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Original Article

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Battling urinary tract infections: Unravelling the etiological spectrum and resistance repertoire of causative non-fermenting Gram-negative bacilli

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ABSTRACT

Objective: To ascertain the microbial landscape of uropathogenic non-fermenting Gram-negative bacilli (NFGNB) and their antimicrobial susceptibility profile in an Indian tertiary care hospital setting.

Methods: A retrospective observational study was conducted from May to October 2024, at a tertiary care hospital in India. Urine specimens were processed and bacterial identification was performed as per standard protocol. Antimicrobial susceptibility testing was performed in accordance with Clinical and Laboratory Standard Institute guidelines. The etiological and antimicrobial susceptibility profiles of the uropathogenic NFGNB isolates were retrieved from laboratory records and analyzed.

Results: Among the 24286 urine specimens received during the study period, 12.3% (2992/24286) showed significant bacteriuria, of which 5.7% (171/2992) were identified as NFGNB. The predominant NFGNB was *Acinetobacter* spp. (48.5%) followed by *Pseudomonas* spp. (48.0%, 82/171), with *Acinetobacter baumannii* being the most common NFGNB (42.1%, 72/171) isolated. *Acinetobacter baumannii* exhibited significantly lower susceptibility to carbapenems (38.9%–44.4%) compared to *Pseudomonas aeruginosa* (69.6%–75.4%) (P=0.001). Among the NFGNB, 56.5% were multidrug-resistant, 34.1% extensively drug-resistant and 15.9% pandrug-resistant. Multidrug-resistant and extensively drug-resistant rates were significantly higher in *Acinetobacter* species than in *Pseudomonas* species (P=0.001 and P=0.002, respectively) with inpatient isolates showing significantly lower antimicrobial susceptibility patterns.

Conclusions: The high prevalence of multidrug-resistant strains of uropathogenic NFGNB, especially in hospitalized patients, underscores the need for strengthened infection prevention and control practices and continuous ongoing surveillance activities to gain a more comprehensive understanding and improve clinical outcomes.

KEYWORDS: Antimicrobial resistance; Multidrug resistance; Nonfermenting Gram-negative bacilli; Uropathogens

1. Introduction

Urinary tract infections (UTIs) are one of the most frequent bacterial infections in the community and in hospital settings, resulting in high morbidity as well as substantial number of deaths[1]. With the evolution and expansion of multidrug resistant

Significance

Non-fermenting Gram-negative bacilli are increasingly recognized as uropathogens, yet their epidemiology and resistance trends remain underreported. This study outlines their prevalence and antimicrobial susceptibility profile in an Indian tertiary-care setting, revealing alarming resistance rates, particularly in hospitalized patients. The findings underscore the need for multicentric surveillance, molecular characterization of resistance, and strengthened infection prevention measures to curb the spread of multidrug-resistant strains and improve patient outcomes.

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pathogens, these infections now present as a therapeutic conundrum with a restricted repertoire of effective antimicrobials available. Nonfermenting Gram-negative bacilli (NFGNB) are ubiquitous organisms that are increasingly implicated in UTIs, accounting for approximately 12%-16% of these infections in India[2,3] and 6.4%-31.5% at the global level[4-6]. These pathogens are unable to ferment or utilize glucose for vital cellular processes, which is why they are aptly classified as nonfermenters[7].

NFGNB are found as commensals in the human gut[8]. However, with advanced microbiological diagnostic tools and accurate identification techniques, these pathogens that were perhaps previously overlooked, are being detected and characterized at an enhanced rate and are now linked to serious health concerns, particularly in children and in immunosuppressed and debilitated patients. Multiple virulence factors like adhesins, flagella, pili, toxic pigments, enzymes and siderophores, have been identified in these NFGNB and play a critical role in the pathogenesis of UTIs caused by them[9,10]. The biofilm producing potential of these organisms is also a pivotal determinant of their virulence, more so in patients with indwelling medical devices like urinary catheters[11-13]. Another grave therapeutic concern is the alarming rise of multidrug resistant NFGNB[4,7,14].

The etiological spectrum and prevalence of NFGNB in UTIs vary across healthcare settings. Regional epidemiology as well as spatial and temporal variations in antimicrobial susceptibility profile of these uropathogenic NFGNB is often not captured, the knowledge of which is otherwise mandatory in order to guide appropriate antimicrobial choices for managing infections caused by them[1]. Moreover, while there is sufficient literature that extensively studies and focuses on the role of Enterobacterales in causing UTIs[15,16], there is a paucity of research that addresses UTIs caused by NFGNB. Therefore, the present study was conducted to ascertain the microbial landscape of uropathogenic NFGNB isolated in a tertiary care setting and to determine their susceptibility profile to various antimicrobial agents.

2. Methods

2.1. Study design and setting

The present observational, retrospective, record-based analysis was conducted at an Indian tertiary care hospital, over a six-month period spanning from May to October 2024.

2.2. Inclusion criteria

NFGNB isolated from urine specimens of patients attending the inpatient and outpatient services of the hospital were included in the study.

2.3. Exclusion criteria

Repeat NFGNB isolates from the same patient, recovered during the same episode of UTIs, were excluded from analysis. Also, mixed bacterial growth with ≥3 organisms isolated on culture was considered as contamination and excluded from the analysis.

2.4. Sample processing

Urine specimens received at the routine bacteriology laboratory at the Department of Microbiology during this period, were processed for microscopy and aerobic bacterial culture by semi-quantitative method as per standard protocol[17]. Bacterial isolates were identified using the matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) platform (VITEK-MS®, BioMerieux, France). Antimicrobial susceptibility testing of the isolates was performed using both Kirby Bauer's disc diffusion method and VITEK-2 Compact system (for minimum inhibitory concentrations testing for *Pseudomonas* spp. other than *Pseudomonas (P.) aeruginosa* and *Burkholderia cepacia* complex) as applicable, in accordance to the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines[18]. Quality control was ensured using standard strains. The isolates were also tested for colistin resistance by colistin agar dilution method and the results further confirmed by colistin broth microdilution method as per CLSI M100 document[18]. The details of the organisms identified as NFGNB and their antimicrobial susceptibility profile were extracted from the laboratory records. All the included isolates were patient de-identified.

2.5. Definitions

Definitions as proposed by Magiorakos *et al.* were used to classify drug resistant organisms. Bacterial isolates that exhibited resistance to at least three or more categories of antimicrobials of different groups were classified as multidrug resistant (MDR), while those resistant to at least one antimicrobial agent in all but one or two antimicrobial categories were designated as extensively drug resistant (XDR). Further, isolates exhibiting resistance to almost all available antibiotics were referred as to pan-drug resistant (PDR)[19].

2.6. Statistical analysis

Data were compiled using the WHONET 2024 and Microsoft Excel software and further analyzed using the SPSS statistics software (Version-25.0, IBM Statistical Product and Service Solutions, NY, USA). Results for quantitative variables were expressed as mean±standard deviation and median, while the qualitative data was depicted as percentages, bar graphs *etc*. Further,

analysis for significance was done using the *Chi*-square test, with statistical significance denoted at $P < 0.05$.

2.7. Ethical statement

The study received approval from the Institutional Ethics Committee of Maulana Azad Medical College and Associated Hospitals, New Delhi, India (Ethical approval number: F1./IEC/MAMC/112/05/2024/No.24). The study involved only retrospectively analysis of data of patient de-identified and anonymized clinical isolates, with no direct contact with patients. The study also did not in any way influence or interfere with patient management.

3.Result

3.1. Bacterial isolation rates from urine specimens

A total of 24286 urine specimens were processed during the study period, among which 2992 (12.3%) urine specimens had bacterial pathogens isolated in significant counts. Of these 5.7% (171/2992) isolates were identified as NFGNB.

3.2. Clinico-demographic profile of patients with NFGNB isolates

The median age of the patients in whom NFGNB were isolated was 32 years (range: 1 month - 80 years). Around 56.7% of these NFGNB isolates were from females. The majority of NFGNB isolates were obtained from hospitalized patients (57.3%, 98/171), with 9.2% (9/98) originating from patients admitted to the intensive care unit. Majority of NFGNB were isolated from patients admitted to the departments of obstetrics and gynecology (41.5%), followed by surgery (32.2%) and medicine (13.5%).

3.3. Species distribution and pathogen profile of uropathogenic NFGNB

With regard to pathogen profile and species distribution of various NFGNB causing UTIs in our set-up, *Acinetobacter* spp.(48.5%, 83/171) and *Pseudomonas* spp. (48.0%, 82/171) were the most prevalent, followed by *Stenotrophomonas maltophilia* (1.8%, 3/171), *Burkholderia cepacia* complex (1.2%, 2/171) and *Sphingobacterium thalpophilum* (0.6%, 1/171) (Figure 1). Overall *Acinetobacter (A.) baumannii* (42.1%, 72/171) was the predominant uropathogenic NFGNB, with a significantly higher rates of isolation in hospitalized patients compared with those in outpatient settings (68.1% *vs.* 31.9%; P=0.015). On the other hand, *P. aeruginosa* was isolated at similar rates in both inpatient and outpatient settings (50.7% *vs.* 49.3%; P=0.152).

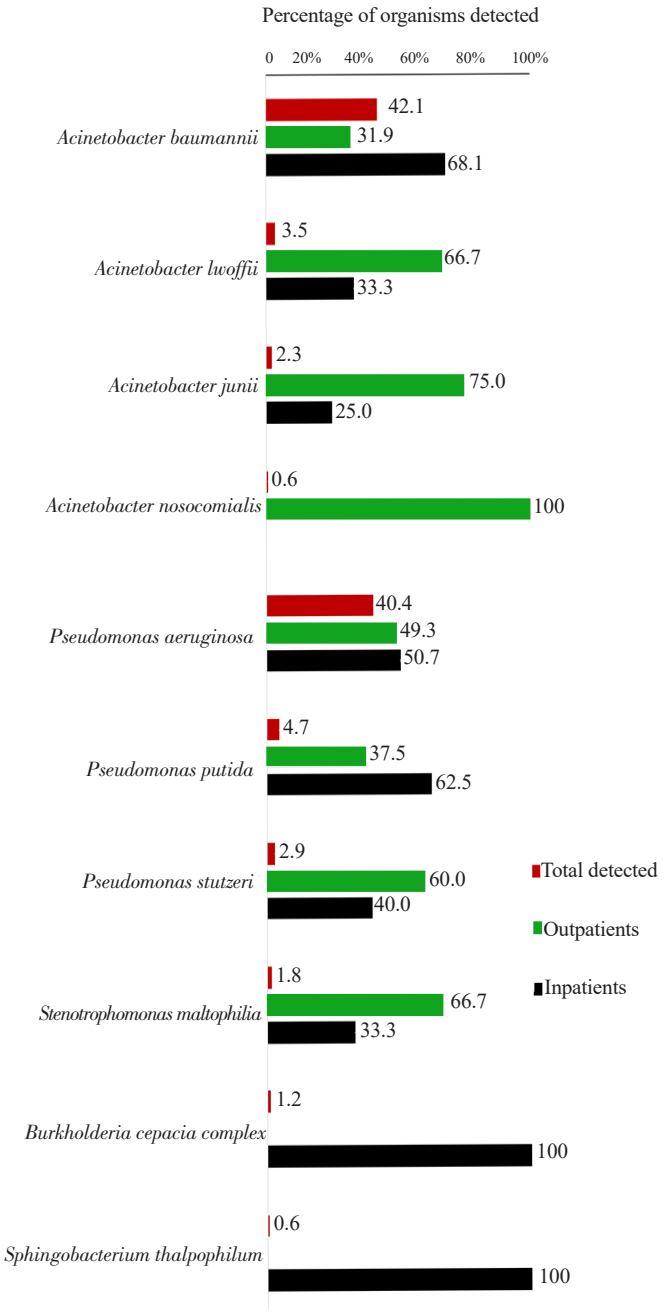

Figure 1. Pathogen profile and species distribution of NFGNB isolated from urine specimens (n=171).

3.4. Antimicrobial susceptibility profile of NFGNB isolates

Antimicrobial susceptibility profile of these uropathogenic NFGNB revealed that *A. baumannii* demonstrated markedly lower susceptibility to various antimicrobials compared to other *Acinetobacter* species. The susceptibility to carbapenems also differed markedly between *A. baumannii* and *P. aeruginosa* (38.9%-44.4% *vs.* 69.6%-75.4%) with the former exhibiting significantly lower susceptibility (P=0.001) (Table 1). All isolates of *Acinetobacter* and *Pseudomonas* species in our study showed no resistance to

Table 1. Antimicrobial susceptibility profile (%) of uropathogenic non-fermenting Gram-negative bacilli (*n*=170).

Organisms / Antibiotics	*Acinetobacter* spp. (*n*=83)	*Acinetobacter baumannii* (*n*=72)	*Acinetobacter* spp. (*non−baumannii*) (*n*=11)	*Pseudomonas* spp. (*n*=82)	*Pseudomonas aeruginosa* (*n*=69)	*Pseudomonas* spp. (non-aeruginosa) (*n*=13)	*Burkholderia cepacia* complex (*n*=2)	*Stenotrophomonas maltophilia* (*n*=3)
Cephalosporins								
Ceftazidime	25.3	18.1	72.7	67.1	71	46.2	NA	NA
Cefepime	42.2	33.3	100	79.3	79.7	76.9	NA	NA
Fluoroquinolones								
Ciprofloxacin	36.1	30.6	72.7	69.5	71	61.5	NA	NA
Levofloxacin	48.2	41.7	90.9	73.2	73.9	69.2	NA	100
Penicillins								
Piperacillin – Tazobactam	16.9	12.5	45.5	76.8	79.7	61.5	NA	IR
Ampicillin - Sulbactam	57.8	51.4	100	IR	IR	IR	IR	IR
Carbapenems								
Imipenem	47	38.9	100	65.9	69.6	46.2	100	IR
Meropenem	51.8	44.4	100	72	75.4	53.8	NA	IR
Aminoglycosides								
Amikacin	48.2	40.3	100	72	75.4	53.8	NA	IR
Gentamicin	51.8	45.8	90.9	-	-	-	NA	IR
Netilmicin	-	-	-	73.2	75.4	61.5	100	IR
Tobramycin	-	-	-	72	75.4	53.8	NA	IR
Tetracyclines								
Tetracycline	45.8	40.3	81.8	-	-	-	NA	IR
Minocycline	72.3	69.4	90.9	IR	IR	IR	100	66.7
Sulfonamides/ Trimethoprim								
Cotrimoxazole	54.2	51.4	72.7	IR	IR	IR	100	100
Monobactams								
Aztreonam	IR	IR	IR	41.5	42	38.5	NA	IR

Note: -: Not tested against the antimicrobial; NA: Not applicable; IR: Intrinsic resistance.

colistin. The antimicrobial susceptibility profile of *Sphingobacterium thalpophilum* (*n*=1) was not tested, as guidelines were not available.

Among the NFGNB tested, 56.5% (96/170) of the isolates were MDR, 34.1% (58/170) were XDR and 15.9% (27/170) were PDR. The Table 2 shows that the differential prevalence of MDR strains among *Acinetobacter* and *Pseudomonas* species was statistically significant (75.9% *vs.* 40.2%; *P*=0.001). Likewise, significantly higher rates of XDR strains were noted in *Acinetobacter* spp. compared to *Pseudomonas* species (46.9% *vs.* 23.2%; *P*=0.002). However, the percentage of PDR strains did not differ significantly between the two pathogens (15.7% *vs.* 17.1% ; *P*=0.415).

It was also observed that percentage susceptibility to different antimicrobials was significantly higher for outpatient isolates of *Acinetobacter* and *Pseudomonas* species in comparison to those recovered from hospitalized patients (Table 3).

Table 2. Resistance patterns in *Acinetobacter* spp. as compared to *Pseudomonas* spp.

Resistance pattern	*Acinetobacter* spp. (*n*=83)	*Pseudomonas* spp. (*n*=82)	*P*
MDR	75.9%	40.2%	0.001*
XDR	46.9%	23.2%	0.002*
PDR	15.7%	17.1%	0.415

Note: *$P<0.05$; MDR: Multidrug resistant; XDR: Extensively-drug resistant; PDR: Pan-drug resistant.

4. Discussion

Life threatening infections caused by NFGNB have emerged as a serious global health concern specifically due to their highly heterogenous virulence factors and extensive antimicrobial resistance. This leads to increased sequelae, morbidity and mortality in the affected patients[4,9]. The increased detection of NFGNB in UTIs combined with their reduced susceptibility to various antimicrobials in our study, reiterates the fact that these pathogens should not be overlooked from an epidemiological perspective and should be brought under the ambit of heightened surveillance practices and tailored infection control strategies.

In the present study, NFGNB were isolated from 5.7% of specimens with significant bacteriuria, comparable to a 10-year retrospective study from Hungary reporting a 6.4% isolation[4]. In contrast, a higher proportion of NFGNB isolates among uropathogens were observed from Nepal (17.5%)[5], Saudi Arabia (31.5%)[6] and other studies from India (33.9%)[20]. The rising incidence of UTIs attributable to NFGNB has been linked to multiple predisposing factors, including metabolic disorders such as diabetes mellitus, weakened immune function, benign prostatic

Table 3. Susceptibility (%) of *Acinetobacter* and *Pseudomonas* species among hospitalized patients and outpatients.

Organism/Antibiotics	*Acinetobacter* spp.		*P*	*Pseudomonas* spp.		*P*
	Outpatients(*n*=31)	Inpatients(*n*=52)		Outpatients(*n*=40)	Inpatients(*n*=42)	
Ceftazidime	38.7	17.3	0.030*	80	54.8	0.015*
Cefepime	58.1	32.7	0.024*	90	69.0	0.019*
Ciprofloxacin	58.1	23.1	0.001*	82.5	57.1	0.013*
Levofloxacin	74.2	32.7	0.000*	85	61.9	0.018*
Piperacillin-tazobactam	35.5	5.8	0.000*	90	64.3	0.006*
Imipenem	87.1	23.1	0.000*	82.5	50	0.002*
Meropenem	87.1	30.8	0.000*	85	59.5	0.010*
Amikacin	83.9	26.9	0.000*	87.5	57.1	0.002*
Gentamicin	83.9	32.7	0.000*	-	-	-
Tetracycline	64.5	34.6	0.008*	-	-	-
Cotrimoxazole	77.4	40.4	0.001*	IR	IR	NA
Minocycline	87.1	63.5	0.020*	IR	IR	NA
Ampicillin-sulbactam	87.1	40.4	0.000*	IR	IR	NA
Netilmicin	-	-	-	85	61.9	0.018*
Tobramycin	-	-	-	82.5	61.9	0.038*
Aztreonam	IR	IR	NA	57.5	26.2	0.004*

Note: *P<0.05; - : Not tested against the antimicrobial; IR: Intrinsic resistance; NA: Not applicable.

hyperplasia, post-menopausal estrogen deficiency, and extended hospitalization particularly in patients with prior antibiotic therapy or those requiring long-term urinary catheterization[1,9,21]. In the current study, females represented a higher proportion of affected patients than males. These findings align with the observations of Berwal *et al*.[20] who reported a similar gender distribution in a south Indian cohort. In our study, UTIs caused by NFGNBs were more commonly observed in hospitalized patients, particularly those from the department of obstetrics and gynaecology followed by surgery. These findings are in line with a study by Yadav *et al.*[5] from Nepal, where the highest proportion of NFGNB isolates were reported from the surgery (32.7%) and obstetrics (26.%) departments, possibly reflecting the greater frequency of invasive procedures and interventions involving urinary tract manipulation in these departments.

The pathogen profile as observed in our study is in agreement with similar research conducted in other Asian countries[6,20,22], with *Acinetobacter* and *Pseudomonas* species being the predominant uropathogenic NFGNB isolated. *A. baumannii* was identified as the most prevalent NFGNB pathogen in our study, with a significantly higher association seen in hospitalized patients compared to outpatients. This is probably attributable to risk factors such as prolonged catheterization and invasive procedures, creating a conducive environment for colonization by multidrug-resistant NFGNB[4,21,23]. Additionally, inadequate disinfection practices and the potential colonization of healthcare personnel may contribute to the persistence and transmission of NFGNB, further exacerbating the infection rates within hospital settings[1,4,23].

A. baumannii exhibited the lowest susceptibility to fluoroquinolones, β-lactams and β-lactam-β-lactamase inhibitor combinations, consistent with findings by Mahajan *et al.*[24] who reported susceptibility rates of 12% and 22% to β-lactams and fluoroquinolones respectively. In the current study, carbapenem susceptibility was significantly lower in *A. baumannii* compared to *P. aeruginosa*, aligning with findings from a 5-year surveillance study by Indian Council of Medical Research[25] which reported a lower proportion of carbapenem susceptible *A. baumannii* (25%-29.8%) than *P. aeruginosa* (46.4%-50.5%) in urine specimens, and a rise in serine and metallo-β-lactamase (MBL) co-producing carbapenem-resistant *A. baumannii*. Similarly, a prior multicentric study conducted in India from 2014 to 2017 observed an increase in the carbapenemase-producing *P. aeruginosa* from 33% to 86%, highlighting the growing threat of multidrug resistant *P. aeruginosa* in clinical settings[26]. Consequently, the WHO has designated these pathogens as critical and high priority pathogens respectively, due to their resistance to multiple antibiotic classes and associated therapeutic difficulties[27]. These findings are consistent with the current study, highlighting the evolving resistance and rising threat of uropathogenic NFGNB.

The burden of MDR and XDR NFGNB in UTIs remains under-reported, with few studies offering a comprehensive overview. In this context, the present study identified 56.5% of uropathogenic NFGNB as multidrug-resistant, including 34.1% XDR and 15.9% PDR, with *Acinetobacter* species demonstrating the highest levels of resistance. High rates of MDR and XDR among NFGNB, particularly *A. baumannii*, have been reported in India[2], Iran[28], and Romania[29]. A cross-sectional study by Namaei *et al.*[28] found a significantly higher prevalence of MDR-NFGNB (66% *vs.* 12.5%) and XDR-NFGNB (55.7% *vs.* 12.5%) in hospitalized patients compared to outpatients. These findings are consistent with the present study, where inpatient isolates exhibited significantly higher resistance. This underscores the role of hospital-associated selective pressure primarily driven by the use of broad-spectrum antibiotics in promoting resistance development, particularly through acquired

mutations or adaptation to prolonged antimicrobial therapy[1,9,14].

Our study captures data from a major tertiary care center in North India and thus offers valuable insights into regional etiological profile and antimicrobial susceptibility patterns of NFGNB causing UTIs. The study, however is fraught with certain limitations. As a retrospective, record-based analysis, the study lacked detailed clinical profiles and patient outcomes, limiting the ability to delineate risk factors or correlate laboratory findings with specific comorbidities or assess the burden of healthcare-associated UTIs. Furthermore, the study's single center design and relatively short duration may hinder its ability to capture a broader snapshot of epidemiological trends in a geographically and demographically diverse country like India, where antimicrobial resistance rates are high and variable[25]. Also, we did not conduct molecular studies to investigate the underlying resistance mechanisms.

5. Conclusions

Our study underscores the imperative for collaborative, multicentric surveillance studies to gain a more comprehensive understanding of UTIs caused by NFGNB. Additionally molecular studies should be conducted to elucidate the genetic mechanisms underlying resistance dynamics. It also highlights the urgent need for strengthened infection prevention and control practices, a robust antimicrobial stewardship programme, and continuous ongoing surveillance activities to curb the spread of MDR-NFGNB and improve clinical outcomes.

Conflict of interest statement

The authors claim there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Mitra S and Mehra B conceived the study, obtained ethical approval, validated, investigated, analyzed the data, reviewed for critical content and contributed in drafting and editing the manuscript. Aggarwal P was responsible for the organization, coordination and management of the study. Guleria A helped in management of the data. Saxena S provided general supervision, project administration and helped in final review and approval of the manuscript. All authors read and approved the final manuscript.

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