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A Glance at the Trend, Bacterial Profile, Demographic Features and Antibiotic Susceptibility of Gram-Positive Uropathogens: A Frequent but Neglected Lot

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A B S T R A C T

Gram-negative organisms are commonest cause of Urinary Tract Infection (UTI). Gram-positive bacteria from urine samples are often disregarded as contaminants. However, Gram-positive organisms are established uropathogens and their numbers are expanding. Materials and Methods: Urine samples received from January 2018 up-to December 2019 and January 2022 up-to December 2023 from suspected UTI cases of our institute were processed for culture and antibiotic susceptibility test. Gram-positive uropathogens isolated and their trend, demographic features and antibiotic susceptibility pattern were analyzed. Result: 28,365 urine samples were processed and 386 Gram-positive bacteria were isolated. Gram-positive uropathogens were 4%, 5%, 9% and 12% in 2018, 2019, 2022 and 2023 respectively. *Enterococcus faecalis* were 39%, *Enterococcus faecium* 25%, *Streptococcus agalactiae* 16%, *Staphylococcus haemolyticus* 7%, *Methicillin Sensitive Staphylococcus Aureus* 5%, *Methicillin Resistant Staphylococcus Aureus* 4%, *Staphylococcus saprophyticus* 2% and *Staphylococcus epidermidis* 2%. The importance of escalating number of Gram-positive bacteria as uropathogens especially in specific age groups and antibiotic susceptibility pattern is highlighted.

Introduction

Urinary tract infections are widely prevalent being associated with significant morbidity and affecting all age groups. Most of the reported Urinary Tract Infections (UTIs) are caused by Gram-negative bacteria mainly Enterobacterales. There are hardly any reports on Gram-positive uropathogens. There is significant difference in antibiotic susceptibility of both these groups of organisms (Liala *et al.,* 2019). The increase in multi-drug resistance among Gram-positive organisms requires strict surveillance. Gram-positive organisms other than *Staphylococcus aureus* and *Enterococcus* have recently been identified as uropathogens (Fernández *et al.,* 2023). Hence, analysis of Gram-positive uropathogens was taken up in our institute across four years which included time period before and after Coronavirus disease 2019 (COVID-19) pandemic. In this brief communication, their trend, bacterial and demographic profile along with antibiotic susceptibility was analysed. This in turn will encourage their accurate reporting and subsequently their treatment, thus contributing to antibiotic stewardship.

Materials and Methods

Clean catch Mid-Stream Urine (MSU) samples, samples from urinary catheter and supra pubic aspirate were processed as per standard protocol. Significant bacteriuria was defined as a count of $\geq 10^5$ Colony Forming Units (cfu)/mL or count of $<10^4$cfu/mL for a single microorganism in the presence of significant number of pus cells in non-centrifuged urine for MSUs and catheterised samples or $>=10^2$ cfu/ml in supra-pubic aspirates (Forbes Betty *et al.,* 2007).

Identification and antibiotic susceptibility testing of Gram-positive bacteria was done using Vitek 2 compact system and CLSI guidelines 2023 (Clinical Laboratory Standard International guidelines, 2023). Patient data was obtained from hospital information system. Values were expressed in absolute numbers and percentage of the group. P value < 0.05 was considered significant.

Results and Discussion

Total of 28,365 urine samples were processed. Gram-positive bacteria causing significant bacteriuria were 386(7%) out of 5160 organisms isolated. In 2018, Gram-positive uropathogens isolated were 45(4%) of 1245 (total growth), 59(5%) out of 1269 in 2019, 104 of 1168 (9%) in 2022 and 178 of 1478 (12%) in 2023 (Fig.1).

Overall, *Enterococcus faecalis* were 149 (39%), *Enterococcus faecium* 97 (25%), *Streptococcus agalactiae* 62 (16%), *Staphylococcus haemolyticus* 26 (7%), *Methicillin Sensitive Staphylococcus Aureus (MSSA)* 18 (5%), *Methicillin Resistant Staphylococcus Aureus (MRSA)* 17 (4%), *Staphylococcus saprophyticus* 9 (2%) and *Staphylococcus epidermidis* 8(2%) [Table 1].

76(51%) and 73(49%) isolates of *E. faecalis* were from Out Patient Department (OPD) and In Patient Department (IPD) respectively. 73(49%), 70(47%) and 6(4%) were from 14-65 years, > 65 years and < 14 years respectively. 79 (53%) isolates were from females while 70 (47 %) from males. 15(15%) and 82(85%) isolates of *E. faecium* were from OPD and IPD respectively. 76 (77%), 21(21%) and 2(2%) isolates were from > 65, 14-65 and < 14 years respectively. 60 (64%) isolates were from females and 37(36%) from males. 42(68%) and 20(32%) isolates of *S. agalactiae* were from OPD and IPD respectively. 48(77%), 13(21%) and 1(2%) were from 14-65, > 65 years and < 14 years respectively. 49 (79%) and 13(21%) were from females and males respectively. Of 26 isolates of *S. haemolyticus*, 15(58%) were from OPD, 18(69%) were from 14-65 years and 14(54%) from females.

Of 18 isolates of MSSA, 13(72%) was from OPD, 10(56%) from 14-65 years and 13 (72%) females. 12(71%) of 17 isolates of MRSA were from OPD, 14(82%) from 14-65 years and 14(82%) from females. 7(78%) of 9 isolates of *S. saprophyticus* were from OPD, 8(89%) from 14-65 years and 100% from females. Of 8 isolates of *S. epidermidis*, 5(63%) was from OPD, 7(88%) from 14-65 years and 6(75%) from females [Table 1]. Susceptibility to ampicillin, vancomycin, teicoplanin, linezolid and nitrofurantoin was 100% in *E. faecalis*, 100 (67%) to levofloxacin and 69(46%) each to ciprofloxacin and norfloxacin.

Amongst *E. faecium,* 100% were sensitive to linezolid and teicoplanin, 93(96%) isolates were sensitive to vancomycin and nitrofurantoin each, 60(62%) to levofloxacin and 35(36%) to ciprofloxacin and norfloxacin. All isolates of *S. agalactiae* were susceptible to the tested drugs. Susceptibility to levofloxacin, ciprofloxacin and norfloxacin was 12(67%), 9(50%) and 9(50%) in *MSSA* isolates and 9(53%), 4(24%) and 4(24%) in *MRSA* isolates [Table 2].

UTIs range from uncomplicated cystitis to complicated pyelonephritis and nephrolithiasis and are a common cause of human infections leading to significant morbidity with Gram-negative organisms in the lead role as uropathogens. Gram-positive bacteria comprise 10-15% of UTIs but their rates are growing due to the lack of information about their resistance pattern and also due to lack of such studies in literature (Liala *et al.,* 2019; Eipa *et al.,* 2023). Gram-negative uropathogens comprise 75% - 95% of UTIs as per Kline *et al.,* (2016). This was comparable to our results where Gram-positive bacteria ranged from 4 % and 5% in 2018 and 2019 but increased to 9% and 12 % in 2022 and 2023 respectively. This depicts their ascending trend especially in the later years which was also the period after the COVID-19 pandemic.

This can be attributed to increase in hospitalization and catheterization involved with the pandemic considering the association of these organisms with such factors (Fernández-Espigares *et al.,* 2023). Females were more susceptible to UTIs by Gram-positive bacteria than males similar to other studies (Fernández *et al.,* 2023; Eipa *et al.,* 2023; Verma *et al.,* 2020).

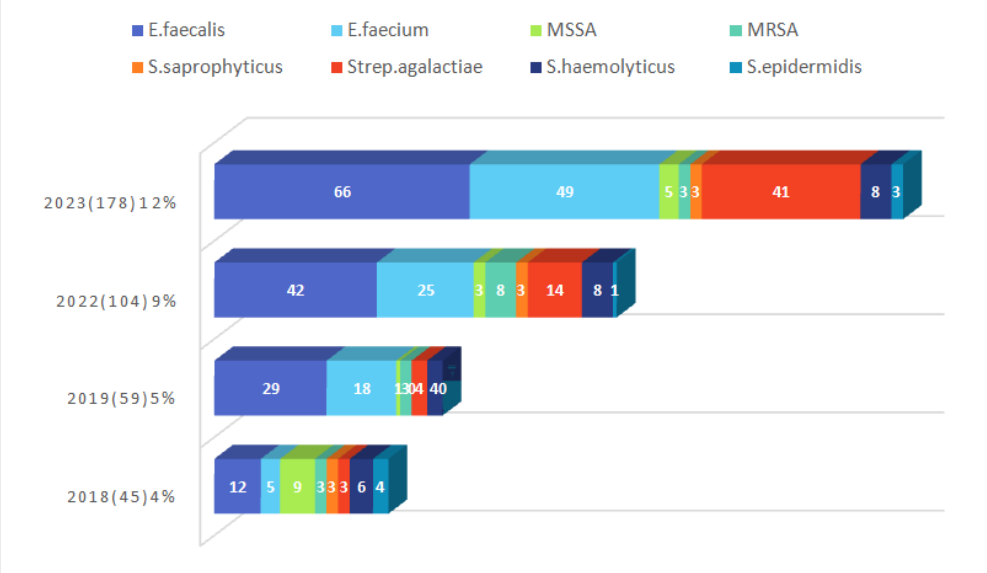
Table.1 Demographic profile of Gram-positive uropathogens.

Organism	OPD No (%)	IPD No (%)	<14 years No (%)	14-65 years No (%)	>65 years No (%)	Females No (%)	Males No (%)	Total 386 No (%)
E. faecalis	76(51)	73(49)	6(4)	73(49)	70(47)	79(53)	70(47)	149(39)
E. faecium	15(15)	82(85)	02(2)	21(21)	76(77)	60(64)	37(36)	97(25)
S. agalactiae	42(68)	20(32)	01(2)	48(77)	13(21)	49(79)	13(21)	62(16)
S. haemolyticus	15(58)	11(42)	0	18(69)	08(31)	14(54)	12(46)	26(7)
MSSA	13(72)	05(28)	0	10(56)	08(44)	13(72)	05(28)	18(5)
MRSA	12(71)	05(29)	0	14(82)	03(18)	14(82)	03(18)	17(4)
S. saprophyticus	07(78)	02(22)	0	08(89)	01(11)	09(100)	0	09(2)
S. epidermidis	05(63)	03(37)	0	07(88)	01(12)	06(75)	02(25)	08(2)

Table.2 Antibiotic susceptibility pattern of Gram-positive isolates

	E.faecalis **(149)**	***E.faecium*** **(97)**	***S.agalactiae*** **(62)**	***S.haemolyticus*** **(26)**	***MSSA*** **(18)**	***MRSA*** **(17)**	***S.saprophyticus*** **(9)**	***S.epidermidis*** **(8)**
Ampicillin	100%	0	100%	13(50%)	100%	0	5(56%)	
Vancomycin	100%	93(96%)	100%	100%	100%	100%	100%	100%
Teicoplanin	100%	100%	100%	100%	100%	100%	100%	100%
Linezolid	100%	100%	100%	100%	100%	100%	100%	100%
Nitrofuran-toin	100%	93(96%)	100%	100%	100%	100%	8(89%)	4(50%)
Levofloxacin	100(67%)	60(62%)	100%	26(100%)	12(67%)	9(53%)	9(100%)	8(100%)
Ciprofloxacin	69(46%)	35(36%)	100%	13(50%)	09(50%)	4(24%)	5(56%)	4(50%)
Norfloxacin	69(46%)	35(36%)	100%	13(50%)	09(50%)	4(24%)	5(56%)	4(50%)
Co-trimoxazole	-	-	-	100%	100%	7(41%)	8(89%)	4(50%)
Tetracycline	-	-	100%	-	-	-	-	-
Clindamycin	-	-	100%	-	-	14(82%)	-	-

Figure.1 Total Gram-positive uropathogens across four years and their bacterial profile.



Incidence of UTIs due to *E faecalis* has risen over the years (Kline *et al.,* 2016). *E faecium* UTIs have multiplied over the years according to Smout *et al.,* (2023). This was in accordance with our findings where rates of UTIs by *E. faecalis* increased from 27% in 2018 to 37%in 2023 and *E. faecium* from 11% in 2018 to 27% in 2023 [Fig.1]. Higher number of *E. faecium* where from IPD patients above 65 years age group comparable to findings of Fernandes *et al.,* (2023). UTIs by *S. agalactiae* increased from 7% in 2018 to 23% in 2023 in 14-65 years age group [Fig.1]. This was akin to that of Bushra *et al*. (2023) where these formed 19% of the uropathogens, Fernandes *et al.,* (2023) who reported *S. agalactiae* from 14-65 years age group and Kline *et al.,* (2016) who reported them in pregnant females (Fernández *et al.,* 2023; Eipa *et al.,* 2023; Kline *et al.,* 2016). Thus, *E. faecalis*, *E. faecium* and *S. agalactiae* dominated the spectrum followed by *Staphylococcal* species. Among the later, *S. haemolyticus* prevailed followed by *MSSA*, *MRSA*, *S. saprophyticus* and *S. epidermidis*. This was commensurate with other studies (Liala *et al.,* 2019; Eipa *et al.,* 2023; Kline *et al.,* 2016; Verma *et al.,* 2020). *S. haemolyticus* UTIs amounted to 10% similar to result of Eltwisy *et al.,* (2022) where these amounted to 10% of UTIs (Eltwisy *et al.,* 2022). *S. aureus* is established cause of UTI as per (Bushra *et al.* 2023; Eipa *et al.,* 2023). Verma *et al.,* (2020) reported *S. aureus* isolates to be 35% (Verma *et al.,* 2020) where-as *MSSA* and *MRSA* isolates as per our results were 5 % and 4% respectively. This could be because prevalence and antimicrobial susceptibility vary as per geographical location (Eipa *et al.,* 2023). *S. saprophyticus* formed 2% of uropathogens comparable to Kline *et al.,* (2016) where it is responsible for 5-20% of community acquired UTIs (Kline *et al.,* 2016). *S. epidermidis* constituted 2% of uropathogens similar results of Bhargav *et al.,* (2022) in which this organism amounted to 2.8% (Bhargav *et al.,* 2022). Resistance to vancomycin among *E. faecium* is 12.4% in India (Smout *et al.,* 2023). However, 96% of *E. faecium* isolates in our study were vancomycin susceptible. This variation can be attributed to the regional variation (Eipa *et al.,* 2023). Susceptibility to ciprofloxacin and norfloxacin was 50% and 24% in *MSSA* and *MRSA* respectively. This was comparable to Bushra *et al.,* who found 58% susceptibility of *S. aureus* isolates to these drugs (Eipa *et al.,* 2023).

Gram-positive bacteria are established pathogens causing UTI. Their numbers are rising and it would be inappropriate to dismiss them as contaminants. *E. faecalis*, *E. faecium* and *S. agalactiae* were isolated more frequently from female patients. Majority of the affected female patients were from community. However, predominance of certain Gram-positive bacteria was observed in specific populations. *E. faecalis* was obtained routinely from adults while *E. faecium* was isolated frequently from hospitalized elderly patients. *S. agalactiae* was commonly reported from reproductive age group. Most isolates of *Enterococci* were susceptible to vancomycin and nitrofurantoin. Resistance to ciprofloxacin among Gram-positive bacteria is alarming. More such studies are the need of the time.

Author Contributions

Sunayana M. Jangla: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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