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THE EMERGING NON- FEREMENTING GRAM NEGATIVE BACTERIA IN URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

BACKGROUND: Urinary Tract Infections (UTIs) are some of the most common infections among patients. There is an emerging appreciation for the pathogenic role of non-fermenting Gram-negative bacteria (NFGNB) in UTIs, particularly in the presence of underlying diseases. The non-fermenter Gram negative bacilli are a divergent set of aerobic, non-sporing, Gram negative bacilli that either withhold utilization of the carbohydrates as a source of energy or degrade them through metabolic pathways other than the fermentation (1). Nowadays, NFGNBs are an emerging cause of UTI's. Antimicrobial treatment of the infection caused by these agents is troublesome. Non-fermenting Gram-negative bacteria present a concerning curative challenge to clinicians, due to their increasing levels of resistance to several antibiotics, acquired either from nosocomial or community settings, ultimately leading to multidrug resistant isolates, leading to prolonged therapy and excess mortality in the affected patient population. The data of our present study may aid the creation of a national surveillance system for urinary tract infections and to ascertain the relevance of non-fermenters as important uropathogens. **MATERIALS AND METHODS:** The present observational study conducted in department of microbiology, SCBMCH and SVPPGIP, Odisha was under taken to find the proportion of various emerging NFGNB in urine samples, their clinical significance and anti- microbial sensitivity pattern. **RESULTS:** Forty-six non-fermenters were isolated from urine samples. A total of 263 NFGNB (15.06%) were isolated from 1746 various clinical specimens. *Pseudomonas aeruginosa* was the most common non-fermenter, accounting for 45.6 % (out of NFGNB isolated from urine samples), followed by *Acinetobacterbaumannii*(17.39 %), while the other emerging species e.g. *Burkholderia cepacia, Chryseobacteriumindologenes, Elizabethkingiameningoseptica, Sphingomonaspaucimobilis* and *Stenotrophomonasmaltophiliawere* isolated in a minority. *P. aeruginosa* showed good sensitivity to Polymyxin B/Colistin (84.9%), Azetronem(67.74%), Piperacillin – Tazobactam(65.59%) Imipenem(62.37%) and Piperacillin(60.22%).*A. baumannii*showed 94.59% sensitivity to Polymyxin B/Colistin, 81.08% sensitivity to Tigecycline and (32.43%) sensitivity to Ampicillin &salbactum. **CONCLUSION:** NFGNB are confirmed to have significant role as critical uropathogens. *P. aeruginosa* and *A. baumannii*were the common NFGNB isolated in our study from patients having urinary tract infection. In this study urinary tract NF GNB found mostly sensitive to drugs Tigecycline and Polymyxin B/Colistin. However, there was no antibiotic to which all strains were susceptible due to MDR pattern in their antibiotic susceptibility testing.

KEYWORDS

Acinetobacterbaumannii, Pseudomonasaeruginosa, NFGNB And UTI

INTRODUCTION

Urinary tract infections (UTIs) are the most prevalent infections among infectious pathologies worldwide, in the human medicine and also represent an important factor of morbidity and mortality .

NFGNBs are extremely prevalent in opportunistic infections, affecting especially immunocompromised, debilitated patients over 60 years of age(2). NFGNB predominantly pseudomonas *spp* and Acienetobacter spp. are emerging nowadays. UTIs are increasingly being caused by multidrug-resistant pathogens, acquired either from nosocomial or community settings (3).

A total of 46 urine samples yielded NFGNB, were also found associated with significant bacteriuria, and presence of inflammatory cells. *P. aeruginosa followed by A. baumannii* were the major urinary pathogens. This included majority cases of ureteric calculi, urinary incontinence, pelvic fracture with ruptured urethra, stricture urethra, benign prostatic hyperplasia (BPH), and prolonged catheterization (4).

TheNon-fermenting Gram negative bacteria (NFGNB) are a glossary varying group of aerobic, non-sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively rather than fermentatively (1).

Approximately 15% of isolates from various clinical samples are NFGNB(5). This group includes diverse genera like *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas, Burkholderia, Flavobacterium, Chrysobacterium, Weeksella, Elizabethkingia, Sphingomonas*, *Moraxella, Psychrobacter, Neisseria, Bordetella, Ralstonia* etc (1).

In recent years, NFGNB have emerged as consequential healthcare-associated uropathogens. The correct initiation of empirical treatment in urinary tract infections (UTIs) can have a major impact on the acquisition of resistance by pathogens such as *A. baumannii and P. aeruginosa.*

However, antimicrobial treatment of the infection caused by these agents is difficult due to their multidrug resistance (MDR) to various groups of antibiotics like Beta-lactam, Aminoglycosides and Fluroquinolones posing problem for both treatment and infection control. In recent years due to the non- restrictive and empirical use of antibiotics, NFGNB have emerged as important health associated pathogens, and their isolation rates are increasing.

Keeping this background knowledge in mind the present study was conducted with the objective of speciating NFGNB isolated from various urine specimens from our hospital, assessing their clinical significance, current trend and the present antibiotic resistance pattern of the isolates.

MATERIAL & METHOD

Our study was conducted in the Department of Microbiology, SCBMCH and SVPPGIP, Odisha over a period of eighteen months from march 2019 to august 2020.The present study was under taken to find the proportion of non-fermenting Gram negative bacteria in urine samples and to know their anti- microbial sensitivity pattern.

Appropriately collected and transported, mid-stream urine samples or samples from indwelling or intermittent catheters of both ipd and opdpateints. The patient was given a sterile, dry, wide-necked, leak proof container and requested for a 10-20 ml specimen (6).Samples were either processed within 24 hour at the laboratory or were refrigerated when immediate processing was not possible.

A wet mount of uncentrifuged urine was examined microscopically and presence of > 1 pus cells in 7 HPF was suggestive of UTI. The samples were then plated by a 2mm loop onto CLED for semi-quantitative analysis. Only those isolates, which were found to be significant in semi quantitative culture of urine that is $>10^5$cfu, were included in the study (6).

Yielding growth of Gram negative bacilli also on MacConkey agar and blood agar 35˚C at after 24-48 hours of incubation which turned out to be non fermenters in OF with glucose were included in the study.

Non-fermenting Gram negative bacteria can grow on routine isolation media (BA,CA). Their growth on MacConkey agar is variable, means while all members of the Enterobacteriaceae grow on MacConkey agar, some non-fermenters do not grow on MacConkey agar. Therefore, a Gram negative bacillus that grows on blood agar but grows poorly or not at all on MacConkey agar should be suspected of belonging to the non-fermentive group. And this property used for their identification. Their optimal temperatures of incubation range from 22-35°C. Incubation period of 24 hrs, sometimes 48-72 hrs is required. Usually they grow much better in an aerobic environment (1).NFGNB are presumptively identified by colonial morphology, Gram stain, oxidase activity and pigment production (7).

Any growth from urine that was identified as non fermenters either by VITEK-2 or by additional conventional tests is included in this study. Their antibiotic sensitivity test was performed by VITEK-2 and Kirby-Bauer disc diffusion method using commercially available discs (Hi-media).The results were interpreted as per the CLSI guidelines and *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strain in AST (6).

Samples yielding mixed growth of organisms and growth other than non-fermenting Gram negative bacteria were excluded from the study.

RESULTS

Two hundred sixty three non-fermenters were isolated from 1746 clinical specimens which were submitted to microbiology laboratory. The isolation rate of total NFGNB was found to be 15.06%, out of which 46(17.49%) were from urine specimens. The most common age group yielding NFGNB was found to be 50-75 years.

Males(69%) more commonly yielded NFGNB related UTI as compared to females (31%) .

Amongst the NFGNB most frequently isolated bacteria was *P.aeruginosa* (45.6%) followed by *Acinetobacterbaumanii*(17.39%). Other species like *P.fluorescence, P.putida* and *P.luteola* along with *Acinetobacterlwofii*and *A. junni*were also isolated from urine.

We were able to identify some rare NFGNB like *Burkholderia cepaciagroup* (10.86 %), *Chryseobacteriumindologenes* (2.17 %), *ElizabethkingiaMeningoseptica* (2.17 %), *Sphingomonaspaucimobilis*(6.5 %) *and Stenotrophomonasmaltophilia*(2.17%). (table 1)

Table 1: Bacterial species isolated in urinary tract infections

Organism Isolated	UTI	percentage
Acinetobacterbaumanii complex	8	17.39%
Acinetobacterlwoffii	1	2.17%
Acinetobacterjunii	1	2.17%
Burkholderia cepacia	5	10.86%
Chryseobacteriumindologenes	1	2.17%
Elizabethkingiameningoseptica	1	2.17%
Psuedomonasaeruginosa	21	45.65%
Psuedomonasfluorescens	2	4.34%
Psuedomonasluteola	1	2.17%
Psuedomonasputida	1	2.17%
Sphingomonaspaucimobilis	3	6.52%
Stenotrophomonasmaltophilia	1	2.17%
Total	46	

P.aeruginosa showed 84.9% sensitivity to Polymyxin B/Colistin followed by 67.74% sensitivity to Azetronem, 65.59% sensitivity to Piperacillin - Tazobactam, 62.37% sensitivity to Imipenum and 60.22% sensitivity to Piperacillin.

P.aeruginosa also showed sensitivity of 58.06% to Ceftazidime, 59.14% to Cefepime.

P.aeruginosa was most resistant to Ciprofloxacin 48.39% followed by Gentamycin 46.23% and Amikacin 41.94% (Fig.1).

P.fluorescence, P.luteola, and *P.putida*showed 66.7% sensitivity to Amikacin&Polymyxim B/ Colistin.

They showed maximum resistance of 88.9% to Ciprofloxacin followed by 77.8% resistance to Aztreonam& Gentamycin *Acinetobacter* spp showed most sensitivity to Polymyxin B/Colistin (94.59%) followed by Tigecycline (81.08%) and Ampicillin &salbactum (32.43%) (Fig.2).

They also found 16.22% sensitive to Imipenum and 13.51 % to both Meropenum & PiperacillinTazobactum.

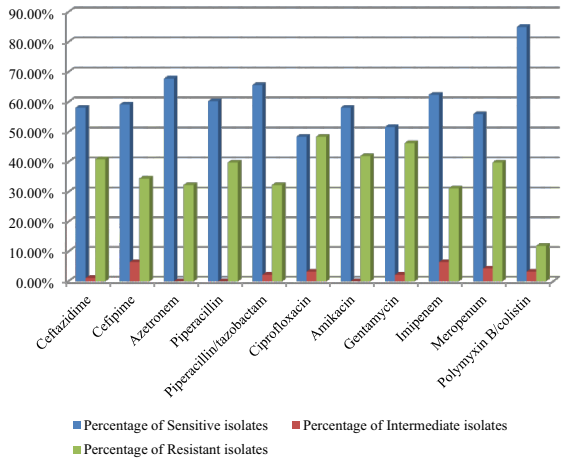

Figure 1: Senstivity pattern of *Psuedomonasaeruginosa.*

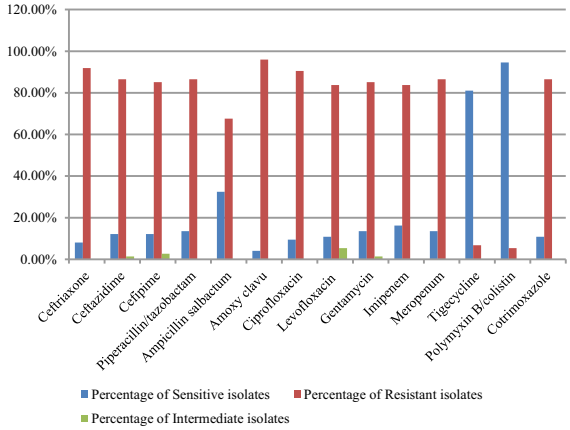

Figure 2: Antibiotics Susceptibility Pattern of *Acinetobacter species*

Acinetobacter spp showed maximum resistance to Amoxycillin & clavulanic acid 95.9% followed by Cefipime (85.1%), Ceftazidime (86.4%), Ceftriaxone (91.8%) .

*Burkholderia*cepacia group showed maximum sensitivity of 77.8% to COT followed by sensitivity of 72.2% to Tigecycline & 61.1% sensitivity to Ceftazidime .

Imipenem showed maximum resistance i.e. 100% followed by resistance of 88.9% to Amikacin, Gentamicin, PIT & Polymyxin B / Colistin.

Sphingomonas Paucimobilis showed maximum sensitivity 72.2% to both Amikacin and Tigecycline followed by 66.7% sensitivity to Piperacillin - Tazobactam,Cefoperazonesalbactum, Meropenem, Levofloxacin &Polymyxim B/Colistin .

It showed most resistance to Ceftazidime (55.6%) followed by Cefepime (50%).

Stenotrophomonasmaltophilia showed sensitivity to Levofloxacin, Cotrimoxazole& Minocycline.

They also showed maximum resistance to Ceftazidime followed by TicarcillinClavulanic acid.

ElizabethkingiaMeningoseptica showed most sensitivity to Vancomycin followed by sensitivity to both Cotrimoxazole&

Clindamycin. It showed maximum resistance to Ciprofloxacin . *Chryseobacteriumindologenes* showed maximum sensitivity to Minocycline. Piperacillin - Tazobactam, Ceftazidime, Cefepime, Amikacin, Gentamicin, Polymyxin B/Colistin & Cotrimoxazole showed 100% resistance.

DISCUSSION

Aerobic Non-Fermenting Gram Negative Bacteria (NFGNB) usually considered as contaminants are emerging as important uropathogens. Their frequent isolation repeatedly from clinical material and their association with disease supports this view (8).

In our study the most common age group yielding NFGNB was found to be 50 -75 yrs which is more or less similar to study of Mahajan et al (9). Since, NFGNB are often opportunistic in nature, their higher isolation rates in elderly also supports this view.

Maximum number of patients found to have NF's infections were males (69%) as compared to females (31%). It was supported by other studies of Rajan et al (10) and Wisplinghoff et al (11) .This male preponderance can be attributed to the fact that it is a male dominate world and males have to go out for lively hood and are more indulged in mechanical labour than females hence many workmen in this area engage in activity that involves strain and which makes them more prone to infections.

In present study *Acinetobacterspp*showed 94.59% sensitivity to Polymyxin B / Colistin , 81.08% to Tigecycline, 32.43% to Ampicillin &Salbactum. *Acinetobacter*spp shows least sensitivity to Amoxycillin & Clavulanic acid 4.05%. Cefipime, Ceftazidine, Ceftriaxone showed sensitivity of 12.16%, 12.16% & 8.11% respectively. Imipenum, Meropenum & Piperacillin - Tazobactum showed sensitivity of 16.22%, 13.51 & 13.51%.

Alike study conducted by Patel et al, found *Acinetobacterbaumanii* were sensitive to Imipenem in 72.9% followed by Amikacin (38.8%), Doxycycline (22.3%), Cotrimoxazole (10%) and for Cephalosporins sensitivity is less than 10% (12).

A recent study conducted by Kakati et al observed that a high resistance to Imipenem by *Acinetobacter*isolates (90.54%) & to Meropenem (95.2%) (13).

In our study *P.aeruginosa* showed 84.9% sensitivity to Polymyxin B/Colistin, 67.74% sensitivity to Azetronem, 65.59% sensitivity to Piperacillin - Tazobactam, 62.37% sensitivity to Imipenum, 60.22% sensitivity. *P.aeruginosa* showed sensitivity of 58.06% to Ceftazidime, 59.14% to Cefepime& 48.39% sensitivity to Ciprofloxacin.

They showed maximum resistance of 88.9% to Ciprofloxacin followed by 77.8% resistance to Aztreonam & Gentamycin.

In a similar study by Patel et al the isolates of *P.aeruginosa, 94%* were sensitive to Imipenem, Piperacillin -Tazobactam (75.9%), Amikacin (39.6%), Aztreonam (39.8%), Cefepime (28.7%) Ceftazidime (24.6%) and Ciprofloxacin (16.53%) (12).

In a study done by Mahajan et al *P.aeruginosa* isolates were highly susceptible to were highly susceptible to Colistin (96.79%), Amikacin, Tobramycin (75%), Piperacillin - Tazobactam (62.85%) and Imipenem (59.61%) which was similar to our study (9).

In a study done by Kotwal et al observed resistance to Ciprofloxacin (82%) in maximum number of isolates followed by that to Gentamycin (77%), Cefepime (74%), Amikacin (60%), and Cefoperazone (56.6%) (14).

In our study *Burkholderia cepacia* group showed maximum sensitivity of 77.8% to COT followed by sensitivity of 72.2% to Tigecycline & 61.1% sensitivity to Ceftazidime. Imipenem showed maximum resistance of 100% followed by resistance of 88.9% to Amikacin, Gentamicin, PIT &Polymyxin B/Colistin .

Mahajan et al also studied 80% sensitivity to COT, 60% to Ceftazidime which is similar to our study (9).

our study *ElizabethkingiaMeningoseptica* showed sensitivity to Vancomycin followed by sensitivity to Cotrimoxazole & Clindamycin. It showed resistance of to Ciprofloxacin and is in accordance with the study done by kakatiet al, who showed their 100% resistance to Imipenem&Meropenam (13).

In present study *Stenotrophomonasmaltophilia* showed sensitivity to Levofloxacin, Cotrimoxazole& Minocycline. TCC and Ceftazidime also showed sensitivity to it.

The results of our study are in accordance with the work done by Rahbar et al who studied that 67% sensitivity to Cotrimoxazole & Ceftazidime showed 81% sensitivity (15).

The reason, for these variation in results of NF's in different studies may be due to existing local difference in emergence of drug resistant bacteria. And can be also be explained by the fact that NF's are found in nature as inhabitants of soil and water. Hence, these findings may be due to differences in the prevalence of NF's in different geographical areas.

The NFGNB have been proved beyond doubt to be uropathogens. Since they differ in their pathogenic potential, transmissibility and antibiotic susceptibility, their accurate identification is important for appropriate patient management.

CONCLUSION

NFGNBs are primarily opportunistic pathogens causing urinary tract infection especially in seriously ill hospitalised and immunocompromised patients. NFGNB are confirmed to have major role as important uropathogens.

Isolation rate of non-fermenting gram negative bacilli in our hospital in UTI was 17.49 %. *Psuedomonasaeruginosa* (45.6 %) was the commonest isolate followed by *Acinetobactercalcoacetecusbaumanii* complex (17.39 %). The most effective antibiotics for urinary tract NFGNB wereTigecycline and Polymyxin B/ Colistin. Most of the NFs isolated were resistant to Penicillin group of drugs, Gentamicin, Ciprofloxacin.

We were able to identify and isolate some infrequent emerging NFGNB like *Burkholderia cepacia group, Chryseobacteriumindologenes, ElizabethkingiameningosepticaSphingomonaspaucimobilis* and *Stenotrophomonasmaltophilia.*

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