by Jawad et al., (1998).

*Burkholderia cepacia*, out of three isolates, all the three showed sensitivity to Ceftazidime & 66.7% sensitivity to cefepime which was similar to study by Gautam (2011), Koneman et al., (2006).

NFGNB isolates showed 39.2% resistance to Ceftazidime, & 46% to Cefepime. In our study *Pseudomonas aeruginosa* showed sensitivity of 64.6% for Ceftazidime, 55.4% to cefepime. In study by Behera B et al., (2008) it was 67% sensitivity to ceftazidime.

NFGNB isolates showed 77% sensitive to Imipenem & 63.5% to Meropenem. *Pseudomonas aeruginosa* showed 21 % resistance to Imipenem in our study however in the last decade there have been increasing reports of resistance of resistance to this life saving antimicrobial in *Pseudomonas aeruginosa* (Gupta et al., 2006).

NFGNB's showed a resistance of 63.5% 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 et al., (2006) Balaji (2011) resistance to Meropenem was 22.16 %. Colistin & Polymyxin-B showed 53.4% & 52% sensitivity respectively. The CLSI recommends discs containing 10µg colistin or 300µg polymyxin B to be used on Mueller-Hinton agar with a 0.5 McFarland inoculum and incubated for 16 - 18 hours. Interpretive values are provided only for *P. aeruginosa*. Almost all studies evaluating the efficacy of the disc diffusion test for polymyxin have consistently reported it to be unreliable for use. The reason attributed to the poor performance of the disc diffusion test is that, polymyxin has large molecules and diffuses inadequately into the medium to produce inconsistent zones of inhibition (Balaji, 2011; Melamed et al., 2003).

Amikacin is showing higher sensitivity (72.3%) among aminoglycosides followed by Netilmycin (66.2%). Higher rate of survival was noticed among patients receiving aminoglycosides and anti-pseudomonal drugs which are similar to other studies by Klaus-Dieter Lessnau (2013).

Co-trimoxazole resistance was 88.5% in our study. In Table 8 we can see that NFGNB showed resistance of 57.4% to Norfloxacin & 36.5% to levofloxacin in our study, whereas in study by Quinn (1998) sensitivity to ciprofloxacin was 89%. So we can say that there is modest increase in resistance to fluoroquinolones.

### Conclusion

NFGNBs are primarily opportunistic pathogens causing infection in seriously ill hospitalised patients, immunocompromised patients. For each of the NFGNB underlying host factors are strongly associated with outcome.

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, catheterization (both urinary and intravenous), diabetes, burns and malignancy. The most common isolates were *Ps.aeruginosa* 43.9% followed by *Ps.fluorescence* 16.2% & *Ac.baumannii* 9.4 %. Most common clinical conditions were local pyogenic infections, post-operative wounds, post traumatic wounds, lower respiratory tract infections & chronic suppurative otitis media.

The most effective antibiotics were Imipenem, Amikacin, Aztreonam, Ticarcillin-clavulanic acid and levofloxacin in our study. Most of the NFs isolated were resistant to Penicillin group of drugs, Gentamicin, Kanamycin & Norfloxacin. 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.

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Organisms are resistant to drugs that are commonly employed in therapy, this 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 infections by broad spectrum intensive treatment and specific therapy is based on laboratory data after identifying the causative agent and antibiotic susceptibility results, Minimized use of available antimicrobials; regular antimicrobial susceptibility surveillance and strict infection control measures are required to contain this emerging antibiotic resistance among NFGNBs.

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