Frequency and antibiotic resistance pattern in gram positive uropathogenes isolated from hospitalized patients with urinary tract infection in Tehran, Iran

Running title: Antibiotic resistance of Gram Positive uropathogenes

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Abstract

Background: Urinary tract infection (UTI) is one of the most frequent types of nosocomial infections. The main purpose of this study was to determine the frequency of gram positive cocci in urine obtained from hospitalized patients and their susceptibility patterns to antimicrobial agents.

Material & Methods: During a 12 months study, 316 clinical isolates of gram positive cocci were recovered from 765 urine specimens of hospitalized patients with UTI. In vitro susceptibility of gram positive cocci isolates to 10 antimicrobial agents Ampicillin; Cephalothin; Ceftriaxone; Nitofurantoin; Erythromycin; Norfloxacin; Gentamicin; Vancomycin; Sulfamethoxazole-trimethoprim and Chloramphenicol was performed using the Kirby-Bauer’s Disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) criteria.

Results: Out of 3233 urine samples of hospitalized patients 765 (23.7%) patients were showed to be urine culture positive. The frequency of Staphylococcus aureus, Enterococcus spp, Coagulase-negative staphylococci (CoNS) and Streptococcus group B were 24.7%; 8.7%, 4.1% and 3.6%. All of gram-positive cocci with the exception of Enterococcus spp (11.9%) were sensitive to vancomycin. All of Streptococcus group B and CoNS isolates were sensitive to nitrofurantoin.
Conclusion: The present study exhibited that *Staphylococcus aureus* is the most common causative agents of nosocomial UTI. Vancomycin and nitrofurantoin seem to be the most effective drugs for treatment of gram positive cocci causing UTI.

**Keywords**: UTI, Antimicrobial susceptibility, Uropathogen

1 Introduction

Nosocomial infections are important public health problems in developing countries. Nosocomial or hospital-acquired infections are usually defined as infections that are identified at least 48-72 hours following admission to hospital and health care facility [1]. Urinary tract infection (UTI) is one of the most frequent types of nosocomial infections and probably affects about one-half of all people during their lifetimes. UTI refers to the existence of microbial pathogens in the urinary tract and is defined as the growth of a single pathogen of $>10^5$ colony-forming units per milliliter from properly collected mid-stream urine specimens [2, 3]. Bacterial agents are responsible for UTI that can be ranged from mild irritative voiding to bacteremia, sepsis and death [4]. The major causatives agents of UTIs are *Escherichia coli* and other *Enterobacteriaceae*. Although relative frequency of the pathogens varies depending upon age, sex, catheterization and hospitalization but in some of hospitalized patients, Gram-negative rods (*Pseudomonas spp*) and gram positive cocci (coagulase negative *Staphylococci*, *Staphylococcus aureus*, *Streptococcus* group B, *Enterococci*) are comparatively more common [5-10]. Although mortality rates associated with UTI are usually low, it is a great concern that requires antimicrobial therapy. Inappropriate use of antimicrobial agents in order to treat patients with UTI has recently led to the spread of antimicrobial resistance and generation of multidrug-resistance (MDR) among urinary pathogens bacteria [11, 12]. During the past decade, gram positive cocci isolates exhibited a remarkable ability to rapidly develop antibiotic resistance. Resistance to antimicrobial agents among gram positive cocci clinical isolates is higher than community isolates. Area-specific monitoring studies in order to detect antimicrobial resistance patterns, adopt effective treatment and decrease mortality rates are necessary [13]. Considering the lack of information about antimicrobial resistance profiles of gram positive cocci from clinical isolates and increasing infection rates, the aim of this study was to investigate the frequency of gram positive cocci urinary infection in hospitalized patients and their susceptibility patterns to commonly used antimicrobial agents.

2 Material and Methods

2.1. Bacterial isolates

The present descriptive study was performed on UTI cases who were hospitalized in different wards of hospital. A total of 316 gram positive cocci clinical isolates were recovered from the 765 urine specimens of hospitalized patients with UTI. The study was performed between September 2012 and October 2013. Urine samples were obtained from the study subjects using a wide mouth sterile container. UTI refers to the existence of microbial pathogens in the urinary tract and is defined as the growth of a single pathogen of $>10^5$ colony-forming units per milliliter (CFU/ml) from properly collected mid-stream urine specimens [14]. All the cases had history of nosocomial UTI and clinical examination was conducted by a physician to exclude community-acquired infections. Proper specimen collection was instructed to all patients. All the urine samples were transported to the laboratory and were processed immediately. Urine specimens were investigated by direct microscopy for white blood cell (WBC) counting. For colony count, urine
samples were cultured according to surface streak procedure using calibrated loops for semi-quantitative method. The plates were incubated in aerobic conditions at 37°C for 24–48 hours. Negative cultures were maintained in incubator up to 2 days. The result of equal or more than $10^5$ CFU/ml was considered as positive UTI and less than $10^2$ CFU/ml was interpreted as negative UTI. Results of $10^2$-$10^4$ CFU/ml were repeated [14].

Urine specimens were inoculated on blood agar (Merck, Germany) for isolation of gram positive cocci. Identification of gram positive cocci isolates was performed by Gram staining, catalase, manitol fermentation and coagulase tests, as well as other conventional biochemical tests. Coagulase test was done using both slide and tube methods [14, 15].

Samples confirmed as gram positive cocci isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C and were subjected to further investigation.

### 2.2. Antimicrobial susceptibility testing

To evaluate antimicrobial susceptibility of isolates, Kirby-Bauer’s Disk diffusion method was done according to Clinical Laboratory and Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) criteria [16]. The following antimicrobial agents were used in this study: Ampicillin; Cephalothin; Ceftriaxone; Nitrofurantoin; Erythromycin; Norfloxacin; Gentamicin; Vancomycin; Sulfamethoxazole-trimethoprim; Chloramphenicol. Antibiotic disks used in this research were supplied by MAST Laboratories Ltd (Bootle, Merseyside, UK). Briefly, the bacterial suspensions were obtained from overnight cultures. The turbidity of each bacterial suspension was adjusted equivalent to a no. 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). Diameter of inhibition zones was measured after incubation at 35°C for 18-24 hours, and data were reported as susceptible, intermediate, and resistant. *Staphylococcus aureus* ATCC 25923 was used as reference strains for susceptibility testing. Data were analyzed using SPSS version 13 software.

### 3 Results

In this study, a total of 3233 urine samples of hospitalized patients were screened in 2013, 765 (23.7%) patients were showed to be urine culture positive (their colony count was equal or more than $10^5$) of which there were 527 (68.9%) females and 238 (31.1%) males. The age range of the patients was from 15 to 65 years with a median of 34.8. The age distribution was 6.4% for patients aged equal or less than 15 years, 66.4% for 16 to 25 years, 15.8% for 26 to 50 years, and 11.4% for equal or greater than 60 years. The isolation percentages of bacterial species were found as gram negative bacteria (53.3%); Staphylococcus *aureus* (24.7%); Enterococcus spp (8.7%), CoNS (4.1%) and Streptococcus group B (3.6%). The gram positive and gram negative bacterial proportion was 316 (41.3%) and 449 (58.7%), respectively. Occurrence of bacterial UTIs associated with gram positive cocci was the highest in the age group 19-39 year (66.4%) and the lowest in the age less than or equal to 15 years (6.4%). The patients were distributed in 5 hospital departments. There were 220 (69.6%) females and 96 (30.4%) males in UTIs associated with gram positive cocci.
Ampicilin, Gentamicin, Ceftriaxone. The level of resistance for isolated bacterial showed wide different level of resistance for tested antibiotics. In this study Frequency and Antimicrobial susceptibilities of 316 gram positive cocci isolates to 10 antimicrobial agents is showed in Table 1, which were common among Enterococcus spp showed the highest susceptibility (35.7%, 32.1%, 39.2%).

The most common gram positive microorganism isolated in all age-groups and both sexes was Staphylococcus aureus. No significant difference was found between isolated bacteria and age of the patients. The profile of isolated bacterial showed wide different level of resistance for tested antibiotics. In this study Frequency and Antimicrobial susceptibilities of 316 gram positive cocci isolates to 10 antimicrobial agents is showed in Table 1, the frequency of gram positive cocci isolated from UTI was 41.3%. Staphylococcus aureus isolates were responsible for about 59.8% UTIs cases and showed high resistance to ampicilin, ceftriaxone and gentamicin, and higher sensitivity to vancomycin, nitrofurantoin and sulfamethoxazole-trimethoprim. All of gram-positive cocci with the exception of Enterococcus spp (11.9%) were sensitive to vancomycin. All of CoNS and Streptococcus group B isolates were sensitive to nitrofurantoin. On CoNS isolates, ampicilin, cephalothin, ceftriaxone, erythromycin, norfloxacin, Gentamicin, sulfamethoxazole-trimethoprim and chloramphenicol showed the highest resistance (100%, 59.3%, 56.2%, 71.8%, 87.5%, 56.2% and 65.6% respectively). The percentage of resistance to ampicilin, ceftriaxone, erythromycin, norfloxacin, gentamicin and sulfamethoxazole-trimethoprim in among Enterococcus spp isolates were more than 50%. On Streptococcus group B isolates, ampicilin, cephalothin, norfloxacin and chloramphenicol showed the highest susceptibility (35.7%, 32.1%, 39.2% and 25% respectively). The percentages of resistance to ceftriaxone, erythromycin, gentamicin and sulfamethoxazole-trimethoprim among Streptococcus group B isolates were more than 50%.

Multidrug-resistant (MDR) was defined as resistance to at least three or more antibiotics [17, 18]. Of 316 isolates tested 151 (47.8%) were MDR. In particular, eighty eight (88%) of isolates were resistant to at least two drugs, fifty nine (59%) of isolates were resistant to at least three drugs and 52 (16.4%) of the isolates were resistant to four drugs. MDR strains to three or more tested antibiotics were isolates from hospitalized patients in infectious, internal medicine, ICU and gastroenterology wards respectively. The predominant resistance profile among our isolates were included resistance to 2 antibiotics (Ampicilin, Gentamicin) and 3 antibiotics (Ampicilin, Gentamicin, Ceftriaxone), which were common among 78 (24.6%) and 69 (21.8%) isolates.

Table 1: Frequency and Antimicrobial susceptibilities of 316 gram positive cocci isolates to 10 antimicrobial agents

<table>
<thead>
<tr>
<th>Isolated Bacteria</th>
<th>Antibiotics No(%)</th>
<th>AM</th>
<th>CF</th>
<th>CRO</th>
<th>FM</th>
<th>E</th>
<th>NOR</th>
<th>GM</th>
<th>V</th>
<th>SXT</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>189(59.8)</td>
<td>189(100)</td>
<td>104(55)</td>
<td>155(82)</td>
<td>15(7.9)</td>
<td>104(55)</td>
<td>47(24.9)</td>
<td>124(65.6)</td>
<td>0(0)</td>
<td>29(15.3)</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td></td>
<td>67(21.2)</td>
<td>67(100)</td>
<td>15(22.4)</td>
<td>34(50.7)</td>
<td>3(4.5)</td>
<td>65(97)</td>
<td>37(55)</td>
<td>60(89.5)</td>
<td>8(11.9)</td>
<td>59(88)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td></td>
<td>32(10.1)</td>
<td>32(100)</td>
<td>19(59.3)</td>
<td>18(56.2)</td>
<td>0(0)</td>
<td>23(71.8)</td>
<td>25(78.1)</td>
<td>28(87.5)</td>
<td>0(0)</td>
<td>18(56.2)</td>
</tr>
<tr>
<td>Streptococcus group B</td>
<td></td>
<td>28(8.8)</td>
<td>10(35.7)</td>
<td>9(32.1)</td>
<td>15(53.5)</td>
<td>0(0)</td>
<td>27(96.4)</td>
<td>11(39.2)</td>
<td>22(78.5)</td>
<td>0(0)</td>
<td>19(67.8)</td>
</tr>
</tbody>
</table>

AM, Ampicilin; CF, Cephalothin; CRO, Ceftriaxone; FM, Nitrofurantoin; E, Erythromycin; NOR, Norfloxacin; GM, Gentamicin; V, Vancomycin; SXT, Sulfamethoxazole-trimethoprim; C, Chloramphenicol

4 Discussion

Recent studies have revealed the importance of gram positive cocci in urinary tract infections. Urinary tract infection (UTI) is one of the most frequent types of nosocomial infections. Many of nosocomial infections are associated with microorganisms that are resistant to antibiotics and can easily spread by hospital environment and personnel. Monitoring of antimicrobial susceptibility can aid clinicians for prescription of appropriate antibiotics and prevention of development of drug resistance [1, 13]. Effective treatment of patients with UTIs commonly relays on the identification of the type of organisms and the selection of an effective antibiotic agent to the organism in question. The pattern of antimicrobial resistance of bacteria producing UTI varies in different regions [17].
Gram-positive organisms recovered from urine have received special attention recently. Staphylococcus aureus, coagulate negative Staphylococcus, Streptococci and Enterococci are seen in small numbers but they are recognized as important causes of UTI [7, 8, 18]. In this study, we investigated the frequency and antimicrobial susceptibility patterns of Gram Positive cocci isolated from patients with nosocomial UTI at a hospital in Tehran, Iran.

In our study the isolation rate of gram positive cocci (41.3 %) from urine was less than gram negative bacteria (58.7%) which is similar to recent studies [19, 20, 21, 22]

The gram positive cocci identified in this study were similar to other studies done in different geographic regions [23]. However different results have been reported. The differences in the type and distribution of bacteria causing UTI may be due to training education, host factors, hygiene practices and healthcare practices in different geographic regions and countries. With regards to the fact that most bacteria causing UTI are commensals perianal and vaginal regions therefore personal hygiene may be important in reducing the incidence of UTI

The sex distribution of patients in our study was female (66.4%) between 16 to 25 years. This result is similar to those reported from many other researches [24]. The higher level of UTI among females could be attributed to anatomy and microflora in genitourinary system.

In our study, among gram positive cocci, Staphylococcus species (28.8%) were by far the most common bacteria isolated from urine samples which is in agreement with the findings of others [25, 26, 27], while in contradiction with some of studies[13].

This study revealed that the occurrence rate of CoNS isolates to be 4.1% which was higher than England (1.5%), Canada (1.3 %) and lower than Iran (11.3%), India (6.3%) and Ethiopia (14.3%). CoNS isolates were mainly isolated from young women [17, 28, 29]. In a study done from 1986 to 2002 in Spain in 2011, Cuevas et al. showed that there were no major shifts in the distribution of CoNS species [30]. This study showed a high prevalence rate of resistance to the commonly prescribed antibiotic agent. Based on our study, a high level of resistance to ampicillin was seen among Staphylococcus aureus, Enterococcus and Coagulase-negative staphylococci isolates. In concordance with other studies, these results implied that ampicillin cannot be used for treatment of UTI [31, 32, 33]

All isolates in our study were fully sensitive to vancomycin with the exception of Enterococcus spp (11.9%). Our findings about vancomycin are in accordance with recent data [28, 34-37]. Resistance to nitrofurantoin among our isolates was very low. Isolates with decrease susceptibility to nitrofurantoin has been confirmed by several investigators [28]

In our study, the highest resistance rate of the CoNS was against ampicillin followed by Gentamicin, Norfloxacin and Erythromycin while all of them were sensitive to vancomycin and Nitrofurantoin. This data is consistent with some earlier reports [34; 35]. The resistance rate to gentamicin, cotrimoxazole among CoNS isolates was higher than other studies [28, 37]. On the other hand, in this study gentamicin, norfloxacin and erythromycin did not have good activity against CoNS isolates.

The data from our investigation exhibited varying antibiotic susceptibility with a significant resistance (more than 80%) of Enterococcus spp isolates to erythromycin, ampicillin, gentamicin and Sulfamethoxazole-trimethoprim. Surprisingly, in our study the susceptibility of Enterococcus spp to gentamicin was low while other studies in Iran reported high sensitivity to this antibiotic [26, 36], the reason for resistance to these antibiotics could be mediated by their widespread use in the hospital and the community. A significant increase in resistance of bacteria causing UTI to cephalothin, ceftriaxone, ampicillin, norfloxacin and SXT has been seen worldwide [37].

A high incidence of MDR strains was found in ICU and Infectious wards in our study. It could be attributable to high usage of antimicrobials agents in ICU. Continued use of antibiotic for treatment of UTI should be supported by monitoring of antimicrobial susceptibility to prevent the spread of resistant isolates and also eliminate the use of antibiotics for a prolonged period [38, 39].

[References]

http://www.ispac...
Although resistance to vancomycin and nitrofurantoin has been seen among our isolates, but it seems that they can 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 UTI. Progressive increase in resistance to these antibiotics and multiple resistances to antibiotics in present study, may be related to increased usage of these antibiotics for treatment of UTI and ability of strains in acquisition of resistance genes.

5 Conclusion

The high level of resistance among gram positive bacteria causing UTI limits the use of antimicrobial agents for therapy and also the spread of MDR isolates is a threat for hospitalized patients. Continuous Surveillance for multidrug-resistant strains is necessary to prevent the further spread of resistant isolates

6 Acknowledgement

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7 Conflict of interest

The authors declare that they have no conflict of interests.

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