Research Article

Antibiogram and Isolation of *S. aureus* from the Urinary Tract Infections: Comparison of Meca Gene Detection and Phenotypic Methods for Detection of Methicillin-Resistant *S. aureus*

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ABSTRACT

Introduction: Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime. *S. aureus* is one of the most widely spread human pathogens.

Objective: To detect Methicillin-resistant Staphylococcus aureus(MRSA) among *S. aureus* causing UTI. Objectives: To know the antibiotic sensitivity pattern of the isolates and comparison of *mecA* gene detection and phenotypic methods for the detection of MRSA.

Methods: This study conducted over 2 years from January 2017 to December 2018. The isolates were identified by standard protocol. All isolates were tested for antimicrobial susceptibility. MRSA was identified by Cefoxitin and Oxacillin disk diffusion method. *mecA* gene was detected by PCR.

Results: UTI were reported more among females in the age group of 21 to 40 with a rate of 42%. Among the male patients, UTI was reported more in elderly patients with 50% of cases occurring between the age group of 40-60 years. Linezolid was found to be the most effective drug overall against *S.aureus*. The highest percentages of resistance were found for penicillin and pefloxacin. Cefoxitin and Oxacillin detected 19 and 15 isolates as MRSA respectively, the PCR detected mec A genes in 20 isolates.

Conclusion: UTIs were more among young females patients and elderly male patients. PCR was the best method for the detection of MRSA. Cefoxitin disc is the best alternatives for PCR for the identification of MRSA. Antibiotic sensitivity revealed MRSA were resistant to many antibiotics but were sensitive to tetracycline, gentamicin, vancomycin and linezolid.

Key Words: Urinary tract infections, *S.aureus*, MRSA, PCR, *mecA* gene

INTRODUCTION

Bacterial resistance to antibiotics increases mortality, the likelihood of hospitalization and the length of stay in hospital. Resistance is related to the increasing usage of antimicrobial agents; growing numbers of patients with impaired immunity; increasing instrumentation, and emphasis on cost control. Furthermore; it no longer remains the domain of Gram-negative bacteria. Antimicrobial usage may be controlled by antibiotic policies, but these can only be formulated if the antimicrobial susceptibility pattern of prevalent bacterial pathogens is known.[1]

Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experi-ence a UTI during their lifetime.[2-4] The infections may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if left untreated. *Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas,* and *Enterococci* species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an out-patient population. *Corynebacterium urealyticum* has been recognized as an important nosocomial pathogen. Anaerobic organisms are rarely pathogens in the urinary tract.[5-8] *Coagulase Negative Staphylococci* are a common cause of urinary tract infection in some reports. *Staphylococci saprophyticus* tends to cause infection in young women of sexually active age.[9,10]

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S. aureus is one of the most widely spread human pathogens. Considering the havoc it causes on life and subsequently on the economy, it became necessary to determine its incidence and antibiogram in our environment for adequate control and treatment.[11] of infections caused by *S. aureus* can be one of the gratifying experiences in clinical practice. Survey of resistant patterns of microbes to drugs has shown a rise in the incidence of microbial resistance to most prescribed antibiotics. The study aimed to detect MRSA among *S. aureus* causing UTI and to know the antibiotic sensitivity pattern of the isolates in our hospital setting.

MATERIALS AND METHODS

Study design, setting
This study conducted in the Department of Microbiology, Shri B M Patil Medical College over 2 years from January 2017 to December 2018.

Sample collection
The samples included midstream urine specimen, catheterized urine samples, supra-pubic aspirates collected in sterile universal bottles. The urine specimens were transported to the bacteriology laboratory within 2 hours of collection.[12]

Statistical analysis
Values were expressed in terms of Mean ± SD. Analysis was done by using SPSS software version 16. $P \leq 0.05$ was considered statistically significant.

Microbiological analysis
All urine samples were examined by routine microscopic examination by the wet mount of urine sediment. All urine samples were cultured over routine culture media with a sterile standard loop. These plates were incubated at 37°C for 2 consecutive days. Culture results were interpreted according to the standard criteria.[13] Cultures with more than three colonies were discarded, as contaminants [12] The isolates were identified by gram staining, colony morphology and standard biochemical tests catalase, slide and tube coagulase, mannitol salt agar test, phosphatase test.[14]

Antimicrobial susceptibility testing
All isolates were tested for antimicrobial susceptibility on Mueller Hinton agar by the standard disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI).[15]

Detection of MRSA
The Cefoxitin Disc Diffusion Test: the test was carried out on Mueller-Hinton agar by using a 30 μg cefoxitin disc. An interpretation was done using the Kirby-Bauer charts.

An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant.[16]

The Oxacillin Disk Diffusion Method: The Oxacillin disk (1 μg) diffusion method was carried out on Mueller-Hinton agar which was supplemented with 4% NaCl to detect MRSA according to the CLSI guidelines. The isolates were considered as resistant when the diameter of inhibition was ≤ 10 mm.[17]

Genotypic detection of MRSA by PCR (mec A gene)
DNA Extraction Procedure was done by Modified Proteinase-K method.[18,19] MRSA strains were amplified by conventional PCR. Following a set of PCR primers were used which were specific to Methicillin-resistant *S.aureus*.[20]

Forward Primer: 5'- TGC TAT CCA CCC TCA AAC AGG -3' Reverse Primer: 3'-AAC GTT GTA ACC ACC CCA AGA -5'
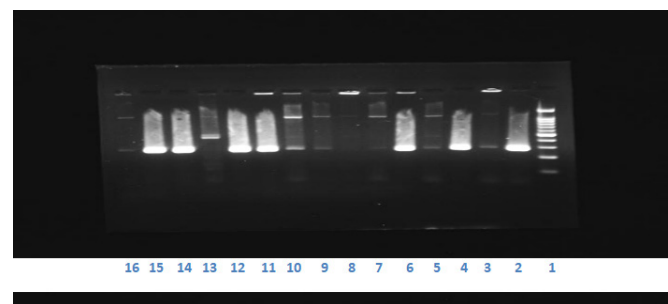

Figure 1: Results of *mecA* gene (left to right), Lane 1: Molecular weight marker, Lane 2: MRSA ATCC 43300, Lane 3: MSSA ATCC 25923, Lane 4, 6, 11,12,14 and 15: MRSA isolates from clinical samples(280 BP), Lane 5,7-10,13,16: MSSA isolates from clinical sample.

We had chosen a primer set that gives an amplified product of size 280 base pair. So the well which gives a DNA band of 280 base pair is considered positive, whereas the well which does not have any DNA band is indicated as negative (Figure 1).

RESULTS

Table 1: Distribution of patients according to age and sex

Age (yrs)	Male		Female		Total
	N	%	N	%	
Below 20	2	8	4	19	6
21-40	6	25	9	42	15
41-60	12	50	6	28	18
Above 60	4	17	2	10	6
Total	24	100	21	100	45

Table 2 : Distribution of MRSA and MSSA among patients with UTI

S. aureus	N	%
MSSA	25	56
MRSA	20	44
Total	45	100

Table 3: Comparison of phenotypic methods with PCR for detection of MRSA

TEST METHODS	MRSA	Sensitivity	Specificity	PPV	NPV	Accuracy
Oxacillin	15	75.0%	100 %	100 %	83.3 %	88.9%
Cefoxitin	19	95.0%	100 %	100 %	96.1 %	97.8%
PCR	20	100 %	100 %	100 %	100 %	100 %

Table 4: Antibiotic resistance pattern of MRSA and MSSA among patients with UTI

Antibiotic susceptibility pattern	MRSA (N=20)		MSSA (N=25)		p-value
	R	%	R	%	
PENICILLIN-G	19	95	23	92	0.688
EYTHROMYCIN	9	45	7	28	0.236
TETRACYCLINE	5	25	4	16	0.453
CEPHALEXIN	7	35	8	32	0.832
CLOXACILLIN	12	60	15	60	1.000
PEFLOXACIN	18	90	18	72	0.134
PIPERACILLIN/TAZOBACTAM	6	30	5	20	0.438
CEFOPERAZONE /SULBACTAM	7	35	6	24	0.419
GENTAMICIN	4	20	4	16	0.727
CIPROFLOXACIN	15	75	18	72	0.821
AMOXICILLIN/CLAVULANATE	13	55	9	36	0.053
CEFUROXIME	5	25	5	20	0.688
AZITHROMYCIN	14	70	12	48	0.138
VANCOMYCIN	3	15	1	4	0.198
LINEZOLID	1	5	0	0	0.258

DISCUSSION

UTI(UTIs) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group.[21,22]

In our study, the UTI was reported more among the age group of 21 to 40 with a rate of 42%. the findings are in agreement with the study conducted by Inaba et al,.[23] and El-Sweih et al,.[24] this can be explained by the fact the structure of the female's urethra and vagina makes it susceptible to trauma during sexual intercourse and pregnancy and or childbirth.[24,25]

S. aureus UTI more often occurs in urinary-catheterized and pregnant individuals. The majority of *S. aureus* UTI isolates are methicillin-resistant and *S. aureus* bacteriuria is associated with subsequent development of invasive infection.[26-28] Like *S. saprophyticus*, *S. aureus* also encodes an active urease enzyme. Two nickel ABC-transporters (Opp2 and Opp5a) have been identified as necessary for urease activity in vitro. These, along with a third ABC-transporter that imports nickel and cobalt when zinc is depleted, are both involved in UTI colonization and virulence in a mouse model.[29-32]

Urinary tract infection is one of the most important causes of morbidity in the general population and is the second most common cause of hospital visits.[32] Among the male patients, UTI was reported more in elderly patients with 50% of cases occurring between the age group of 40-60 years. Our finding is in agreement with a study conducted by Das et al.[33]

With advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder.[34] Recurrent infections are common and can lead to irreversible damage of the kidneys, resulting in renal hypertension and

renal failure in severe cases.[33] UTIs have been reported to be the majority caused by Gram-negative bacteria with *E. coli* being the most prevalent. However, there is an increasing prevalence of *S. aureus* as a UTIs etiological agent with an alarming rate of developing antimicrobial resistance (Table 1 and 2).[35]

Linezolid was found to be the most effective drug overall against *S. aureus* followed by vancomycin, tetracycline, gentamycin and cefuroxime. The highest percentages of resistance were found for penicillin, pefloxacin. these results are basically in agreement with other studies carried out around the world. Our findings illustrate that antimicrobial therapy needs to be selected based on actual culture findings and antimicrobial sensitivity patterns of isolates (Tables 3 and 4).

Antibiotic susceptibility pattern revealed a high resistance to routinely used antibiotics. Resistance to quinolones I,e. ciprofloxacin and pefloxacin were high in this study. This is comparable to the study done by Sanjana et al,[36] in Nepal. Majumder et al,.[37] also revealed that resistance to various antibiotics with methicillin-resistant strains was s higher in comparison to methicillin-sensitive isolates. Factors responsible for drug resistance in MRSA are as follows. Antibiotics are available without a prescription at drug stores or even at general stores and injudiciously used in communities, animal husbandries, and fisheries and use of allopathic drugs by traditional practitioners.[38]

In our study, the *mecA* gene PCR detected 20 isolates as MRSA and the 25 isolates as MSSA. Detection of *mecA* gene is considered the best method for MRSA confirmation.The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*.[39] This higher sensitivity to cefoxitin can be explained by the increased expression of the *mecA*-encoded protein PBP2a, cefoxitin being an inducer of the *mecA* gene.[39] Our study reveals that cefoxitin disc is better than oxacillin disc for the detection of methicillin resistance.

CONCLUSION

To conclude, we found that UTIs were more among young females patients and elderly male patients. PCR was the best method for the detection of MRSA but in peripheries where it is not available Cefoxitin disc diffusion test is the best alternatives for PCR for the identification of MRSA. Antibiotic sensitivity revealed MRSA were resistant to many antibiotics but were sensitive to tetracycline, gentamicin, vancomycin and linezolid.

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