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Non-Fermenting Gram Negative Bacteria as Uropathogens in Causing Urinary Tract Infection and its Antimicrobial Susceptibility Pattern at A Tertiary Care Centre of South India

Anupam Berwal[1], K.L. Shobha[1]*, Rohit Gupta[1], Kriti Gupta[2], Asem Ali Ashraf[1], Abishek Kumar B[1], Raghav Chawla[1], Prerna Aggarwal[3], Bhawna Sharma[3] and Sonali[4]

[1]Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy Of Higher Education, Manipal, Karnataka, India.
[2]Dr. Niraj Kumar Gupta MicroPath Lab, Bijnor (U.P.) India.
[3]Department of Microbiology, Kalpana Chawla Govt. Medical College, Karnal, India.
[4]Department of Pathology, Kalpana Chawla Govt. Medical College, Karnal, India.

Abstract

Non fermenting gram-negative bacilli (NFGNB) are recently striving as uropathogens. The present study was conducted to isolate the common species of bacteria in NFGNB causing urinary tract infection (UTI) and its correlation with comorbid conditions and to study the antibacterial susceptibility pattern. This retrospective study was done at the diagnostic Microbiology laboratory of a tertiary care hospital. Urine samples were collected for the period of six months. These samples were plated on blood agar and MacConkey agar and incubated at 37°C for 18–24 hr under aerobic conditions. Identification of NFGNB was done by Gram staining and MALDI-TOF (Matrix- Assisted Laser Desorption/ Ionization-Time of Flight, Biomerieux- Diagnostics). Antibiotic sensitivity testing was done by Vitek® 2 system (Biomerieux- Diagnostics) using N 281 card. Data was analyzed using SPSS IBM version 16. Out of the total 16,413 non repetitive urine samples that were received in the laboratory, 318 had significant bacteriuria. NFGNB were identified in 108 (33.9%) of all the urine samples with significant bacteriuria. Prevalence of non-fermenters in our study was 0.6%. NFGNB were more frequently isolated in the females and also in the age group of more than 50 years. Eighty five (78.70%) had comorbid conditions. *P. aeruginosa* and *A. baumannii* were the most common organism isolated among NFGNB. *Pseudomonas aeruginosa* isolates showed high susceptibility to imipenem (80.2%) and amikacin (66.6%). NFGNB although seen frequently in females and in age group of 50 years and above, clinical correlation with comorbid condition is essential to label it as uropathogens. Amikacin or imipenem may be the empirical drug of choice.

Keywords: NFGNB, comorbid condition, Antibiotic susceptibility, Bacteriuria, Urinary tract infection

***Correspondence:** shobha.kl@manipal.edu; 9945271648 (Received: March 10, 2020; accepted: August 07, 2020)

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INTRODUCTION

Non fermenting gram-negative bacilli (NFGNB) are gram negative bacilli that can survive and grow in oxygenated environment and are incapable of fermenting glucose & utilizing it as a sole source of energy[1]. Hospital surroundings and intestines of human beings provide a niche for them to survive as saprophytes[2,3].

They may pose a serious problem by causing infections associated with indwelling medical devices like urinary catheter or ventilator. Around 12-16% of all bacterial species isolated from the laboratory are identified as non-fermenting gram negative bacilli[4]. High degree of resistance to various biocides are present in these isolates. Widespread patient-to-patient transmission occurs either via contaminated surfaces or through the hands of the health care providers especially in immunocompromised patients[2,5]. NFGNB include numerous organisms like *Pseudomonas* spp, *Acinetobacter* spp, *Alkaligenes* spp, *Stenotrophomonas maltophilia*, *Burkholderia cepacia complex* (BCC). Presently, *P. aeruginosa* and *A. baumannii* are the most frequently isolated non-fermenters[6].

Factors that pose a risk of NFGNB infection include immunosuppression (cancer patients, organ transplant patients and AIDS), neutropenic patients, diabetes, trauma, foreign body like indwelling catheters, ventilators and implants, overuse and inappropriate consumption of broad-spectrum antibiotics, intravenous saline infusion[7] and Foley's catheter.

Multidrug resistance among NFGNB is very common and is constantly rising. Carbapenem resistance among *Pseudomonas* and *Acinetobacter* leaves very less treatment options[8]. Carbapenemase activity in *A. baumannii* is mainly due to carbapenem-hydrolyzing class D b-lactamases (CHDLs) and in case of *P. aeruginosa* the predominant mechanism of resistance to carbapenem is loss of OprD2 which is a substrate-specific protein molecule that facilitates the diffusion of carbapenems into the cell[9]. Recently few studies conducted in India have identified the antimicrobial sensitivity pattern for these organisms[3,10,11].

Other organisms usually were ignored and were conveniently presumed to be contaminants because identification of species of these organisms is time-taking and hence very difficult in a busy routine diagnostic centre[12]. But introduction of the automated identification systems like Vitek 2 Compact and MALDI-TOF has made this task possible.

NFGNB have now been given a status of more than just a contaminant because of their increased isolation from urine samples with significant bacteriuria indicating their pathogenic role in causing UTI. Clinical correlation is required in order to label them either as colonizer or as pathogen[13-15].

Rationale of the Study: To isolate various species of NFGNB causing UTI, its associated comorbid conditions and their antimicrobial susceptibility pattern in the recent years.

MATERIALS AND METHODS

Urine specimens collected from patients attending tertiary care hospital for routine culture and susceptibility test over a study period of 6 months were included in the present study. The urine samples included midstream urine samples, urine from Foley's catheter or from DJ stenting. These specimens were processed by culturing on 5% sheep blood agar and MacConkey agar. Those midstream urine samples yielding a monomicrobial culture of $\geq 10^5$ CFU/ml were noted as cases having significant bacteriuria according to Kass concept, while any number of colonies in samples collected from Foley's catheter or DJ stenting were considered significant. Identification was done by Gram staining and MALDI-TOF. Antibiotic susceptibility testing was done by Vitek® 2 system using N 281 card. The antibiotics used for sensitivity testing were piperacillin-tazobactam, ceftazidime, cefepime, cefoperazone-sulbactam, imipenem, meropenem, aztreonam, amikacin, gentamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, tigecycline and colistin.

Details of the patients such as demography, signs and symptoms, radiological impression, ICU/ward admission, hospitalisation and prognostic outcomes were retrospectively collected from the medical record department. The case sheet of individual patients were analysed for relevant laboratory and clinical criteria in order to access the clinical significance of the isolates. The criteria in the laboratory for the diagnosis of

UTI caused by NFGNB included the presence of pus cells along with gram-negative bacilli in the stained smear from the sample, monomicrobial infection, isolation of the same organism from a repeat sample, raised total leucocyte count, and significant radiological findings (in patients with complicated UTI)5. The clinical criteria included burning micturition, lower abdominal pain, pyuria, ureteric stone and urinary retention.

Statistical analysis

Statistical analysis was done using software SPSS version 16.

RESULTS

Among the total number of 16,413 urine samples that were received in the laboratory, 318 had significant bacteriuria. NFGNB were isolated in 108 (33.9%) the urine samples with significant bacteriuria. Prevalence of non-fermenters out of the total urine samples collected was 0.6%.

It was found that all the samples that grew NFGNB were from the patients who were admitted in the hospital. Maximum number of isolates were found from patients within the age range of 51-60 Years (Table 3). Ratio of males, 44(40.47%,) to females 64 (59.25%) from which NFGNB were isolated was 1:1.55 (Table 2). Seven (6.5%) out of 108 samples, were from pregnant women. Previous hospitalization was from 43 cases (39.8%). Nine samples (8.3%) were from the patients admitted in ICU.

Among the NFGNB cases, samples collected from the mid-stream urine were 75(69.4%), indwelling catheter (Foley's catheter) were 19 (17.6%) and samples from DJ stent were 14 (12.9%). Pyuria was seen in 63 (58.3%) patients and recurrent UTI was a complaint in 38(35.1%) cases. Among the comorbid conditions, patients with diabetes were 27(25%), hypertension were 35 (32.4%), chronic kidney disease were 12(11.1%), and immunosuppression were 11 (10.2%). Total number of patients with comorbid conditions were 85/108 (78.70%) Out of 108 patients in which NFGNB were isolated, three patients did not survive. Rest all were successfully treated with antibiotics.

Pseudomonas aeruginosa was the most predominant isolate growing in 77 (71.29%) urine samples followed by *Acinetobacter* spp 22(20.37%).

DISCUSSION

Out of 16,413 urine samples that were received in the laboratory, 318 had significant bacteriuria. NFGNB were isolated in 108 (33.9%) of the 318 urine samples with significant growth.

Table 1. Antibiotic susceptibility pattern of NFGNB isolated in the study

Antibiotics	*Pseudomonas spp* (n=81) Sensitivity %	*Acinetobacter spp* (n=22) Sensitivity %	*Stenotrophomonas maltophila* (n=5) Sensitivity %
Amikacin	66.67	63.64	-
Ceftazidime	64.20	0.09	-
Ciprofloxacin	51.90	31.82	100
Gentamicin	58.02	27.27	-
Aztreonam	20.99	0	-
Cefoperazone-sulbactum	62.96	63.64	-
Cefepime	72.84	63.64	-
Imipenem	80.25	77.27	-
Meropenem	91.36	90.91	-
Piperacillin-Tazobactam	82.70	63.64	
Tigecycline	88.89	95.45	
Colistin	100	100	
Trimethoprim-sulfamethoxazole	-	-	100

Pseudomonas spp included *Pseudomonas aeruginosa* (n=77) and *Pseudomonas putida* (n=4).
Acinetobacter spp included *Acinetobacter baumanii* complex (n=21) and *Acinetobacter junii* (n=1). (-) indicates not applicable.

Table 2. Gender distribution of patients in which NFGNB were isolated

Gender	*Pseudomonas* spp. (n=81)	*Acinetobacter* spp. (n=22)	*Stenotrophomonas maltophilia* (n=5)
Females (64) 59.25%	54	7	3
Males (44) 40.74%	27	15	2

Table 3. Demographic details of patients in which NFGNB were isolated

Age group (in years)	*Pseudomonas* spp.	*Acinetobacter* spp.	*Stenotrophomonas malophilia*
>=10	2	3	0
11-20	4	2	0
21-30	9	2	0
31-40	6	1	0
41-50	5	1	0
51-60	15	6	1
61-70	11	3	2
71-80	14	4	2
81-90	15	0	0
Total no. of isolates	81	22	05

Out of the 108 patients, females were 59.25% and males 40.74%. A similar study conducted by Rachana Solanki et al.[16] in Kolar, Karnataka reported 1.6% NFGNB. In the previous study done by Shobha KL et al.[17] at Manipal, out of 1271 midstream urine isolates with significant bacteriuria, NFGNB were found in 120 (9.44%) cases and was higher in female patients. Higher isolation rate of non-fermenters in our study may be attributed to the fact that majority of the samples collected in our study were from patients who were admitted in the hospital either in ward or ICU due to some underlying causes and were on Foley's catheter or DJ stenting.

Among 108 patients from which NFGNB were isolated, 67.6 % were above fifty years of age. This finding was in concordance to a study done in Telangana where 69.04 % NFGNB were isolated in the age group of >50 years of age.

In the present study, 71.3% of the non-fermenters were identified as *P. aeruginosa* which was similar to the study done by Rachana Solanki et al.[16] in Kolar, Karnataka where the same organism was isolated in 56.5% urine samples. A similar finding was found in a study conducted in Hungary where *Pseudomonas* spp. (outpatients: 78.7%; inpatients:85.1%) and *Acinetobacter* spp. (outpatients: 19.6%, inpatients: 10.9%), were the most prevalent organisms isolated[19].

NFGNB that were earlier considered to be contaminants have been implicated in causing many health care associated infections[20]. *P. aeruginosa* and *Acinetobacter* species were most commonly associated with such health care associated infections[20]. Present study showed *Pseudomonas* species *and Acinetobacter* species accounted for 95.3% of the isolates. They comprised 87% of the total non-fermenter isolated in the study done in Kolar, Karnataka[3].

Antimicrobial susceptibility pattern of one region differs from other; further it changes with time according to the adaptability of the infecting organism[21]. High grade of susceptibility to imipenem (80.2%) and amikacin (66.6%) was found in our *Pseudomonas* isolates which were similar to the study done in Kolar, Karnataka3 where susceptibility to imipenem was 94% and to amikacin was 69%. In a study conducted at Bangalore, 60-70% isolates of *P. aeruginosa* showed resistance to amikacin, ceftazidime, and ciprofloxacin[22]. In a similar study done at Chandigarh 42% of *P. aeruginosa* isolates showed

resistance to imipenem[23]. Most of the patients in our study were admitted in wards, while very few of them needed ICU care. *Pseudomonas aeruginosa* isolates were fairly sensitive to imipenem, amikacin, and cefoperazone. *A. baumannii* were sensitive to imipenem and piperacillin.

NFGNB though earlier regarded as contaminants have now being increasingly implicated in causing both hospital- and community-acquired infection. In our study, patients with comorbid conditions were 85/108 (78.70%) who definitely needed treatment for urinary tract infection due to NFGNB infection, where NFGNB is uropathogen. Therefore clinical correlation is necessary to determine this pathogenic role of NFGNB before ignoring them as mere contaminant. Identification of these organisms as uropathogens with assessment of their antibiotic sensitivity is necessary for appropriate treatment of this infection .

CONCLUSION

Pseudomonas aeruginosa and *Acinetobacter baumannii* were the most frequent non-fermenters isolated in our study. Though NFGNB are important etiological agents causing urinary tract infection more frequently in females and in the age group of >50 years, the clinical correlation with comorbid conditions of the patients should be made to identify NFGNB as uropathogen. This would prevent the use of unnecessary antibiotics in patients having NFGNB as colonizer and emergence of drug-resistant strains. Amikacin or imipenem may be the empirical drug of choice in these cases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

SKL: Planned the study and helped in editing the manuscript. AB and RG: Conducted the study and manuscript writing. AKB and RC: Helped in data entry. KG and AAA: Manuscript writing and ethical approval of study.

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None.

ETHICS STATEMENT

Ethics approval was taken and ethics approval number is IEC: 349/2019.

DATA AVAILABILITY

All datasets generated of analyzed during this study are included in the manuscript.

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