Evaluation of Resistant Urinary Tract Infections by Gram-positive Bacteria in Medina, Saudi Arabia

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Abstract Background: Gram-positive uropathogens have become common, associated with serious underlying illnesses and increasingly resistant to available antibiotics. Objectives: The goal of this study was to investigate the incidence and risk factors of gram-positive cocci UTIs in hospitalized patients in Medina, KSA and their susceptibility patterns to widely used antimicrobial agents. Methods: During a 12-month study, 165 clinical isolates of gram-positive cocci were recovered from 1137 culture-positive urine specimens at a tertiary hospital. Antimicrobial susceptibility of gram-positive cocci isolates was tested with the disk diffusion and E test methods. Molecular typing of some VRE isolates was done to detect the predominant Van genotypes. Results: Out of 8600 reviewed cases, 1137 (13.2%) were culture positive, 165 cases (14.5%) were gram positive cocci. E. faecalis formed 53.3% (88/165) of isolated gram-positive cocci, followed by E. faecium (17.6%), S. agalactiae (23.6%) and S. aureus (5.5%). Multidrug resistant positive cocci formed 9.7% of gram-positive isolates including VRE (8.5%) and MRSA (1.2%). 75% of E. faecalis and 50% of E. faecium isolates were sensitive to nitrofurantoin, all VRE strains were sensitive to linezolid. All S. aureus isolates were sensitive to cefazolin, nitrofurantoin, sulfamethoxazole and vancomycin. 75% of MRSA strains were sensitive to sulfamethoxazole and all were sensitive to vancomycin. All isolates of S. agalactiae were sensitive to cefazolin and nitrofurantoin, and all were resistant to Trimethoprim-sulfamethoxazole. Van B genotype was detected. Conclusion: Vancomycin and nitrofurantoin seem to be effective drugs for treatment of gram positive UTIs. vanB genotype was detected.

Keywords: urinary tract infection, gram positive bacteria, antibiotic resistance, genotyping


1. Introduction

UTI refers to the presence of urinary tract microbial pathogens and is characterized as the growth of a single pathogen from properly collected mid-stream urine specimen of > 10^5 colony-forming units per milliliter [1,2]. UTIs account for more than 100,000 hospital admissions annually, most often for pyelonephritis [3]. They also account for at least 40% of all hospital-acquired infections and are, in most cases, catheter associated [4]. Bacteriuria develops in up to 25% of patients who require a urinary catheter for > 7 days, with a daily risk of 5% [5].

Furthermore, bacteria, including, are continuously exposed to the selective pressure from antibiotics or antiseptic agents in the nosocomial environment. Approximately 25% of the patients who have had an episode of acute cystitis clinically manifest recurrence [6]. Basically, most acute UTIs are confined to the lower urinary tract, but persistence and recurrence of the infection might lead to renal progression, producing pyelonephritis which can cause kidney damage or even failure if left untreated for an extended period of time [7].

UTIs during pregnancy affect mothers and fetus, causing premature labor, preeclampsia, anemia and transient renal insufficiency, intrauterine growth retardation (IUGR), and low birth weight. Nonetheless,
not everyone with UTI (i.e., having a significant number of bacteria in urine) would necessarily develop recognizable signs and such a condition is defined as asymptomatic bacteriuria (ASB) [8].

Relative frequency of the pathogens varies depending upon age, sex, catheterization and hospitalization. Escherichia coli and other Enterobacteriaceae are the main causative agents of UTIs. Other gram-negative rods (Pseudomonas spp) and gram-positive cocci (coagulase-negative Staphylococci, Staphylococcus aureus, Streptococcus group B, Enterococci) are comparatively more common in some hospitalized patients, however [9].

Nosocomial UTIs comprise perhaps the largest institutional reservoir of nosocomial antibiotic resistant pathogens (Maki et al. 2001). Gram positive cocci isolates have demonstrated a remarkable capacity to rapidly establish antibiotic resistance over the past decade. Area-specific monitoring studies are required to identify trends of antimicrobial resistance, to implement successful treatment and to reduce mortality rates [10].

2. Subjects, Materials and Methods

2.1. Study Design, Period and Area

A prospective cross-sectional study was conducted on UTI cases attending Maternity and Child Hospital, Medina, Kingdom of Saudi Arabia, from April to June 2016.

2.2. StudySubjects

The research subjects who did not obtain antimicrobials during the previous seven days gave a total of 8600 clean catchmidstream urine samples. Study subjects included catheterized patients more than 72 hours admitted to Medical, Surgical, Gynecology, and Maternity wards and non-catheterized patients from the outpatient departments with symptoms of UTI.

2.3. Sample Collection, Handling, and Transport

A freshly voided midstream urine samples (10-20 ml) were collected from non-catheterized patients in wide mouth sterile containers. Catheter urine specimens (10-20ml) were transferred to sterile containers after cleansing the outlets of the catheters with appropriate disinfectant. The urine specimens were delivered to the laboratory to be processed within one hour. If a delay was suspected, boric acid was used as a preservative (0.1 g/10 ml of urine).

2.4. Culture and Identification

Standard microbiological techniques are used in the culture of all urine specimens and in the identification of the isolates.

The uropathogens were isolated by a surface streak procedure on both blood and CLED agar (cysteine lactose electrolyte deficient medium) (Oxoid, Ltd., Basingstoke, Hampshire, England) by using calibrated loop (0.001/ml). Cultures were incubated in aerobic atmosphere at 37°C for 24 hrs. A positive urine culture was defined as colony count > 10^5 CFU/ml for mid-stream urine and > 10^2 CFU/ml for catheter urine. Gram positive isolates were identified using catalase test, bile esculin test and phenotypic API strept, API staph methods according to the manufacturer’s instructions (BioMérieux, Marcy L’Etoile, France). Isolates were identified by the Vitek2 system [11].

2.5. Preparation of Stock Cultures

Stock cultures are prepared of all Gram-positive isolates using glycerol as the osmotic protector. 8 to 10 colonies from an overnight incubated plate are inoculated in 1 ml freeze broth (500 µl 30% glycerol +500 µl broth). The broth was homogenized on a vortex mixer and stored at -70°C. Control plates are inoculated and incubated at 37°C overnight to check for contamination. If the control plate shows contamination, the strain is purified by reincubating it on CLED plate.

2.6. Antimicrobial Susceptibility Testing of Uropathogens

The antimicrobial susceptibility testing of Gram-positive isolates was done by the standard disk diffusion method of the Clinical and Laboratory Standards (13). The antibiotic discs and their concentrations were ampicillin (10µg), Cefazolin (30µg), ceftriaxone (30µg), norfloxacin (5µg), gentamicin (10µg), erythromycin (15µg), nitrofurantoin (300µg), TMP-SMX (25µg), and vancomycin (30µg). All the antimicrobials used for the study were obtained from Oxo Ltd. Basingstore Hampaire, UK. Standard inoculae adjusted to 0.5 McFarland were spread with swabs on the surface of Muller- Hinton agar plates (Oxoid Ltd. Basingstore Hampaire, UK); antibiotic discs were dispensed after drying the plates which were then incubated for 24 hours at 37°C.

Reference strains of Enterococcus faecalis (ATCC 29212) and S. aureus (ATCC 29213), sensitive to the antimicrobial agents tested were used as a quality control throughout the study. Multi-drug resistance was defined as resistance to three or more of the antimicrobials tested [12].

2.7. E-test

E-test (bioMérieux) was used to determine the minimum inhibitory concentration (MIC) of linezolid. The test was performed on Mueller Hinton agar (Oxoid, Basingstoke, UK) with incubation for 16-18 h at 36°C. Susceptibility testing was performed, and breakpoints were established according to the recommendations of the CLSI. Resistance to oxacillin is measured by E-test using species-related breakpoints with resistance defined as an MIC of ≥ 2.0 µg/ml [13].

2.8. Vancomycin Resistance Gene Typing by PCR

Enterococci were first incubated overnight at 37°C in Todd-Hewitt broth, and then 1-ml volumes were microcentrifuged and the pellet was resuspended in 200 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). The
suspensions were heated at 95°C for 20 min and then microcentrifuged for 2 min. Five-microliter volumes of the supernatant were subjected to PCR amplification in 50-ml reaction mixtures containing each deoxynucleoside triphosphate at a concentration of 200 mM, each primer at a concentration of approximately 1 mM, and 1 U of Taq polymerase (Boehringer Mannheim) in 10 mM Tris-HCl (pH 8.3)-50 mM KCl-2 mM MgCl2-0.1% gelatin-0.1% Tween 20-0.1% Nonidet P-40.

The samples were subjected to 35 PCR cycles, each consisting of 1 min of denaturation at 94°C, 2 min of annealing at 60°C, and 2 min of elongation at 72°C. PCRs were analyzed by electrophoresis on 2% agarose gels and were stained with ethidium bromide. The oligonucleotide primers used for detection of vanA, vanB, vanC1, and vanC2 or vanC3 sequences were designed with reference to the sequences deposited in GenBank by P. Courvalin and colleagues under accession numbers X56895, L06138, M75132, L29638, and L29639, respectively. Primer sequences and specificities are presented in Table 1.

For each sample, two PCRs were set up. One contained primers VanABF, VanAR, and VanBR, which direct amplification of 231- and 330-bp fragments from the vanA and vanB genes, respectively. The other contained primers VanC1F, VanC1R, VanC23F, and VanC23R, which direct amplification of 447- and 597-bp fragments from the vanC1 gene and either the vanC2 or the vanC3 gene, respectively. Multiplex PCRs were tested in duplicate. Known positive and negative controls were also included with each PCR run [14]. Genotype-negative VRE isolates, for which vancomycin MICs were 4 mg/ml, were also tested for the presence of vanD by using the primers described by Perichon et al. 1997 [15].

2.9. Statistical analysis

The statistical analyses will be performed using Statistical Package for the Social Sciences (SPSS for Windows Version 22.0). All the results will be tabulated and presented by figures and histograms using the one-way ANOVA repeated measures, and paired T-test.

### Table 1. PCR primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Specificity</th>
<th>Location within gene (strand) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>VanABF</td>
<td>GTAGGCTGCGATATTCAAGC</td>
<td>vanA</td>
<td>358-378 (++)</td>
</tr>
<tr>
<td>VanAR</td>
<td>CGATTCAATTCGATGTTCAAA</td>
<td>vanA</td>
<td>568-588 (−)</td>
</tr>
<tr>
<td>VanBR</td>
<td>GCGGACAACTCAGATCATCCTC</td>
<td>vanB</td>
<td>664-684 (−)</td>
</tr>
<tr>
<td>VanC1F</td>
<td>TGGATATGGATCAAGGAAC</td>
<td>vanC1</td>
<td>139-160 (−)</td>
</tr>
<tr>
<td>VanC1R</td>
<td>AGATGGGACTGCTGTGTTGTC</td>
<td>vanC1</td>
<td>565-585 (−)</td>
</tr>
<tr>
<td>VanC23F</td>
<td>CAGCAGCATTGGCGGTACCA</td>
<td>vanC2 and vanC3</td>
<td>431-450 (−)</td>
</tr>
<tr>
<td>VanC23R</td>
<td>CAAGCAGTTTTTGATAGTTC</td>
<td>vanC2 and vanC3</td>
<td>1006-1027 (−)</td>
</tr>
</tbody>
</table>

*aPosition in nucleotide sequence relative to the initiation codon. +, positive strand; −, negative strand.

3. Results

Out of 8600 reviewed cases, 1137 (13.2 %) were culture positive. Gram-positive cocci represented 165 (14.5%) whereas 972 (85.5%) were gram-negative bacilli. (Figure 1)

<table>
<thead>
<tr>
<th>Type of the isolate</th>
<th>Number (%) (N=1137)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative Isolates</strong></td>
<td>972</td>
</tr>
<tr>
<td>E. coli</td>
<td>620 (63.8)</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>180 (18.5)</td>
</tr>
<tr>
<td>Kelebsiella species</td>
<td>130 (13.4)</td>
</tr>
<tr>
<td>Proteus species</td>
<td>51 (5.2)</td>
</tr>
<tr>
<td>Morganella species</td>
<td>12 (1.2)</td>
</tr>
<tr>
<td><strong>Acinetobacter species</strong></td>
<td>8 (0.8)</td>
</tr>
<tr>
<td><strong>Gram positive Isolates</strong></td>
<td>165</td>
</tr>
<tr>
<td>Children</td>
<td>109</td>
</tr>
<tr>
<td>Adult females</td>
<td>56</td>
</tr>
</tbody>
</table>

| **Enterococci** | 117 (70.9) |
| **E. faecalis** | 88 (53.3)  |
| **E. faecium**  | 29 (17.6)   |
| Streptococcus agalactiae | 39 (23.6) |
| Staphylococcus aureus | 9 (5.5)   |

Figure 1. Distribution of the isolated organisms from urine
Out of 165 Gram positive cocci culture, the isolation rate of Enterococcus spp was 70.9% (117/165). Enterococcus faecalis was isolated from 88 cultures (53.3 %) whereas Enterococcus faecium was isolated from 29 cultures (17.6 %). Streptococcus agalactiae represented 39 isolates (23.6 %) then Staphylococcus Aureus 9 (5.5 %) isolates. (Table 2)

Frequency of isolated gram-positive cocci were higher among children (109/ 165) (66%) than adult females (56/165) (34%). The frequency of different gram-positive spp isolated from children and adult females are represented in Table 2. Enterococcus faecalis isolated from children were 62 out of 109 (56.9 %) and 26 out of 56 in adult females (46.4%) while Staphylococcus aureus was the least isolated organism in children (3/109) (2.7%) and Enterococcus faecium was the least isolated organism among adult females (1/56) (1.8%) (Figure 2).

Regarding children sex and age distribution, no significant difference in the frequency of isolation of gram-positive cocci from urine of male children (57/109) compared to female children (52/109) aged from 1 month to 13 years (52.3% and 47.7% respectively). However, gram-positive cocci were isolated from 28 infants ≤ 2 years and percentage of gram positive UTIs was significantly higher in male (71.4% “20/28”) than female (28.6% “8/28”) infants (Figure 3). Among children those with isolated gram-positive cocci from their urine, 41 (37.6%) were presented with only urinary tract infection while the rest of cases (68/109) (62.4%) presented with other clinical manifestations and anomalies associated with urinary tract infections including chest infections, polycystic kidney, hydronephrosis, sepsis, epilepsy, colitis and sickle sick anaemia (Figure 4). The majority of paediatric cases (55%) were admitted in inpatient paediatric departments, while 36.7% were from paediatric emergency department and 6.4% in neonatal intensive care units. Minority of cases were from other departments as nephrodialysis and behaviour and growth units (Figure 5).
Regarding adult females, 42 out of 56 (75%) diagnosed after urine culture as urinary tract infection were pregnant. 19 of those pregnant females presented as asymptomatic bacteruria (GBS were isolated from 9 out of 19 cases (47.4%) of asymptomatic bacteruria). The remaining 25% (14/56) of females included in the study presented with UTI associated with premature rupture of membranes, vaginitis, vaginal bleeding or fever (Figure 6).

Multidrug resistant positive cocci formed 9.7% (16/165) of total gram-positive isolates (Figure 7). VRE formed 87.5% (14/16) of multidrug resistant positive cocci, while MRSA formed 12.5% (2/16) (Figure 8).

### Table 3. Description of multidrug resistant gram-positive cocci cases

<table>
<thead>
<tr>
<th>Point of description</th>
<th>Number</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>10</td>
<td>62.5</td>
</tr>
<tr>
<td>- Female</td>
<td>6</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ≤2 years</td>
<td>15</td>
<td>93.7</td>
</tr>
<tr>
<td>- &gt;2 years</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Site of Admission:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Paediatric department</td>
<td>14</td>
<td>87.4</td>
</tr>
<tr>
<td>- PICU</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>- NICU</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Clinical presentation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Pure urinary tract infection</td>
<td>5</td>
<td>31.2</td>
</tr>
<tr>
<td>- Other presentations</td>
<td>11</td>
<td>68.8</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>(5)</td>
<td>(45.4)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>(2)</td>
<td>(18.2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>(2)</td>
<td>(18.2)</td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>(1)</td>
<td>(9.1)</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>(1)</td>
<td>(9.1)</td>
</tr>
<tr>
<td><strong>Length of hospital stay:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ≤25 days</td>
<td>15</td>
<td>93.7</td>
</tr>
<tr>
<td>- &gt;25 days</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Outcome:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cure</td>
<td>15</td>
<td>93.7</td>
</tr>
<tr>
<td>- Death</td>
<td>1</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Regarding antibiotic sensitivity results for isolated gram-positive cocci, all Enterococcus faecalis isolates were sensitive to ampicillin, vancomycin and linezolid while, 75% of them were sensitive to nitrofurantoin and 18% were sensitive to gentamycin. All Enterococcus faecium isolates were sensitive to linezolid, 50% of them were sensitive to nitrofurantoin drug and all were resistant to ampicillin. Data from our investigation showed varying susceptibility to antibiotics with significant resistance of Enterococcus spp isolates (more than 80%) to erythromycin, ampicillin, cefazolin, ceftriaxone, norfloxacin, and sulfamethoxazole-trimethoprim. VRE isolates were all resistant to vancomycin and all sensitive to linezolid drugs. All staph aureus isolates were sensitive to cefazolin, nitrofurantoin and sulfamethoxazole drugs while, all MRSA isolates were resistant to penicillin, ampicillin, cefazolin, and nitrofurantoin. 75% of MRSA isolates were sensitive to sulfamethoxazole and all were sensitive to vancomycin drugs. All isolates of streptococcus agalactiae were sensitive to cefazolin and nitrofurantoin drugs, while
52% of them resistant to ampicillin and all were resistant to sulfamethoxazole drugs (Figure 9). Molecular typing of VRE strain in this study revealed vanB genotype.

4. Discussion

Bacterial infection of the urinary tract is one of the common causes for seeking medical attention in the community. Effective management of patients suffering from bacterial UTIs commonly relays on the identification of the type of organisms that caused the disease and the selection of an effective antibiotic agent to the organism in question [16].

In this study, the isolation rate of bacteria from urine was 13.2%. This finding is in line with a study done in Addis Ababa where the prevalence of urinary tract infection was 48/414 (11.6%) [17] and one from Iran which had a UTI rate of 13.2% [18]. This is possibly because UTI symptoms are not a reliable indicator of infection and in children younger than 2 years of age are non-specific.

In our investigation, most of the urine samples were collected from patients who did not have classic UTI symptoms, and most of the subjects had been referred by general practitioners not specialist physicians. These results indicate that urine culture is necessary in the majority of cases for a definitive diagnosis of UTI. On the other hand, our result is comparatively lower than reports within the country and other parts of the world. A study conducted in the King Fahad National Guard Hospital in Riyadh reported 24.4% prevalence of hospital acquired UTI [19]. In another study in Kenya, 24% prevalence of UTI was detected [16], this might have been either due to sample size variation or the studies might have been based on retrospective survey.

In this study, the most frequent uropathogens were Gram negatives which made up to 85.5% of all isolates (Figure 1). Our result is in concordance with that published by Angoti et al, 2016 [20] who isolated gram negatives from 80% of positive urine cultures. *E. coli* was by far the most common bacteria isolated from urine samples in both outpatients and inpatients of both sexes (63.8%), followed by *Enterobacter* spp (18.5%), *Klebsiella* spp (13.4%) and *Proteus* spp. (5.2%) while *Acinetobacter* spp were the least isolated among gram-negative organisms (only 8 isolates i.e., 0.8%) (Table 2). This finding was in agreement with other studies [18,21] where *Escherichia coli* was the most common etiological agent of UTI (74.6%, 80.5% respectively), followed by *Klebsiella* spp (11.7%) (18) and *Proteus* spp. (6.1%) [21].

Recently, gram-positive species recovered from urine have gained special attention and are identified more frequently. Symptoms associated with uncomplicated UTI caused by Gram-positive uropathogens are similar to those caused by Gram-negative organisms. Complicated UTIs often occur in nosocomial settings, particularly in individuals with structural or functional alterations of the urinary tract (often associated with urinary catheterization), or other underlying renal, metabolic, or immunological disorders [22]; those populations are at greater risk of Gram-positive and polymicrobial UTI of [23]. In spite of being usually seen in small numbers of cultured urine samples, *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Streptococci* and *Enterococci* are recognized as important causes of UTI [24,25].

In this study, we investigated the frequency and antimicrobial susceptibility patterns of Gram-positive cocci isolated from patients with nosocomial UTI at the maternity and child hospital (MHC) in Medina, KSA. In this study, 14.5% of our urine isolates were gram-positive cocci (165/1137) (Figure 1). Higher isolation rates of gram-positive cocci from urine were reported in other studies [18,21,26]. The variations in the type and distribution of UTI-causing bacteria in various geographic regions and countries may be due to training, host conditions, hygiene practices, and healthcare practices.

*Enterococcus faecalis* isolated from children were 62 out of 109 (56.9%) and 26 out of 56 in adult females (46.4%) while *Staphylococcus aureus* was the least isolated organism in children (3/109) (2.7%) and *Enterococcus faecium* was the least isolated organism among adult females (1/56) (1.8%) (Figure 2). This result is different from that of Tayebi et al, 2014 [27] who reported that *S. aureus* was the most common gram-positive cocci isolated in both sexes and all age-groups (*S. aureus* strains were responsible for 59.8% of UTI cases).

The incidence of UTI varies according to the age and sex of children. It is known that UTI is more frequent in boys in the first 2 years of life (period of infancy). In the present study this was also true. Gram positive cocci were isolated from 28 infants ≤ 2 years and percentage of gram positive UTIs was significantly higher in male (71.4% “20/28”) than female (28.6% “8/28”) infants (Figure 3). This was very close to the value arrived at in the study done by Saeed and coworkers, 2015 [28] who reported that the percentage of UTIs was significantly higher in males (60.9%) than females (39.1%) during the first 3 months of life. This male to female preponderance during infancy, may be due to congenital obstruction, phimosis and high frequency of urethral malformations in male infants [29]. In addition to high frequency of UTI in uncircumcised boys [30]. Another study [31] found that there are almost equal numbers of cases of UTI for the first two years of life in male and female patients, and then a marginally increasing rate of UTI was found among female children older than two years.

Among children with isolated gram-positive cocci from urine, only one third of cases (37.6%) were presented with urinary tract infections while most cases (68/109) (62.3%) were presented with other conditions including chest infections, polycystic kidney, hydronephrosis, sepsis, epilepsy, colitis and sickle sick anaemia (Figure 4). Multiple studies mentioned that the classical features of UTI are absent in young children, who often present with few signs or symptoms other than fever [32,33,34]. Urinalysis in febrile babies, especially boys younger than 12 months, is important to consider because febrile urinary tract infections (UTI) have long been considered amongst the most common serious bacterial infections in childhood with renal scarring a frequent outcome [35]. The majority of paediatric cases (55%) in the current study were admitted in inpatient paediatric departments, while 36.7% were from paediatric departments.
emergency department and 6.4% in neonatal intensive care units. Minority of cases were from other departments as nephrodialysis and behaviour and growth units (Figure 5).

The rate of UTIs gradually increases with age to 30 years in women (average 20-40 years which is the childbearing period) [36]. Regarding adult females in our study, 42 out of 56 (75%) presented with urinary tract infection associated with pregnancy while the other 14 (25%) presented with UTI associated with other conditions such as vaginitis, abnormal vaginal bleeding and fever (Figure 6). In addition, we had a 34% (19/56) prevalence of asymptomatic bacteriuria in our study population. Our value is much higher than the range of 2-10% reported elsewhere [37,38,39]. Variation in studies may be due to differences in geographical location, socioeconomic status, setting of study (primary care, general hospital and community), sample size and variation in screening tests (cut-off point for the detection of pathogens).

For long time, enterococci were frequently considered to be commensal organism and was ignored when isolated in clinical laboratory. But recently its capability of causing variety of infections, especially in hospitalized patients, and difference in antimicrobial sensitivity of each species to varying antibiotics have led to understanding the importance of identification of Enterococcus to species level. Enterococcus species were by far the most common gram-positive cocci isolated from urine samples in our study (71.9%). E. faecalis was the predominant species (53.3% of isolates) (Table 2), which is not surprising, in light of global epidemiological reports on the causative agents for UTIs [10] while was contrary to the findings of others [40,41] who reported Staphylococcus species as the most commonly isolated gram positive uropathogen. The high incidence of enterococcal UTI may be attributed to the underlying condition, longer hospital stay, colonization of Foley's catheters and increasing use of broad-spectrum antibiotics particularly cephalosporins as the case in our hospital.

The increased incidence of enterococcal UTI is alarming; resistance to most commonly used antimicrobial agents is a typical characteristic of these bacteria. In the current study, 75% of Enterococcus faecalis and 50% of Enterococcus faecium isolates were sensitive to nitrofurantoin (Figure 9). This result was comparable with that published by Al-Tawfiq and Anani, 2009 [42] who found that the susceptibility of HCA and CA isolates of E. faecalis to nitrofurantoin were 78.3 and 93.6%, respectively. Several other investigators have reported isolates that have reduced sensitivity to nitrofurantoin [43].

18% of Enterococcus faecalis isolates were sensitive to gentamicin. Gajdać et al, 2020 reported that high-level aminoglycoside resistance in enterococci was noted in 31.0-46.6% of cases [44].

Data from our investigation showed varying susceptibility to antibiotics with significant resistance of Enterococcus spp isolates (more than 80%) to erythromycin, ampicillin, cefazolin, ceftriaxone, norfloxacin, and sulfamethoxazole-trimethoprim. The motivation for resistance to these antibiotics could be their abuse in the hospitals and the community. All the enterococcal isolates (other than VRE isolates) were susceptible to vancomycin and linezolid. This finding is similar to that published in the study done by Kaur et al., 2014 in India [45].

S. aureus is an important uropathogen and was responsible for 10.7% (6/56) of UTI cases among adult females in our study (Figure 2). This result matched with that of Tchente Nguefack et al, 2019 who reported that S. aureus accounted for 8.6% of pathogens involved in bacteriuria of pregnant women [46]. Similarly, Baraboutis et al, 2010 stated that in contrast to S. saprophyticus, which is a predominant cause of community-acquired UTI, S. aureus UTI more often occurs in urinary-catheterized and pregnant individuals [47]. It has been emphasized that any amount of this bacterium should be subjected to antibiogram test [48].

Over the last decade there has been a substantial increase in resistance of S. aureus strains to antibiotics. In this study, fortunately resistant strains to cefazolin, nitrofurantoin, sulfamethoxazole and vancomycin were not observed (Figure 9). Similar findings were present in the study of Tayebi et al, 2014 [41]. Based on our study, a high level of resistance to ampicillin was seen among Staphylococcus aureus isolates. In concordance with other studies, these results implied that ampicillin cannot be used for treatment of UTI [49,50].

Streptococcus agalactiae, otherwise known as group B Streptococcus (GBS) is a gram-positive β-hemolytic chain-forming coccus that is a common asymptomatic inhabitant of the lower gastrointestinal and female reproductive tracts. In our study, GBS was estimated to cause 3.4% (39/1137) of all monomicrobial UTIs (Table 2). Our result approached to that reported by Foxman, 2003 [51]. Asymptomatic bacteriuria (ASB) caused by GBS is common among pregnant females with higher risk of ascending pyelonephritis that can progress to bacteremia and/or urosepsis [52].

In addition, GBS is the leading cause of sepsis and meningitis in newborns and can be acquired by the newborn in utero or during passage through the colonized birth canal [53]. This was apparent in our study in which GBS were isolated from 9 out of 19 cases (47.4%) of asymptomatic bacteriuria among pregnant females included in the study.

All isolates of Streptococcus agalactiae in our study were sensitive to cephalocin and nitrofurantoin, while 52% of them were resistant to ampicillin and all were resistant to Trimethoprim-sulfamethoxazole (Figure 6). This result was matched with that arrived by Alhambra et al, 2004 [54] who found that S. agalactiae isolates were susceptible to the β-lactams and nitrofurantoin used in the treatment of UTIs caused by this species. However, levels of resistance to macrolides and lincosamides were significant (20%) in their study and most isolates (83.5%) were resistant to tetracyclines. (MDR) was defined as resistance to at least three or more antibiotics [12]. In the current study, multidrug resistant positive cocci formed 9.7% (16/165) of total gram-positive isolates (Figure 7) including vancomycin resistant enterococci (VRE) (8.5%) and methicillin resistant staph aureus (MRSA) (1.2%) (Figure 8). Recent studies have reported the increasing prevalence of multi drug resistant S. aureus especially MRSA in UTIs [55].
Tayebi et al, 2014 reported of 316 isolates tested 151 (47.8%) were MDR [27]. All the 16 MDR stains were isolated from infants (15 of them were less than 2 years); 10 males and 6 females and all were admitted in the hospital. It could be attributable to high usage of antimicrobial agents in the hospitals. Monitoring of antimicrobial susceptibility helps prevent the spread of resistant isolates and eliminate the use of antibiotics for a prolonged period [56,57].

Only one third of our MDR-infected infants presented with symptoms of UTI (31.2%) and the mortality rate among them was 6.3% (1/16) (Table 3). VRE colonization is seen more frequently in newborns who have medical problems during follow-up. Therefore, surveillance cultures that performed routinely in NICUs, would be helpful to detect VRE colonization in time and to implement isolation precautions rapidly to prevent dissemination of the organism and decrease the incidence of bacteremia and death [58].

Among the strains of Enterococcus spp. investigated in this study, 14/117 (11.9%) were VRE. The 14 VRE isolates were Enterococcus faecium. This result matched the study of Gajdács et al, 2020 in which all urinary VRE isolates were exclusively from E. faecium [44]. Among E. faecium isolates, vanB genotype was detected. The predominance of E. faecium vanB was similarly reported in an Australian paper [59] and suggested an epidemiology different from that in either Europe or the United States. On the other hand, a study held in a tertiary hospital in Saudi Arabia and reported that vanA+/vanB with vanB phenotype were predominant [60]. Another study conducted in King Faisal Hospital in Riyadh showed predominant vanA genotype and provided the first report of vanB phenotype- vanA genotype incongruent E. faecium in the Middle East region [61]. Molecular typing indicates clonal spread and high occurrence of virulence genes associated with epidemic clones.

5. Conclusion

Gram-positive organisms recovered from urine are found more often with increasing levels of antibiotic resistance. Continuous Surveillance for multidrug-resistant strains is necessary to prevent the further spread of resistant isolates and to provide physicians with knowledge about the most effective empirical treatment of UTIs. From the present study, vancomycin and nitrofurantoin seem to be effective drugs for treatment of UTI associated with gram positive cocci. According to our findings, Ampicillin, Ceftriaxone, erythromycin and Sulfamethoxazole-trimethoprim are not effective drugs for treatment of gram positive UTI. Molecular typing of VRE strain in this study revealed vanB genotype.

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Ethics Approval and Consent to Participate

1. The study protocol was approved by the Studies and Research Committee, MMCH, General Directorate of Health, Madinah, Saudi Arabia.
2. Maintaining confidentiality of information obtained from subjects under the study.
3. Results of samples collected were donated to physicians of patients included in the study for treatment prescription.

Conflict of Interest

The authors declare that they have no conflict of interests.

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