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Isolation, Characterisation and Antibiogram Profile of *Staphylococcus aureus* in the Urine of Tertiary Care Hospital Patients of District Quetta, Pakistan

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Authors' contributions

This work carried out in collaboration between all the authors; author RA was the primary researcher conceived the study, collected data and drafted the manuscript for publication. Author MS, MAK assisted in data collection and manuscript drafting. Author KBA assisted in study design, data collection and in manuscript drafting. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most common infectious diseases in community practice and a significant public health problem regarding morbidity and financial cost worldwide.
Objectives: This study was conducted to determine the prevalence of *Staphylococcus aureus (S. aureus)* among UTI patients of District Quetta Pakistan and to observe the antibiogram profile of the bacterial isolates.
Materials and Methods: One hundred urine samples were collected from both male and female UTI patients. These mid-stream urine samples were taken early in the morning in sterile, wide mouth containers from the pathological section of a tertiary care hospital (Bolan Medical College

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Hospital BMCH), District Quetta. Samples were further processed at the Microbiology laboratory of Centre for Advance Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan from August to December 2011.

Results: Only five samples were found positive for *S. aureus* on methyl red, Voges-Proskauer, catalase and coagulase, while negative on indole, citrate and motility. Positive samples showed cream and yellow coloured colonies on *Staphylococcus* medium 110 and mannitol salt agar, respectively. Infection was more common in female (60%) as compared to males (40%); while the overall infection rate was highest in the age group of 26-45 years (80%). During antibiotic sensitivity test, *gentamycin* showed 100% susceptibility against *S. aureus*.

Conclusion: It was concluded that only 5% of patients of UTI were caused by *S. aureus* that can be eliminated by treating the patients with antibiotic *gentamycin*. In the local scenario, routine urine culture tests and surveillance program is encouraged to be implemented.

Keywords: Staphylococcus aureus; Urinary tract infection; prevalence; antibiogram; Quetta.

1. INTRODUCTION

Worldwide urinary tract infections are amongst the main causes of urinary system inflammations that include kidneys, bladder and urethra and are more common in women than men [1]. These infections can be painful and cause inconvenience in routine affairs and are easily treatable if their signs such as increase or decrease in urination, dysuria and urgency; occasionally suprapubic tenderness and lower body pain are recognised [2]; when *S. aureus* is introduced into the urinary system, normally through the urethra [3]. UTI includes a variety of clinical syndromes such as acute and chronic *pyelonephritis* (kidney and renal pelvis), *cystitis* (bladder), *urethritis* (urethra), *epididymitis* (epididymis) and *prostitis* (prostate). The incidence rate is equal between the sexes in the first year of life, in the elderly, it is between 1 and 3% and increases with age, co-existent diseases or institutional care [4].

Staphylococcus is a group of bacteria that can cause a number of diseases. The name *Staphylococcus* comes from the Greek *staphyle*, meaning a bunch of grapes, and *kokkos*, meaning berry. It is gram +ve, facultatively anaerobic, usually un-encapsulated *cocci* and appearing as singles, paired, clusters or in chains [5]. Over 30 different types of *Staphylococci* can infect humans, but most infections are caused by *S. aureus*, it is the most pathogenic specie implicated in a variety of infections. Clinically common species of *staphylococci* other than *S. aureus* are often referred to as coagulase-negative *staphylococci*. These *staphylococci* are normal flora of the skin and, as such, frequently act as opportunistic pathogens, especially in the compromised host. Staph-related illness ranged from mild with no treatment to severe and potentially fatal. It can be found normally in the nose and on the skin (and less commonly in other locations) of 25 to 30% healthy adults. In the majority of the cases, bacteria do not cause disease; however, damage to the skin or another injury may allow the bacteria to overcome the body's natural protective mechanisms leads to infection [6].

S. aureus causes most staph infections, including skin infections, pneumonia, food poisoning, toxic shock syndrome and blood poisoning (bacteremia). It is also the causative agent of supportive infections such as boils and wound infections, superficial infection such as a skin pustule, subcutaneous and sub-mucosa abscesses, *osteomyelitis*, bronchopneumonia and food poisoning, a common cause of vomiting and diarrhoea [7,8]. It is the commonest cause of infection in hospitals and is most liable to infect newborn babies, surgical patients, old and malnourished persons, and patients with diabetes and other chronic diseases [8], it is also the leading cause of UTI [9,10]. However, if the bacterium does enter the bloodstream, it can spread into the urinary tract, lungs and heart of a person ultimately resulting in a very deadly situation. The illnesses caused by these bacteria can range from minor skin infections such as pimples, boils and *cellulitis* to much more serious and life-threatening illnesses such as pneumonia, *meningitis, septicemia, osteomyelitis* and *endocarditis* [11,12]. It is an opportunistic pathogen affecting both immune-competent and immune-compromised individuals, frequently resulting in high morbidity and with complications which constitute problems to health care institutions [13]. This study was designed to know the *S. aureus* prevalence and investigate it through different biochemical and antibiotics sensitivity tests.

2. MATERIALS AND METHODS

2.1 Study Area

The present study was carried out in the pathological laboratory of a tertiary care hospital (Bolan Medical College Hospital BMCH) that caters patients from rural and urban areas of the district and microbiology laboratory of Centre for Advance Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan. The study was carried out from August to December 2011 in District Quetta.

2.2 Study Population

A total of 100 adults both (male and female) patients with an age group ranging from 20 to >60 years were enlisted having signs and symptoms of urinary tract infections. These patients were attended by the outpatient and inpatients departments of the BMCH for this study. Prior to specimen collection, consent was taken from all these patients.

2.3 Sample Collection

Patients were provided dry, labelled, wide mouth, screw-capped and leak-proof containers for the collection of early morning mid-stream specimens of urine. For microbiological examination samples were immediately transported to CASVAB laboratory in cold chain for processing within two hours as per the guidelines of Monica Cheesbrough and Lynn, et al. [14,15].

2.4 Sample Processing

The collected samples were centrifuged, and the pellet was streaked on nutrient agar media plates and incubated at 37°C for 24 hrs. Later on, to study *S. aureus*, the appeared growth colonies on plates were transferred through sterile wire-loop on *Staphylococcus* medium 110 (DIFCO) and Mannitol Salt Agar **(**MSA).

2.5 Preparation of Media and Reagent

To study the primary isolation, purification and biochemical characterization of *S. aureus*; the basic, selective and differential media and reagents used were Nutrient Agar (Lab), *Staphylococcus* 110 Medium (DIFCO), Mannitol Salt Agar (Lab), Tryptone broth (Oxoid), Methyl Red and Voges-Proskauer Broth (Merck), Simmon Citrate Agar. As per the directions of manufacturers, the commercially available media were reconstituted in distilled water and pH was adjusted with one molar HCl and one Molar NaOH. Media were autoclaved at 15 lb/in^2 pressure per square inch (PSI) for 15 minutes at 121°C and was then allowed to cool down to 50°C. Liquid media were dispensed in clean pre-sterile test tubes having cotton wool plugs, aseptically poured into Petri plates and allowed to solidify. Agar medium was also dispensed in clean pre-sterile tubes in 5 ml quantities and allowed to solidify in slant positioned for preparation of slants. Plates, slants and tubes of liquid media were incubated at 37°C for 24 hrs to confirm sterility of the media. Sterile media were stored at 4°C until further use. Meanwhile, if any medium showed bacterial or fungal growth, it was not used and discarded away.

2.6 Gram Staining

The smear was prepared from fresh culture (20 to 24 hrs) by placing a drop of autoclaved normal saline and loop touched with *S. aureus* colony from MSA on pre-cleaned glass slides. The smear was fixed by passing slides 2 to 3 times through flame. Heat-fixed smear was gram stained as described by Arora, D. R. [16]; later on, it was stained with crystal violet for one minute and washed with tap water. Iodine solution was slowly applied for one minute and washed with tap water. De-colourised with 95% ethanol for 30 seconds and rinsed with tap water. Counterstained with safranin solution for one minute and washed with tap water. Stained slides were air-dried and examined under oil immersion objective lens (100X) of the microscope. The staining reaction and morphology were then studied.

2.7 Sample Biochemical Testing

Six most common biochemical tests such as Indole Production Test (IPT), Citrate Utilization Test (CUT), Catalase Test (CT), Coagulase Test (CoT), Methyl Red Test (MRT) and Voges-Proskauer Test (VPT) were used to characterise the *S. aureus* from urine samples. The Mannitol Fermenting organisms on Mannitol Salt agar producing yellow colonies were suspected as *S. aureus* and further investigated by using biochemical tests for identification of *S. aureus.*

Table A. Types of antibiotic discs (Oxoid) their concentration and codes

S. No.	Types of antibiotics	Codes of antibiotics	Concentration of antibiotics (µg)
1.	*Gentamicin*	CN	10
2.	*Cotrimoxazole*	SXT	30
3.	*Chloramphenicol*	C	30
4.	*Erythromycin*	E	15
5.	*Oxacillin*	O	1
6.	*Tetracycline*	TC	24

2.8 Motility Test

Motility test was performed to differentiate the motile organism from non-motile organism. One litre of the medium was prepared, and the test was conducted as described by Arora, D. R. [16], 3 to 4 ml of the medium was dispensed into small tubes and autoclaved. All the tubes were refrigerated until further use. Tubes containing test medium were stabbed with a straight platinum loop and then incubated at 37°C for 24 hrs, and the result was recorded.

2.9 Antibiotic Sensitivity Test

Disc diffusion test was performed to test antibiotic sensitivity. One litre Muller Hinton agar (oxoid) was prepared and autoclaved at 121°C for 15 minutes. After cooling up to 45°C the medium was poured in Petri plates and allowed to solidify. An antibiotic sensitivity test was performed against *S. aureus* by adopting the following procedures:

i. **Inoculum Preparation:** 0.5 standard McFarland test inoculums contain approximately 1.5 x 10^8 colony forming a unit (CFU)/ml, test inoculum were vortexed, and tubes held side by side against a white card with heavy black lines. If lines looked same through tubes, the inoculum was satisfactory.
ii. **Agar Plate Inoculation:** A sterile cotton swab was dipped into inoculums, the swab was raised above the liquid and rotated against the wall of the tube to remove excess liquid. The inoculum was applied to plate, covering the entire surface and rotated on 60 degrees thrice.
iii. **Antimicrobial Disc Application**: Not more than 12 Discs were applied in this procedure, on a 150 mm plate and five discs were used on a 100 mm plate.
iv. **Plate Incubation:** Plates were inverted (agar side up) and incubated at 37°C for18 to 24 hrs.
v. **Measurement of Zone of Inhibition:** After 24 hrs, agar was checked for the confluent lawn of growth and uniform, circular zones of inhibition, holding plates on agar side up over a black, non-reflective surface using reflected light directed from above. The diameter of the zone of inhibition was measured to the nearest mm.
vi. **Interpretation of Results:** Zone of inhibition was measured with scale in mm these measurements were then used to categorise the sensitivity as under:
    - Susceptible–infection may be treated with an antimicrobial agent at the recommended dosage.
    - Resistant–organism not likely inhibited by usual antimicrobial agent doses.
    - Intermediate–therapy handled in a variety of ways.

3. RESULTS

Out of the total 100 samples collected from UTI patient of BMCH (Table 1); 5% shows the substantial presence of *S. aureus* pathogen. It is considerably lower than the value of 8.56% found in a tertiary care hospital of Tamil Nadu India [17], main reason may be due to different hygienic practices, socio-economic status and geographical locations of the patients.

Age distributions of UTI patients affected by *S. aureus* revealed that majority of the patients were found in the age group of 26 to 45 years in both male and female patients. Out of the total 100 UTI urine samples from clinically suspected patients were analysed for *S. aureus*. Of these, 3 (60%) samples in female and 2 (40%) were found in male patients were culture positive showing significant bacteriuria (*S. aureus)*; while remaining 95 samples were either non-significant bacteriuria or had sterile urine. Female UTI patients were more at risk than male, while the prevalence of UTI caused by *S. aureus* was highest among the age group of 26 to 45 years (Table 1).

Table-1. Effect of gender and age on prevalence of urinary tract infection

S. No.	Gender	Sample size	Infected Patient Age Group (Years)		
			26 to 35	36 to 45	46 to 55
1.	Male	50	01	01	00
2.	Female	50	01	01	01
TOTAL		**100**	**02**	**02**	**01**

3.1 Colony Characteristics of Isolates on the Growth Media

i. **Growth on Nutrient Agar.** Colonies of *S. aureus* were circular, 2 to 3 nm in diameter with a smooth shiny surface when grown on Nutrient Agar after 24 hrs incubation at 37°C (Fig. 1).

ii. **Growth on *Staphylococcus* Medium 110.** *S. aureus* showed *c*ream coloured colonies with acid production and gelatin liquefaction when grown on *Staphylococcus* medium 110 after incubation of 24 hrs at 37°C (Fig. 2).

iii. **Growth on Mannitol Salt Agar (MSA).** Isolates produced yellow colonies of *S. aureus* on Mannitol Salt Agar after incubation for 24 hrs at 37°C (Fig. 3).

3.2 Morphological Characteristics of the Isolates

3.2.1 Gram Staining of the Isolates

Test organisms were found in clusters like structure showing Gram +ve reaction under oil immersion (100X) lens (Fig. 4).

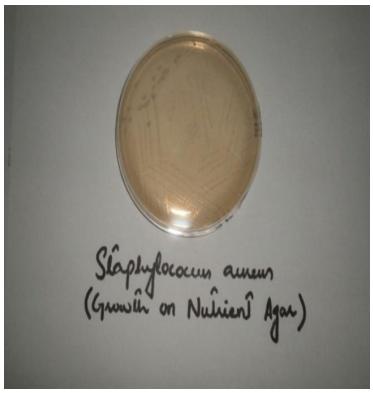

Fig. 1. Colonies of *S. aureus* on Nutrient Agar Media plate

Fig. 2. Colonies of *S. aureus on Staphylococcus* medium 110

Fig. 3. Colonies of *S. aureus* on Mannitol Salt Agar

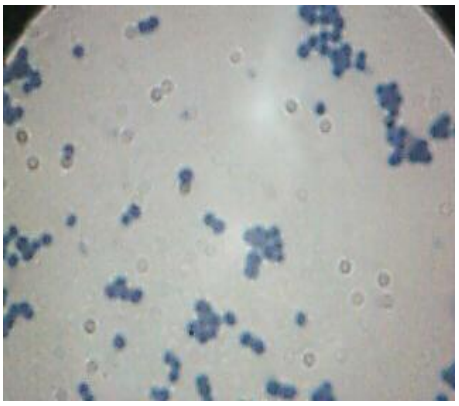

Fig. 4. Structure and staining characteristics of *S. aureus* under the microscope

3.3 Biochemical Tests

The entire well-grown colonies on selective media such as *Staphylococcus* 110 medium and Mannitol Salt Agar and Gram +ve cocci were further characterised by a series of biochemical tests like indole, methyl red, Voges-Proskauer, citrate utilisation, catalase and coagulase for final confirmation as described by [18].

i. **Indole Production Test.** *S. aureus* did not possess the enzyme tryptophanase that cleaves tryptophan with the production of indole as end-product. Therefore, *S. aureus* shows the negative result with no colour change (Fig. 6).
ii. **Methyl Red (MR) and Voges-Proskauer (VP) Test.** It is an acid sensitive dye that is yellow at pH above 4.5 and red at pH below 4.5. It is used to indicate whether glucose has been broken down completely to highly acidic end products or only partially to less acidic end products; while VPT is used to detect the presence of acetylmethylcarbinol, one of the end products of glucose metabolism. In our study, *S. aureus* gave both MR and VP positive as it converts acidic products and can convert those acidic products into some neutral/basic products side by side. Hence, it gives both positive results (Fig. 7, 8).
iii. **Citrate Utilization Test.** No colour change was visible within 24 to 48 hrs, indicating that *S aureus* was not able to utilise the citrate contained in the medium (Fig. 9).
iv. **Catalase Test.** Catalase enzyme produced by *S. aureus* that decomposes hydrogen peroxide into oxygen and water results bubble formation (Figure 10).
v. **Coagulase Test.** Coagulase enzyme catalyses the conversion of fibrinogen to fibrin in blood plasma, and such test was performed to check the organism whether it forms clots on rabbit plasma or not. Clumping of rabbit plasma shows a positive reaction. Coagulation within 24 hrs is indicative of *S. aureus* (Left negative, Right positive) (Fig. 11).

3.4 Motility Test

S. aureus is non-motile bacteria that do not move away from the stab line. Heavy growth appears only along the stab, and the red colour indicates where the bacteria are growing (Fig. 5).

Fig. 5. Motility test for *S. aureus*

3.5 Antibiotic Sensitivity Test

After incubation, different antibiotics described in Table A for varying degree of inhibition zones (Fig. 12) shows highly effective antibiotics that inhibited the growth of organism while others were found resistant. Table.3 represents percentage sensitivity, resistivity and diameter of the zone of inhibition profile of each antibiotic on *S. aureus* [19]. Isolates were considered as sensitive, intermediate or resistant to particular antimicrobial agents on the basis of the inhibitory zone that matches the criteria of the manufacturer interpretive table which follow the recommendation of National Committee for Clinical Laboratory Standard [20].

Antibiotic sensitivity test results show that *gentamycin* was highly sensitive whereas *Chloramphenicol, Oxacillin, Tetracycline* showed intermediate sensitivity and *Erythromycin* and *Cotrimoxazole* were resistive against *S. aureus.*

Table 2. Biochemical and motility tests for the identification of *S. aureus*

Organism	Indole (Ind)	Methyle Red (MR)	Voges-Proskauer (VP)	Citrate (Cit)	Catalase (Cat)	Coagulase (Coag)	Motility (Mot)
S. aureus	-ve	+ve	+ve	-ve	+ve	+ve	-ve

S. aureus Findings through Biochemical Tests

Fig. 6. Indole Test for *S. aureus*

Fig. 7. Methyl Red (MR) Test for *S aureus*

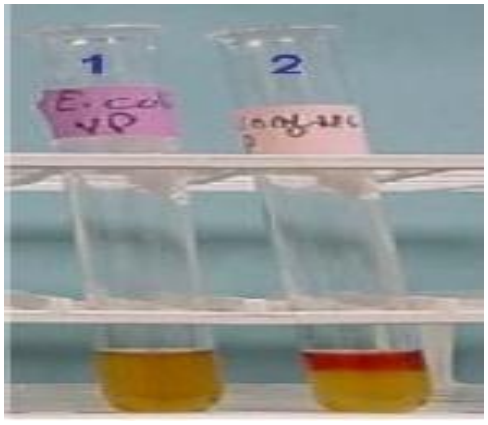

Fig. 8. Voges-Proskauer (VP) Test for *S. aureus*

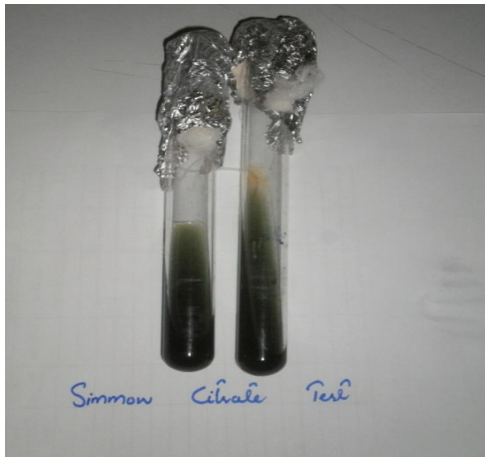

Fig. 9. Citrate Test for *S aureus*

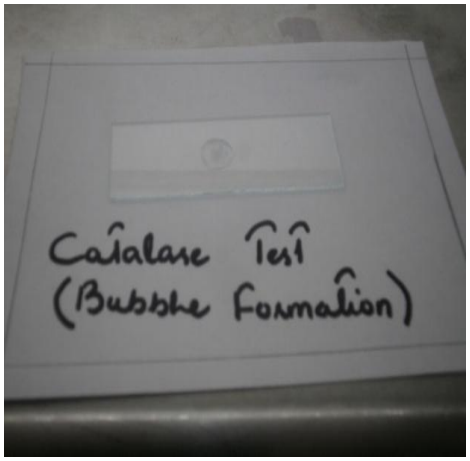

Fig. 10. Catalase Test for *S aureus*

Fig. 11. Coagulase Test for S. aureus

Table 3. Effect of different antibiotics on *S. aureus*

S. No.	Antibiotics types	% Sensitivity	% Resistivity	Zone of inhibition (mm)
1.	*Gentamycin*	100.0	0.0	16
2.	*Chloramphenicol*	0.0	100.0	14
3.	*Oxacillin*	66.7	33.3	16
4.	*Tetracycline*	0.0	100.0	13
5.	*Cotrimoxazole*	55.6	44.4	14
6.	*Erythromycin*	33.3	66.7	14

Table 4. Antibiotics sensitivity by disc diffusion

S. No.	*Gentamycin* (CN)	*Chloramphenicol* (C)	*Oxacillin* (OX)	*Tetracycline* (TC)	*Cotrimoxazole* (SXT)	*Erythromycin* (E)
1.	S	R	R	R	R	R
2.	S	S	S	S	R	R
3.	S	S	S	S	R	R
4.	S	R	R	R	R	R
5.	S	R	R	R	R	R

S = Sensitive, R = Resistance

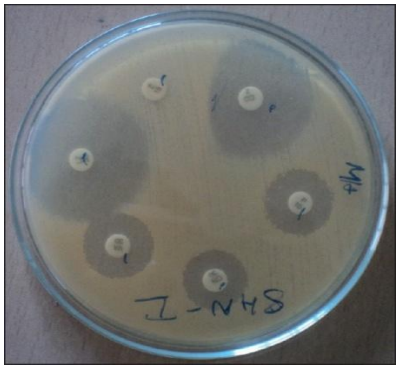

Fig. 12. Antibiotic sensitivity test for *S. aureus* by Disc Diffusion Method

Study findings in Table 1 regarding the antibiotic sensitivity test for *S. aureus* show that it was highly sensitive to *Gentamycin* (100%), *Chloramphenicol* (66.7%), *Oxacillin* (55.6%) and is resistant to *Erythromycin* (100%) *Cotrimoxazole* (100%), *Tetracycline* (66.7%) and *Amoxicillin* (59.3%). Strains are normally sensitive to the majority of antibiotics (26), but antibiotic resistance is becoming a problem, so it is advisable to perform antibiotic sensitivity test to minimise the hazards of drug resistance and to avoid economic losses on treatment.

4. DISCUSSION

S. aureus is one of the most widely spread human pathogens. This could be due to its minimal growth requirements, ability to survive long in most unfavourable environments and to find a susceptible host. *S. aureus* is the causative agent of wide variety of disease of supportive infections such as boils and, wound infections, superficial infections such as skin pustules, subcutaneous and sub-mucosa abscesses, osteomyelitis, broncho-pneumonia and food poisoning, a common cause of vomiting and diarrhea and also a leading cause of UTI [9,10]. UTI is a heterogeneous disease which can be divided into several types of infections such as acute, uncomplicated bacterial *pyelonephritis*, complicated UTI, recurrent *cystitis* and asymptomatic bacteriuria [21]. Urinary tract generally has a hostile environment for bacteria, and except for the distal urethra, it is usually sterile. Infections result when the bacterial virulence factor overcomes the numerous host defence mechanisms [21].

S. aureus is an opportunistic pathogen affecting both immune-competent and immune-compromised individuals, frequently resulting in high morbidity and with complications which constitute problems to health care institutions [13]. It was noticed from this study that the isolation frequency of *S. aureus* vary from patient to patient as it is a relatively infrequent urinary tract isolate in the general population [22].

Out of hundred, five samples produced cream colour colonies on *Staph* 110 medium (Fig. 4), while on Mannitol Salt Agar the isolates produced yellow colour colonies (Fig. 5) after 24 hr incubation at 37°C. These results relate to the findings of Hare Krisna, Tiwari & Malay R. Sen and Sarasu, V.P., & Rani, S. R. [23,24].

All positive five samples colonies were verified by different biochemical tests such as Indole, MR-VP, Citrate, Catalase and Coagulase tests. In the present study all the biochemical tests results for *S. aureus* were same as described by Shaw, C. [18], such as methyl red, Voges-Proskauer, catalase, coagulase tests positive while negative for indole and citrate utilisation tests. The motility of the organisms was determined as described by Freney et al. [25]; while positive samples for *S. aureus* were non-motile.

Results regarding UTI infection in female patients was higher (60%) than male (40%), while patients age group ranges from 26 to 45 years in both male and female patients; these findings are in line with the findings of Ghadage et al. and Sanjee et al. [26,27].

Antibiotics sensitivity test of this study showed that positive samples were resistant to *Erythromycin* (100%), *Cotrimoxazole* (100%) and *Tetracycline* (66.7%), respectively. It is widely accepted that the prevalence of antibiotic-resistant bacteria is due to the indiscriminate use of antibacterial drugs in human and livestock. This accounts for a high level of resistance in clinical isolate is also reported by Hafeez and Kumari et al. [28,29]. The study also showed that they were sensitive to *gentamycin*, *chloramphenicol* and *oxacillin,* so these antibiotics can be used to treat these infections in District Quetta.

5. CONCLUSION

The second most common type of infection in the human body is UTIs, caused by microbes (fungi, viruses and bacteria); while the well-known causing bacteria include *Escherichia coli*, *Enterococcus*, *Klebsiella ssp.*, *Pseudomonas ssp.*, *Proteus ssp.,* and *S. aureus*, respectively. In the present study *S. aureus* was isolated from 5% UTIs affected patients. Isolates of *S. aureus* were subjected to antibiotic sensitivity by disc diffusion method and *Gentamycin*, *Chloramphenicol and Oxacillin* were found to be very effective and are recommended to the patients having UTI due to *S. aureus* in District Quetta.

CONSENT

These patients were attended by the outpatient and inpatients departments of the BMCH for this study. Prior to specimen collection, consent was taken from all these patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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