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Prevalence of non-fermenter as uro pathogen in tertiary care hospital in Chidambaram, Tamil nadu

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ABSTRACT

Background and Objectives: Aerobic, nonfermenting gram-negative bacilli (non-fermenters) are now emerging as important uropathogens. This study was done to know the prevalence of non-fermenters in Urinary Tract Infections (UTI) along with their antibiotic sensitivity.

Method: 1361 mid stream urine specimens received in 18 months period from December 2012 to July 2014 were subjected to Gram, staining, semi-quantitative culture, biochemical tests and antibiotic sensitivity testing as per the standard procedures in the routine microbiology laboratory and the results were noted.

Results: Out of the 317 culture positive cases with significant bacteremia, 131 (9.7%) were caused by non-fermenters. Among the non-fermenters, *Pseudomonas* spp. was the most common (80.6%) followed by *Acinetobacter* spp. (19.3%). Majority of patients belonged to the age group of 15 to 50 years and most of them were females. Antibiotic sensitivity testing revealed that, among *Pseudomonas* spp., 77.4% isolates were sensitive to Piperacillin, 71.13% sensitive to Amikacin, 41.9% sensitive to Ciprofloxacin, 35.4% sensitive to Ceftazidime and 90% sensitive to Imipenem. In the case of *Acinetobacter* spp., 68% isolates were sensitive to Amikacin, 33.3% sensitive to Norfloxacin and all were sensitive to imipenem. Out of the 6 non-fermenter isolates which were resistant to commonly used antibiotics, 4 were from inpatients indicating the possibility of nosocomial infection.

Conclusion: The non-fermenters are emerging as important cause of Urinary Tract Infections. In this study, *Pseudomonas* spp. was the most common non-fermenter isolated in significant numbers in MSU specimens. But, *Acinetobacter* spp. appeared to be more resistant to invitro antibiotics in general.

Key Words: Non-fermenters, Uro pathogen, Microbiology.

INTRODUCTION

The non-fermentative Gram negative bacilli (NF GNB) are aerobic and non spore forming microorganisms. They

do not utilize carbohydrates or breakdown them through oxidative metabolic pathway. Previously NF GNB were considered to be non pathogenic and of very little

significance. Recently, rate of infection by NF GNB is rising, especially in hospitalized and immunocompromised patients. NF GNB infection constitutes about one – fifth of all Gram negative bacilli infections.

These organisms can remain viable on medical devices and are resistant to many commonly used antibiotics. Therefore, they play an important role in hospital acquired infection. Although, rate of isolation of NF GNB from clinical specimens is increasing rapidly, very few laboratories in India identify these organisms routinely. Sometimes, NF – GNB is difficult to identify phenotypically. Most of them are multidrug resistant (MDR).¹ Malini et al² from Karnataka, India observed 4.5 % infection rate of NF GNB in their hospital. Their infection are endogenous or exogenous origin, depending on several factor such as: use of immunosuppressant substance, abusive use of wide spectrum antimicrobial agents, prolong surgical wide spectrum antimicrobial agents, prolong surgical procedure and inadequate instrumentation.³ Infections of the urinary tract are the most frequently encountered serious bacterial illness affecting humans throughout their life time. They are second only to infections of the upper respiratory tract as infectious disorders for which medical intervention is sought.

Urinary tract infections cannot be diagnosed on clinical grounds alone. The hallmark of a UTI has been the presence of a single microorganism of $>10^5$ Colony Forming Units (CFUS) per ml in a clean catch or midstream urine specimen, with lower numbers usually indicating contamination. *Escherichia coli* are the commonest urinary pathogen accounting for over 80% of community-acquired infection. Far less commonly *Klebsiella* spp, *Proteus* spp and *Staphylococcus saprophyticus* are responsible for community-acquired infection. The distribution of urinary pathogens in hospitalized patients is different with *Escherichia coli* accounting for about 50% of infections. *Enterococcus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Pseudomonas aeruginosa*, *Providencia*, and *Staphylococcus epidermidis* account for most of the rest. (Bryan et al 1984)⁽¹⁾. Aerobic nonfermenting gram-negative bacilli (nonfermenters) 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. (Koneman

EWet al 1988)⁽⁴⁾ This group includes organisms from diverse genera like *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacter*, *Oligella*, *Flavimonas*, *Agrobacter* *Weeksiella*, etc.

These organisms are common inhabitants of soil and water. They also exist as harmless parasites on the mucus membranes of humans and animals. Though primarily regarded as contaminants or incidental organisms, they are becoming increasingly important as opportunistic pathogens in immunocompromised patients. They can also cause infection by gaining access to normally sterile body sites through trauma. (Baron E.J et al 1990)⁽²⁾. Though these organisms vary in their potential to cause infection and antibiotic susceptibility, there are common disease manifestations and risk factors. *Pseudomonas aeruginosa* is the predominant and most well known organism out of this heterogeneous group (Katsumi Shigemura et al 2006)⁽¹³⁾ (Jombo G.T.A et al 2008)⁽¹⁴⁾. This is partly due to its easy recognition in the laboratory as it produces pyocyanin, a blue-green pigment. Other organization usually get ignored as contaminants because identification and not possible in a busy routine microbiology laboratory (Stansfeld JM 1966)⁹. It is also well-known that most of these non-fermenter gram negative bacilli are resistant to many commonly used antibiotics for treatment (Gupta V et al 2002)⁽¹⁰⁾ (Jamal W et al 2009)⁽¹¹⁾ (Nenek A et al 2008)⁽¹²⁾ (Takeynama K et al 2008)⁽¹⁵⁾. Evaluation of significance of isolating these organisms is complicated by the fact that they are usually present in mixed culture and the clinicians are rather unfamiliar with their names. This retrospective study was done to know the prevalence of non-fermenters in UTI along with their antibiotic susceptibility.

MATERIALS AND METHODS

Mid stream urine (MSU) specimens collected from both inpatients and outpatients attending Rajah Muthiah Medical College and Research Institute, Annamalai University and Government Hospital, Chidambaram from December 2012 to July 2014 were included the study. These MSU specimens were studied for significant bacteruria those urine specimens containing organisms and pus cells on grams staining and yielding a pure culture of 10^5 or more CFU/ml are considered to be significant bacteruria cases.

All urine samples were plated on blood agar, MacConkey agar and nutrient agar, and incubated at 37°C for 18-48 hours and growth recorded, and lactose non-fermenting colonies were followed. Morphology and motility of the organisms were determined by Gram staining and hanging drop method respectively and oxidase test was done.

All the Gram-negative bacilli grew on Mac Conkey agar or blood agar, whether oxidase positive or negative were inoculated on Triple sugar iron agar medium (TSI). Organisms grew on Triple Sugar Iron and produced an alkaline reaction were provisionally considered to be nonfermenter gram negative bacilli, and were inoculated into Hugh and Leifson's medium for glucose, lactose, sucrose and maltose to find out whether a particular organism was oxidizer or non-oxidizer and identified particular organism its biochemical reactions characteristics.

- a) Lack of evidence of glucose fermentation.
- b) Positive cytochrome oxidase reaction.
- c) Failure to grow on MacConkey agar.

In-vitro antibiotic sensitivity test was performed by Kirby Bauer's disc diffusion method using Muller Hinton Agar as per Clinical Laboratory Standards Institute (CLSI) guidelines and susceptibility pattern was noted. (Quinn PJ 1988)⁽⁶⁾ In this study, we have included only the non-fermenters causing UTI. First and second line antibiotics used for oxidase positive non-fermenters were gentamicin (10mg), tobramycin (10mg), netilmycin (30mg), amikacin (30mg), ciprofloxacin (5mg), ceftazidime & piperacillin (100mg), where as for oxidase negative non-fermenters gentamycin (10mg), netilmycin (30mg), amikacin (30mg), norfloxacin (10mg), ampicillin (10mg), amoxicillin-clavulanic acid (10mg), cefuroxime (30mg) & cefotaxime (30mg). Isolates resistant for all first & second line antibiotics were tested against third line antibiotics which included aztreonam (30mg), ceftiprome (30mg), ticarcillin-clavulanic acid (30mg), piperacillin –tazobactam (30mg), cefoperazone-sulbactam & imipenem (30mg) for both oxidase positive & negative non-fermenters. All culture media and antibiotic discs were from Himedia Laboratories Pvt Ltd, Mumbai, India.

RESULTS

Out of the one thousand three hundred and sixty one MSU specimens, no significant growth was recorded in one thousand forty four (76.7%) specimens where as three hundred and seventy one (23.2%) specimens showed

significant growth. This indicates that approximately ¼ of the total specimens are significant bacteremia cases. Out of urinary isolates with significant bacteremia, number of fermenter gram negative bacilli was 256 (80.2%), non-fermenters gram negative bacilli was 31 (9.7%), gram positive cocci was 17 (5.3%) and yeasts 13 (4.1%). Thus fermenter GNBs caused more than cases of significant bacteremia where as the remaining cases were caused by non-fermenter GNBs, GPCs and yeasts. In case of non-fermenter GNBs, the number of *Pseudomonas* spp. was 25 (80.6%) and *Acinetobacter* spp. 6 (19.3%), in *Acinetobacter* species - *Acinetobacter baumannii* 5 (83.3%), *Acinetobacter lwoffii* – 1 (16.6%).

DISCUSSION AND CONCLUSION

Urinary tract infections are found in all age groups. The incidence is higher in male infants due to presence of congenital anomalies (Stansfeld J.M 1966)⁽⁸⁾. From adulthood to the age group of 65 years, the incidence of UTI is very high in women as compared to men. In older patients there is a progressive decrease in the female to male ratio due to development of obstructive uropathy from the prostatic enlargement. The results of this study are in line with the general observation regarding the age groups (Stansfeld J.M 1966)⁽⁸⁾.

In the present study, non-fermenter GNBs were found to be significant in 9.7% cases, second only to the fermenters (80.2%). *Pseudomonas aeruginosa* is an established pathogen of urinary tract. (Rambaugh K.P et al 1999)⁽⁷⁾. *Pseudomonas* spp. was the commonest non-fermenter isolate in the present study being significant in 7.8% of cases. *Acinetobacter* are ubiquitous in environment and in hospitals (Quinn P.J. 1988)⁽⁶⁾. They account for 1 to 3 percent of nosocomial infections, being second only to *P.aeruginosa* as non-fermenters in their recovery from clinical specimens. (Hall G.S 1995)⁽³⁾ *Acinetobacter* have been implicated in causation of a variety of illness including UTI. (Pederson M.B et al 1970)⁽⁵⁾. They accounted for 01.80% cases in this study. A high level of drug resistance was observed in general for majority of strains of these bacterial species in different studies (Gupta V et al 2002)⁽¹⁰⁾ (Jamal W et al 2009)⁽¹¹⁾ (Nenek A et al 2008)⁽¹²⁾ (Jombo G.T.A et al 2008)⁽¹⁴⁾ (Takeyama K et.al., 2008)⁽¹⁵⁾ which is in line with the results of this study. Out of the 6 non-fermenter urinary isolates which were resistant to all first and second line antibiotics invitro, 4 were from inpatients. This could be due to the fact that most of these isolates

may be from UTI with nosocomial origin (Marcus Net.al.)⁽¹⁶⁾

Age Distribution in Male and Female with UTI (31 Isolates)

S.No.	Age Groups (Years)	Total Number	Male	Female
1.	>15 Years	6 (19.3%)	4 (66.6%)	2 (33.3%)
2.	15 – 50 Years	13 (41.9%)	5 (38.4%)	8 (61.5%)
3.	>50 Years	12 (38.7%)	7 (58.3%)	5 (41.6%)

Non-fermenters were found to be significant in 31 cases, in which 20 were in-patients and 11 were attending Outpatients Department. In this, 17 were males and 14 females. Children below 15 years of age were four in

which three were males and two are females. In the remaining 25 cases, 13 were in the age group between 15 to 50 years (5 males & 8 females) and 12 were above the age of 50 years (7 males & 5 females).

Antibiotic sensitivity pattern of non-fermenter in urine samples (31 Isolates)

S.No.	Antibiotics	P.aeruginosa n=25	A. Baumannii n=5	A. lowffi n=1
1.	Amikacin (30mg)	18 (72%)	4 (66.6%)	1 (100%)
2.	Gentamicin (10mg)	11 (44%)	4 (66.6%)	1 (100%)
3.	Ciprofloxacin (5mg)	13 (52%)	2 (33.3%)	(0%)
4.	Ceftazidime (10µg)	11 (44%)	(0%)	(0%)
5.	Piperacillin (100mg)	19 (76%)	(0%)	1 (100%)

10 Isolates		6 Isolates		
6.	Imipenem (30mg)	9 (90%)	5 (100%)	1 (100%)
7.	Piperacillin and Tazobactam (30mg)	4 (40%)	3 (60%)	1 (100%)
8.	Cefaperazone and Sulbactam (30mg)	5 (50%)	2 (40%)	(0%)
9.	Aztreonam (30mg)	(0%)	(0%)	(0%)
10.	Cefpirome (30mg)	(0%)	(0%)	(0%)
11.	Ticarcillin – Clavulanic Acid (30mg)	(0%)	(0%)	(0%)

This study revealed that for Pseudomonas spp., Piperacillin and Amikacin are most effective (Sussman M 1998)⁽⁹⁾ followed by Ciprofloxacin and Ceftazidime in the group of first and second line antibiotics and also Imipenem to be the most effective followed by Cefoperazone-Sulbactam and Piperacillin-Tazobactam in the group of third line reserved antibiotics. For Acinetobacter spp., Aminoglycosides in the group of first and second line antibiotics and Imipenem in the group of third line reserved antibiotics are found to be most effective. A previous study has reported that for Pseudomonas aeruginosa, amikacin, ceftazidime and piperacillin are the recommended antibiotics (Gupta V et al 2002) ⁽¹⁰⁾ which is in accordance with the results of our study. But other studies reported multiple drug resistance in Pseudomonas aeruginosa isolates

(Takeyama K et al 2008) ⁽¹⁵⁾, (Jombo G.T.A et al 2008) ⁽¹⁴⁾, (Katsumi Shigemura 2006) ⁽¹³⁾. It has been also reported that Acinetobacter infection in hospitalised patients showed high degree of resistance to almost all the antibiotics used routinely necessitating its susceptibility testing for newer drugs (Gupta V et al 2002) ⁽¹⁰⁾ which is supported by our study. Also, all strains of Acinetobacter spp. were found sensitive to imipenem in our study though emergence of resistance to this group of antibiotics has been reported in previous studies (Jamal W et al 2009) ⁽¹¹⁾ (Nenek A et al 2008) ⁽¹²⁾. From the present study it can be concluded that non-fermenter gram negative bacilli though regarded as contaminants are important bacteria causing hospital and community acquired UTI. If recovered in pure culture, a repeat urine specimen should be taken to confirm their presence, and the strain should be identified up to the

species level along with the antibiotic susceptibility testing for routinely used antibiotics.

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