iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 8 **Issue:** VII **Month of publication:** July 2020

DOI: https://doi.org/10.22214/ijraset.2020.30289

www.ijraset.com

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 8 Issue VII July 2020- Available at www.ijraset.com

Antimicrobial Screening of *Staphylococcus Aureus* in different Clinical Specimen of Urine in Duhai

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Abstract: ***Diseases caused by Staphylococcus aureus are of major global health concern. There are various bacteria which cause toxicity and create major health issues. It can even destroy one's immune system and result in death. Out of all these bacteria, "Staphylococcus aureus" is one of the most prevalent bacterium which causes UTI diseases in human. S. aureus forms complex communities with unwanted bacteria in multispecies biofilms. To enhance the safety we need to work on improving hygiene conditions, especially in the less educated sectors. Symptoms of Staphylococcus aureus UTI include burning feeling when you pee, frequent or intense urge to pee, cloudy, dark, bloody, or strange-smelling pee. Preventive measures include maintaining personal hygiene, adequate cleaning and disinfection of equipment and prevention of cross-contamination in home and outside. This study aims to evaluate the presence and contamination rate of Staphylococcal aureus UTI. There is a dramatic increase in multidrug-resistant bacterial pathogens across the globe over the years. the present study was conducted specially to characterize the Indian S. aureus isolates at phenotypic level, to find out the existing antimicrobial pattern of the isolates obtained from clinical specimens of human urine.***

Keywords: UTI, antibiotics, multi drug resistance, S. aureus

I. INTRODUCTION

Staphylococcus aureus is a well-known pathogen of man and animals (Johnson R.C et al., 2016). *Staphylococcus aureus* is Gram-positive, spherical coccus having a diameter of 1µm – 1.3µm. It is non-motile, non-spore forming and occasionally capsulated, aerobic or facultative anaerobic organism. *Staphylococcus aureus* belongs to order Bacillales, family *Staphylococcaeae* and is a part of the genus *Staphylococcus.* There are approximately 39 species and 21 subspecies in genus Staphylococcus (Tille, 2014). On microscopic examination, the organisms appear in clusters, like bunches of grapes.It also causes serious infections in man including deep seated abscesses, bacteraemia, endocarditis, osteomyelitis, food poisoning etc. (JhalkaKadariya et al., 2014, and Jacques-Antoine Hennekinne et al., 2012). An area of primary concern with *S. aureus* biofilm infections is the rapid increase in the use of medical implants and prostheses and the concomitant rise in device-related infections (McConoughey SJ et al., 2014). Other species of *Staphylococcus* genus are also implicated in similar disease conditions. For example, *S. epidermidis* is involved in bacterial endocarditis, prosthetic heart valve endocarditis, bacteremia, surgical wound infections, intravascular catheter infections, postoperative endophthalmitis, conjunctivitis and keratitis. The coagulase negative Staphylococci (CoNS) species have been implicated at low incidence in a variety of infections. For example, *S.saprophyticus* is often regarded as a more important opportunistic pathogen than S. epidermidis in human urinary tract infections (UTIs), especially in young sexually active females. Other staphylococcal species such as *S. haemolyticus, S. hominis* and *S. lugdunensis* are usually found as contaminants of blood cultures but these organisms could also be associated with a variety of infections (Martineau et al., 2001). Besides several commercially available systems that allow strains to be biochemically characterized, have also been developed. Various diseases conditions caused by this organism are; wound infections, septicaemia and toxic shock syndrome. Besides, skin pustules, impetigo, osteomyelitis, renal abscess, pneumonia, endocarditis, meningitis, gastroenteritis and sometimes serious conditions in patients with undergoing hemodialysis, diabetic mellitus etc, may also be caused by *S. aureus* (Lewis and Jorgensen, 2005). Several methods such as Gram's staining, cell morphology, production of catalase and coagulase enzymes, pigment production, susceptibility to lysostaphin and lysozyme, and anaerobic production of acid from glucose are used for identification of *S. aureus* (Paul et al., 2009).
Urinary tract infections (UTIs) are a serious health problem affecting millions of people every year (John L Brusch,2019),Infection of the urinary tract is one the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Kibret and Abera, 2014; Swetha et al., 2014). It has been estimated that globally symptomatic UTIs result in as

many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually (10 Amin et al., 2009; Razak and Gurushantappa, 2012; Swetha et al., 2014).

Uncomplicated urinary tract infection (UTI) is a bacterial infection of the bladder and associated structures. UTIs are a common burden in patients with diabetes mellitus. Cystitis, ascending infection leading to pyelonephritis, impaired leucocyte function, recurrent vaginitis, emphysematous complications and renal/perinephric abscesses are well recognized in this group of patients if glycemic control is poor (Mahesh et al., 2011; Prakasam et al., 2012). Indwelling catheters are a common cause of bacterial colonisation and urinary tract infections (Jarvis et al., 2014; 4 www.lef.org, 2014).

The etiological agents and their susceptibility patterns of UTI vary in regions and geographical location. Besides, the etiology and drug resistance change through time. Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made (Kibret and Abera, 2014; Chu M.Churistine, Loder Ljerry; 2018).

UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities; these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immune suppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices. Catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes.

A. Symptoms of UTIs

1) A burning feeling when you pee
2) A frequent or intense urge to pee, even though little comes out when you do
3) Cloudy, dark, bloody, or strange-smelling pee
4) Feeling tired or shaky
5) Fever or chills (a sign that the infection may have reached your kidneys)
6) Pain or pressure in your back or lower abdomen

B. Types of UTIs

An infection can happen in different parts of your urinary tract. Each type has a different name, based on where it is.

1) *Cystitis (bladder):* You might feel like you need to pee a lot, or it might hurt when you pee. You might also have lower belly pain and cloudy or bloody urine.
2) *Pyelonephritis (kidneys):* This can cause fever, chills, nausea, vomiting, and pain in your upper back or side.
3) *Urethritis (urethra):* This can cause a discharge and burning when you pee.

Our strategy has been to elucidate molecular mechanisms of UTI sequentially, from the initial contact of bacteria to the mucosa to the immune response and genetic susceptibility profiles. Our main goals have been to identify key, virulence factors that distinguish pathogens from commensals.To study antibiotics patterns among UTI strains.

II. REVIEW OF LITERATURE

S. aureus is a relatively infrequent urinary tract isolate in the general population. In a multicenter, community-based study conducted in Great Britain, *S. aureus* accounted for only 0.5% of isolates. 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. 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). Isolation of *S. aureus* from urine samples in the absence of bacteremia is therefore often considered to represent colonization.

A. General Taxonomical Description Of Staphylococcus Aureus

Staphylococcus aureus belongs to the Domain: bacterium, Kingdom: Eubacteria, Phylum: Firmicutes, Class: Bacilli, Order Bacillales, Family: Staphylococcaceae, Genus: Staphylococcus and Species: aureus Binomial name: *Staphylococcus aureus*, Abbreviation: *S. aureus.*

Taxonomically the staphylococci belong to the Family Micrococcaceae. Classification of the micrococci and staphylococci based on physiological and biochemical tests has been proposed by Baird-Parker (1963), where the Family Micrococcaceae divided into Group I (Staphylococcus Rosenbach emend. Evans) and Group II (Micrococcus Cohn emend. Evans). Staphylococcus can be differentiated from the other three members in the family, Micrococcus, Stomatococcus and Planococcus, based on the guanine plus cytosine content of the DNA, cell wall composition, and the ability to grow and ferment glucose anaerobically. Out of 37 only three species of Staphylococcus (*S. aureus, S. epidermidis and S. saprophyticus*) were included in the genus in 1974. These species were differentiated on the basis of the ability to produce coagulase, ferment mannitol (both aerobically and anaerobically) and produce heat-stable endonuclease and by the cell wall composition. Kloos and Schleifer outlined a simplified method for the routine identification of human Staphylococcus species in 1975. They divided Staphylococcus species into 11 species on the basis of coagulase activity, heamolysis, nitrate reduction, and acid production from several sugars. 40 Since then the number of species and sub-species had increased to 32 as of 1994. 41 Currently (2015), according to the List of Prokaryotic Names with Standing in Nomenclature, the genus Staphylococcus comprises 52 species and 428 subspecies.

B. Virulence Factors And Their Role In Pathogenesis

S. aureus is an opportunistic pathogen able to persist and multiply in various environments and cause a diverse range of diseases in both humans and animals. Disease causing ability has been attributed to two major mechanisms: 1) Invasion and inflammation and 2) Toxin production. The ability of the bacterium to produce an array of virulence factors that contributes effectively to establish and maintain the pathogenicity.

C. Antimicrobial Resistance And Its Global Threat

There is a dramatic increase in multidrug-resistant bacterial pathogens across the globe over the years. The World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) are considering infections caused by antibiotic resistant bacteria as a major public health problem. WHO defines antibiotic resistance as resistance of a microorganism to an antibiotic that was originally effective for treatment of infections caused by it. The emergence of resistant microorganisms is mediated either by mutation or by the acquisition of mobile genetic elements carrying resistance genes. The exposure to antibiotics provides the necessary selective pressure for the rise and spread of resistant pathogens. Therefore, the driving force behind the increasing rates of resistance can ultimately be found in the overuse of antibacterial agents, whether in patients or livestock.

The continuing emergence of nosocomial pathogens such as multidrug-resistant Pseudomonas aeruginosa, Klebsiella pneumoniae, vancomycin resistant enterococci and *Staphylococcus aureus (S. aureus*) with intermediate susceptibility to vancomycin and other glycopeptide antibiotics threaten the lives of hospitalized individuals. Even the common community acquired infections, such as Streptococcus pneumoniae, Mycobacterium tuberculosis, Salmonella, Campylobacter species, Neisseria gonorrhoeae and Human Immunodeficiency virus are developing resistance to standard therapies. Infections caused by antibiotic resistant microorganisms fail to respond to the standard medical treatments, resulting in prolonged illness, higher health care expenditures and a great risk of death. For example, people infected with methicillin resistant *S. aureus* (MRSA) are estimated to be 64% more likely to die than people with a methicillin sensitive *S. aureus* (MSSA). The annual economic burden associated with the treatment of antibiotic resistant infections have been estimated to be between $21,000 and $34,000 million in the United States alone, and around €1500 million in Europe. In the Indian context, Chandy et al., 2014 estimated the economic burden and health consequences due to antibiotic resistance in hospital inpatients in a tertiary care hospital at Vellore. Results showed the median difference between ‘resistant’ and ‘susceptible’ groups in overall costs, antibiotic costs and pharmacy costs as INR 41,993 (P =0.001); 8,315 (P =0.011) respectively. 88 Studies, looking only at part of the impact of antimicrobial resistance, show that a continued rise in resistance by 2050 would lead to an estimated 10 million people dying every year and a reduction of 2 to 3.5% in Gross Domestic Product (GDP). It would cost the world up to $100 trillion. Antimicrobial resistance is no longer a medical issue instead, it has become a global threat that will require the action of many different stakeholders (i.e. policy makers, public health authorities, regulatory agencies, pharmaceutical companies and the scientific community at large) to tackle antibiotic resistance and come up with a coordinated set of strategies to fight antimicrobial resistance in a multifaceted approach. If resistance rates continue to rise at the present rate, there is a growing concern that therapeutic choices will be limited. After the development of penicillin, there was a ‘golden age’ of antibiotic discovery during the 1950s and 1960s. However, after 1985 there has been a sharp falloff in new class of antibiotic

discovery. Unfortunately during this same time-period MRSA, multidrug-resistant as well as extensively drug resistant Mycobacterium tuberculosis, multiple cases of hospital-acquired infections with Clostridium difficile, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii have emerged across the globe.

A diagnosis of symptomatic urinary tract infection for the purposes of this study, however, required recovery of 105 bacteria/mL from a voided specimen or 104 bacteria/mL from a catheter specimen, no clinical evidence of a non–urinary tract site of infection, and at least 2 of the following symptoms: temperature of >38.5°C, change in mental status, gross hematuria, suprapubic discomfort, dysuria, or flank pain. We intentionally chose a strict definition requiring at least 2 symptoms, because fever alone has a low predictive value in localizing infection to the urinary tract in elderly patients with bacteriaurian.

III. MATERIALS AND METHODS

The clinical samples were obtained from different pathological labs and were tested in the Department of Microbiology, IAMR College, Ghaziabad.

The samples were collected in sterile containers according to standard techniques as per the instruction given by Clean Catch and for the isolation of isolates standard techniques prescribed by Mackie and McCartney were used.

A. Collection of Samples

The sample collection approach used was the clean catch method to minimize contamination midstream urine was collected from each patient into a 20 mL calibrated sterile screw-capped container which was initially distributed to the patients. The specimen was appropriately labeled, transported to the laboratory, and analyzed within 2 hours after collection. Prior to sample collection, all patients were well instructed on how to collect the urine sample aseptically to avoid contamination.

A total of 150 urine samples included in this study were collected from the various pathological labs. The samples were collected in sterile containers according to standard techniques (Mackie and McCartney. 2000) for the isolation of *Staphylococcus aureus.*

B. Laboratory Procedures

1) *Direct Microscopic Examination:* Smears were prepared from all the clinical samples on clean sterile glass slides for Gram's staining. The smears were allowed to dry and then fixed by passing through flame and stained by Gram's technique. The smears were examined under oil immersion to look for Gram positive cocci in clusters.

C. Processing of Clinical Sample

1) *Media:* The media used were: nutrient agar (NA) from Biotec Limited, while nutrient broth (NB), MacConkey agar (MCA), blood agar (BA) and cystine lactose electrolyte deficient (CLED) agar were supplied by Hi-media Limited. Media were prepared according to the manufacturer’s specifications and sterilized by autoclaving at 121 °C for 15 min (Amin et al., 2009; Kibret and Abera, 2014).
2) *Microscopic:* The urine samples were mixed and aliquots centrifuged at 5000 rpm for 5 min. The deposits were examined using both 10X and 40X objectives. Samples with 10 white blood cells/mm3 were regarded as pyuric. A volume of the urine samples were applied to a glass microscope slide, allowed to air dry, stained with Gram stain, and examined microscopically.
3) *Motility:* Sulphate indole motility (SIM) medium with bacterial isolate incubated overnight at 37C. Motility was shown by diffused turbidity in the medium.
4) *Culturing of Urine Sample:* This was carried out as described by Cheesbrough (2002; 2004; 2006), Prescott et al (2008) and Amin et al (2009). Tenfold serial dilutions were made by transferring 1.0 ml of the sample in 9.0 ml of sterile physiological saline. One ml was then poured into molten nutrient agar in Petri dishes and rotated gently for proper homogenization. Left to allowed to set and the plates were then incubated at 37 °C for 24 h.

Bacterial colonies appearing on the plates after the incubation period were enumerated to determine urine samples with significant bacteriuria. A loopful of each urine sample was also streaked on blood agar plate for the isolation of the bacteria present in the urine. After incubation, plates with pure growth were selected, the colonies were isolated using inoculating loop and subsequently sub cultured on agar slants for use in further tests.

D. Phenotypic Tests for Identification / Characterization

The methods used in the identification and characterization of isolated bacteria include Gram stain followed by microscopic examination, motility test and biochemical tests according to Mackie and McCartney and Cheesbrough (2000; 2004 and 2006). The isolates were identified by Bergey s Manual for determinative bacteriology (Buchanan and Gribbons, 1974).

E. Colony Morphology: On Nutrient agar and Sheep Blood Agar

The circular, smooth, 1-3 mm, low convex, glistening and opaque colonies, which were easily emulsifiable. butrous in consistency golden yellow/creamy colour, surrounded by zone of p-hemolysis on blood agar were identified as the colonies of *S. aureus*. On Baird-Parker medium distinct black colored colonies were found.

F. Biochemical Test

All the isolates were subjected to various biochemical tests:

1) *Production of acid from Sugars:* Each isolate was tested for production of acid from glucose and mannitol (both aerobically and anaerobically) and from trehalose (aerobically). Carbohydrate test solution were inoculated by test culture from nutrient agar plate and incubated overnight. Reddish pink colour of the medium indicates positive reaction,
2) *Hugh and Leifson's O/F test:* Hugh and Leifson's O/F test was performed to see the ability of test strain to produce acid from glucose aerobically and / or anaerobically.
3) *Kligler Iron Agar Test:* The medium (KIM) was inoculated with colonies of bacteria isolate thoroughly the surface of slant and then stab down into the center of the butt. These were then incubated at 37ºC for 24 - 36 hours. Blackening of the medium strip indicates hydrogen sulphide production (Al-Afifi, 2009).
4) *Bacitracin Sensitivity:* Bacitracin sensitivity was done to differentiate between Micrococci and Staphylococci. *S. aureus* were resistant to bacitracin. Resistance to bacitracin was tested on Mueller-Hinton agar medium (Hi Media) with 0.04 units of bacitracin disc (Hi Media). Resistance to bacitracin was reported when zone of inhibition of growth was found less than 10 mm.

G. Enzymatic Tests

1) *Catalase Test:* A drop of H2O2 was placed on a clean glass slide. Using a clean glass rod, small amount of colony to test was picked up from nutrient agar plate and immersed into H2O2 drop. Production of gas bubbles immediately indicates positive reaction.
2) *Coagulase Test:* This test is done by both Slide coagulase and Tube coagulase method.

Antimicrobial drug sensitivity test

a) Antibiotic sensitivity test was done by the disc diffusion method as proposed by Kirby Bauer (1961).

Requirements

- Media:

Mueller-Hinton Agar (Hi Media)

Mueller-Hinton agar supplemented with an additional 5% NaCl (for Methicillin sensitivity)

- Antibiotic discs (Hi-Media): Commercially available antibiotic discs obtained from (Hi Media) were used for antibiotic sensitivity testing. The antibiotics and their contents were:

Antibiotics	Abbreviation	Disc potency
Penicillin G	P	10 units
Amoxycillin	Ac	30µm
Chloramphenicol	C	30µm
Ciprofloxacin	Cf	5µm
Cefoclor	Cj	30 µm
Ceftriaxone	Ci	30 µm
Cefepime	Cpm	30 µm
Ceftazidime	Ca	30 µm
Tetracycline	T	30 µm

Gentamycin	G	10 µm
Oxacillin	Ox	1unit
Vancomycin	Va	30 µm
Teicoplanin	Te	30 µm

All antibiotic discs were stored in, refrigerator. On removal from the refrigerator for use, the vials were left at room temperature for about an hour to allow the temperature to equilibrate, thus preventing the amount of condensation that occurs immediately after. Before use each lot of antibiotic disc was tested with standard strain of *S. aureus* 6571.

The results were interpreted according to the standard table provided by the supplier.

- Standard control Strains: Oxford *S. aureus* 6571 *S. aureus* (MRSA) in-house control
- Test strains (Bacterial inoculum)
- Opacity Standard (0.5 McFarland)
- Sterile Nontoxic cotton swabs.

H. Inoculum

Test strains of *Staphylococcus aureus* and standard oxford *S. aureus* 6571 were isolated on nutrient agar. 4-5 identical colonies were picked up from both strains (standard and test strains) and inoculated in 5ml of nutrient broth separately and incubated at 37 °C for 4-6 hours. The density of the suspension was compared with the opacity standard tube i.e. 0.5 McFarland standard.

I. Method

Drug sensitivity test for all antibiotics \vas carried out on Mueller Hinton agar plate (Hi Media). While, for melhicillin resistance oxacillin discs were used on Mueller-Hinton agar supplemented with 5% NaCl and incubated for 18-24 hour at 35°C.
The test strain was applied on the surface of the Mueller-Hinton agar plate using sterile swabs and allowed to dry for 10 minutes at room temperature. The antibiotic discs having the standard strength were lightly pressed on the surface of agar so is to ensure firm even contact of the disc with the seeded agar. Care was also taken to make sure that the placed discs straddle the gap uniformly at both sides and are about 1 cm away from the rim of the plate. The plates were then incubated for overnight at 37 °C.

IV. RESULTS

In the present study the characterization of *S.aureus* was carried out. The clinical samples were obtained from different pathological labs and were tested in the department of microbiology IAMR college, Ghaziabad. Out of 150, 102 were found positive for *S. aureus*.
All the isolates were subjected to direct microscopic examination and 102samples showed Gram-positive cocci in clusters. 76 showed golden yellow pigmentation on sheep blood agar (fig-2)**.** All were found positive for catalase test,coagulase test negative for oxidase test. All the 102 isolates showed fermentation of glucose, mannitol and trehalose. All the isolates were found resistant to bacitracin and sensitive to Novobiocin.

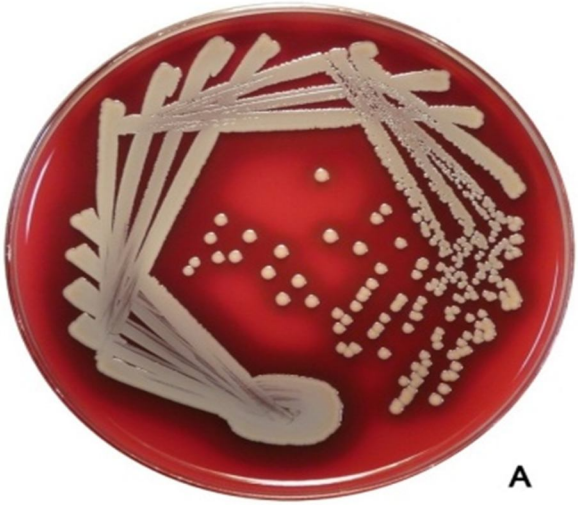

Fig-1: Samples showing the staphylococci showed pigmentation

Among 102 clinical isolates maximum number of isolates were resistant to penicillin 100 (98.03%) followed by cotrimoxazole cotrimoxazole 71 (69.61%), tetracycline 70 (68.62%), amoxycillin 66 (64.7%), ciprofloxacin 62 (60.79%), erytliromycin 56 (54.9%), amikacin 35 (34.3%), oxacillin 33 (32.35%), cefaclor 33 (32.35%), ceftriaxone 33 (32.35%), ceftazidime 33 (32.35%), cefepime 33 (32.35%), chloramphenicol 24 (23.53%) and gentaniicin 28 (27.45%). While none of the isolates were found resistant to vancomycin and teicoplanin.

A total of 102 clinical isolates were tested for oxacillin resistant by disc diffusion method and by screen agar plate method. Out of 102 clinical isolates 33 strains were oxacillin resistant by disc diffusion method, 32 strains by screen agar plate method.

The drug resistance pattern of clinical isolates showed resistance to two drugs in 3 (2.94%), three drags in 10 (9.8%) isolates, four drugs in 18 (17.65%) isolates, five or six drugs in 25 (24.51%)) isolates, seven or eight drugs in 16 (15.67 %)) isolates whereas resistance to more than ten or more drugs was found in 30 (29.47%) isolates. No isolate was resistant to only one drug.

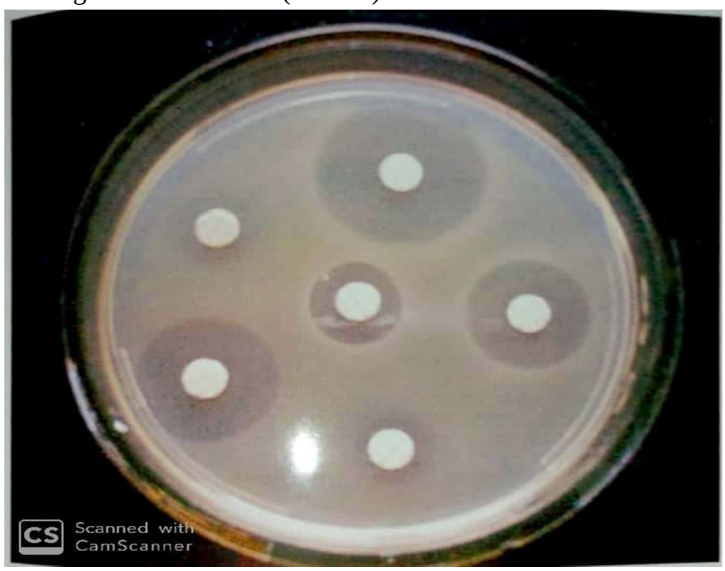

Fig:2 Photograph showing antimicrobial sensitivity on Muller-Hinton agar medium

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 8 Issue VII July 2020- Available at www.ijraset.com

Fig-3: Picture showing oxacillin resistant and sensitive strain

V. DISCUSSION

Staphylococcus aureus is an important microorganism producing various diseases in man and animals. A variety of exoproteins produced by *Staphylococcus aureus* contribute to the pathogenesis in human and animal host (Salasia et al, 2004). In man, it is an important cause for both nosocomial and community acquired infections like post-operative wound infections, pneumonia, bacteraemia, food poisoning and other infections. Therefore the present study was conducted specially to characterize the Indian *S. aureus* isolates at phenotypic level, to find out the existing antimicrobial pattern of the isolates obtained from clinical specimens of human urine. Hemolysis is considered to be one of the important virulence markers of *Staphylococcus aureus*. The workers have reported beta-hemolysis ranging from 86 to 97% (Kotrzyiiski and Kozanecki, 1990). In our study 77.4% human isolates showed beta-hemolysis on sheep blood agar. The clinical isolates showing beta-hemolysis also had multidrug resistance character.

The ability of Staphylococci to produce free coagulase, an extra cellular enzyme which activates a coagulase reacting factor (CRP) is normally present in plasma and clots plasma by conversion of fibrinogen to fibrin, and contributes to its pathogenicity It has been proposed that coagulase may inhibit phagocytosis and protect the cocci from antibacterial substances in tissue fluids laying down a fibrin barrier around them and rendering them resistant to opsonization and phagocytosis. Its production is used by the clinical microbiologists for the identification of *S. aureus* strains from human infections. Staphylocoagulase a majoi phenotypic determinants of *S. aureus* exists in multiple allelic forms, partly because of the existence of gene variants within the 3'-end coding region. In our study, All of the human clinical isolates included in the present study, were found positive for coagulase enzyme by slide and tube coagulase test. Out of 102 *S. aureus* and studied from clinical specimens, 98.03% Clinical isolates were found positive for slide coagulase test whereas they showed 100% positivity by tube coagulase test.

Among 102 clinical isolates maximum number of isolates were resistant to penicillin 100 (98.03%) followed by cotrimoxazole cotrimoxazole 71 (69.61%), tetracycline 70 (68.63%), amoxycillin 66 (64.7%), ciprofloxacin 62 (60.79%), erytliromycin 56 (54.9%), amikacin 35 (35.3%), oxacillin 33 (32.35%), cefaclor 33 (32.35%), ceftriaxone 33 (32.35%), ceftazidime 33 (32.35%), cefepime 33 (32.35%), chloramphenicol 24 (23.53%) and gentaniicin 28 (27.45%). While none of the isolates were found resistant to vancomycin and teicoplanin. The drug resistance pattern;} of clinical isolates showed resistance to two drugs in 3 (2.94%), three drags in 10 (9.8%) isolates, four drugs in 18 (17.65%) isolates, five or six drugs in 25 (24.51%)) isolates, seven or eight drugs in 16 (15.67 %)) isolates whereas resistance to more than ten or more drugs was found in 30 (29.47%) isolates. No isolate was resistant to only one drug. Antimicrobial resistance has been noticed as one of the paramount microbial threats -first century.

Staphylococcus aureus has always been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of *Staphylococcus aureus*. Therefore, continuous surveillance on the resistance patterns and characterization of *S. aureus* in understanding new and emerging trends in human and animal from India is of utmost importance.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide; and is often difficult to detect due to the heterogeneous nature of expression of oxacillin resistance. A total of 102 clinical isolates were tested for oxacillin resistant by disc diffussion method, by screen agar plate. Out of 102 clinical isolates 33 strains were oxacillin resistant by disc diffusion method, 32 strains is by screen agar plate.

In the present study, 98.03% of *S. aureus* isolates from human clinical specimens showed resistance to penicillin. Most of the reports from previous studies have revealed that, *S. aureus* from various sources had resistance to penicillin ranging between 59.89% to 98.96% (Das, 1988; Sanjeev and Mahadeva Iyer, 1988; Kar et al. 2003; Norazah et al. 2003; Shamsuzzarman et al. 2003 and Audu and Kudi, 2004). In an study, Murugan et al, (2008) noted 100% penicillin resistant strains among 2314 clinical isolates of *S. aureus*.

Resistance to chloramphenicol was observed in 23.53% strains in the present study. This is comparable to 23.1% resistance obtained by Archer et al. (1991), Orrett (2008) noted 13.3% resistance but Refsahl et al. (1992), reported higher resistance (79.4%) to chloramphenicol. In our study 64.7% strains were resistant to amoxycillin. AssaduUah et al. (2003), and Vidhani et al. (2001) reported 89.3% and 100% resistance for amoxycillin. Among cephalosporins, in the present study, 32.35% strains were resistant to cefaclor, cefotaxime, ceftazidime and cefepime.

Amongst aminoglycosides in the present study, 27.45% and 35.3% strains were resistant to gentamicin and amikacin respectively. Mathur et al (2000), reported 44.5% and 38% and Orrett (2008)noted 96.8% and 94.1% resistance to gentamicn and amikacin respectively. In other studies, variable resistance to entamicin has been reported (Assadullah et al. 2003; and Anupurba ei al. 2003). Among the fluoroquinolones, 60."9°/o strains were found resistant to ciprofloxacin. This is in comparison to the study of Mathur et al. (2000), Assadullah et al. (2003), Anupurba et al. (2003) and Orrett (2008) who reported resistance in 50.8%, 45.8%, 84.1% and 95.2% isolates respectively.

Resistance to erythromycin was observed in 54.9°/3 of strains. This was in accordance with the findings ol Majumder et al. (2001), who reported resistance to erythromycin in 33.5%. Mathur et al. (2000), reported resistance to erythromycin in 92% isolates respectively. Orrett (2008) noted 98% resistance erythromycin strains.

Methicillin resistant *Staphylococcus aureus* (MRSA) strains were identified as early as 1961 immediately after the introduction of methicillin in clinical settings (Barber, 1961). These strains have probably arisen by a succession of methicillins and the acquisition of resistance plasmids. MRSA strains have emerged worldwide as a major cause of nosocomial infections (Michel et al., 1997). Methicillin resistant *Staphylococcus aureus* is also an emerging issue; in veterinary medicine (Defra, 2008). MRSA strains are difficult to eradicate because of their multiple drug resistance, thereby complicating the management of infections. The need to differentiate MRSA from MSSA in clinical specimens. This demands a rapid and accurate method of MRSA detection for proper management and prevention of transmission of *S. aureus* in clinical settings. Though various methods for detection of MRSA have been recommended, the heterogeneous nature of resistance to methicillin and oxacillin by many strains make their recognition problematic. In this study for detection of Methicillin resistance various conventional methods disc diffusion and screen agar plate were used. Among the 102 human clinical isolates, percentage of MRSA detected by disc diffusion method, screen plate agar methods were used. In this study, 33 and 32%were found resistant to oxacillin. Vidhani et al. (2001) found 57.6% methicillin resistant *Staphylococcus aureus*, Anupurba et al. (2003) reported 54.8% MRSA, Majumder et al. (2001) reported 52.9% MRSA from patients and healthy carriers. Whereas in a study done by, Murugan et al, (2008), noted 42.86% MRSA among patients.

These variations might be because of variations in the phenotypic expression of oxacillin resistance gene such as heterogenous expression of methicillin-resistance.

VI. CONCLUSION

Staphylococcus aureus is an adaptable, opportunistic pathogen, its ability to persist and multiply in a variety of environments leads to wide spectrum of diseases in both humans and animals. In

human *Staphylococcus aureus* is the causative agent of many infections, ranging from superficial skin suppurations to life-threatening septicaemia associated with visceral or bone infections. Successful treatment is often hindered by the increasing prevalence of methicillin-resistant strains and by antibiotic inefficacy against the bacteria involved in chronic infections.

Various global studies are available characterizing the *Staphylococcus aureus* at molecular level, however, the studies are fragmentary from India on the current aspect Therefore, the present study is undertaken with the aim to evaluate the phenotypic characters of *Staphylococcus aureus*, which might help to understand its characters of Indian *S. aureus* isolates obtained from human infections. A total of one hundred and fifty clinical specimens were collected during Nov 2019 to Feb 2020 were randomly selected for further study. Out of which 102 were found as *S. aureus* and studied from clinical specimens, 98.03% Clinical isolates were found positive for slide coagulase test whereas they showed 100% positivity by tube coagulase test Among 102 clinical isolates maximum number of isolates were resistant to penicillin 100 (98.03%) followed by cotrimoxazole 71 (69.61%), tetracycline 70 (68.63%), amoxycillin 66 (64.7%), ciprofloxacin 62 (60.79%), erytliromycin 56 (54.9%), amikacin 35 (35.3%), oxacillin 33 (32.35%), cefaclor 33 (32.35%), ceftriaxone 33 (32.35%), ceftazidime 33 (32.35%), cefepime 33 (32.35%), chloramphenicol 24 (23.53%) and gentaniicin 28 (27.45%). While none of the isolates were found resistant to vancomycin and teicoplanin. The drug resistance pattern; of clinical isolates showed resistance to two drugs in 3 (2.94%), three drags in 10 (9.8%) isolates, four drugs in 18 (17.65%) isolates, five or six drugs in 25 (24.51%)) isolates, seven or eight drugs in 16 (15.67 %)) isolates
whereas resistance to more than ten or more drugs was found in 30 (29.47%) isolates. No isolate was resistant to only one drug. Antimicrobial resistance has been noticed as one of the paramount microbial threats -first century.

Staphylococcus aureus has always been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant forms of *Staphylococcus aureus*. Therefore, continuous surveillance on the resistance patterns and characterization of *S. aureus* in understanding new and emerging trends in human and animal from India is of utmost importance.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide; and is often difficult to detect due to the heterogeneous nature of expression of oxacillin resistance. A total of 102 clinical isolates were tested for oxacillin resistant by disc diffussion method, by screen agar plate. Out of 102 clinical isolates 33 strains were oxacillin resistant by disc diffusion method, 32 strains is by screen agar plate.

VII. SIGNIFICANCE

Various global studies are available to see the prevalence and characterizing the *Staphylococcus aureus* from food sample, however, the studies are fragmentary from India on the current aspect and especially in *Staphylococcus aureus* isolates obtained from samples of urine. Therefore, the present study undertaken with the aim to evaluate the phenotypic characters of *Staphylococcus aureus*, which might help to understand the characters and prevalence of Indian *S. aureus* isolates obtained from clinical samples.

VIII. FUTURE PROSPECTS

Staphylococcus aureus is an adaptable, opportunistic pathogen, its ability to persist and multiply in a variety of environments leads to wide spectrum of diseases in both humans and animals. In humans *Staphylococcus aureus* is the causative agent of many infections, ranging from superficial skin suppurations to life-threatening septicaemias associated with visceral or bone infections. Successful treatment is often hindered by the increasing prevalence of methicillin-resistant strains and by antibiotic inefficacy against the bacteria involved in chronic infections.

S.aureus is a cause of urinary tract infection among patients with urinary tract catheterization. The majority of isolates are methicillin-resistant *S.aureus* bacteriuria can lead to subsequent invasive. The efficacy of antistaphylocooccal therapy in preventing late-onset staphylococcal infection in patient with persistent staphylococcal bacteriuria should be tested in controlled trials.

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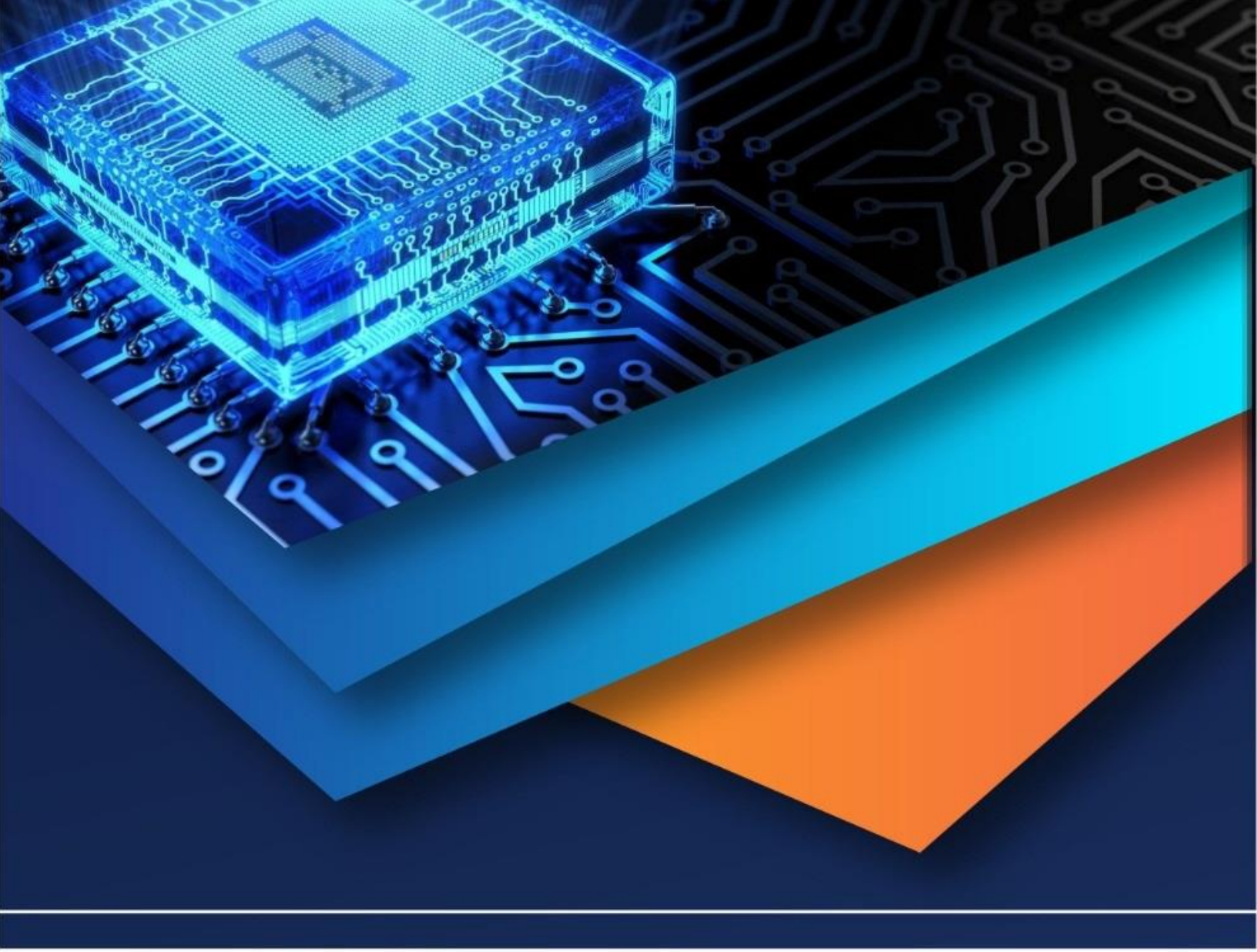

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