



Isolation and Identification of *S. aureus* in Urinary Tract Infection

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ARTICLE INFO

Keywords: Aflatoxin, Ciprofloxacin, Isolation, Urinary Tract Infection (UTI), Teicoplanin.

Received : 28 January 2023

Revised : 15 May 2023

Accepted : 28 June 2023

ABSTRACT

Urinary Tract Infection (UTI) defines a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing, and occasionally life-threatening condition. *Staphylococcus aureus* is frequently isolated from urine samples obtained from long-term care patients. The significance of staphylococcal bacteriuria is uncertain. We hypothesized that *S. aureus* is a urinary pathogen and that colonized urine could be a source of future staphylococcal infection. **Methods:** This study focused on the frequency of *S. aureus* and its antibiotic susceptibility in different gender in the Derna District. A total of 200 UTI cases were reviewed in this study from Augustus 2022 to October 2022. Midstream specimens of urine, preferably of the first-morning void were collected from known UTI patients. Cultural and biochemical characterization of *S. aureus* **Results:** During this study period 200 samples were received, and from these samples, 170 bacterial isolates were obtained. Out of 170 isolates 160 were identified as *S. aureus*. Out of this predominant numbers were that of *S. aureus* 160 (94%), followed by other bacterial isolated 10 (6%). Also, it is shown that the highest number of *S. aureus* isolated were from married females (57.3%) followed by a male (42.7%). And it is showing 69.3% sensitivity toward Ciprofloxacin. Followed by Teicoplanin which shows 25.3% sensitivity, piperacillin, and Ofloxacin showed the lowest sensitivity. Thus our study showed that there were statistically significant differences between the isolated *S. aureus* and age, gender, and marital status. We concluded that ciprofloxacin is a better antibiotic used for *S. aureus*.

INTRODUCTION

Urinary tract infections (UTIs; ranging from uncomplicated cystitis to severe pyelonephritis and nephrolithiasis) are the third most common type of infection in human medicine worldwide (after respiratory tract infections and infections of the alimentary tract), and the second most commonly occurring infections in developed countries, with 100–180 million cases/year (Flores-Mireles, 2015–Sobel, J, 2018). *Staphylococcus aureus* (SA) is an unusual cause of urinary tract infection (UTI) whose prevalence ranges between 0.15 and 4.3% (Flores-Mireles, 2015). SA bacteriuria has been described predominantly in patients with predisposing conditions for ascending SA colonization (e.g., history of urinary obstruction, urinary catheter, recent urological surgical

procedures, malignancy, and recent hospitalization) (Sobel, J, 2018). Nevertheless, it is commonly interpreted as a genitourinary infection (Wiedemann, B. 2014; Hooton, T. M. 2009). In up to 34% of cases, SA bacteriuria is associated with SA bacteremia (Flores-Mireles, 2015, Wiedemann, B. 2014; Gupta, 2011; Abbo, 2014).

Staphylococcus aureus is a relatively uncommon cause of urinary tract infections in the general population (Flores-Mireles, 2015; Wiedemann, 2014). Although isolation of *S. aureus* from urine samples is often secondary to staphylococcal bacteremia arising elsewhere (e.g., in cases of endocarditis) (Sobel, 2018), in certain patients, *S. aureus* causes ascending urinary tract colonization and infection. Urinary tract instrumentation and the presence of an indwelling

catheter increase the risk of *S. aureus* carriage in the urinary tract (Flores-Mireles, 2015; Hooton, 2018).

The majority of cases of *S. aureus* bacteriuria are not associated with symptoms of urinary tract infection (Flores-Mireles, 2015). Because bacteriuria nearly universally occurs concomitantly with long-term urinary catheterization (Gupta, 2011; Abbo, 2014), the clinical significance of isolation of *S. aureus* from the urine is undefined in such patients. Clear differentiation between asymptomatic bacteriuria and clinical urinary tract infection is difficult in the elderly population (Callan, 2018; Stefaniuk, 2016). Although urinary *S. aureus* may be the source of staphylococcal bacteremia (Behzadi, 2015; Murray, 2013), the proportion of patients with chronic *S. aureus* bacteriuria who subsequently become bacteremic is unknown. In the absence of risk factors for SA colonization, SA bacteriuria may be related to deep-seated SA infection, and specifically to infective kidney embolisms associated with underlying infective endocarditis (Sobel, 2018; Abbo, 2014).

The general objective of this study was to isolate and identification of the *S. aureus* in urinary tract infections. While the specific objectives were (1) to determine the relationship between *S. aureus* according to demographic (age, gender, and marital status); (2) to determine the best antibiotics for isolated *S. aureus*.

METHODS

This prospective cross-over study was performed in the Department of Microbiology Lab, College of Medical Technology Derna from Augustus 2022 to October 2022.

Sample Collection

A total of 200 UTI (Urinary Tract Infection) cases were reviewed in this study from Augustus 2022 to October 2022. Midstream specimens of urine, preferably of the first-morning void collected from known UTI patients.

Urine Culturing

Urine samples were cultured on Nutrient agar, blood agar and McConkey agar medium and incubated overnight at 37°C. Significant growth was evaluated as ≥ 105 colony-forming units CFU/mL of midstream urine.

Isolation

Urine samples were cultured on different media including Blood agar, MacConkeys agar and Nutrient agar, and incubated at 37°C for 24 hours. Thereafter bacteria were isolated and purified by streaking two times on the same media. In Figure 1,



2

Figure 1. Samples were cultured on media including Blood agar, MacConkeys agar



Figure 2. Media preparation.

Characterization and identification

Each of the color, size, elevation, margins, and texture of colonies was screened. Pure isolates were examined microscopically, according to gram staining technique; isolates were cultured on Mannitol selective and differential media to find out their color.



Figure 3. a. *S. aureus* on blood agar; b. *S. aureus* (left) and *S. epidermidis* (right) - colonies on blood agar

Microscopic examination

The suspected colonies were stained using the gram stain method, and their shapes, colors, and arrangements were observed under a light microscope.

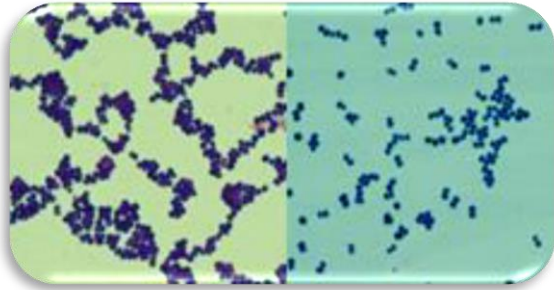


Figure 4. *S. aureus* by Gram stain.

Biochemical tests

All bacteriological isolates were examined and confirmed by biochemical tests according to Baron and Bergey’s manual of determinative bacteriology and other references (Difco’s Manual, 1984). Selected colonies were identified and differentiated according to their cultural characteristics; microscopical examination and microbiological analysis were tested biochemically for further confirmation of isolated bacteria, such as; catalase, oxidase, and coagulase test.



Figure 5. *S. aureus* - Catalase test positive

Antimicrobial sensitivity testing (Kirby-Bauer method)

The susceptibility of isolates to antibiotics was demonstrated by using nine specific antibiotics, including prescribed antibiotics that have been given by physicians. Isolates were placed on Mueller-Hinton agar by swabbing. After drying for about 5-10 min, Plates were incubated for about 10-15 min at 37°C. Furthermore, interested antibiotic discs were adjusted on cultured plates using sterile forceps and incubated as inverted for 24 h at 37°C. After overnight incubation, the diameter of each inhibition zone (including the diameter of the disc)

was measured and recorded in mm. The antibiotics used were (Teicoplanin, Piperacillin, Ciprofloxacin, and ofloxacin) (Thornsberry, 1983; Garner, 1988).

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. The significance of the obtained results was judged at the 5% level.

RESULTS AND DISCUSSION

The management of UTIs is very important because the prevalence of the pathogenesis and development of drug resistance caused by UTIs are increasing in a higher magnitude. As per the reports documented by different countries, *S.aureus* was found to be the most predominant uropathogenic isolated from the patients with UTI and the development of multi-drug resistance among uropathogens that causes a complicated UTI. (Garner, 1988).

During this study period, 200 samples were received, and from these samples, 170 bacterial isolates were obtained. Out of 170 isolates 160 were identified as *S.aureus*. Out of this predominant numbers were that of *S.aureus* 160 (94%), followed by other bacterial isolated 10 (6%) Figure 6.

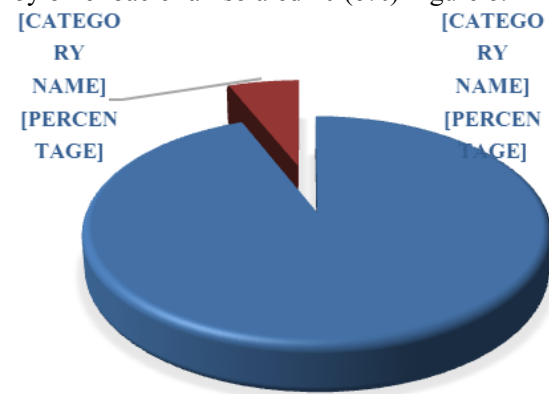


Figure 6. Percentage of *S.aureus* from a total 190 examined urine samples.

The results of the distribution of the studied samples according to demographic data (n = 160) show that the highest *S. aureus* isolated were from females (57.3%) followed by a male (42.7%). Figure 7.

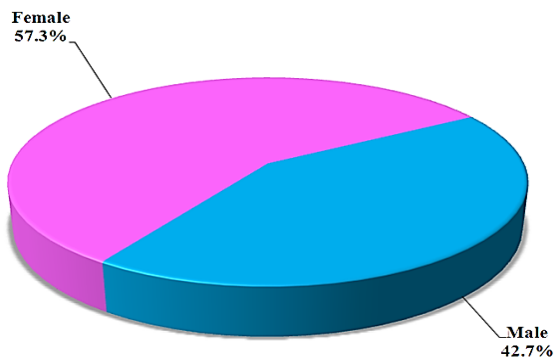


Figure 7. Distribution of the studied samples according to gender.

It is clear from Table 1 the patients in the age group ≤ 30 years showed the presence of the highest number of *S. aureus* isolated 42.7%. Age groups 30-40 years and ≥ 40 years showed the lowest number of *S. aureus* isolated (32.0, and 25.3%) respectively.

Table 1. Distribution of the studied samples according to age.

Age (years)	No.	%
< 30	64	42.7
30 – 40	48	32.0
> 40	38	25.3

It is evident from Figure 8 that, the highest percentage of *S. aureus* isolated was revealed from married statute (59.3%).

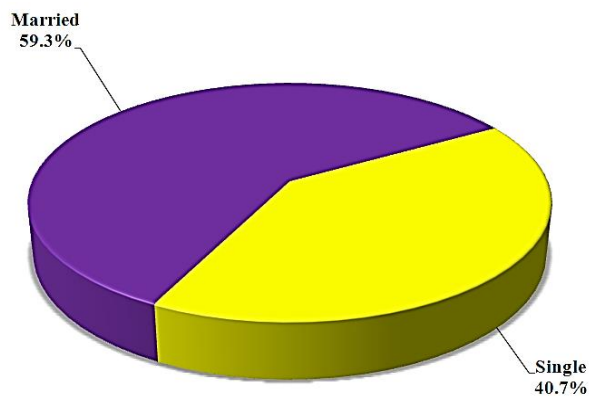


Figure 8. Distribution of the studied samples according to marital status.

The plates were observed for zone formation around the discs and the diameter of the growth inhibition zone was measured and recorded in mm. The isolated *S. aureus* was sensitive to various antibiotics. It is shown from Table 2 (69.3%) sensitivity towards Ciprofloxacin. Followed by teicoplanin which shows 25.3% sensitivity,

piperacillin and ofloxacin showed the lowest sensitivity.

Table 2. Distribution of the studied samples according to the sensitivity of antibiotics.

Antibiotics	No.	%
Tecioplanin	38	25.3
Piperacillin	6	4.0
Ciprofloxacin	104	69.3
Ofloxacin	2	1.4

Urinary tract infections (UTIs) are serious infections worldwide (Demuth PJ. 1979). *S. aureus* is a relatively uncommon cause of urinary tract infections in the general population (Barrett SP, 1999- . Lee BK, 1978). Although isolation of *S. aureus* from urine samples is often secondary to staphylococcal bacteremia arising elsewhere (e.g., in cases of endocarditis) (Coll PP, 1994), in certain patients, *S. aureus* causes ascending urinary tract colonization and infection. Urinary tract instrumentation and the presence of an indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract (Barrett SP, 1999, Breitenbacher RB, 1984). During this study period, 200 samples were received, and from these samples, 170 bacterial isolates were obtained. Out of 170 isolates 160 were identified as *S. aureus*. Out of this predominant numbers were that of *S. aureus* 160 (94%), followed by other bacterial isolated 10 (6%). In a multicenter, community-based study conducted in Great Britain, *S. aureus* accounted for only 0.5% of isolates (Barrett SP, 1999). A similar laboratory-based study conducted in France found that *S. aureus* accounted for only 1.3% of isolates from urine specimens submitted from the community (Goldstein, 2000). Prior studies suggest that isolation of *S. aureus* from the urine is often secondary to staphylococcal bacteremia originating at another site (e.g., in cases of endocarditis) (Musher, 1977). Isolation of *S. aureus* from urine samples in the absence of bacteremia is therefore often considered to represent colonization. The results of the distribution of the studied samples according to demographic data (n = 160) show that the highest *S. aureus* isolated were from females (57.3%) followed by a male (42.7%). In contrast, numerous studies have reported sex bias in UTIs. *S. aureus* is the most common cause of skin and soft tissue infections (SSTI), yet sex bias in susceptibility to *S. aureus* SSTI has not been

described. A search of electronic health records revealed an odds ratio of 2.4 for *S. aureus* SSTI in males versus females. (Musher, 1977; Araki, 2002; Jones, 1999).

The patients in the age group ≤ 30 years showed the presence of the highest number of *S. aureus* isolated was 42.7%. Age groups 30-40 years and ≥ 40 years showed the lowest number of *S. aureus* isolated (32.0, 25.3%) respectively the relationship between sexual activity and UTI established in younger women (Capitano, 2003). Also in this study, the highest percentage of *S. aureus* isolated was revealed from married statute (59.3%). Perhaps during intercourse bacteria gain access to the UT by colonizing and ascending. (Araki, 2002) The isolated *S. aureus* was sensitive to various antibiotics. It shows a 69.3% sensitivity toward Ciprofloxacin. Followed by teicoplanin which shows 25.3% sensitivity, piperacillin and ofloxacin showed the lowest sensitivity.

The reasons for the enumerated resistance spread of antibiotics in the study may be traced to the random and illogical use of these antibiotics by the temporary and permanent patients of the hospital (Jones, 1999; Muder, 1991). The active way to prevent the enumerated resistance of the drug by pathogens is the logical use of antibiotics in addition to being restricted to the specialized physician orders which supply the best benefit of antibiotics, in addition to the financial expenditures (Mody, 2003).

CONCLUSIONS

S. aureus is a cause of urinary tract infection among patients with urinary tract in married females. Thus our study showed that there were statistically significant differences between the isolated *S. aureus* and age, gender, and marital status. We concluded that ciprofloxacin is a better antibiotic used for *S. aureus*.

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