

Prevalence and antibiogram of pseudomonas aeruginosa and acinetobacter baumannii in the clinical samples from tertiary care hospital

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Abstract

Introduction: The present study was undertaken to determine the prevalence of non-fermenting gram negative bacilli and antibiogram of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Materials and Methods: Conventional bacteriological methods were used for identification non fermenting gram negative bacilli and susceptibility testing was performed with the help of the Kirby-Bauer disc diffusion method.

Results: A total 195 non fermenting gram negative bacilli were isolated during the study period. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates represent 89.23% of all isolated bacteria. Therefore, antibiotic resistance rates were performed specifically against these two bacteria. *P. aeruginosa* showed good sensitivity to imipenem(94.29%) followed by amikacin(86.67%) and piperacillin/tazobactam(68.57%). *A. baumannii* showed good sensitivity to imipenem(82.61%) followed by amikacin(76.81%).

Conclusion: *P. aeruginosa* and *A. baumannii* were the most common NFGNB isolated in our study. Both showed good susceptibility towards imipenem and amikacin. Alarming resistance rate was found towards commonly used antibiotics. Control measures which include the judicious use of antibiotics, antibiotic cycling, the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done, to prevent the spread of these strains.

Keywords: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Antibiogram

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Introduction

Taxonomically, they are diverse group of aerobic, nonsporing bacteria that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathway than fermenting or utilizing it oxidatively.¹ They have been isolated from soil, water and medical devices as well. NFGNB can exist as normal commensal.² Previous studies reported upto 15% NFGNB isolation rate from clinical specimens.³ Non-fermenting gram-negative bacilli (NFGNB) such as *Pseudomonas* spp. And *Acinetobacter* spp. are most frequently encountered pathogens in the health-care environment.^{4,5} Involvement of other species in causing human infections are very rare.⁶ *P.fluorescens*, *A.lwoffii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *Cryseomonas* species, *Sphingomonas* species, and *P.stutzeri*. Infections caused by *P.aeruginosa* ranges from trival to fatal.⁷ *Acinetobacter boumanii* is responsible for an increasing number of cases of blood stream infection, urinary tract infection, and healthcare and ventilator-associated pneumonia.⁸ Additionally, it is reported as a cause of outbreaks worldwide,

especially in personnel involved in military operations in Iraq and Afghanistan.⁹

In recent years, the problem is further compounded by the emergence of resistance to antimicrobial agents which are widely used against the non-fermenters especially *pseudomonas aeruginosa* and *Acinetobacter baumannii*, making them as an important healthcare associated pathogens. Understanding the spectrum and resistance patterns may guide effective empirical antibiotic therapy, decrease treatment failure and costs. Resistance pattern of microorganisms vary widely.¹⁰ There are only few studies from India that provide identification and antimicrobial susceptibility pattern of NFGNB.¹¹

Hence, there is a need to conduct region wise study on susceptibility patterns of various pathogens with which clinician can choose the correct empirical treatment. Therefore, the present study was undertaken to determine the prevalence of NFGNB and antibiogram of dominant pathogens.

Materials and Methods

This was a prospective, observational study conducted in a tertiary care teaching hospital over a period of ten months from March 2015 to December 2015. A total of 2259 clinical specimens were processed in the department of clinical microbiology. 195 clinical specimens yielded the growth of non-fermenting gram negative bacilli. All the clinical specimens were plated on blood agar and Mac Conkey's agar and incubated at 37°C for 48 hours before being reported as sterile. The isolates that

showed non lactose fermenting colonies on Mac Conkey agar 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.¹

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

Exclusion/inclusion criteria:

The clinical significance of the NFGNB was assessed by laboratory and clinical criteria. The laboratory criteria included, repeat isolation of same bacteria from the sample concerned, leukocytosis and relevant radiological evidence (respiratory samples). The clinical criteria included the presence of risk factors such as underlying diseases (chronic renal failure, malignancy, hepatitis, bronchiectasis, cystic fibrosis, pneumonia and other immunosuppressive conditions) and presence of intravenous or urinary catheters.

Antimicrobial susceptibility testing of the isolated organisms was performed by the disk diffusion method as recommended by clinical laboratory and standards institute (CLSI).¹²

Antimicrobial susceptibility of the isolates was determined against the following antibacterial agents, Co-trimoxazole (25 ug), ciprofloxacin (5 ug), amikacin (30 ug), ceftazidime (30 ug), ceftriaxone (30 ug), cefotaxime (30 ug), piperacillin(100ug), piperacillin/tazobactam (100 ug/10 ug), imipenem (10 ug), aztreonam(30ug)

Quality control strains

Escherichia coli ATCC 25922

Pseudomonas aeruginosa ATCC 27853

All the analysis was performed using simple percentage method.

Results

During the study period, 195 non fermenters were isolated out of 2259 clinical samples accounting for an isolation rate of 8.63%. Monomicrobial growth was seen in 119 (61.03%) specimens whereas 76 (38.97%) specimens showed polymicrobial growth where non fermenters were isolated with other organisms. Most commonly associated bacteria were E.coli and Klebsiella species. P. aeruginosa was the predominant isolate, 105 (53.85%) followed by Acinetobacter baumannii69 (35.38%). Other NFGNB isolated were Pseudomonas fluorescens13(6.67%), Acinetobacter lowffii 6(3.08%) Burkholderia cepacia 1 (0.51%) and Stenotrophomonas maltophilia1(0.51%). The spectrum and clinical sources of these isolates are shown in Table 1.

Table 1: Profile prevalence of NFGNB

Bacteria isolated	Pus	Respiratory specimens	Urine	Blood	Body fluids	Ocular specimens	Stool	Total
P.aeruginosa	54	27	7	7	4	6	-	105
A.baumannii	11	39	11	5	2	1	-	69
P.fluorescens	9	2	2	-	-	-	-	13
A.lwoffii	1	1	-	4	-	-	-	6
Burkholderia cepacia	-	1	-	-	-	-	-	1
Stenotrophomonas maltophilia,	-	-	-	-	-	-	1	1

Both Pseudomonas aeruginosa and Acinetobacter baumannii isolates represent 89.23% of all isolated bacteria. Therefore, antibiotic resistance rates were performed specifically against these two bacteria. Table 2

Table 2: Antibiotic sensitivity of P. aeruginosa and A.baumannii

Antibiotic	P.aeruginosa(n=105)	A.baumannii(n=69)
Imipenem	99(94.29%)	57(82.61%)
Amikacin	91(86.67%)	53(76.81%)
Ciprofloxacin	29((27.62%)	11(15.94%)
Cetazidime	48(45.71%)	9(13.04%)
Cefepime	57(54.29%)	11(15.94%)
Ceftriazone	52(49.52%)	13(18.84%)
Piperacillin	50(47.62%)	5(7.25%)
Piperacillin/Tazobactum	72(68.57%)	25(36.23%)
Co-Trimaxazole	27(25.71%)	7(16.14%)
Aztreonam	17(16.19%)	5(7.25%)

Discussion

Non fermenting Gram negative bacilli considered to be contaminants in the past have now emerged as important major pathogenic organisms. Previous studies reported varied isolation rates of NFGNB. In our study, isolation rate of NFGNB was 8.63% which is in agreement with the study conducted by Juyal, where the isolation rate was 9.32%.¹³ Our study is not in agreement with the studies conducted by Sidhu (45.9%)¹⁴ and Vijaya (21.80%)¹⁵ where very high isolation rate was reported. However, studies conducted by Malini and Bruno showed very low isolation rate of 4.5% and 2.18% respectively.^{16,17} In the present study, highest number of the NFGNB isolates were from pus sample, similar to the observations made by others.¹⁸ *Pseudomonas aeruginosa* was found to be commonest non fermenter in previous studies¹⁶ followed by *Acinetobacter baumannii* and this is in concordance to our finding.

In our study, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were isolated from ocular specimens. Three isolates of *Pseudomonas aeruginosa* were from corneal scrapings. In India, second most important cause of bacterial keratitis after gram positive cocci is *Pseudomonas aeruginosa*. Pili or fimbriae plays an important role in adhesion to the corneal surface.¹⁹ Remaining three isolates were from the eyes with acute dacryocystitis. *Acinetobacter baumannii* was isolated from endophthalmitis. As bacterial biofilm formation has been implicated in the pathogenesis of bacterial endophthalmitis.²⁰

Majority of *A. baumannii* (56.52%) were isolated from respiratory specimens such as endo tracheal tube, trans tracheal tube and sputum. This is comparable with the study conducted by Shanti and Shekar²¹ who reported 41.8% isolation rate of *A. baumannii* from respiratory specimens as dominant pathogen. *A. baumannii* have emerged as important pathogen in intensive care units (ICUs), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs in recent years.

P. aeruginosa and *A. baumannii* are resistant to various antimicrobials which are commonly being used to treat infections. Outer membrane impermeability, increased activity of multidrug efflux pumps, target site alterations, or enzymatic degradation could be the reason for antimicrobial resistance (e.g., aminoglycoside-modifying enzymes and β -lactamases). Resistance to noncarbapenem β -lactams in *P. aeruginosa*.²² and *A. baumannii*²³ is due to excessive production of cephalosporinases.²⁴

P. aeruginosa presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome. Even more

problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care.^{25,26}

P. aeruginosa isolates in our study were highly susceptible to imipenem (94%), followed by amikacin (86.67%) and piperacillin/tazobactam (68.57%). This contrasts with the study which was conducted by Gladstone,²⁷ where 42.8% of *P. aeruginosa* isolates were resistant to imipenem. *P. aeruginosa* showed a lower range of sensitivity against fluoroquinolone and this may be attributed to their widespread use particularly ciprofloxacin.

According to the study conducted by Karlowsky,²⁸ *P. aeruginosa* showed high degree of susceptibility to amikacin and piperacillin-tazobactam followed by cefepime, ceftazidime, imipenem, and meropenem.²⁸ The resistance patterns of *A. baumannii* towards various antimicrobial agents were determined. In the present study, *A. baumannii* exhibited the highest susceptibility to imipenem (82.61%) followed by amikacin (76.81%). Less susceptibility was exhibited to piperacillin/tazobactam (36.23%) compared to *P. aeruginosa* (68.57%). However, in other studies susceptibility rate of *A. baumannii* to imipenem, piperacillin-tazobactam and amikacin was 50.7%, 42.1% and 38.2%, respectively. The lower resistance rate of *A. baumannii* to imipenem in our study may be due to its recent introduction. The important risk factors for the acquisition of imipenem-resistant *A. baumannii* include previous carbapenem use, longer duration of hospital stay until infection, ICU stay, urgent surgery, total parenteral nutrition, having a central venous catheter, endotracheal tube and urinary catheter or nasogastric tube.²⁹

In conclusion, *P. aeruginosa* and *A. baumannii* were the most common NFGNB isolated in our study. High frequency of drug resistance to commonly used antibiotics was observed. Imipenem and amikacin were found to be the most effective antibiotics against. Knowledge on the antibiotic susceptibility pattern of NFGNB from clinical specimens is crucial for planning the appropriate treatment.

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