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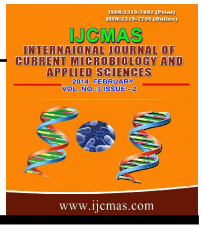

Original Research Article

In vitro Antimicrobial susceptibilities and pathogenic attributes of *Staphylococcus aureus* isolates from UTI patients in Punjab, India

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A B S T R A C T

Staphylococcus aureus is known to cause urinary tract infections (UTI), pathogenic factors help to resist the opsonisation and immune response of host and in enhancing the resistance against antibiotic therapy. Moreover being deficient in adequate information about antimicrobial susceptibilities, an inappropriate use of antimicrobial agents is causing spread of resistance among microorganism causing UTI. Realizing the developing antibiotic resistance in pathogenic isolates of *S. aureus*, in the present study clinical samples (vaginal swabs) from urogenital tracts of women with UTI from Punjab region were collected, isolated colonies of *S. aureus* were studied for pathogenic attributes and resistance against a panel of 24 antibiotics. Morphological and biochemical characterization of *S. aureus* isolates from the samples (vaginal swabs) was carried out by colony morphology, Gram staining, catalase test, capsule formation and haemolysin test. Antibiotic resistance test was performed using disc diffusion assay for 24 different antibiotics of different groups. All the recovered distinctive colonies were positive for Gram staining, catalase and capsule test. 50% samples were positive for Mannitol salt agar test while 40%, 22% samples were positive for α-haemolysin, β-haemolysins respectively. Among the Cifixime, Ofloxacin, rifampicine Gentamycine, Amikacin and Vancomycine were found most successful.

K e y w o r d s

Staphylococcus aureus; MRSA (Methicillin resistant *S aureus*); UTI (urinary tract infection); Antimicrobial Susceptibility.

Introduction

Urinary tract infection (UTI) is the most common infection after upper respiratory tract infections (Hryniewicz et al., 2001). Even though several different microorganisms can cause UTIs, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases (Bonadio et al., 2001). As studied by Manikandan et al (2011) *S. aureus* was responsible for 20.5% of UTI cases. Multi drug resistant *Staphylococcus aureus* is of great concern with high virulence (Chamber, 2005), ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions (Lowy, 2003).

Barka *et al* (2014) reported *Staphylococcus* to be responsible for 24.16% of the genital damage and 20.83% of urinary manifestations respectively of the total samples studied.

The empirical therapy of urinary tract infections (UTI) relies on the awareness of antimicrobial susceptibility patterns of the agents causing UTI. The antimicrobial resistance patterns of the causes of UTI are highly variable and continuous surveillance of trends in resistance patterns of uropathogens is important (Farajnia et al., 2009), regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy (Grude et al., 2001; Kripke, 2005). In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganism causing UTI (Manikandan et al., 2011). Hence current study was performed for developing an antibiogram of *S. aureus* isolates against a panel of 24 antibiotics.

Materials and Methods

All the chemicals and reagents used in the study were of analytical and molecular grade and procured from Himedia Laboratories Pvt. Ltd (India), Bangalore Genei Ltd. (India), Merck Co., Inc. (USA), Bio-Rad Laboratories Inc. (USA), etc.

Collection of samples

The present study was carried out by collecting samples from women suffering from UTI including hospitalized, un-hospitalized, rural and urban areas of Punjab. 45 samples in total were collected covering different hospitals, testing labs, rural and urban areas of Punjab, India. Samples were taken as vaginal swabs, collected in sterilized glass test tubes containing 2 ml of 0.5% peptone water as it prevents the cell wall damage of the bacteria. Then collected samples were incubated for 2-3 h at 37 ºC for proper bacterial growth, to be used for isolation of *S. aureus*.

Isolation of *S. aureus* from the collected samples

For the isolation of presumptive colonies of *S. aureus* Baird Parker Agar (BPA) was used. Sterile egg yolk potassium tellurite emulsion was poured aseptically to media and after a proper mixing poured in petri plates.

Thereafter 100 µl from the collected sample was poured, spread over the petri dish and incubated overnight at 37ºC for proper growth of the bacterium. *Staphylococci* produces black colony with brownish ring outside on the BPA plate. The distinct colonies of *Staphylococci* were selected, taken for Gram staining, Catalase test and for morphological and biochemical characterization.

Biochemical characterization of isolates

Capsule test, mannitol salt agar test (MSA), haemolysin, biofilm and slime production tests were performed to characterize the isolates (APHA, 1998). Antimicrobial Susceptibilities (Antibiogram) of *S. aureus* isolates Antibiogram of *S. aureus* isolates was prepared using disc diffusion method on Mueller-Hinton agar according to the National Committee on Clinical Laboratory Standards (NCCLS) recommendations (NCCLS, 2000), using Mueller-Hinton medium. On the basis of the diameter of the zone, isolates were classified as susceptible and resistance. The detail and the concentration of antibiotic used are given in the Table 2.

Results and Discussion

Isolation, Identification and Biochemical characterization

Isolates from all the samples were found positive for Gram staining, catalase, haemolycine test and the presence of capsule while 50% were found positive for Mannitol Salt Agar test (Table 1 and Fig. 1). Results of biochemical characterization have summarized in Table 1.

Antimicrobial Susceptibilities (Antibiogram) of *S. aureus*

The rates of resistance of isolates against the panel of antibiotics, including penicillins, cephalosporins, aminoglycosides, and trimethoprim etc., which are routinely used to treat UTI infections, are shown in Table 2.

The present study was on pathogenic attributes and antibiogram of *S. aureus* isolates from UTI women patients. For the isolation and identification of *S. aureus* isolates attributes *e.g.* colony morphology, Gram staining, Catalase production and capsule formation were studied. Further biochemical characterization involved α, β-haemolysin that correlates to those by Aarestrup et al l999). Surface factors inhibit phagocytic engulfment, protection against host defence and antibiotic tolerance (capsule), biochemical properties (Table 1) enhance their survival in phagocytes (catalase production) and membrane-damaging toxins that lyses eukaryotic cell-membranes *e.g.* haemolysins, come under virulence factor (Suheyla and Osman, 2006). UTI are caused by various virulence factors/pathogenicity of *S. aureus* include enzymes such as haemolysins; coagulase and acid production from mannitol (Murray et al., 2003; Lowy, 1998; Cheung et al., 2002). Slime formation has been shown to play a crucial role to facilitate the adherence and colonization of isolates of *S. aureus* to the host. Haemolysins, capsule and slime formation of the staphylococci species were regarded as pathogenicity criterions in laboratories (Turkyilmaz and Kaya, 2006; Otto, 2004). Results of present study on pathogenic attributes correlates with the facts mentioned.

As far the susceptibility of *S. aureus* against antimicrobial agents is concerned, scientists have studied the presence of drug resistant *S. aureus* and MRSA at different times in the urine sample and high vaginal swabs (Daniyan et al., 2010; Kumari et al., 2008; Anbumani et al., 2006; Anupurba et al., 2003). Resistance rates among *S. aureus* strains are increasing, and a major part of this species has become resistant to β-lactam antibiotics (Karbasizaed et al., 2003). Research findings have confirmed that methicillin resistant *S. aureus* (MRSA) infections in the community have been increasing in Asian countries; various MRSA clones have spread between the community and hospitals as well as between countries (Song et al., 2011).

In the present study large number of isolates showed resistance to multiple antibiotics (Table 2) that may be due to several factors like indiscriminate use of antibiotics, lack of awareness and unethical treatment before coming to the hospital (Anbumani et al., 2006). Inappropriate antimicrobial use due to availability of antimicrobials without prescription or prescribed by non-skilled practitioners can lead to inadequate therapy and contribute to further drug resistance (Yilmaz et al., 2009).

Table.1 Results of biochemical characterization

S. No.	Type of study	No. of Positive samples for the test (Total=45)	%age (n=45)
1	Gram Staining	45	100
2	Catalase	45	100
3	Capsule	45	100
4	MSA	23	50
5	Haemolysis	45	100
	α-haemolysin	18	40
	β- haemolysin	10	22
	γ- haemolysin	17	37
6	Slime production	27	60

Table.2 Percentage of antibiotic resistance among the *S. aureus* isolates

Antibiotic Group	Name of the Antibiotic	Concentration (µg/disc) used	% of resistant sample (n=45)
Beta Lactam	Methicillin (MET^5)	5	33.3
	Methicillin (MET^{10})	10	15.5
	Penicillin-G	10	53.3
	Cloxacillin	5	20.0
	Ampicillin	10	24.4
	Amoxicillin	10	2.2
Aminoglycosides	Gentamycine	10	0
	Streptomycine	10	2.2
	Amikacin	30	0
Fluoroquinolone	Ciprofloxacin	5	11.1
	Ofloxacin	5	0
Lincosamide	Clindamycine	2	46.6
	Lincomycine	15	51.1
Amino-penicillin	Ampicillin/Sulbactum	10/10	37.7
Cephalosporins	Cifixime	5	0
Streptogramins	Pristinomycine	15	68.8
Tetracyclines	Tetracycline	30	2.2
Amphenicols	Chloramphenicol	30	4.4
Macrolide	Erythromycine	15	35.5
Rifampin	Rifampicine	5	0
Glycopeptide	Vancomycine	30	0
----	Trimethoprim	5	66.6
----	Amoxyclave	30	4.4
----	Azithromycine	15	15.5

MET^5- Methicillin 5 µg/disc
MET^{10}- Methicillin 10 µg/disc

In the present investigation, *S.aureus* isolates were typed for antibiotic resistance to obtain important information that could help in evolving a strategy for prevention and treatment of UTI in women.

33.3% isolates is found resistant to methicillin (MET^{5}) in this study that correlates to 34.2% as reported by Diekema et al (2004) however Prashini et al (2004) reported methicillin resistance by 56.25% of the isolates tested. When the concentration of the methicillin doubled ($MET^{1\circ}$) the percent resistance reduced to less than half i.e. 15.5%. Against the antibiotics among the panel e.g. Amikacin (0%), gentamycine (0%), vancomycine (0%), ciprofloxacin (11%) the isolates showed no resistance or a little resistance that correlates with the study by Farajnia et al (2009) which reports Amikacin (0%), gentamycine (0%), vancomycine (0%) and ciprofloxacin (0%). In comparison to Manikandan et al (2011), resistance of the isolates experienced against ciprofloxacin and gentamycine is found comparable whereas trimethoprim (66%), Ampicillin (24.4%) and oxacillin (20%) are very less than that reported in their study. Tetracycline, erythromycin, pristinomycine, amoxicillin has been more effective than that reported by Tyagi et al (2008) however a higher resistance (68.8%) is shown against Cifixime. The regional variations of resistance to antibiotics may be explained in part by different local antibiotic practices (Sannes et al, 2004; Bell et al., 2002). However vancomycine has been 100% effective which correlates with the results different studies reported by Anupurba et al (2003), Kumari et al (2008), Tyagi et al (2008). In the present study Cifixime, Ofloxacin, rifampicine Gentamycine, Amikacin and Vancomycine are found 100% effective in contrast to reports of S. aureus isolates with reduced susceptibility to vancomycine by Centres for Disease Control and Prevention, USA (CDCP-USA 1997). Resistance of S. aureus against vancomycine has also been reported by other workers in the past (Cosgrove et al., 2004; Hiramatsu et al., 1997). However routine testing of other glycopeptides like teicoplanine should be done.

Conclusively, as the pattern of bacterial sensitivity to antibiotics varies over time and in different geographical regions, antibiotic treatment of infections should be based on local experience of sensitivity and resistance patterns. In the present study Cifixime, Ofloxacin, rifampicine Gentamycine, Amikacin and Vancomycine were found most appropriate parental antibiotics, for the empirical therapy of UTIs. Regular surveillance of hospital-associated and community associated infections including monitoring of antibiotic susceptibility pattern of MRSA and formulation of definite antimicrobial policy may be helpful for reducing the infections. Further public awareness, only use of prescribed antibiotics, safe sex, and personnel hygiene are among critical preventive measures. Knowledge about MRSA and carrier status needs to be raised among the public. A further study of MRSA may be done for epidemiological mapping of the UTI.

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