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Non- Fermenters as Mysterious Pathogens or Contaminants- Continuing Dilemma

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Abstract: Non-fermenters (NFs) account for about 15% of all bacterial pathogens isolated from a clinical microbiology laboratory. Primarily regarded as contaminants, they have been implicated in causing various infections. They are innately resistant to many antibiotics and emerging as important nosocomial pathogens**.** To isolate non-fermenters from Urinary Tract Infections and assess their clinical significance and anti-microbial sensitivity pattern.Five thousand nine hundred and thirty four (5934) non-repetitive consecutive samples of urine from patients diagnosed of culture-proven UTI of a tertiary-care teaching hospital over a period of four-years (2014-2017) were selected. A urinary sample was subjected to presumptive screening followed by semi-quantitative culture on CLED (Himedia, Mumbai) agar. Identification and antibiogram was done by Vitek 2 compact (bioMérieux, France) automated systems. Clinical history and laboratory parameters were utilized for clinical correlation.Out of 5934 samples showing significant growth 6212 (15.6%) bacterial isolates were grown, Non fermenters being in 5.6%. *P. aeruginosa* (55.17%)and *Burkholderia cepacia* (17.42%) were predominant NFs. Post- operative UTI (23.3%) and history of Diabetes (17.5%) were the two most common risk factors associated. *Pseudomonas spp., Burkholderia spp., Acinetobacter spp. and Sphingomonas spp.* gave variable susceptibilities to normally recommended drugs compared to *Myroides* spp, *Achromobacter* spp.,and other NFs.Appropriate identification and susceptibility patterns of NFs is vital for management of infections, implementation of appropriate Infection Control interventions, and avoidance of irrelevant antibiotics use preventing emergence of drug resistance and thus morbid complications.

Keywords: Non-fermenters, Sphingomonas, Myroides, Contaminats, Pathogen.

INTRODUCTION

Non-fermenting gram-negative bacilli (NF), are saprophytes and some are commensals in the human intestine and they have now emerged as important healthcare-associated pathogens [1, 2]. These are a taxonomically diverse group of aerobic, non-sporing, bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively [3].NF account for approximately 15% of all bacterial isolates from a clinical microbiology laboratory [4]. These NFs include organisms from diverse genera like *Pseudomonas,Acinetobacter, Alcaligenes, Myroides, Oligella, Flavimonas, Agrobacter,Weeksiella,* etc. Because these are saprophytes and some are commensals in the human intestine, they are generally considered as contaminants. However in immune-compromised patients they are opportunistic pathogens, by gaining access to normally sterile body sites through traumatic or other routes [5]. In recent years, due to the liberal and empirical use of antibiotics, NF have emerged as important healthcare-associated pathogens.NF are innately resistant to many antibiotics and they exhibit resistance not only to beta lactam, carbapenems and the other groups of antibiotics [4].

Pseudomonas aeruginosa is the most well-known organism in the NFs group, relatively due to its easy recognition in the laboratory, as it produces pyocyanin (a bluegreen pigment). Other organisms are usually get overlooked as contaminants because their identification up to species level is cumbersome, laborious and difficult in a conventional diagnostic microbiology laboratory. With the introduction of the automated identification systems like Vitek 2 Compact, NFs are being routinely identified up to species level and are being increasingly isolated in various infections

such as, septicemia, meningitis, pneumonia, urinary tract infections (UTI), and surgical site infections. They are being increasingly isolated in significant bacteriuria cases in a routine bacteriology laboratory over the years indicating their potential to cause serious UTIs. Various studies have been done to know the prevalence of these organisms from various samples. Clinical correlation is very essential for the confirmation of these organisms in urine culture as these are sometimes considered as contaminants [6-8].

Rationale of the Study

The present study was done to know the significance of non-fermenters including the routinely isolated organisms like *Pseudomonas aeruginosa* and *Acinetobacter spp.* in UTI and to assess their clinical significance and their anti-microbial sensitivity pattern.

MATERIALS AND METHODS

Five thousand nine hundred and thirty four (5934) non-repetitive consecutive inpatients and outpatients diagnosed of culture-proven UTI amongst 39826 clinically suspected patients, after approval from Institutional Ethic Committee of a tertiary-care teaching hospital over a period of four-years (2014-2017) were selected for the study. Diagnosis was done using standard microbiological techniques and guidelines. A single midstream clean-catch urinary sample from patients presenting with urgency, hesitancy, frequency, dysuria, burning micturition and/or fever were included. Suprapubic aspirates and urinary catheters were not accepted for processing. UTI screen in asymptomatic patients at-risk of UTI such as antenatal patients, fever of unknown origin, bacteremia and sepsis etc. were also included. All samples were subjected to presumptive screening followed by semi-quantitative culture and antimicrobial susceptibility. Presumptive screening of wet mounts for ≥1 leucocyte per seven 400X magnification fields as well as ≥1 bacteria per 1000X magnification field from un-centrifuged samples was done. Semi-quantitative cultures were done on cysteine lactose electrolyte deficient (CLED) agar (Himedia, Mumbai, India) and the plates were aerobically incubated overnight at 37^0C. The organisms were identified and antibiogram was done by Vitek 2 compact (bioMérieux, France) automated systems wherein identification percentage >85% and antibiogram/AES consistent and consistent with correction utilizing inbuilt standards were taken as cutoffs for final validation. Antimicrobial susceptibility was done by Vitek 2 using N281 card (BioMerieux, France) which has a panel of antimicrobials on a single card to which susceptibility results can be tested. Wherever required manual antimicrobial sensitivity was done 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. Clinical history and laboratory parameters (Total count, Urine microscopy: Pus cell and RBC) were utilized for clinical correlation and further analysis was carried out.

Statistical analysis

Statistical analysis was done in case comparison of two or more set of variables, p value was calculated by using software SPSS version 17. If p value was ≤0.05 then it was taken as significant.

RESULTS

A total of 39826 urine samples were processed for quantitative culture. Out of which 5934(14.9%) showed significant growth and 6212 (15.6%) bacterial isolates were grown. Predominantly *Enterobacteriacae* (40.38%)were isolated from culture positive samples (Table1). Non fermenters were isolated in 5.6% of the culture positive samples. Amongst NF *P. aeruginosa* (55.17%)and *Burkholderia cepacia* (17.42%) were the predominant organisms isolated (Graph-2).

Various risk factors associated with different NFGNB are mentioned in Table-2. Post- operative UTI (23.3%) and history of Diabetes (17.5%) were the two most important risk factors associated with Non fermenters UTI. *Pseudomonas spp.* (63.1%) *Burkholderia spp* (15.2%) and *Acinetobacter spp.* (14%) were the most common organisms associated with various risk factors.

Antimicrobial susceptibility pattern of various organisms associated with different risk factors is shown in Table-2. Compared to *Myroides* spp, *Achromobacter* spp., *Chryseobacterium spp*,and *S. maltophila*, *Pseudomonas spp., Burkholderia spp., Acinetobacter spp. and Sphingomonas spp.* gave variable susceptibilities to normally recommended drugs.

Table-1: Distribution of the urine samples processed in the laboratory during the study period (Jan 2014-December 2017)

Total number of urine sample tested in four years	Total number of culture positive sample	Percentage	Total number of organisms isolated in four years	Percentage
39826	5934	14.9%	6212	15.6%

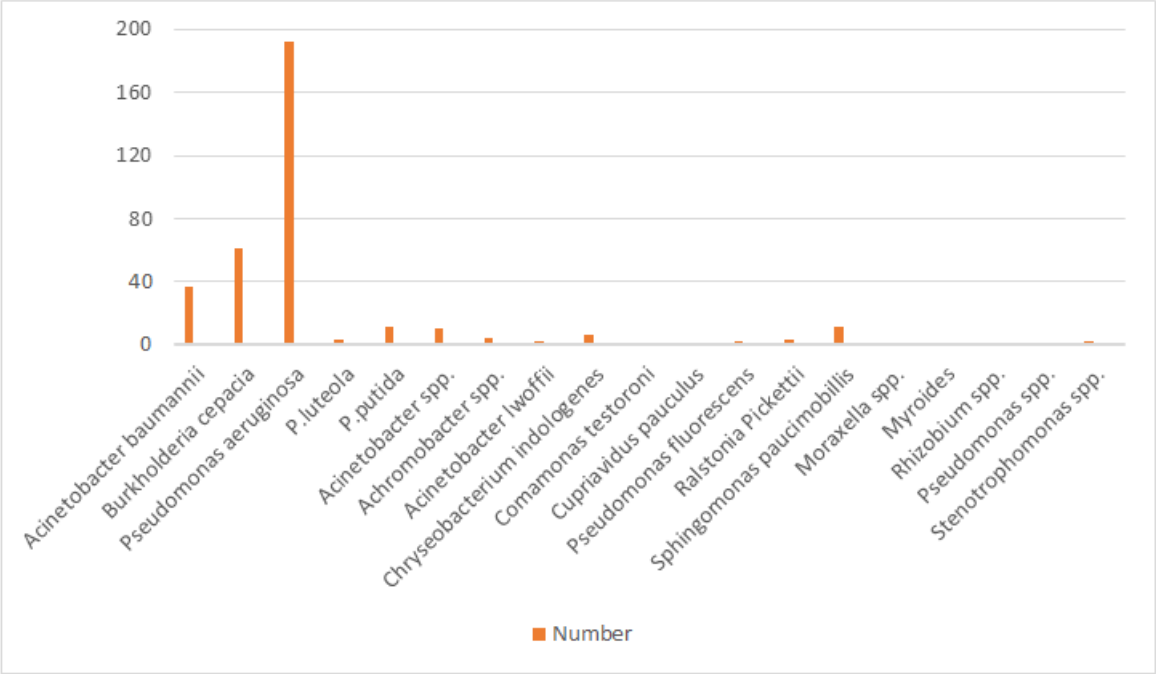

Fig-1: Distribution of different Non-fermenters in the Urine samples (N=350)

Table-2: Various risk factors associated with different Non fermenters

Risk Factors	*P.aeuginosa*	*Acinetobacter spp.*	*Burkholderia spp.*	*Myroides spp.*	*Sphingomonas spp.*	*Achromobacter spp.*	*Chryseobacterium Spp.*	*Stenotrophomonas spp.*
Catheterization {23 (13.4%)}	18	4	1	-	-	-	-	-
ICU stay{ 23 (13.4%)}	11	4	5		1	-	-	2
Post-Operative {40 (23.3%)}	25	3	8	-	-	3	1	-
Dialysis {14 (8.18%)}	10	2	1		1	-	-	-
Diabetes Mellitus {30 (17.5%)}	14	7	5	-	2	-	2	-
Immunosuppression{ 17 (9.35%)}	11	1	5	-	-	-	-	-
Burn{24 (14.0%)}	19	3	1	1	-	-	-	-
Total {n=171(49.1%)}	**108 (63.1 %)**	**24 (14%)**	**26 (15.2%)**	**1 (0.58 %)**	**4(2.33%)**	**3(1.75%)**	**3 (1.75%)**	**2(1.16%)**

Table-3: Antibiotic Resistance Pattern of the Non-fermenters associated with various risk factors (N=350)

Organism	**G**	**TOB**	**AK**	**AT**	**CAZ**	**CTR**	**CEF**	**NIT**	**IM**	**MR**	**PIT**	**COT**	**LE**	**CIP**	**C**
Pseudomonas spp.	34	35	34.5	5.2	3.1	-	39.2	26	43	41	37	-	32.4	29.3	99.5
Acinetobacter spp.	16.2	29.7	10.8	16.2	10.8	16.2	18.9	0	21.6	24.3	13.5	16.2	18.9	18.9	100
Burkholderia spp.	-	-	18	16.3	3.2	16.3	1.6	-	-	54	-	42.6	40.9	14.7	0
Myroides spp.			0	0	-	0	0							0	0
Sphingomonas spp.	63.6	54.5	63.6	18.1	-	63.6	63.6	45.4	72.7	72.7	54.5	63.6	-	63.6	-
Achromobacter spp.	0	0	0	0	0	0	0	0	75	75	100	0	0	0	100
Chryseobacterium Spp.	0	0	0	0	0	-	0	0	0	0	0	33.3	0	33.3	0
Stenotrophomonas spp.	0	-	0	-	100	-	0	-	-	-	-	100	-	100	100

G- Gentamicin, **TOB**- Tobramycin, **AK**- Amikacin, **AT**- Aztreonam, **CAZ**- Ceftazidime, **CTR**- Ceftriaxone, **CEF**- Cefepime, **NIT**- Nitrofurantoin, **IM**- Imipenem, **MR**- Meropenem, **PIT**- Piperacillin-tazobactam, **COT**- Cotrimoxazole, **LE**- Levofloxacin, **CIP**- Ciprofloxacin, **C**- Chloramphenicol

DISCUSSION

Non-fermenters account for about 15% of all bacterial pathogens isolated from a clinical microbiology laboratory [4]. They have been implicated in various infections. Though primarily regarded as contaminants or incidental organisms, these organisms have been implicated in causing septicemia, meningitis, osteomyelitis, wound infections, pneumonias and urinary tract infections. Various risk factors include immunosuppression, trauma, foreign body, broad-spectrum antibiotic use and infused body fluids like saline irrigations [9]. Post- operative UTI (23.3%) and history of Diabetes (17.5%) and history of burn (14%) were the most important risk factors associated with Non fermenters UTI. Solanki *et al*.,[5] in their study found the most common risk factors as ICU stay, previous hospitalization, catheterization and Diabetes Mellitus, while Meharwal *et al*., [9] in their study found , Postoperative period (42.6%), followed by obstructive uropathy (32%) and surgery on urinary tract (8%) as the commonest risk factors. Non-fermenters mostly considered as contaminants but they are important pathogens causing UTI in community as well as hospital set up, both in immune-compromised and in immune-competent individuals. Appropriate identification of the Non- fermenters is required along with a detailed history to confirm their clinical relevance. Amongst various Non-fermenters in the current study 41.9% of our isolates were clinically significant. It was similar to that found by Solanki *et al.*, [5].

Prevalence and Clinical significance of Different NFs

In the current study *P.aeruginosa* (63.1%), *Burkholderia spp*.(15.2%) and *Acinetobacter spp.* (14%) were the most commonly isolated non-fermenters associated with different risk factors.*P. aeruginosa is* an established pathogen of urinary tract [10]. It is the commonest isolate in the present study. *Pseudomonas spp*. like *P. fluorescence* and *P. putida* and *P. luteola*have been recovered from urine samples but their clinical relevance in the present study has not been found to be significant.

Acinetobacters are abundant in environment and in hospitals and have been associated in causation of a variety of illness including UTI [11, 12].They account for 1 to 3 percent of nosocomial infections, being second only to *P. aeruginosa* in their recovery from clinical specimens [13]. In a study by Panda *et al*.,[14]out of 300 samples of UTI *P. aeruginosa* (16.6%) and *Acinetobacter spp* (3.3%) were the predominant non-fermenters isolated. In another study by Khan *et al*., [15] in Pune, *Pseudomonas spp* (17.86%). and *Acinetobacter spp*. (12.5%) were the most common NFs isolated amongst the renal transplant recipients. *Pseudomonas spp*. and Acinetobacter spp. have a high potential to form biofilm that is responsible for their high survival potential in the environment, antibiotic resistance and virulence [16].

Burkholderia cepacia is high virulent organism usually causing lower respiratory tract infections especially in Cystic fibrosis (CF) and post lung transplant patients. Urinary tract infections with *Burkholderia cepacia* have been associated after bladder irrigation or use of contaminated hospital objects [17]. There have been rare reports of urinary tract infection (UTI) caused by *B. cepacia*. Hosts with predisposing factors, such as post renal transplant, vesico-ureteral reflux (VUR), neurogenic bladder, bladder irrigation, or use of contaminated medical devices, have been reported to be susceptible to *B. cepacia* UTI [18]. In our study 15.2% of the *Burkholderia spp.* were associated with various risks factors causing UTI, out of which Post-operative cases

contacting Burkholderia UTI (20%) were maximum (Table-2).

Myroides spp. inhabits hospital water supply and colonize surfaces to form biofilms [19].It has been also reported that *Myroides spp*. are highly resistant to almost all the routinely used antibiotics [5].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 [20].In our study however, only one isolate of *Myroides spp.* was isolated in burn patient (Table-2).

The *Achromobacter xylosoxidans* has been isolated from various clinical samples of infected patients from the blood, peritoneum, pleural liquid, sweat, respiratory secretions and urine. *A.xylosoxidans* display resistance to the aminoglycosides, quinolones, narrow-spectrum penicillins, first-and second-generation cephalosporins, some third-generation cephalosporins (cefotaxime and ceftriaxone) and aztreonam [5].In the current study three isolates of *Achromobacter spp.* were isolated from post- operative UTI cases.

Several reports of human infections caused by *Chryseomonasluteola* are there. It is a saprophyte found in the soil and water. Various factors predisposing to infections caused by *Chryseomonasluteola* include immunosuppressive therapy, previous history of long-term antibiotic use, chronic alcoholic abuse with liver cirrhosis, chronic renal failure, malignancy and bone marrow transplant patients [21]. It causes bacteremia, pneumonia, biliary tract infections, surgical wound infections, abscesses, peritonitis, subdural empyema, and infections associated with the presence of prosthetic devices [22]. In our study three isolates were found to be clinically relevant in Post- operative UTI patients and those with Diabetes. *Chryseomonasluteola*, could emerge as a potential pathogen with any of the risks factors, therefore should never be ignored as laboratory contaminants.

Sphingomonas paucimobilis, a nonfermenting Gram-negative bacillus, considered as of lesser clinical significance. However, it is found to be associated with *S. paucimobilis* include primary bacteraemia, intravascular catheter infections, peritoneal dialysis-associated peritonitis, UTI, biliary tract infection, ventilator associated pneumonia, meningitis, etc. The origin of *S. paucimobilis* nosocomial infections may be endogenous or exogenous. *Sphingomonas paucimobilis* causing UTI is a rarely encountered entity [23]. In the current study it was found to be associated with Diabetes, ICU stay, and Post –Dialysis. This emerging pathogen should be dealt more cautiously and should not just be regarded as contaminate.

S. maltophilia is ubiquitous in aquatic or humid environment and can survive there for extended periods. It can survive and multiply in respiratory secretions, urine or intravenous fluids [24]. Immuno-compromised patients are more susceptible to *S. maltophilia* infection. In our study it was found to be associated with prolonged ICU stay. *S. maltophilia* can cause bacteraemia, endocarditis, pneumonia, meningitis, infections of bones and joints, urinary tract, soft tissues, and wounds. The bacterium is intrinsically resistant to β-lactams and is often resistant to other antimicrobials as well and hence should never be ignored when isolated in a clinical microbiological sample [25].

Significance of Antibiotic Sensitivity

The Antibiotic Sensitivity pattern of different Non-fermenters is variable. Proper identification of the Non-fermenters is essential till the species level, as it is mandatory in clinical situation to monitor their sensitivity patterns for the appropriate management of various infections caused by them, for better patient prognosis and appropriate infection control interventions [26].In most of the studied isolates, amikacin, carbepenems and piperacillin –tazobactum were the most effective antibiotics. Quinolones were the least effective antibiotic which might be due to their extensive use.

Strength of the current study

There is a need to validate various study results on NFs, paying more attention to each of them isolated in various clinical samples and conducting more frequent studies. Our study is thought-provoking for usage of advanced methodologies/ Technologies in a routine clinical microbiology laboratory where more numbers and varieties of non-fermenters are frequently being detected from various clinical samples. It is needed to pay more attention to identify them and ascertain the resistant pattern of these emerging pathogens, giving a platform to modulate the therapies accordingly.

Future Research Direction

Polymicrobial Urinary Tract infections is also an emerging concern amongst patients with Nosocomial UTI. In case of immune-compromised recipients it offers a diagnostic and therapeutic challenge due to sublime clinical presentation, weak immunological reaction and antimicrobial resistance. Polymicrobial infections may be involved with prolong morbidity, increase hospital stay, reduce graft survival and promote further antimicrobial resistance leading to complications such as sepsis and mortality [27].

CONCLUSION

The different species of Non- fermenters have shown varied sensitivity pattern and associated with variety of risks factors in the current study. Evaluating the significance of isolating these organisms is difficult,

by the fact that these organisms are usually present in mixed culture and most of the clinicians are unfamiliar with their names. Therefore, identification of Non-fermenters, and checking their susceptibility patterns, is important for managing properly the infections caused by them and thus implement appropriate Infection Control interventions. The current study highlights that the clinical significance and relevance of these organisms should be assessed before considering them as normal contaminants, to avoid irrelevant use of antimicrobial agents leading to emergence of drug resistance followed by morbid complications. The significance of polymicrobial flora in cases of UTI is not ignored by the authors and further study on this emerging problem is in queue.

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