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Synul FOR Reserve	Original Research Paper	Microbiology
international	ESTIMATE THE PROPORTION OF MRSA AMONG STAPHYLOCOCCUS UREUS ISOLATED FROM PATIENTS OF URINARY TRACT INFECTION ALONG WITH ITS ANTIBIOGRAM IN TERTIARY CARE HOSPITAL AT AGARTALA, TRIPURA	
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ABSTRACT Background: Generally, gram-negative bacteria are the most common uropathogenic bacteria causing		

UTI, as per recent findings gram-positive bacteria also equally responsible for the UTI. As they have a plethora of pathogenic virulence factors that are highly responsible to give rise to severe UTI infection. Moreover, a betalactamase-producing gram-positive organism such as *Staphylococcus aureus* is often associated with high resistance to a wide class of antibiotics. There are few studies regarding the UTI infection-causing by the multi-drug resistant strain of *Staph aureus* called MRSA in Tripura Northeast India. This study aimed to determine the proportion of MRSA causing UTI in Tertiary care hospitals at Agartala Tripura.

Materials and method – Mid-stream urine specimens were collected from patients suspected to be having UTI. Well, the mixed sample of urine was inoculated on specific culture media and all the isolates were identified as *staphylococci* by Gram staining, catalase test, coagulase test, and other biochemical reactions required for the identification of *S.aureus* and as per the CLSI guidelines we have studied the antibiogram of *S.aureus* and we have detected the MRSA by cefoxitin disc diffusion method.

Result: in our present study 650 midstream urine samples were collected and processed. Out of the total 650 urine samples 91(14%) yielded growth on culture media and out of the 91 positive cases *Staphylococcus aureus* infected UTI cases were 12(13%) and among the 12 S.aureus positive isolates 6(50%) isolates were MSSA and other 6(50%) isolates were MRSA. Here in our study vancomycin(100%), doxycycline(83%), Nitrofurantoin(100%), Linezolid(83%) found to be very effective drugs for the treatment of MRSA, and most importantly here in this region of northeast only 6.5% of the total UTI patients affected by the MRSA which means the ratio of UTI infection by MRSA is low in this region.

CONCLUSION: This study will be useful to update the policy of the empirical drugs in our hospital and at the same time, it will also help to control the unnecessary use of antibiotics to treat UTI patients.

KEYWORDS : MRSA, CONS, UTI, CLSI

INTRODUCTION

UTI has become the most common and serious health problem both in hospital and community settings, UTI causes fullblown infection with a high rate of morbidity in hospitals as well as in the community domain $^{\scriptscriptstyle [2,3]}$. The prevalence of UTI was estimated to be 150million per year worldwide and it is one of the most common microbial diseases encountered in medical practice^{[4].} One study found that Staphylococcus aureus is the common cause of UTI, it colonizes the urinary tract In immunocompetent and immunocompromised patients and it could be the source of future staphylococcal infection^[5]. Staph aureus gives rise to multi-drug resistance capacity against a wide range of drugs termed MRSA and this strain carries multi-drug resistance mecA gene on their plasmid which is responsible for the ability of multi-drug resistance among other organisms^[6]. MRSA is a troublous pathogen and it is difficult for a clinician to treat because it resistant to most useful antibiotics like Amoxicillin, clindamycin, Quinolones, Cotrimoxazole and Cephalosporins, Vancomycin is the only most useful drug for the treatment of MRSA associated infections, this ability of resistance to many efficient drugs occur in bacteria by the action of resistance genetic material present on a plasmid ^[8]. In many studies, we found that MRSA resistance to a large amount of highly efficient drugs such as Aminoglycosides, Macrolides, Tetracycline, Chloramphenicol, and many other disinfectants also but the thing is that the capacity or ability to resistance towards a large number of drugs by MRSA is varying region to region.^(9,10). here in this region of the Northeast, only a few studies have been done on the proportion of MRSA causing UTI infection. Continuous Surveillance for multidrug-resistant strains is necessary to prevent the further spread of resistant isolates.

This is a hospital-based cross-sectional study, was carried out in the department of microbiology of Agartala government medical college, Agartala, Tripura for a period of 3 months from Oct 2020 to Dec 2020. A total 650 midstream urine samples were collected and processed from UTI patients who attended the hospital with UTI symptoms during our study period. Among the 650 isolates of UTI only 91cases(14%) showed significant growth and out of 91 isolates only 12 (13%) isolates were *Staphylococcus aureus*.

SAMPLE PROCESSING-

Midstream urine samples preferably early morning specimens were collected in a sterile container. All patients were clearly instructed about the procedure of collection of a midstream urine specimen to avoid contamination.

Urine microscopy was done for pus cells and cultured on CLED agar, MacConkey agar, and blood agar media. All the inoculated plates were incubated at 37 C for 24-48 hours, then after the incubation period the colony characteristics, pigment production, and hemolysis were observed.

Then we went through Gram staining and another biochemical confirmatory test mainly the coagulase test and the catalase test to identify *Staphylococcus aureus*.

Detection of MRSA

In our study, a phenotypic detection method was followed for the detection of MRSA.

Screen test

Phenotypic detection of MRSA among the S.aureus was carried out by using the cefoxitin disc diffusion method. As per

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CLSI guidelines, 0.5 Mc Farland suspension of the isolate was made and culture was done on the MHA plate. A 30 microgram cefoxitin disc was placed and plates were incubated at 37 C for 18 hours and after the incubation period, the zone diameter was measured. The zone diameter must be measured in reflected light with the help of scale. If the Zone diameter of Staph aureus is \leq 21 mm was reported as methicillin-resistant (MRSA) and \geq 22 mm was considered as methicillin-susceptible.

Here we use mainly the Cefoxitin disc diffusion test for the detection of MRSA because Cefoxitin can detect only MRSA with a mecA mediated resistance mechanism.

OBSERVATION & RESULT

Among the 650 isolates of UTI, only 91 cases(14%) showed significant growth. Out of 91 positive cases, 37 (41%)isolates were gram-positive and 54(60%) isolates were Gram-negative.

Among 91 isolates only 12 (13%) isolates were Staphylococcus aureus. Out of these 12 patients, the most common age group which was affected was 60-80 years of age. A total of 4 (66%)MRSA associated UTI patients were present in this group.

Out of 12 Staphylococcus aureus isolates, 5(42%) were isolated from males and 7(58%) were isolated from females.

Among 12 Staphylococcus aureus isolates 6 isolates were MSSA and the other 6 were MRSA, which is confirmed by the Cefoxitin disc diffusion test (Fig no.1).



Fig no.1

Antibiotic susceptibility pattern to Methicillin resistant Staphylococcus aureus showed highest sensitivity to Vancomicin(100%), Nitrofurantin(100%), Doxycycline(83%) and Linezolid(83%) (Fig no. 2).

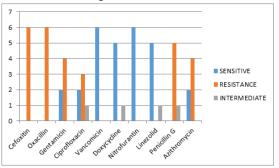
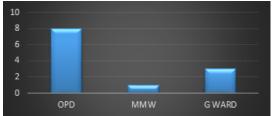


Fig no. 2

Fig no. 3

In ward wise distribution of patients the most cases of MRSA were found in OPD patients.



DISCUSSION :

In our study, we investigated "The proportion of MRSA among Staphylococcus aureus causing UTI and showing their antibiogram in Tertiary care hospital at Agartala, Tripura" Our reports highlight that UTI infection by MRSA needs to strictly monitor in communities as well as in hospitals to prevent the Nosocomial and Community-acquired UTI infection in our societies.

In our research, we analyzed the rate of Urinary tract infection in this specific geographical area is 14%, among them a percentage of *Staphylococcus aureus* associated UTI cases are 13%, and in total *Staphylococcus aureus* isolates 50% were MSSA and other 50% were MRSA. The faster rate at which *Staphylococcus aureus* developing resistance to many antibiotics is reducing their usefulness in the empiric treatment of UTI. Because of this large number of multidrugresistant isolates the empirical treatment of UTI patients has become a serious threat in our society.

In our study, we got only 13% isolates of Staphylococcus aureus cause UTI, were 58% cases come from females and 42% were from the male which means females were more infected in contrast to males, and this result a closely related to a study done by Dharmendra Singh et. a July 2019 women's are mainly getting an infection which could be due to the anatomical structure of a female genital organ or else it could be due to the close proximity to urethral meatus to anus^{77.}

In this study, we found that among all the Gram-positive organisms *Staph aureus*, CONS, *Enterococci* is relatively common causing UTI which resembles some studies done by T.A schlager and another study done by N.Kumari, A Rai C p Jaiswal^(11,12).

Many studies showed that Gram-negative bacteria very highly causing UTI as compared to Gram-positive bacteria^[12], but here in our specific region of Tripura we found that 41% of Gram-positive bacteria causing UTI. which is similar to a study done by Zahra Tayebi, sima Sadat seyedjavedi^[13], Though different research articles in different areas should show various results which could be due to the climate, hygiene, and waste management in our society.

In this study, we observed that the presence of MRSAassociated UTI infection is comparatively higher in outdoor patients as compare to indoor patients.

This condition might be the result of not drinking enough water, holding urine for a long period, or else it could occur if the patient has any injuries on their spinal cord or if they have a tumor on that urinary passage, kidney stone, enlarged prostate or sexual intercourse.

This Staphylococcal UTI can be cured by initial antibiotic treatment such as Trimethoprim, Fostomycin, Cephalexin, Ceftriazone but in several articles, we analyzed that the increasing incidence of UTI caused by *S.aureus* is resistant to the number of antibiotics is varying region to region worldwide. One study done by Murugan's, Mani KR, Uma Devi et al Aug 2008¹⁴ showed that 42.86% of the total *S.aureus* was MRSA which showed resistance to the wide number of antibiotics such as penicillin, ampicillin, cloxacillin, OmniTRAX, cephalexin, ciprofloxacin, norfloxacin, amikacin, linezolid, and Vancomycin was found to be 100% sensitive to MRSA.

Another study was done by Dr, Adebola onaniga et al 2012 at yenagoa Nigeria found that all the *Staphylococcus aureus* isolates from UTI patients were resistant to cefoxitin or methicillin and they showed full resistance to 97% Ampicillin, 97.8% to tetracyclin, 80.4% to chloramphenicol and

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cotrimoxazole, 73.9% gentamycin, 69.6 % to augmentin and vancomycin, 54.3% to cefuroxime, 39.1% to nitrofurantoin, 34.8% to of loxacin and 32.6% to ciprofloxacin $^{\scriptscriptstyle [15]}$

But in the present study we found that vancomicin(100%), doxycycline(83%), Nitrofurantin(100%) and Linezolid(83%) are the most effective drugs for the treatment of UTI associated with MRSA, and as per our study Gentamycin, CIP, penicillin G, Azithromycin is a less effective drug for the treatment of UTI associated with MRSA.

According to our study 60-70 years age group is getting more infected with MRSA associated UTI as compared to other age groups, here in our study out of 6 isolates of MRSA 4 were belongs to this age group it might be due to lowered immune response in aged persons, or because of their improper medication or prolonged hospital stay (nosocomial infections).there was one research conducted on MRSA as a uropathogen in an Irish setting by Looney, April 2014^[16] concludes that MRSA associated UTI infection was high in the aged between 70 to 90 years of the age group which closely resembles with this study.

CONCLUSION AND SUMMARY

Staph aureus was relatively uncommon causing UTI but nowadays Staph aureus was the commonest agent responsible for urinary tract infections. In this study among all the isolates, the percentage of gram-negative bacteria was quite higher(59%) as compared to gram-positive bacteria (41%) causing urinary tract infection. Females are most frequently affected with UTI than males. Here we found around 50% of S. aureus was Methicillin-resistant (MRSA) and 50% of S. aureus were Methicillin sensitive. The age group of 60-70 years was the most commonly affected with urinary tract infections. Doxycyclin and Nitrofurantoin, Linezolid Seem to be a reasonable alternative to vancomycin for the treatment of urinary tract infection caused by methicillinresistant Staph aureus. The proportion of MRSA in 91 Urinary tract infected patients was 6.5%.

The high level of resistance among gram-positive bacteria causing UTI limits the use of antimicrobial agents for therapy. Continuous surveillance for MDR strains is necessary to prevent the further spread of resistant isolates.Regular surveillance will show changes in the organism distribution, antibiotic sensitivity patterns and MICs of the commonly used drugs. This will help in formulating a working antibiotic policy for our hospital which will aid the clinicians in prescribing proper antibiotics. The knowledge of the proportion of MRSA and MSSA strain in Urinary tract infections and the antibacterial resistance pattern of the bacterial strain in this geographical area will help us to provide improved empirical antibacterial therapies.

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