Characterization and Determination of Antimicrobial Sensitivity Pattern of *Staphylococcus aureus* Associated with Urinary Tract Infection

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MA and SUD designed the study. Writing of the manuscript was done by all authors. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: The aim of this study is to characterize and determine the antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from urine samples of urinary tract infection patients.
Place and Duration of Study: A total of 20 urine samples were collected from Murtala Muhammad specialist hospital Kano Nigeria, from September to November 2015.
Methodology: Using Gram staining, biochemical characterization and bacteriological method, 16 isolates were confirmed as *S. aureus* from the 20 different urine samples.
Results: All the 16 isolates were able to ferment Mannitol, showed Golden yellow coloration on

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Nutrient agar and produce β-haemolysis on blood agar. They also found to be positive for both Catalase and Coagulase test. On sensitivity, the isolates were found to be resistant to Augmentin, Ofloxacin and Amoxicillin. On the other hand, the isolates were sensitive to Ciprofloxacin, Streptomycin, Erythromycin and Neomycin.

Conclusion: This study showed that *S. aureus* is one of the most frequent aetiologic agents of urinary tract infection.

1. INTRODUCTION

A number of different microorganisms have been known to cause urinary tract infections; these include those of the normal flora of the skin, genital areas, anus and those from exogenous sources that maybe contacted through bad sanitary habits especially of underwear's [1]. Some of the risk factors of UTI include gender, sexual activity, immune system disorder, urinary tract anatomical malformations, disruption of normal flora of the genital area with antiseptics and antibiotics, urinary catheter and instrumentation [1,2]. About 95% of urinary tract infection occurs when bacteria ascend the urethra to the bladder or ascend the ureter to the Kidney [3]. Urinary tract infection occurs much more frequently in female than male due to the proximity of the urethra to the anus. Approximately 50% of all women will have at least one UTI in her life time with many women having several infections through their life time [2].

Members of the genus *staphylococcus* are Gram positive cocci that tend to be arranged in grape-like cluster [4]. *Staphylococcus aureus* is a Gram-positive spherical bacterium approximately 1 µm in diameter. Its cells form grape-like clusters, since cell division takes place in more than one plane. It is often found as a commensal associated with skin, skin glands, and mucous membranes, particularly in the nose of healthy individuals [5]. It has been estimated that approx. 20–30% of the general population are S. *aureus* carriers [6]. On a rich medium, *S. aureus* forms medium size "golden" colonies. On sheep blood agar plates, colonies of *S. aureus* often cause β-haemolysis [4]. The golden pigmentation of *S. aureus* colonies is caused by the presence of carotenoids and has been reported to be a virulence factor protecting the pathogen against oxidants produced by the immune system [5]. Staphylococci are facultative anaerobes capable of generating energy by aerobic respiration, and by fermentation which yields mainly lactic acid.

Staphylococcus sp. is catalase-positive, a feature differentiating them from *Streptococcus sp.*, and they are oxidase-negative and require complex nutrients, e.g., many amino acids and vitamins B, for growth. *S. aureus* is very tolerant of high concentrations of sodium chloride, up to 1.7 molar. *S aureus* may be pathogenic or non-pathogenic and the pathogenic strains are usually coagulase-positive and cause disease in their hosts. The infection may manifest as abscesses or mastitis to a severe toxic shock syndrome.

The present study was aimed to characterized and determine antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from urine samples of UTI patients attending Murtala Muhammad Specialist Hospital Kano. This report will be useful for the identification of the bacteria and its sensitivity pattern to some antibiotics, which will provide vital information on how to prevent the disease and as well to improve public health system and sanitation in Kano State and Nigeria at large.

2. MATERIALS AND METHODS

2.1 Study Population

The study population was drawn from outpatients attending Murtala Muhammad Specialist Hospital, Kano city, Kano State, Nigeria. Urine samples were collected from total of 20 UTI patients (10 male and 10 female) from September to November 2015.

2.2 Samples Collection

A total of 20 clean urine samples were collected in sterile universal containers from 10 male and female patients of all age groups respectively with suspected cases of UTI. At the point of collection, samples were labeled appropriately and immediately investigated for microscopy, culture and sensitivity analysis.

2.3 Culture

A loop full of the urine from each samples were inoculated into plates of Nutrient agar (Life save Biotech Trade San Diego, USA.) using sterile platinum wire loop. All plates were incubated at 37℃ aerobically for 24 hrs. The plates examined macroscopically for bacterial growth.

2.4 Isolation of *Staphylococcus aureus*

The presumptive colonies of *S. aureus* on Nutrient agar plates were further cultured on Nutrient agar plates and repeatedly sub-cultured to get pure culture. These isolates recovered were preserved for further bacterial identification.

2.5 Bacterial Characterization

The isolates were identified as *S. aureus* on the basis of Gram staining, colony morphology on Mannitol salt agar (MSA) and haemolysis patterns on Blood Agar (Life save Biotech-Trade San Diego, USA). Biochemical tests include Catalase, Oxidase and Coagulase tests which were done on the basis of conventional biochemical test with reference to Cheesbrough, [7] and Prescott et al. [8] while Gram staining was done according to the method described by Cheesbrough, [7].

2.6 Antibiotic Sensitivity Test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method as described by Bauer et al. [9]. Mueller Hilton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37℃ for 24 hours. Sensitivity pattern of the isolates to Augmentin (30 µg/disc), Erythromycin (10 µg/disc), Streptomycin (30 µg/disc), Amoxicillin (30 µg/disc), Gentamicin (20 µg/disc), Oxacillin (10 µg/disc), Ofloxacin (30 µg/disc), Neomycin (20 µg/disc), Ciprofloxacin (10 µg/disc) and Septrin (30 µg/disc), produced by Abtek pharmaceutical limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [10].

3. RESULTS AND DISCUSSION

Table 1 present the distribution of *S. aureus* from the samples collected. A total of 20 samples were collected 10 from male and female respectively. The results showed that *S. aureus* were isolated from 7 out of the 10 samples collected for men and 9 out of 10 samples collected from female patients.

Table 2 shows the result of Gram staining and some biochemical tests, the result revealed that the pure cultures exhibited clusters of Gram-positive cocci. These isolates were positive for catalase and coagulase test while negative for Oxidase test.

Table 2 shows the sensitivity pattern of the isolate against some antibiotics, the result shows that in the present investigation high resistance was recorded in Augmentin, Amoxicillin and Ofloxacin with mean zone of inhibition 3 mm each. On the other hand, high sensitivity was observed in Neomycin, Streptomycin and Ciprofloxacin each with average zone of inhibition of 21, 20 and 19 mm respectively. This is followed by Erythromycin and Oxacillin with average zones of inhibition of 16 and 15 mm respectively.

Infections due to staphylococci are of major importance to veterinary and human medicine. *S. aureus* is one of the most significant pathogens causing diseases worldwide [11]. *S. aureus* is the leading cause of nosocomial infections and is responsible for a wide range of human diseases, including endocarditis, food poisoning, toxic shock syndrome, septicemia, skin infections, soft tissue infections and bone infections, as well as bovine and bovine mastitis [12]. The present study was designed for the characterization of *S. aureus* from urine samples of urinary tract infected (UTI) patients. Our results indicated that 16 samples of urine collected out of 20 samples were positive for *S. aureus* which accounted for 80% of samples collected. Identification of *Staphylococcus aureus* was based on cultural characteristics, Gram staining and biochemical properties. All the 16 isolates fermented Mannitol with the color change of Mannitol Salt Agar (MSA) and produced yellow colony, they also showed β-haemolysis on blood agar media enriched with 5% sheep blood (Table 1). Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci. These isolates were positive for catalase and coagulase test while negative for Oxidase test (Table 2). In

catalase test; Hydrogen peroxide was broken-down into water and oxygen. Production of oxygen was indicated by bubble formation [13]. The isolates were identified as *S. aureus* by coagulase test. The positive result of coagulase test was confirmed by the formation of curd like clotting compared to negative control [13]. The isolates were also identified as Oxidase negative, the result was confirmed due to its inability to give blue color on reaction with oxidase reagent. Earlier findings by Amengialue et al. [14]; Yabaya et al. [15]; Jahan et al. [13] identified and characterized *Staphylococcus aureus* on the basis of cultural characteristics, Gram staining and Biochemical characterization.

This study showed the presence of *Staphylococcus aureus* as one of the possible etiologic agents of Urinary tract infection cases. This finding was inconformity with that of Amengialue et al. [14] who reported *Staphylococcus aureus* as the most prevalent bacteria among UTI patients which accounted for 28% of all isolates. Similar reports were earlier reported by Akortha and Ibadin [16]; Obiogbolu et al. [17]; Ojo and Anibijuwon [18]. This finding agrees with the reports of Sule [19]; Akere and Ahonkhai [20] that *S. aureus* is the most frequent organisms isolated from urine cultures and it is the leading etiologic agent in urinary tract infection in Benin city, Edo state Nigeria [14]. The study also revealed that there is higher incidence of *S. aureus* in female urine than men urine samples. This is may be due to possibility of pelvic inflammatory disease in female.

Based on antibiotic sensitivity test, most of the *S. aureus* isolates recovered were found multidrug resistant (Table 3). The in the present investigation high resistance was recorded in Augmentin, Amoxicillin and Ofloxacin with mean zone of inhibition 3 mm each. On the other hand,

high sensitivity was observed in Neomycin, Streptomycin and Ciprofloxacin each with average zone of inhibition of 21, 20 and 19 mm respectively. This is followed by Erythromycin and Oxacillin with average zones of inhibition of 16 and 15 mm respectively. These findings are slightly correlated to that of Amengialue et al. [14] which found *Staphylococcus aureus* is resistant to Augmentin and ofloxacin, In an another study in Bangladesh conducted by Foxman [5] revealed that *S. aureus* was resistant to Penicillin-G and Amoxicillin, respectively; however, in this study, it has been found that both the Amoxicillin and Ofloxacin were highly resistant to *S. aureus*, indicating increasing resistance of the organism against Ofloxacin and Amoxicillin. This work also supported that of Yabaya et al. [15], they observed that *Staphylococcus aureus* is sensitive Ciprofloxacin, Streptomycin and Erythromycin. On other hand this result is contrary to that of Jahan et al. [13] who revealed that *Staphylococcus aureus* is resistant to Erythromycin.

Table 1. Distribution of *Staphylococcus aureus* recovered

Sex	No. of samples	No. of isolates recovered	% Occurance
Male	10	7	70%
Female	10	9	90%
Total	20	16	80%

Table 2. Biochemical characteristics of *Staphylococcus aureus*

S/N	Test	Result
1	Gram staining	Gram positive Cocci
2	Catalase test	Positive
3	Oxidase test	Negative
4	Coagulase test	Positive

Table 3. Mean zone of inhibition (with standard error) and status of antibiotics against *Staphylococcus aureus*

S/N	Antibiotics	Conc.(µg/disc)	Mean zone of INHIB. (mm)	Status
1	Augmentin	30	03 ±0.27	Resistant
2	Erythromycin	10	16±0.34	Susceptible
3	Streptomycin	30	20±0.27	Susceptible
4	Amoxicillin	30	03±0.00	Resistant
5	Gentamicin	20	10±0.27	Intermediate
6	Oxacillin	10	15±0.34	Susceptible
7	Ofloxacin	30	03±0.27	Resistant
8	Neomycin	20	21±0.00	Susceptible
9	Ciprofloxacin	10	19±0.34	Susceptible
10	Septrin	30	06±0.27	Resistant

4. CONCLUSION AND RECOMMENDATION

Urine samples from different Urinary tract infected patients at Murtala Muhammad specialist hospital Kano Nigeria were tested. Isolates were confirmed as *S. aureus* by Cultural characteristic, Gram staining and Biochemical tests. The isolates were resistant to Augmentin, Amoxicillin and Ofloxacin. On the other hand, the isolates were found to be sensitive against Ciprofloxacin, Streptomycin and Neomycin. This study showed that *S. aureus* is the most frequent etiologic agent of urinary tract infection. Resistance pattern against broad spectrum antibiotic depicts an alarming situation, which needs special attention. It is recommended that antibiotic susceptibility test should be conducted to Urinary tract infection patient before prescription in order to avoid antibiotic resistance.

ETHICAL APPROVAL

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urology Clinical North American. 2008;53(1):1–2.
2. Foxman B. Epidemiology of UTI: Incidence morbidity and economic costs. Public Medical Government. 2003;49(2):53–70.
3. Farooqui BJ, Khurshid M, Alam M. Urinary tract infection. JPMA Journal Pakistan Medical Association. 1989;39(5):129–131.
4. Ryan KJ, Ray CG. Sherris medical microbiology: An introduction to infectious diseases. 4th Edition. McGraw Hill Publishers; 2004.
5. Crossley KB, Archer GL. The Staphylococci in human disease. Churchill Livingstone; 1997.
6. Heyman D. Control of communicable diseases manual. 18th Edition. American Public Health Association, Washington DC; 2004.
7. Cheesbrough M. District laboratory practice in tropical countries. Cambridge United Press, UK. 2000;27:105.
8. Presscott W, Harley S, Klein. Microbiology 7th Edition McGraw Hill; 2008
9. Bauer AW, Kirby MM, Sherris JC, Turch M. Antibiotics susceptibility testing by standardized single disc method. American Journal Clinical Pathology. 1996;36:493-496.
10. CLSI. Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement. Clinical and Laboratory Standards Institute. 2007;27(1):M100-S17.
   Available:www.clsi.org
   (Accessed on October 01, 2014)
11. Zadoks R, Van Leeuwen W, Barkewa H, Sampimon O. Application of pulsed-filed gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *S. aureus* isolates. Journal Clinical Microbiology. 2000;38(5):1931-1939.
12. Koreen L, Srinivas V, Ramaswamy EA, Graviss S, Naidich JM, Musser, Kreiswirth N. SpA typing method for discriminating among *S. aureus* isolates: Implications for use of a single marker to dentect genetic micro-and macrovariation. J. Clinical Microbiology. 2004;42(2):792-799.
13. Jahan M, Rahman M, Parvej S, Shah M, Chowdhury ZH, Haque M. Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. J. Adv. Vet. Anim. Res. 2005;2(1):49-55.
14. Amengialue OO, Osawe FO, Edobor O, Omoigberale MNO, Egharevba AP. Prevalence and antibiogram pattern of *staphylococcus aureus* in urinary tract infection among patients attending specialist hospital, Benin City, Nigeria. G.J.B.A.H.S. 2015;2(4):46-49.
15. Yabaya A, Jeremiah MY, Manga SS, Umar A. Prevalence and antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from the skin and Nasal cavity of students and staff of Kaduna state University, Kaduna, Nigeria. Best Journal. 2011;8(2):191-194.
16. Akortha EE, Ibadan OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* among patients with urinary tract Infection (UTI) in UBTH Benin City, Nigeria. African Journal Biotechnology. 2008;11:1637-48.

17. Obiogbolu CH, Okonko ID, Anyamere CO, Adedeji AO, Akanbi AO, Ogun AA, Ejembi J, Faleye TOC. Incidence of urinary tract infections (UTIs) among pregnant women in Akwa metropolis, South Eastern Nigerian. Science Research Essay. 2009;4(8):820–824.

18. Ojo OO, Anibijuwon II. Urinary tract infection among female students residing in the campus of the University of Ado Ekiti, Nigeria. African Journal Microbiology Research. 2010;4(12):1195-1198.

19. Sule OAO. Bacterial flora of surgical wounds. Tropical Journal Obstetrics Gyneacology. 2015;9(2):4-9. Stanley CN, Ugboma H, Ibezim E, Attama A. Prevalence and antibiotic susceptibility of *Staphylococcus aureus* and other staphylococcal infections in pregnant women attending antenatal clinic in a tertiary hospital in Port Harcourt, Nigeria. J Infec. Disease and Therapy. 1991;2:1.

20. Akerele J, Ahonkhaia IE. Urinary pathogens and antibacterial susceptibility. A retrospective study of private diagnostic laboratory in Benin City Nigeria. Journal Medical Laboratory Sciences. 2000;9:47–48.

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