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Gram Positive Cocci Associated Urinary Tract Infections, their Prevalence and Antibiotic Susceptibility Patterns

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary tract infections (UTIs) are the third most common type of infection in humans globally. Gram-positive bacteria are said to be responsible for ten percent of urinary tract (UTI) infections. The study's goal was to profile gram-positive cocci-associated UTIs and their antibiogram, as they were observed at LASUTH.
Methods: This was a retrospective assessment of the Medical Microbiology Laboratory records of the LASUTH to review the in vitro antibiotic susceptibility patterns of gram-positive urinary bacterial isolates between April 2020 and March 2021. The bacteria were isolated and identified from routine

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urine samples using standard bacteriological methods and the API. In vitro antibiotic susceptibility test (AST) was routinely performed by the modified Kirby-Bauer disk diffusion test and susceptibility breakpoints were determined using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: 2,253 urine samples were processed in the medical microbiology laboratory over the one year and 662 (29.4%) samples yielded Positive cultures. Of the 662 isolates, 494 (74.6%) were gram-negative bacteria. 164 (24.8%) were gram-positive cocci while 4 (0.6%) were gram positive rod. Among the gram-positive cocci's isolated Enterococcus faecalis had the highest frequency 58 (35.4%). Aminoglycosides (Gentamycin and Amikacin) and Linezolid antibiotics were found to be the most effective drugs against gram-positive cocci bacteria except Enterococcus *spp.* For empirical treatment of *Enterococcus spp* in our facility Fosfomycin and Tigecycline are the best options, while for Streptococcus agalactiae associated UTI, Amikacin, Cefuroxime, Linezolid, and levofloxacin can be used for empirical treatment.

Conclusion: The prevalence rate of gram-positive cocci associated UTI in this study was 7.3% (164/2253). The emergence of drug resistance in these pathogens to commonly used antibiotics is a thing of concern. Therefore, efficient antimicrobial stewardship programmes must be in place.

Keywords: UTIs; uropathogens; gram-positive cocci; enterococcus; MRSA.

1. INTRODUCTION

When compared to other uropathogens, bacterial urinary tract infections are the most common and dangerous infections in humans, and they frequently occur in both the community and in hospitals [1-4].

Most Gram-negative bacteria that cause illness are classified as pathogenic bacteria; they include *Escherichia coli, Klebsiella species*, *Enterobacter species, Proteus species, Pseudomonas species, Acinetobacter species, Serratia species,* and *Citrobacter species.* 90% of UTI cases are caused by them. The remaining ten percent of UTI infections are caused by gram-positive bacteria, specifically *Enterococcus species*, group B streptococci, and *Staphylococcus species* [3,4].

Although coagulase-negative staphylococci (CoNS) and *S. aureus* were previously thought to be uncommon etiological agents in ascending UTIs in outpatients, they may play a more significant role in hospitalised, immunocompromised patients. When S. aureus is isolated from urine, it can also be a sign of a more serious illness (like endocarditis or bacteraemia), in which the germs spread hematogenous and end up in the kidneys [5]. The literature indicates that the isolation frequency of *S. aureus* from UTIs ranges from 0.5 to 13% [6,7]. Conversely, *S. saprophyticus i*s a well-studied pathogen in catheter-associated UTIs as well as simple cystitis. Afte*r S. saprophyticus's* pathogenic role in UTIs—also known as "honeymoon cystitis"—was identified in the 1960s, an increasing amount of information about the pathophysiology of this illness has been discovered [8].

S. saprophyticus is thought to be the etiological agent in 5–20% of UTIs according to epidemiological research, but a Swedish study discovered that it was the cause of more than 40% of females' uncomplicated UTIs [9]. *Enterococcus species* are among the few Gram-positive bacteria that are resistant to bile, and they are widely distributed in the gut microbiota of both humans and animals [10]. Given their high prevalence in aquatic habitats, enterococci should be considered as a sign of faecal contamination in urban areas [11]. The most frequent species in bacteraemia, endocarditis, infections of the central nervous system, and urinary tract infections are *Enterococcus faecalis and E. faecium*; nevertheless, the appearance of non-faecalis enterococci should be considered [12,13]. These organisms are important in nosocomial infections worldwide, much like *Staphylococcus aureus* [14].

Staphylococcus haemolyticus is the second-most frequently isolated CoNS (S. epidermidis is the first) and it is considered a relevant hospital-acquired pathogen [15]. It is associated with the insertion of foreign devices into the human body like Urinary catheters, prosthetic valves and Cerebrospinal stunts [16,17,18].

Streptococcus agalactiae or Lancefield group B Streptococcus (GBS), a gram-positive ß-haemolytic chain-forming coccus, is not a common bacteria cause of UTI. It's estimated to cause approximately 1%–2% of all single microorganism source UTIs [19,20]. Pregnant, diabetic, immunocompromised, and persons with pre-existing urologic abnormalities are also susceptible to GBS-caused UTIs. In these cases, there is an increased risk of ascending pyelonephritis, which may develop into bacteraemia and/or urosepsis [20,21,22,23].

Gram-positive cocci have numerous virulence factors, such as maintaining their high affinity for the epithelial cells of the urinary tract, allowing for their survival. These virulence factors include fibrillar proteins (Ssp) mediatingcell–cell interactions, fibronectin-binding proteins, elastin-binding protein, adhesins, hemagglutinin, elastase, and lipase. Furthermore, urease is produced by the majority of *S. saprophyticus* and over 90% of *S. aureus* strains, which breaks down carbamide (urea) in the urine [5-14,24,25,26]. Because *Enterococcus spp* are found in faecal matter and may colonise the rectum, their anatomical closeness to the urinary system may further increase their ability to cause UTIs [11, 27]. Biofilm production in these species is another important factor for the emergence and persistence of UTIs, with some reports suggesting that some 80% of uropathogenic Gram-positive cocci are biofilm producers [19]. The presence of biofilm in urethral stents and catheters may lead to obstruction.

Furthermore, microorganisms embedded in biofilm may survive 1000-times higher concentrations of antibiotics, compared to non-embedded cells [28,29,30].

2. MATERIALS AND METHODS

2.1 Study Setting

The study was conducted in the department of Medical Microbiology of the Lagos State University Teaching Hospital, an 800-bedded tertiary centre located in Ikeja, Lagos southwest Nigeria. The hospital is dedicated to teaching, research and specialist services and serves Lagos State and neighbouring States in southwest Nigeria.

2.2 Study Design

This was a retrospective study that involved a review of the medical microbiology laboratory records to analyse the antimicrobial susceptibility profiles of Gram-positive cocci urinary tract infections isolates obtained between April 2020 to March 2021.

2.3 Isolation and Antibiotic Susceptibility Pattern of Bacterial Isolates

Normal processing of urinary samples in the laboratory during the period of the review involved macroscopic and microscopic examination. And then, urinary samples were inoculated into Cystine Lactose Electrolyte Deficient (CLED) and Blood agar plates and incubated aerobically at 35-37^{0}C for 18-24 hours. Isolates were identified by conventional biochemical tests and using Analytical Processing Index (API) and antimicrobial susceptibility testing (AST) was performed using the modified Kirby-Bauer disk diffusion method. The break points for Susceptibility were determined using the Clinical and Laboratory Standards Institute (CLSI) guidelines [31].

2.4 Data Analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 22.

3. RESULTS

In the 1-year period under consideration, a total number of 2,253 urine samples were processed in the medical microbiology laboratory and 662 (29.4%) samples yielded Positive cultures (Table1).

Of the 662 isolates, 494 (74.6%) were Gram-negative bacteria, while 164(24.8%) were gram positive cocci and 4 (0.6%) were gram positive rod (Table 2).

The 164-gram positive cocci came from 74(45.1%) inpatients urine samples and 90(54.9%) outpatients urine samples (Table 3).

Among the gram-positive cocci's isolated Enterococcus *faecalis* had the highest frequency 58(35.4%), followed by *Staphylococcus epidermidis* 48(29.3%), and *Enterococcus faecium* 24 (14.6%). MRSA and *Streptococcus agalactiae* were 10(6.1%) respectively, Staphylococcus *aureus* 6(3.7%), while *Staphylococcus saprophyticus* and *Streptococcus agalactiae* had a frequency of 4 (2.4%) each (Table 4).

Females had the greater incidence of gram-positive cocci associated UTI with a frequency of 120(73.2%) while males had a frequency of 44 (26.8%). This is in a ratio of 3: 1 in favour of the females (Table 5).

In terms of age, among the males age group 51-60, 31-40 and 21-30 years were mostly affected in decreasing order, while for the females, age group 31-40years were mostly affected, followed by age group 41-50 and 21-30 years (Table 5).

Table 1. Total urine samples, culture outcome and frequency

		Percentage
Total urine samples	2,253	100%
Positive culture	662	29.4%
Negative culture	1601	70.6%

Table 2. Positive culture bacteria and frequency

Positive culture	Frequency	Percentage (%)
Gram negative	494	74.6
Gram positive cocci	164	24.8
Gram positive bacilli	4	0.6
Total	662	100%

Table 3. Frequency distribution of gram-positive cocci isolates betweeen inpatients and outpatients

Gram positive cocci isolate	Frequency	Percentage (%)
Inpatient	74	45.1
Outpatient	90	54.9

From Table 6, Most species of *Enterococcus faecalis* and *Enterococcus faecium* were sensitive to Fosfomycin (70% -100%), Tigecycline (70 -79.3%), and Meropenem (86-93.1%). These species of Enterococcus had the greatest resistance to Streptomycin (51-58.3%), Cefotaxime (50-56.9%) and Amoxicillin-clavulanate (36-41.7%).

Furthermore, *Staphylococcus aureus* isolates were most sensitive to Amoxicillin, Amoxicillin -clavulanate, Linezolid and Gentamycin, with a sensitivity of 66.7% each. *Staphylococcus aureus* were most resistant to Clindamycin and Trimethoprim-sulfamethoxazole (66.7% each). While for Methicillin Resistant *Staphylococcus aureus* Linezolid was the most sensitive drug with a sensitivity of 80%.

For *Staphylococcus epidermidis* isolates, Nitrofurantoin and Levofloxacin had the most sensitivity with sensitivity rates of 83.3% and 54.2% respectively. Their greatest resistance was to Amoxicillin (83.3%) and erythromycin (62.5%).

Staphylococcus haemolyticus isolates were most sensitive to Gentamycin (75%) and Linezolid (50%). They were most resistant to Clindamycin (50%).

Again, *Staphylococcus saprophyticus* isolates were most sensitive to Nitrofurantoin (75%), levofloxacin (75%), Linezolid and gentamycin (50% each). *S. saprophyticus* isolates were most resistant to erythromycin (50%).

Streptococcus agalactiae isolates were most sensitive to Cefuroxime (90%), Amikacin and linezolid (80% each) and erythromycin and levofloxacin (60% each). They were most resistant to Nitrofurantoin (60%), Amoxicillin (40%) and Amoxicillin-clavulanate (30%).

4. DISCUSSION

In this Study a total number of 2,253 urine samples were processed in the medical microbiology laboratory and 662 (29.4%) samples yielded Positive cultures while 1591(70.6%) had negative cultures. The reason for the no bacterial growth among a good number of the urine samples may be because some of the patients have been on antibiotic therapy before reporting to the hospital or laboratory. These antibiotics may have inhibited bacterial growth [32].

The prevalence rate of gram-positive cocci Uropathogens causing UTI from this Study was 7.3% (164/2253). This was lower than that of a study done in Indian [33] that got a prevalence of 18.35%.

The 164-gram positive cocci came from 74(45.1%) inpatients urine samples and 90(54.9%) outpatients urine samples.

Females had the greater incidence of gram-positive cocci associated UTI with a frequency of 120(73.2%) while males had a frequency of 44(26.8%). This is in a ratio of 3: 1 in favour of the females. This may be due to the shortness of the Female urethra when compared with that of males. Furthermore, the moist vaginal introitus, which the urethral meatus opens into, is colonised by both pathogenic bacteria and normal flora, some of which could induce cystitis. UTI in females is also influenced by other significant factors, such as pregnancy, postmenopausal status, and sexual activity [34].

Table 4. Gram positive cocci isolates and their frequency

	Gram positive cocci	Frequency	Percentage (%)
1.	E. faeccum	24	14.6
2.	E. faecalis	58	35.4
3.	S. aureus	6	3.7
4.	MRSA	10	6.1
5.	S. saprophyticus	4	2.4
6.	S. epidermidis	48	29.3
7.	S. agalactiae	10	6.10
8.	S. haemolyticus	4	2.40
Total		164	100

Table 5. Gram positive cocci bacteria occurrence by age group and sex

Age range (Year)	Male	Female	Total
0-10	1	6	7
11-20	2	13	15
21-30	10	26	36
31-40	12	40	52
41-50	3	28	31
51-60	14	3	17
>60	2	4	6
	44(26.8%)	120(73.2%)	164(100%)

Table 6. Isolates and antibiotics subsceptility pattern

Organisms	Antibiotics	Sensitivity	Intermediate	Resistance	Total
Enterococcus faecium	Cefotaxime	10(41.7%)	2(8.3%)	12(50%)	24
	Amoxicillin – Clavulante	14(58.3%)	——	10(41.7%)	
	Levofloxacin	10(41.7%)	2(8.3%)	12(50%)	
	Tigercycline	17(70.8%)	2(8.3%)	5(20.8%)	
	Amoxicillin	20(83.3%)	3(12.5%)	1(4.3%)	
	Fosfomycin	17(70.8%)	5(20.8%)	2(8.3%)	
	Streptomycin	10(41.7%)	——	14(58.3)	
	Tetracycline	14(58.3%)	4(16.7%)	6(25%)	
	Meropenem	21(87.5%)	2(8.3%)	1(4.2%)	
Staphylococcus epidermidis	Amoxicillin	8(16.7%)	——	40(83.3%)	48
	Amoxicillin-Clavulanate	18(37.5%)	20(41.7%)	10(20.8%)	
	Cefoxitin	48(100%)	——-	——-	
	Nitrofurantion	40(83.3%)	———	8(16.7%)	
	TMP	10(20.8%)	8(16.7%)	30(62.5%)	
	Clindamycin	20(41.7%)	4(8.3%)	24(50%)	
	Erythromycin	16(33.3%)	2(4.2%)	30(62.5%)	
	Gentamicin	22(45.8%)	2(4.2%)	24(50%)	
	Levofloxacin	26(54.2%)	2(4.2%)	20(41.7%)	
	Linezolid	22(45.8%)	8(16.7%)	18(37.5%)	
Enterococcus faecalis	Amoxicillin-Clavulanate	20(34.5%)	16(33.3%)	22(37.9%)	58
	Amoxicillin	28(48.3%)	——	30(51.7%)	
	Cefotaxine	15(25.9%)	10(20.8%)	33(56.9%)	
	Tigercycline	46(79.3%)	12(20.7%)	——	
	Fosfomycin	58(100%)	——	——	
	Meropenem	54(93.1%)	2(3.4%)	2(3.4%)	

Organisms	Antibiotics	Sensitivity	Intermediate	Resistance	Total
	Erythromycin	30(51.7%)	6(10.3%)	22(37.9%)	
	Levofloxacin	38(65.5%)	2(3.5%)	18(31.0%)	
	Tetracycline	40(83.3%)	18(16.7%)	——	
	Streptomycin	20(34.5%)	8(13.8%)	30(51.7%)	
Staphylococcus aureus	Amoxicillin	4(66.70%)	1(16.65%)	1(16.65%)	6
	Amoxicillin-Clavulanate	4(66.7)	2(33.3%)		
	Cefoxitin	6(100%)	——	——	
	Nitrofurantion	3(50%)	2(33.3%)	1(16.7%)	
	Clindamycin	1(16.6%)	1(16.7%)	4(66.7%)	
	Linezolid	2(66.7%)	2(33.3%)	——	
	TMP	1(16.7%)	1(16.7%)	4(66.6%)	
	Gentamycin	4(66.7%)	2(33.3%)	——	
	Rifampicin	3(50%)	2(33.3%)	1(16.7%)	
	Levofloxacin	2(33.3%)	2(33.3%)	(33.3)	
Staphylococcus haemolyticus	Levofloxacin	2(50%)	2(50%)	0(0%)	4
	Amoxicillin	2(50%)	1(25%)	1(25%)	
	Cefoxitin	4(100%)	——	——	
	Nitrofurantion	2(50%)	1(25%)	1(25%)	
	Clindamycin	1(25%)	1(25%)	2(50%)	
	Linezolid	2(50%)	2(50%)	——	
	Gentamycin	3(75%)	1(25%)	0	
	TMP	2(50%)	1(25%)	1(25%)	
	Erythromycin	2(50%)	1(25%)	1(25%)	
	Rifampicin	2(50%)	1(25%)	1(25%)	
Staphylococcus saprophyticus	Cefoxitin	4(100%)	——		4
	Nitrofurantion	3(75%)	1(25%)	0	
	Amoxicillin	2(50%)	1(25%)	1(25%)	
	TMP	2(50%)	1(25%)	1(25%)	
	Levofloxacin	3(75%)	0	1(25%)	
	Linezolid	2(50%)	2(50%)	——	
	Gentamycin	2(50%)	2(50%)	0	
	Erythromycin	1(25%)	1(25%)	2(50%)	
	Clindamycin	1(25%)	2(50%)	1(25%)	
	Rifampicin	2(50%)	2(50%)	——	
Streptococcus agalactiae	Levofloxacin	6(60%)	2(20%)	2(20%)	10
	Amoxicillin	4(40%)	2(20%)	4(40%)	
	Linezolid	8(80%)	1(10%)	1(10%)	
	Amikacin	8(80%)	2(20%)	——	
	Amoxicillin	4(40%)	2(20%)	4(40%)	
	Erythromycin	6(60%)	1(10%)	3(30%)	
	TMP	4(40%)	3(30%)	3(30%)	
	Nitrofurantoin	2(20%)	2(20%)	6(60%)	
	Cefuroxime	9(90%)	——	1(10%)	
	AMC	5(50%)	2(20%)	3(30%)	
MRSA	Amoxicillin	2(20%)	2(20%)	6(60%)	10
	Cefoxitin	--	--	10(100%)	
	Nitrofurantoin	4(40%)	2(20%)	4(40%)	
	Clindamycin	6(60%)	2(20%)	2(20%)	
	Linezolid	8(80%)	2(20%)	--	
	Gentamycin	6(60%)	3(30%)	1(10%)	
	Rifampicin	8(80%)	1(10%)	1(10%)	
	Amoxicillin-Clavulanate	6(60%)	2(20%)	2(20%)	
	Erythromycin	2(20%)	4(40%)	4(40%)	
		3(30%)	4(40%)	3(30%)	

Among the group of gram-positive cocci, E. faecalis was the predominant species (35.4%), which is not surprising, in view of worldwide epidemiological reports identifying the causing agents of UTI's. [35].

The prevalence of MRSA isolates from urinary samples in our study was low (0.4%), and the levels of these isolates were in contrast to report from other literature [36,37].

Staphylococcus aureus in total (MRSA included) accounted for 16(9.8%) of the gram-positive cocci associated UTI's. This may be because *Staphylococcus aureus i*s implicated in UTI in many sexually active females, as reported by some studies [38,39].

In our study, Aminoglycosides (Gentamycin and/ Amikacin) and Linezolid antibiotics were found to be the more effective drug against gram-positive cocci bacteria except Enterococcus *spp*. This was contrary to another Study [40] that found out that Teicoplanin, and Nitrofurantoin were most sensitive to gram positive cocci (GPC) urinary isolates.

Most *Streptococcus agalactiae* and all Enterococcus spp had the common resistance; Amoxicillin -clavulanate, while Staphylococcus *saprophyticus and Staphylococcus epidermidis* have come resistance to erythromycin. Furthermore, *Staphylococcus aureus* and *Staphylococcus haemolyticus* have similar resistance to Clindamycin. These findings where Contrary to a study [40], where the most resistant drugs were Penicillin, Ampicillin, and Ciprofloxacin in all GPC (only Penicillin except *Enterococcus spp.*).

5. CONCLUSION

The prevalence rate of gram-positive cocci associated UTI in this study was 7.3% (164/2253). Although urinary tract infections are mainly caused by gram-negative bacteria, gram-positives cocci have emanated as important agents of UTIs, particularly among elderly patients, mostly associated with co-morbidities, pregnant women, and catheterized patients, both in low- and high-income countries. In our study, Enterococcus spp. had the highest prevalence (3.6% (82/2253)) among other gram-positive cocci cause of UTI.

The emergence of drug resistance in these pathogens to commonly used antibiotics is a thing of concern.

The resistance rates for fluoroquinolones are worrisome and as such these agents are not recommended to be used empirically. In contrast, the use of nitrofurantoin for staphylococci may still be regarded as safe in our setting and the tested isolates are almost uniformly susceptible to the available last-resort antibiotics.

Generally, for empirical treatment of Enterococcus spp in our facility Fosfomycin and Tigecycline is our best option, while for Streptococcus agalactiae associated UTI Amikacin, Cefuroxime, Linezolid and levofloxacin can be used for empirical treatment.

To ensure the proper use of antibiotics for treating urinary tract infections, efficient antimicrobial stewardship programmes must be in place.

ETHICAL APPROVAL

Ethical approval for the study was obtained from Lagos State University Teaching Hospital Research and Ethics Committee. As data were retrospectively obtained from the laboratory records and did not involve contact with patients nor was recruitment of patients, informed consent not deemed necessary. However, privacy and confidentiality of patients' data were protected.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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