

Nonfermenting Gram-Negative Bacilli and Urinary Tract Infection -Sorting the Mystery of Infections Caused

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Abstract

Background: Aerobic nonfermenting gram-negative bacilli (NFGNB) are now emerging as important uropathogens.

Methods: This study was done to know the significance of NFGNB other than *P. aeruginosa* and *Acinetobacter* spp. in Urinary Tract Infections (UTI) along with their antibiotic sensitivity pattern. Total 10,198 urine specimens received in eight months period from October 2013 to May 2014 were subjected to quantitative culture as per the standard procedures in the routine microbiology laboratory and the results were noted. Detailed clinical history and laboratory parameters (Total count, Urine microscopy: Pus cell and RBC) were gathered to know the significance of the organism.

Results: Total 40.9% of the NFGNB isolates were clinical significant. Common risk factors associated with these NFGNB are ICU stay, previous hospitalization, catheterization and Diabetes Mellitus.

Conclusion: Clinical correlation of NFGNB from urine is required before considering them clinically significant or contaminants.

Keywords: Urinary tract infection; Nonfermenting gram-negative bacilli; Risk factors

Introduction

Urinary tract infection (UTI) is the second most common bacterial infections affecting humans throughout their lifetime [1]. *Escherichia coli* is the commonest urinary pathogen accounting for over 80% of community-acquired infection. Aerobic nonfermenting gram-negative bacilli (NFGNB) are a heterogeneous group of organisms that are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative rather than fermentative pathway [2]. These nonfermenters include organisms from diverse genera like *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Myroides*, *Oligella*, *Flavimonas*, *Agrobacter*, *Weeksiella*, etc. Because of these being the common inhabitants of soil, water and harmless parasites on the mucus membranes of humans and animals, they are generally considered as contaminants or incidental organisms. However in immunocompromised patients they can cause opportunistic infections, by gaining access to normally sterile body sites through trauma [3].

Pseudomonas aeruginosa is the predominant and most well-known organism out of this heterogeneous group [4,5]. This is partly due to its easy recognition in the laboratory as it produces pyocyanin, a blue-green pigment. Other organisms usually get ignored as contaminants because identification up to species level is cumbersome, laborious and not possible in a busy routine microbiology laboratory [6]. But with the introduction of the automated identification systems like Vitek 2 Compact and now MALDI-TOF, NFGNB negative bacilli are being easily identified up to species level. It has been noted that the NFGNB are being increasingly isolated in significant bacteriuria cases in routine urinary microbiology over the years indicating their potential in causing urinary tract infections. It is also well-known that most of these NFGNB are resistant to most of the commonly used antibiotics for treatment [7-9]. Various studies have been done to know the prevalence of these organisms. Isolation rates for NFGNB in urine samples was 10.8% -11% with the distribution of isolates as 72%-87% *Pseudomonas aeruginosa*, followed by 10.4%-18% *Acinetobacter baumannii*, and 0.77% *Acinetobacter lwoffii* [10,11]

Presence of these organisms in urine culture require a clinical correlation as these can be contaminants. This study was done to know the significance of nonfermenters other than *Pseudomonas aeruginosa* and *Acinetobacter* in UTI.

Material and Methods

Urine specimens (mid-stream and catheter catch) collected from both inpatients and outpatients attending Indraprastha Apollo Hospital, New Delhi (750 bed capacity) for culture and sensitivity test, from October 2013 to May 2014 were included in this study. Quantitative culture was performed on Mac Conkey Agar and Blood Agar (BioMerieux, France) plates. Plates were incubated for 48 hrs at 37° C. Identification of the isolates was done with the help of MALDI-TOF (Vitek MS, BioMerieux, France). Vitek MS uses matrix-assisted laser desorption ionization to ionise a sample into the gas phase. Briefly target slide with the bacteria is prepared and introduced to a high-vacuum environment. A precise laser burst ionizes the sample to generate a "cloud" of proteins which passes through a ring electrode. The protein's Time of Flight is recorded using a formula. Proteins are detected with a sensor to create a spectrum that represents the protein makeup of each sample. The VITEK MS system reads each spectrum as a series of peaks that are detected and sorted by mass and intensity to give exact identification of the isolate.

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Antimicrobial susceptibility was done by Vitek 2 using N280 card (BioMerieux, France) which has a panel of antimicrobials on a single card to which susceptibility results can be tested. The antibiotics Polymixin-B (300 µg), vancomycin (30 µg) and teicoplanin (30 µg) were put in addition for testing by Kirby –Bauer disc diffusion method on Muller-Hinton agar. The results were interpreted as per Clinical Laboratory standards Institute guidelines. *Escherichia coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

To know the significance of the isolated organism detailed clinical history was taken from patients (Table 1). Clinical history and laboratory parameters (Total count, Urine microscopy: Pus cell and RBC) were utilized for clinical correlation and further analysis was carried out.

Results

A total of 10,198 urine samples were processed for quantitative culture. 15.6% cultures showed significant growth. Predominantly *Enterobacteriaceae* were isolated from culture positive samples. Non fermenters were isolated in 2.1% of the culture positive samples. Among NFGNB *P. aeruginosa* was the predominant one.

Percentage of various NFGNB was mentioned in Table 1. Various risk factors associated with different NFGNB are mentioned in Table 2.

Compared to *Myroides* spp and *Achromobacter* spp., *B. cepacia*, *S. maltophilia* and *P. putida* gives variable susceptibilities to routinely prescribed drugs. Antimicrobial susceptibility of various NFGNB is mentioned in Table 3.

Discussion

NFGNB have been related with the wide spectrum of disease like septicemia, meningitis, osteomyelitis, wound infections, pneumonias and urinary tract infections. These infections can be endogenous or exogenous depending from various risk factors such as: immunosuppression, trauma, foreign body, broad-spectrum antibiotic use, infused body fluids like saline

irrigations [12] and urinary catheterization [4]. In our study the most common risk factors are ICU stay, previous hospitalization, catheterization and Diabetes Mellitus (Table 2).

NFGNB though regarded as contaminants are important bacteria causing hospital and community acquired UTI in both immunocompromised and in immunocompetent individuals. Thus, proper identification of the NFGNB is the need of the day. If recovered in pure culture, a detailed clinical history is required to confirm their significance. Among the studied NFGNB in the present study 40.9% isolates were clinical significant.

Because of the varied sensitivity pattern related to the different species of NFGNB their proper identification is required. Monitoring their susceptibility patterns is important for the proper management of the infections caused by them.

Myroides spp. colonizes water in hospital environment [13] and display a strong tendency to colonize surfaces and form biofilms [14]. It has been also reported that *Myroides* spp. showed high degree of resistance to almost all the routinely used antibiotics [2] which is supported by our study. The production of chromosome-encoded metallo-β-lactamases -I has been documented in *Myroides* spp which is capable of hydrolyzing cephamycins, penicillins, cephalosporins, aztreonam, imipenem, and meropenem make these bacteria resistant to many antimicrobial agents, which may favor nosocomial infections or infections in immunocompromised individuals [8]. Direct measurement of MICs rather than use of the disk diffusion method is mandatory for *Myroides* spp because of the discrepancies of the results by various other susceptibility methods [15].

The *Achromobacter xylosoxidans* has been isolated from infected patients from the blood, peritoneum, pleural liquid, sweat, respiratory secretions and urine [16,17]. Antibiotic susceptibility patterns for *A. xylosoxidans* are characterized by resistance to the aminoglycosides, quinolones, narrow-spectrum penicillins, first-and second-generation cephalosporins, some third-generation cephalosporins (cefotaxime and ceftriaxone) and aztreonam [9].

Treatment can be difficult due to the high level of antibiotic resistance. Trimethoprim-sulfamethoxazole may be useful for treatment, particularly in outpatients with community acquired infections. Piperacillin-tazobactam and meropenem are other alternatives for treatment.

Out of the 11 isolates of *Pseudomonas putida* 36% isolates were sensitive to amikacin, 27% to carbapenems and piperacillin – tazobactam was effective against 18% of the isolates. Quinolones were the least effective (9%). The increase in the resistance rate of *Pseudomonas* isolates to quinolones is due to their extensive use.

However, only a relatively small number of NFGNB isolates were examined in this study, hence the need to validate these results on more strains. It will be interesting to test this approach in a routine laboratory in which NFGNBs are frequently detected.

Organism	No	Details available	Clinical significant
<i>Myroides</i> spp	19	13	5
<i>P. putida</i>	11	6	2
<i>B. cepacia</i>	2	2	1
<i>Stenotrophomonas maltophilia</i>	2	-	-
<i>A. denitrificans</i>	1	1	1

Table 1: Distribution of NFGNB isolated from Urine and their clinical significance.

Risk Factors	<i>Myroides</i> spp (13)	<i>P. putida</i> (6)	<i>B. cepacia</i> (2)	<i>A. denitrificans</i> (1)
Catheterization	9	2	1	1
DJ Stenting	2	-	-	-
Previous Hospitalization	10	2	2	1
ICU stay	13	5	-	1
Immunosuppression	6	1	1	-
Diabetes Mellitus	7	3	-	-

Table 2: Risk factors associated with Various NFGNB.

Organism	TZP	CFS	FEP	CA	IMP	MEM	PB	AK	GEN	TOB	COT	CIP	MINO	TEI	VA
<i>Myroides</i> spp	0	0	0	0	0	0	-	0	0	0	0	0	-	0	0
<i>P. putida</i>	18	18	9	9	27	27	100	36	27	18	18	9	-	-	-
<i>B. cepacia</i>	-	-	-	100	-	100	-	-	-	-	100	-	100	-	-
<i>A. denitrificans</i>	0	0	0	0	0	0	100	0	0	100	100	0	-	-	-
<i>Steno. maltophilia</i>	-	-	-	0	-	-	-	-	-	-	0	50	100	-	-

Table 3: Antibiotic susceptibility pattern of NFGNB.

Conclusion

Our study highlights the fact that though NFGNB are important bacteria causing hospital and community acquired UTI the clinical relevance of the isolated NFGNB should be assessed before they are considered as pathogens. This would prevent misuse of antibiotics and emergence of drug-resistant strains.

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Conflict of interest: Nil

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