Antimicrobial resistance pattern of Uro-Pathogens emphasizing non-lactose fermenting gram negative bacilli

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Abstract

Objective: To identify various species of non-lactose fermenting gram-negative bacilli involved in urinary tract infections, and to determine their antimicrobial resistance pattern.

Method: The retrospective, descriptive, cross-sectional study was conducted from January 1 to April 1, 2022, at the Dow University of Health Sciences, Karachi, and comprised data from the institutional diagnostic laboratory that was related to urine samples regardless of age and gender from January 1, 2020, to December 31, 2021. Data was analysed using SPSS version 25.

Results: Of the 103,887 urine samples, 41,280(39.7%) were positive, 51,146(49.2%) showed no bacterial growth, 11,000(10.6%) had non-significant bacterial growth and 461(0.4%) had mixed bacterial growth. Of the positive samples, 18359(44.5%) were positive in 2020, and 22,921(55.5%) in 2021. Gram-negative lactose fermenting bacteria included escherichia coli 23,123(22.3%) and klebsiella pneumoniae 2,993(2.9%), gram-negative non-lactose fermenting bacteria included pseudomonas aeruginosa 1,110(1.07%), and gram-positive bacteria included enterococcus 8,008(7.7%). Pseudomonas aeruginosa was most resistant against tobramycin 880(79.3%) and least resistant against piperacillin-tazobactam 146(13%).

Conclusion: Piperacillin-tazobactam was highly sensitive drug against non-lactose fermenting uro-pathogens.

Key Words: Antimicrobial resistance, Non-lactose fermenting, Gram-negative bacteria, Urinary tract infection.

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Introduction

Gram-negative bacilli are common pathogens associated with urinary tract infections (UTIs). These gram-negative bacilli are becoming more resistant to several antibiotics with time.[1] The gram-negative organisms that cause frequent onset of UTIs are mainly escherichia (E.) coli and klebsiella (K.) pneumonia.[2] UTI is among the most common bacterial infections affecting all age groups. UTI is one of the major public health concerns in terms of illness and financial burden, contributing greatly to healthcare expense. It has been recently estimated in the United States that around 8.6 million healthcare visits were made annually, resulting in serious global economic burden[3]. Prevalence of UTI is affected by multiple factors, such as old age, poor personal hygiene, pregnancy, urinary catheterisation, genitourinary tract abnormalities, and co-morbidities, including diabetes and human immunodeficiency virus (HIV) infections. Clinically, UTI may present as asymptomatic, acute, chronic, complicated, or uncomplicated, depending upon the type of infectious agent, part of the urinary tract involved, and patient's immune response.[4] Most UTIs are treated empirically based on the culture and sensitivity pattern generated by microbiology laboratories. The spectrum of antimicrobial susceptibility of UTI pathogens varies from time to time and in different geographic areas. Therefore, regular monitoring of the antibiotic susceptibility pattern of these uro-pathogens is required for reduction and eradication of antimicrobial resistance. The antimicrobial resistance against broad-spectrum antibiotics is increasing with every passing day owing to excessive and irrational utilisation, self-medication, mal-drug disposal practices, and genetic proliferation among bacterial species.[5]

UTIs are more common in females than males due to anatomical proximity of urethra to the gut opening.[6] UTI signifies common illnesses confronted in medical practice today, with an expected 150 million UTIs each annum globally. Around 20% of UTIs are documented to be found in males. In healthy women, uncomplicated UTIs have a prevalence of 50/1000/time.[7] Besides, 50% of females experience UTI incidence at least once in their lifetime, and 20-40% females have chronic episodes.[8]

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Most incidents of UTI are triggered by E. coli, while K. pneumoniae, staphylococcus (S.) saprophyticus and proteus species represent the remaining infections.[2] Susceptibility data from regional microbiological services help in the empirical range of antibiotics for treatment of UTI, though such data is restricted to complex UTIs as uncomplicated UTI samples are hardly sent to the laboratories. The growing prevalence of drug resistance amongst uro-pathogens is considerable for public health interest, requiring continuous antibiotic susceptibility screening for bacteria causing UTI. Additionally, antimicrobial sensitivity for UTI-causing bacteria is documented to change with time and location. Thus, screening for sensitivity is significant for creating up-to-date epidemiological information.[9] The heterogeneous group of proteobacteria, non-lactose-fermenting gram-negative bacteria (NLF-GNB) is distinguished by the failure to ferment carbohydrates for energy production to carry out their essential cellular purposes. In UTIs, NLF-GNBs must not be overlooked as they are essential bacteria from the epidemiological perspective with greater prevalence.[10] These contain, in descending order of occurrence, pseudomonas, acinetobacter, Burkholderia cepacia complex (BCC), and stenotrophomonas (xanthomonas) maltophilia. Additionally, there are achromobacter, alcaligenes, brevimundas, elisabethkingia, flavobacterium, ralstonia and others. Some less predominant members, for instance Burkholderia (B.) mallei and B. pseudomallei, acquire significance as bioterrorism representatives.[11] They are very common in cunning infections, impacting harshly immunocompromised and weakened patients aged around 60 years.

The current study was planned to identify various species of NLF-GNB involved in UTIs, and to determine their antimicrobial resistance pattern.

Materials and Methods

The retrospective, descriptive, cross-sectional study was conducted from January 1 to April 1, 2022, at the Dow University of Health Sciences (DUHS), Karachi, and comprised data from the institutional diagnostic laboratory that was related to urine samples regardless of age and gender from January 1, 2020, to December 31, 2021.

Duplicate samples, catheter specimens, specimens with growth of candida species, data of antibiotic susceptibility patterns of all isolated gram-positive bacteria and lactose-fermenting gram-negative bacteria of other sources, like blood, pus, pleural fluids, etc., were excluded.

After approval from the institutional ethics review committee, midstream urinary specimen was inoculated on Cystine lactose electrolyte deficient (CLED) agar (Oxoid, United Kingdom) by colony count quantitative method using 0.001ml loop by sterile microbiological method. After 18-24 hours of incubation at 37°C oxygen (O2) plate was observed for growth, if any. Re-incubations were performed for the plates with no growth after another 24 hours of incubation. Existence of 105CFU/ml was considered significant.

Growth of non-lactose fermenting colonies on MacConkey agar was later identified by colony's morphology, gram staining and various biochemical tests, including catalase test, oxidase reaction, citrate test, sulfur indole motility (SIM) test, triple sugar iron (TSI) test and urease test, while the final confirmation was done by Analytical Profile Index ()API 20E and API 20NE (bioMerieux, France), as needed. Antibiotic susceptibility test was done using the Kirby-Bauer disk diffusion test protocol with 0.5 McFarland turbidity standard.[12]

Antimicrobial discs (Oxoid, UK; Bioanalyse, Turkey) included amikicin (AMK; 30µg), gentamicin (CN; 10µg), tobramycin (TOB; 10ug), ampicillin (AMP; 10µg), amoxiclav (AMC; 20/10µg), cefuroxime (CXM; 30ug), ceftriaxone (CRO; 30µg), cefixime (CFM; 5ug), ceftazadime (CAZ; 30µg), pipracillin-tazobactam (TZP; 100/10µg), meropenem (MEM; 10µg), levofloxacin (LEV; 5ug), ciprofloxacin (CIP; 5ug), trimethoprim/sulphamethoxazole (SXT; 1.25/23.75µg). The inverted plates were incubated at 37°C for 16-18 hours. The zone of inhibition was measured by scale and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines[13].

Data was analysed using SPSS version 25.

Results

Of the 103,887 urine samples, 41,280(39.7%) were positive, 51,146(49.2%) showed no bacterial growth, 11,000(10.6%) had non-significant bacterial growth and 461(0.4%) had mixed bacterial growth.

Of the positive samples, 18,359(44.5%) were positive in 2020, and 22,921(55.5%) in 2021. Of the positive sample 29,954(72.6%) were from females and 11,326(27.4%) from males, and 10,283(26.2%) from those aged 41-60 years, followed by 9124(22%) from those aged 61-80 years (Table 1).

Gram-positive isolates included enterococcus (2020: 3,286[73%], 2021: 4,722[73%]}, streptococcus group D (2020: 481[11%] 2021: 635[10%]), streptococcus species (2020: 381[8%] 2021: 584[9%]), S. aureus (2020: 180[4%]

Table-1: Mean organ weights (g) and ventricle thicknesses (cm) of the study population.

Number of Isolates	Year 2020		Year 2021	
Gender wise age groups (Year)	Male (n, %)	Female (n, %)	Male (n, %)	Female (n, %)
0-20	998 (35%)	1648 (65%)	882(26%)	2555 (74%)
20-40	861 (15%)	5273 (85%)	1217 (17%)	5777 (83%)
41-60	1297 (27%)	3409 (73%)	1776 (30%)	4341 (70%)
61-80	1709 (40%)	2572 (60%)	1940(41%)	2903 (59%)
>80	268 (45%)	324 (55%)	378 (25%)	1152 (75%)
Total	5133 (28%)	13226 (72%)	6193 (27%)	16728 (73%)

2021: 236[3.6%]), coagulase-negative staphylococcus species (2020: 173[3.8%], 2021: 240[3.7%]) and S. saprophyticus (2020: 28[0.6%], 2021: 23[0.3%]). Gram-negative lactose fermenters comprised E. coli (2020: 10,873[86%], 2021: 12,250[82%]), K. pneumoniae (2020: 1,226[9.7%], 2021: 1,767 [11.8%]), enterobacter species (2020: 233[2%], 2021: 565[4%]), klebsiella species (2020: 188[1%], 2021: 36[0.2%]), K. oxytoca (2020: 20[0.15%], 2021: 222[1.49%]), citrobacter species (2020: 12[0.09%], 2021: 12[0.80%]), citrobacter frendi (2020: 7[0.03%], 2021: 1[0.006%] and citrobacter koseri (2020: 0[0%], 2021: 1[0.006%]). Non-lactose fermenters numbered 1,271 in

Table-2: Antibiotic resistance pattern of uro-pathogens.

	Pseudomonas aeruginosa		Pseudom-onasspp		Acineto-bacterspp		Proteus mirabilis		Proteus vulgaris		Morganella morganii		Providenciaspp		Sternophomasm altophila		Aeromonassp	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Anti biotics	(n=498)	(n=612)	(n=30)	(n=35)	(n=332)	(n=465)	(n=185)	(n=190)	(n=44)	(n=39)	(n=88)	(n=82)	(n=6)	(n=4)	(n=10)	(n=17)	(n=14)	(n=32)
AMK	93 (19%)	131 (21.4%)	3 (10%)	10 (28%)	32 (9.6%)	69 (15%)	7 (3.7%)	21 (11%)	2 (4.5%)	3 (7.6%)	7 (7.9%)	8 (9.7%)	2 (33%)	1 (33.3%)	NT	NT	NT	NT
CN	135 (27.23%)	153 (25%)	4 (15%)	10 (28%)	65 (19.57%)	93 (20%)	35 (18.9%)	57 (30%)	15 (34%)	17 (43.5%)	29 (32.9%)	22 (26.8%)	2 (33%)	2 (50%)	NT	NT	NT	NT
AMP	NT	NT	NT	NT	NT	NT	112 (60%)	143 (75%)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
AMC	NT	NT	NT	NT	NT	NT	60 (32.4%)	82 (43%)	27 (61.3%)	31 (89.4%)	NT	NT	NT	NT	NT	NT	7 (50%)	24 (75%)
TZP	60 (12%)	86 (14%)	2 (6.6%)	6 (17%)	40 (12.12%)	92 (20%)	7 (3.7%)	7 (3.7%)	2 (5.0%)	5 (12.8%)	5 (5.6%	5 (6%)	0 (0%)	0 (0%)	NT	NT	2 (14.2%)	5 (15.6%)
CFM	NT	NT	NT	NT	NT	NT	105 (56.7%)	116 (61%)	42 (95%)	36 (92.3%)	81 (92%)	63 (76.8%)	3 (50%)	2 (50%)	NT	NT	0 (0%)	16 (50%)
CAZ	115 (23%)	159 (26%)	2 (7.6%)	7 (20%)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2 (20%)	6 (35%)	NT	NT
CRO	NT	NT	NT	NT	196 (59%)	299 (64%)	76 (41%)	83 (43%)	32 (72.7%)	27 (69.2%)	50 (56.8%)	40 (48.7%)	2 (33%)	2 (50%)	NT	NT	2 (14.2%)	6 (18%)
CXM	NT	NT	NT	NT	NT	NT	107 (57.8%)	117 (61%)	NT	NT	NT	NT	2 (33%)	3 (75%)	NT	NT	4 (28.5%)	19 (59.3%)
MEM	254 (51%)	263 (43%)	10 (33%)	8 (22%)	54 (16.2%)	95 (20.4%)	5 (2.7%)	5 (2.6%)	0 (0%)	2 (5.1%)	6 (6.8%)	7 (8.5%)	0 (0%)	0 (0%)	NT	NT	0 (0%)	1 (0.09%)
LEV	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1 (10%)	1 (5.8%)	0 (0%)	2 (6.25%)
CIP	137 (28%)	181 (30%)	7 (23%)	14 (40%)	98 (30%)	137 (30%)	57 (30.8%)	79 (42%)	29 (66%)	26 (66.6%)	57 (64.7%)	52 (63.4%)	2 (33%)	3 (75%)	NT	NT	0 (0%)	4 (12.5%)
SXT	NT	NT	NT	NT	130 (39 %)	166 (35.6%)	146 (79%)	143 (75.2%)	29 (66%)	28 (74%)	58 (66%)	48 (59%)	2 (33%)	2 (50%)	2 (20%)	4 (23.5%)	0 (0%)	8 (25%)
TOB	403 (81%)	477 (78%)	23 (76%)	35 (100%)	226 (68%)	350 (75.2%)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

AMK: Amikacin, CN: Gentamicin, AMP: Ampicillin, AMC: Amoxicillin / Clavulanic acid, TZP: Piperacillin-tazobactam, CFM: Cefixime, CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, MEM: Meropenem, LEV: Levofloxacin, CIP: Ciprofloxacin, SXT: Co-trimoxazole. TOB: Tobramycin, NA: Not applicable NT: Not tested.

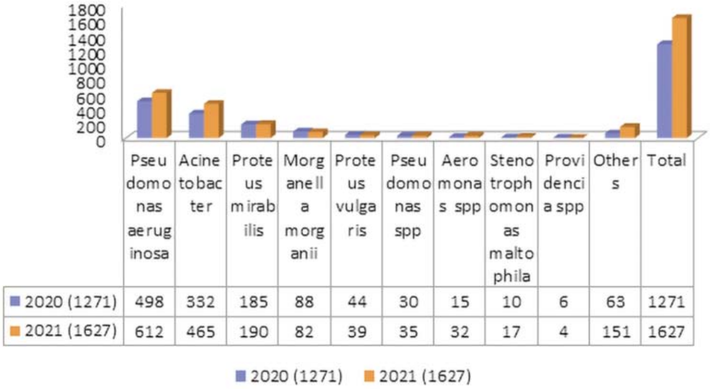

Figure: Frequency of various species of non-lactose fermenting gram-negative uro-pathogens.

2020 and 1,627(%) in 2021 (Figure). Among them, Pseudomonas (P.) aeruginosa (2020: 498[3%], 2021: 612[3%]) was the most prevalent organism. followed by acinetobacter (2020: 332[26%], 2021: 465[29%)], proteus mirabilis (2020: 185[15%], 2021: 190[12%]), morganella morganii (2020: 88[7%], 2021: 82[5%] and proteus vulgaris (2020: 44[3%], 2021: 39[2.3%]).

P. aeruginosa was most resistant against TOB 880(79.3%) and least resistant against TZP 146(13%). Comparison of antibiotic susceptibility pattern of 2020 and 2021 was noted in detail (Table 2).

Discussion

UTI is the most prevalent infection affecting people worldwide, and is rated as the third most common infectious disease. Uncomplicated UTIs are cured with short courses of antibiotics, but urine samples are sent in several cases for microbiological assessment following treatment failure or chronic or worsening infection.[14] The most consistently associated infectious agents of UTIs are enterobacterales, including E. coli and klebsiella, citrobacter, enterobacter, serratia, proteus, morganella and providencia species, gram-positive cocci, including enterococcus species, S. aureus and S. saprophyticus, and NLF-GNBs, like P. aeruginosa.[11]

The current study had 41,280 urine samples positive for bacterial growth. Females of reproductive age formed the primary group of patients with UTI, which was in line with literature that has extensively reported that women have a greater occurrence of UTIs than males, primarily due to anatomic and physical issues.[15] Similarly, a study documented that elderly woman aged >61 showed lower UTI incidence (22%) that males. This was perhaps because with age, males develop prostate swelling and neurogenic bladder.[16]

In the present study, among gram-negative uro-pathogens, E. coli had the highest prevalence, followed by K. pneumonia, which has been reported earlier as well.[10,17] The enterococci species was the dominant pathogen compared to other gram-positive isolates, having a slightly higher prevalence than a study in 2016.[18] NLF-GNBs are frequently correlated with uro-pathogenecity.[19] Among NLF-GNBs, most of the urine infections were caused by P. aeruginosa and acinobacter species, which is similar to the data reported earlier.[20] These two isolates showed increased resistance pattern against AMK, TZP, CAZ and CIP. However P. aeruginosa was found to be more resistant towards MEM and TOB,. Acinetoobacter isolates wereresistant against CRO. The findings closely resembled those reported earlier.[21] However, contradictory findings have also been reported.[22] In the present study, proteus mirabilis and proteus vulgaris showed resistance against several antibiotics, but higher resistance was observed with CFM and CRO. This was in line with another study.[23]

LF-GNBs represent a challenge to clinicians because of their rising resistance against various antibiotics classes, ultimately leading to multidrug resistance (MDR), extensive drug-resistance (XDR), or even pan-drug resistance (PDR).[24] The resistance in these clinical isolates has developed owing to various mechanisms, such as intrinsic non-susceptibility processes, alterations or through plasmids/integrons during the extended course of therapy although the same treatment was effective primarily. The resistance mechanism contains porin deficiency and mutations impacting outer membrane absorbency (β-lactam antibiotics), changes in target locations (aminoglycosides, fluoroquinolones), energy-reliant efflux pumps (fluoroquinolones), as well as the making of drug-inactivating enzymes.[25]

The current study has limitations as it did not deal with UTI prevalence in the local population. Besides, the current data could not correlate antimicrobial resistance with the duration and cause of UTI because it was retrieved from the laboratory records in which clinical data is not recorded.

Conclusion

Among NLF-GNBs, P. aeruginosa and acinobacter species were the most frequent cause of uro-pathogenecity, with great resistance towards MEM, TOB, AMK, TZP, CAZ and CIP. The There is an urgent need for antibiotic stewardship and infection control measures in healthcare settings.

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Author's Contributions

AF: Conception, synthesis, planning of research, active participation in methodology, interpretation, analysis critical review, final approval.

MR: Introduction, discussion, review of literature, referencing.

MS: Data collection, interpretation, analysis.

HG: Data Interpretation, analysis, critical review.

WI: Data collection, active participation in methodology.

RB: Introduction, referencing, critical review.