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"Molecular Characterization and the Prevalence of Urinary Tract Infection its Antibiogram with Special Reference to Drug Resistance *Mec A* gene in *Methicillin-resistant Staphylococcus aureus* (MRSA) isolates at a Tertiary Care Centre, India"

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ABSTRACT

Introduction: Most hospital visits worldwide are caused by urinary tract infections (UTIs), which are one of the primary causes of morbidity and comorbidity in patients with underlying illnesses. As *Methicillin-resistant Staphylococcus aureus* (MRSA) emerges in the community and infections with this pathogen become a common issue among UTI patients, the emergence of antibiotic-resistant bacterial strains is a serious problem and the biggest challenge in public health care.

Aim and Objective: To study the prevalence of urinary tract infection its bacteriological profile and the drug resistance pattern of the Patients with the molecular characterization of MecA gene in MRSA isolates.

Material and Methods: This was a Cross sectional study carried out in the Department of Microbiology at Rama Medical College Hospital and Research Centre, Mandhana Kanpur for a period of 1 year i.e, between August 2022 to August 2023. A total of 526 freshly voided mid-stream urine sample were collected in a sterile wide mouth container from the individuals preliminary routine urine tests positive for pus cells and albumin. All the urine samples were processed within one hour after the collection for aerobic bacterial culture. If delayed, samples were refrigerated and processed within 4 - 6 hours. The identification , biochemicals and the AST pattern was done accoding to the CLSI guidelines 2022. The DNA was extracted by using Qiagen DNA Extraction kit, which was further proceeded for the MecA gene detection in MRSA isolates by the conventional PCR.

Results: In the present study a total of 600 urine samples were received out of which 130 (21.6%) urine samples were showing significant growth for UTI. The ratio of females 79 (60.7%) were more as compared to that of the males 51 (39.2%) with the maximum age of 21-30 (40%) been affected the most followed by 31-40 (24.6%) years of age and least in the age group above 61 years of age. It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. It was observed that the maximum number of isolates were from the *E.coli* 60 (46.1%) followed by *Klebsiella pneumonia* 21 (16.1%) and least for *Proteus vulgaris*, *Acinetobacter baumannii*. Among the gram positive isolates the *S.aureus* (7.6%) was observed to be the maximum with 3 (30%) isolates resistant for MRSA . Our study showed a very high rate of resistance (>70%) among *E. coli* isolates to

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

piperacillin. Among *Klebsiella* isolates, no resistance was found for meropenem and low resistance was found for ciprofloxacin (9.52%), norfloxacin(9.52%) , and cefotaxime(23.80%) but high for nitrofurantoin (95.23%) and trimethoprim/sulfamethoxazole (42.85%) . The molecular characterization of MRSA confirmed the detection of MecA gene among the UTI patients.

Conclusion: To determine and elucidate the genetic mechanism behind antibiotic resistance and prevalence, phenotypic and genotypic research are required. Therefore, regular exams and strict adherence to antibiotic stewardship initiatives can minimise the cost of UTI prevention. Antibiotic abuse also highlights the need for improved prescription procedures.

Keywords: UTI, Antibiotic sensitivity testing, CLSI, Molecular characterization, DNA, Mec A , PCR

INTRODUCTION

One of the most prevalent bacterial infections in regular clinical practice are urinary tract infections (UTIs), which can show clinically as anything from asymptomatic to severe sepsis. UTIs are the second most common reason for hospital visits and one of the major causes of morbidity in the general population. It also makes up around 35% of all hospital-acquired infections and is frequently the most frequent nosocomial infection in many hospitals [1].

Urinary tract infections (UTIs) are the inflammatory disorders of the urinary tract caused by the abnormal growth of pathogens [2,3]. Urinary tract infection is known to cause short-term morbidity in terms of fever, dysuria, and lower abdominal pain (LAP) and may result in permanent scarring of the kidney [4]. Urinary tract infections can be community acquired or nosocomial. Community-acquired urinary tract infections (CA-UTIs) are defined as the infection of the urinary system that takes place in one's life in the community setting or in the hospital environment with less than 48 hours of admission. Nosocomial urinary tract infections (N-UTIs) are the infection of the urinary tract that occurs after 48 hours of hospital admission, and the patient was not incubating at the time of admission or within 3 days after discharge [5].

The clinical manifestations of urinary tract infections (UTIs) vary depending on the urinary tract portion affected, the etiologic organisms, the severity of the infection, and the patient's capacity to mount an immune response to it. UTIs can be asymptomatic, acute, chronic, complicated, or uncomplicated [6]. The age of the infected individual and the location of the urinary tract infection are the main determinants of the symptoms of urinary tract infections (UTIs), which include fever, burning sensations during urination, LAP, itching, blisters and ulcers forming in the vaginal area, genital and suprapubic pain, and pyuria [3].

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. There are many bacteria responsible of causing UTI infection like *E.coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp [7-10].

The incidence of urinary tract infection is increasing because patients are more frequently fitted with various urinary catheters as endourology progresses technologically. In complicated urinary tract infections and hospitalized patients, Gram positive bacteria such as MRSA are comparatively more common [11].

Patients are being fitted with different types of urinary catheters more often, which is contributing to an increase in the prevalence of urinary tract infections caused by MRSA [12]. Since MRSA is a newly discovered pathogen that can resist the effects of many antimicrobials, particularly those strains that form a biofilm in both hospitalised patients and the general public, early detection of this resistance can aid in the effective eradication of the infection. *S. aureus* develops resistance to a wide range of antibiotics due to its capacity to acquire resistance genes [13, 14].

UTI is treated with broad spectrum antibiotics empirically to start with, which are de-escalated to specific antibiotic based on information obtained from the antimicrobial susceptibility pattern of the urinary pathogens [15].Antibiotic-resistant bacteria have emerged as a result of the widespread use of antimicrobial treatments, and the market for new medications is rising. Uropathogen distribution and antimicrobial sensitivity patterns can vary by location, thus it's important to research and gather information on them in specific contexts [16].

Antibiotic overuse has led to the emergence of drug resistance in bacteria, which is a global health concern [17, 18].

Therefore, the present study was undertaken to study the prevalence of urinary tract infection its bacteriological profile and the drug resistance pattern of the patients with the molecular characterization of MecA gene in MRSA isolates at a tertiary care centre in Uttar Pradesh.

MATERIAL AND METHODS

This was a Cross sectional study carried out in the Department of Microbiology at Rama Medical College Hospital and Research Centre, Mandhana Kanpur for a period of 1 year i.e, between August 2022 to August 2023. A total of 600 freshly voided mid- stream urine sample were collected in a sterile wide mouth container from the individuals preliminary routine urine tests positive for pus cells and albumin. All the urine samples were processed within one hour after the collection for aerobic bacterial culture. If delayed, samples were refrigerated and processed within 4 - 6 hours.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

The patients presenting or highly suspcious of having UTIs and ready to give consent were included in the study. Any patient who was terminally ill, who fails to give urine samples, with a history of antibiotic administration in the last two weeks and any female who was in their menstruation period were excluded from the study [19].
The patients demographic details including age, gender, tribe, residence, level of education, and history of medical conditions were included in the study.

Microscopic Study

One of the diagnosis criteria of UTI was based on microscopic findings of more than 10 pus cells/ high power field (40×) in urine were included in the study.

Collection and process of urine samples

Mid-stream urine samples were collected in a sterile container and were processed within 2 h of collection time. These urine samples were also centrifuged and urine sediment was used for direct microscopic examination of red blood cells (RBCs), leukocytes, epithelial cell, casts, crystals, and parasites. In the normal urine sediment, a few count of RBCs, pus cells (0–5/high power field), and epithelial cells may present. Epithelial cell count reported as "few," "moderate," or "many" per low-power field.

Isolation and Identification of Uropathogens

The Urine sample was inoculated on a standard culture media Cystine–Lactose– Electrolyte-Deficient (CLED) agar using a calibrated (1 μL) loop.
Culture plates were incubated at 35–37°C ambient air incubator for 18 h. After the allocated time period, the culture plates were visualized for the presence of bacterial colonies. They were reported as significant or non-significant growth on the basis of colony count method. Isolated colonies were further characterised based on cultural characteristics by growing on differential media, such as MacConkeys agar and blood agar [20]. Further, the isolates were identified by cultural, morphological and biochemical tests. The method used in the identification and characterisation of isolated bacteria included Gram staining, motility test and biochemical tests like, TSI and IMViC according to Cheesbrough [21, 22, 23]. Isolated and characterized uropathogens were then preserved in nutrient broth containing 25% glycerol at −20°C.
The plates were incubated at 37°C for 24 hrs and extended to 48 hrs in culture (growth) negative cases. The identification , biochemicals and the AST pattern was done accoding to the CLSI guidelines 2022 [24]. All chemicals required for culture media and reagents were procured from HiMedia laboratories Pvt Ltd., Mumbai.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Molecular Detection of MecA Gene by Polymerase Chain Reaction (PCR) in Methicillin resistant *Staphylococcus aureus*

Bacterial DNA was extracted by QIAamp DNA Kit by following manufactures guidelines. *S. aureus* previously extracted DNA was used for the amplification of Mec A gene. The primers were purchased from "**Saha gene'** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

Figure No.1: The DNA Extraction kit Figure No.2: The Reagents used for the DNA Extraction

Figure No.3: The MecA gene primers from the Saha gene Figure No. 4: Run of Amplified product

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

All *S. aureus* isolates that were resistant to cefoxitin 30 µg and positive on ORSAB examination were then subjected to a PCR test to detect the presence of the mecA gene [25]. The DNA extraction process was carried out according to the QIAamp DNA Mini Kit protocol, where previously the isolates were purified on MSA (HiMedia Pvt. Ltd, M118) and inoculated on MHA (Oxoid, CM0337).

Gene	Primer	Base Pair	Reference
MecA	F: 5′-AAA ATC GAT GGT AAA GGT TGG C-3′ R: 5′-AGT TCT GCA GTA CCG GAT TTG C-3′.	533	[26]

Table No. 1: Primer used for the MecA gene detection

Molecular Characterization of MecA gene

The PCR master mix used GoTaq Green Master Mix (Promega, 9PIM712) which is a ready-to-use solution mixture containing Taq DNA polymerase, dNTPs, $MgCl_2$, and a reaction buffer. DNA was amplified using a Thermal Cycler T100 machine (Bio-Rad, 186-1096) for 40 cycles in 25 µl of the reaction mixture with the following steps: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 min with a final extension at 72°C for 5 min. A total of 10 µl of PCR product were analyzed by 2% agarose gel electrophoresis, and the gel was visualized under ultraviolet light [27]. A positive test indicated a PCR product in the 533-base pair (bp) band [27].

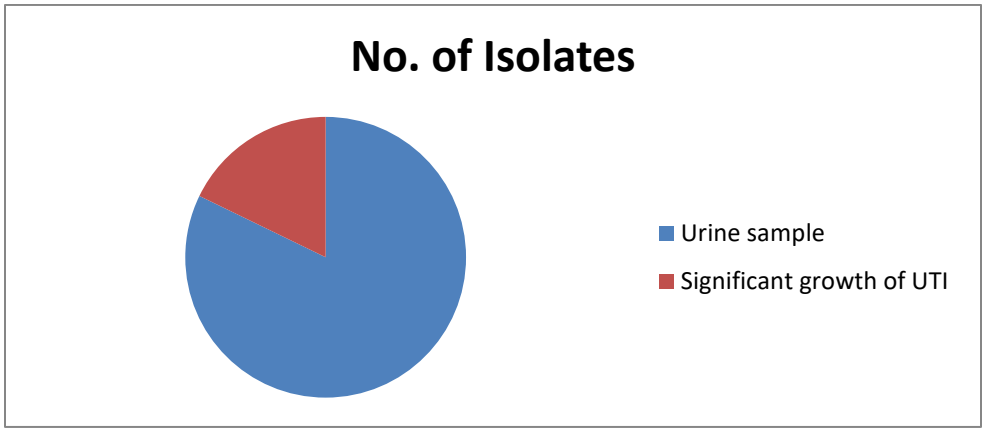
RESULTS

A total of 600 urine samples were received in the Microbiology Laboratory at RMCH&RC out of which 130 (21.6%) urine samples were showing significant growth for UTI [Table No. 2]. The ratio of females 79 (60.7%) were more as compared to that of the males 51 (39.2%) [Table no. 3] . The maximum age of 21-30 (40%) was affected the most followed by 31-40 (24.6%) years of age and least in the age group above 61 years of age [Table no.4].

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Type of Clinical Isolates	Number of Isolates	Percentage
Urine samples	600	78.3%
Significant growth for UTI	130	21.6%

Table No. 2 : Samplewise distribution of the clinical isolates

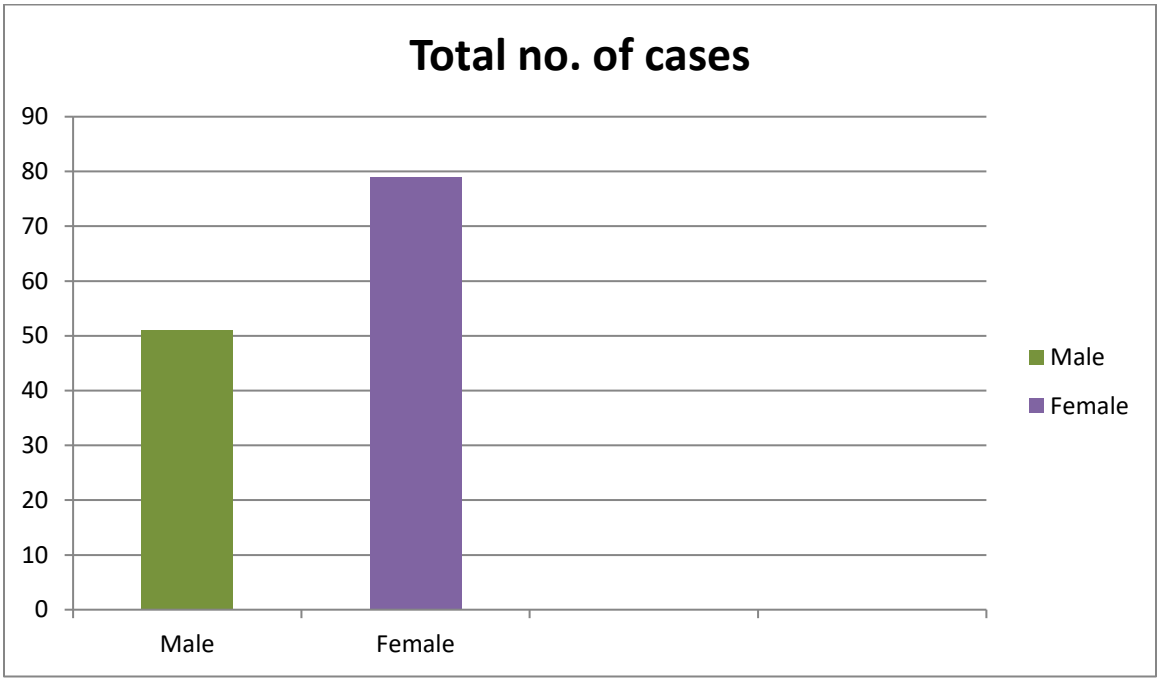


Graph No. 1: Graphical Representation of Samplewise distribution of the clinical isolates

Gender	Total no. of Cases studies (N=130)	Percentage
Male	51	39.2%
Female	79	60.7%

Table No. 3: Gender wise distribution of the UTI cases

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021



Graph No. 2: Graphical Representation of Genderwise distribution of the UTI cases

S.N.	Age group (Years)	Male N= 51	Female N= 79	Percentage (%)
1.	0-10	-	-	-
2.	11-20	8	11	14.6%
3.	21-30	18	34	40%
4.	31-40	14	18	24.6%
5.	41-50	5	7	9.2%
6.	51-60	3	5	6.1%
7.	61-70	2	4	4.6%
8.	≤ 80	1	-	0.7%

Table No. 4 : Agewise distribution of the UTI cases

It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates.

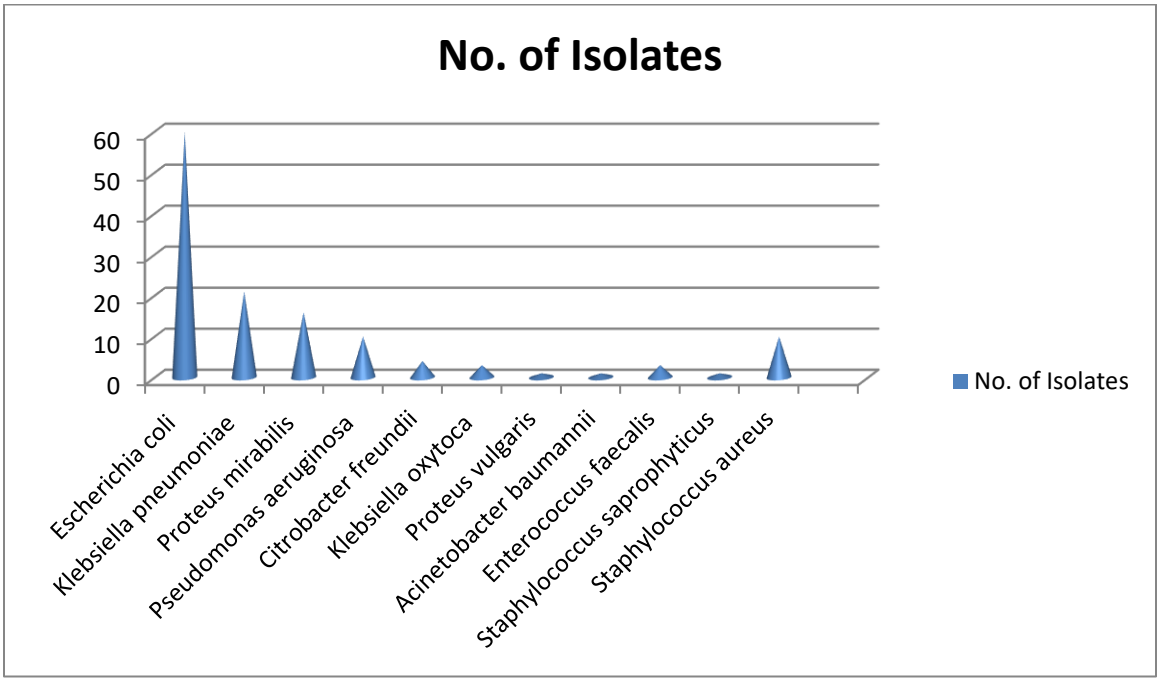
It was observed that the maximum number of isolates were from the *E.coli* 60 (46.1%) followed by *Klebsiella pneumonia* 21 (16.1%) and least for *Proteus vulgaris*, *Acinetobacter baumannii*, *Staphylococcus saprophyticus* with 1 (0.76%). Among the gram positive isolates the *S.aureus* (7.6%) was observed to be the maximum with 3 (30%) isolates resistant for MRSA isolates [Table no.5].

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Type of Organism Isolated	**No. of Isolates**	**Percentage**
Escherichia coli	60	46.1%
Klebsiella pneumoniae	21	16.1%
Proteus mirabilis	16	12.3%
Pseudomonas aeruginosa	10	7.6%
Citrobacter freundii	4	3%
Klebsiella oxytoca	3	2.3%
Proteus vulgaris	1	0.76%
Acinetobacter baumannii	1	0.76%
Enterococcus faecalis	3	2.3%
Staphylococcus saprophyticus	1	0.76%
Staphylococcus aureus	10	7.6%
Total	**130**	**100%**

Table No. 5: The Frequency of bacteria isolated from the UTI cases

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021



Graph No. 3: Graphical Representation of Frequency of bacteria isolated from the UTI cases

In the present study it was also observed that high degree of drug resistance among bacterial isolates was observed . Our study showed a very high rate of resistance (>70%) among *E. coli* isolates to piperacillin. Among *Klebsiella* isolates, no resistance was found for meropenem and low resistance was found for ciprofloxacin (9.52%), norfloxacin(9.52%) , and cefotaxime(23.80%) but high for nitrofurantoin (95.23%) and trimethoprim/sulfamethoxazole (42.85%) .

Antibiotics	***Escherichia coli***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Pseudomonas aeruginosa***
Ampicillin	53 (88.3%)	21 (100%)	11 (68.75%)	IR
Amoxicillin/clavulanic acid	29 (48.3%)	13 (61.9%)	4 (25%)	IR

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Piperacillin	50 (83.3%)	20 (94%)	4 (25%)	10 (100%)
Piperacillin/tazobactam	19(31.6%)	7 (33.33%)	4 (25%)	10(100%)
Cefalotin	42 (70%)	7 (33.33%)	5 (31.25%)	IR
Cefuroxime	6 (10%)	7 (33.33%)	5 (31.25%)	IR
Cefoxitin	5 (8.3%)	2(9.52%)	4 (25%)	IR
Cefpodoxime	5 (8.3%)	5 (23.80%)	4 (25%)	IR
Cefotaxime	2(3.3%)	5 (23.80%)	4 (25%)	IR
Ceftazidime	3 (5%)	5 (23.80%)	4 (25%)	10 (100%)
Cefepime	3 (5%)	5 (23.80%)	4 (25%)	8 (80%)
Meropenem	5(8.3%)	0	0	5 (50%)
Amikacin	9 (15%)	3 (14.28%)	0	2 (20%)
Gentamicin	5 (8.3%)	3 (14.28%)	0	2 (20%)
Tobramycin	10 (16.6%)	3 (14.28%)	0	2 (20%)
Ciprofloxacin	19 (31.6%)	2 (9.52%)	0	2 (20%)
Norfloxacin	19 (31.6%)	2 (9.52%)	4 (25%)	2 (20%)
Nitrofurantoin	5 (8.3%)	20 (95.23%)	IR	-
Trimethoprim/sulfamethoxazole	30 (50%)	9 (42.85%)	4 (25%)	IR

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Total number of isolates	**60**	**21**	**16**	**10**

Table No.6 : Number (%) of common Gram-negative urinary pathogens resistant (R) to antimicrobial agents

Antibiotics	***Citrobacter freundii***	***Klebsiella oxytoca***	***Proteus vulgaris***	***Acinetobacter baumannii***
Ampicillin	IR	IR	IR	IR
Amoxicillin/clavulanic acid	IR	3 (100%)	1 (100%)	IR
Piperacillin	4 (100%)	3 (100%)	1 (100%)	0
Piperacillin/tazobactam	1 (25%)	3 (100%)	1 (100%)	0
Cefalotin	IR	3 (100%)	IR	IR
Cefuroxime	IR	IR	IR	IR
Cefoxitin	IR	2(66.6%)	1 (100%)	IR
Cefpodoxime	IR	3 (100%)	1 (100%)	-
Cefotaxime	2 (50%)	3 (100%)	1 (100%)	0
Ceftazidime	2 (50%)	3(100%)	1 (100%)	0
Cefepime	1 (25%)	3 (100%)	1 (100%)	0
Meropenem	0	2 (66.6%)	0	0

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Amikacin	2 (50%)	3 (100%)	0	0
Gentamicin	2 (50%)	2(66.6%)	0	0
Tobramycin	2 (50%)	3 (100%)	0	0
Ciprofloxacin	4(100%)	3 (100%)	1 (100%)	0
Norfloxacin	4 (100%)	3 (100%)	1 (100%)	0
Nitrofurantoin	2(50%)	2 (66.6%)	IR	-
Trimethoprim/sulfamethoxazole	1 (25%)	2 (66.6%)	1 (100%)	1 (100%)
Total number of isolates	**4**	**3**	**1**	**1**

Table No. 7: Number (%) of less common Gram-negative urinary pathogens resistant (R) to antimicrobial agents

Antibiotics	***Enterococcus faecalis***	***Staphylococcus saprophyticus***	***Staphylococcus aureus***
Benzyl penicillin	3 (100%)	1 (100%)	10 (100%)
Cefoxitin	-	0	3 (30%)
Gentamicin	IR	0	0
Tobramycin	IR	0	0
Levofloxacin	0	0	3 (30%)
Clindamycin	IR	0	0
Linezolid	0	0	0

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Teicoplanin	0	0	0
Vancomycin	0	0	0
Fosfomycin	3 (100%)	IR	0
Nitrofurantoin	0	0	0
Rifampicin	3(100%)	0	0
Trimethoprim/Sulfamethoxazole	IR	0	0
Total number of isolates	**3**	**1**	**10**

Table No.8 : Number (%) of common Gram-positive urinary pathogens resistant (R) to antimicrobial agents

The frequency of *Acinetobacter*, *Citrobacter*,*Klebsiella* and *proteus* is mentioned in Table 7 due to their clinical relevance. The number (percentage) of common Gram-negative urinary pathogens resistant (R) to antimicrobial agents is shown in Table 7 and Table 8 refer to the Gram positive isolate .

The DNA Extraction was performed by the Qiagen DNA kit and the DNA was isolated from the samples. From the 9 isolates of *S.aureus*, there were 3 isolates observed positive for MRSA. The DNA Extraction and the gene detection of the isolate 3 isolates was performed for the detection of Mec A gene among the MRSA isolates.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

Figure No. 5: The DNA Extraction

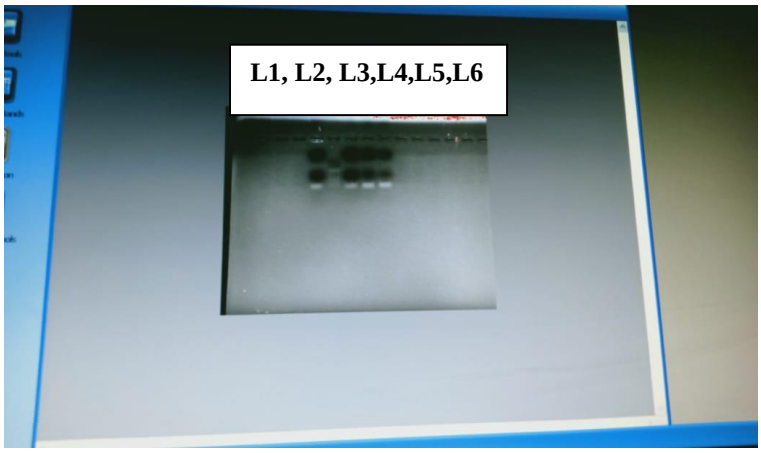


Figure No. 6: Photograph of amplified MecA gene in MRSA; the amplified DNA band size was obtained 533bp, L1 corresponds to the Negative control and L2 corresponds to the Positive control , L3 corresponding to 100bp ladder used ;Lane 4-6 are the sample positive for MecA

In the current study it was observed that there were 3 isolates found positive for the MecA gene.

GTTGTAGTTGTCGGGTTTGGTATATATTTTTATGCTTCAAAAGATAAAGAAATTAAT
AATACTATTGATGCAATTGAAGATAAAAATTTCAAACAAGTTTATAAAGATAGCAGT
TATATTTCTAAAAGCGATAATGGTGAAGTAGAAATGACTGAACGTCCGATAAAAAT

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

ATATAATAGTTTAGGCGTTAAAGATATAAACATTCAGGATCGTAAAATAAAAAAAG
TATCTAAAAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGT
AACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAG
TCTAAAAAGCATGTAAAAGAATTTGCGACCAGATTGCAAAATCTGCAACGAGCTTT
GGGTTTACTCCCCCCGGTGGAGATGGATATAAAAATGCTCAAAAAAGTACCACCAC
TATATTTTCCTAAGAAGCTATCAAATAATTATAATCA

Figure No.7: Obtained gene sequences of MecA gene in *S. aureus*

DISCUSSION

UTIs are among the most serious infections that are reported worldwide. Many conditions, including urethritis, cystitis, and pyelonephritis, are classified as UTIs. 50% of women had a history of urinary tract infections, according to reports. UTIs are serious health problems that affect 150 million people worldwide each year [28]. According to reports, bacteria are most frequently responsible for UTIs. Although Staphylococcus aureus is not known to be a prominent bacterium that causes UTIs, recent studies have found that its prevalence has grown [29].

In the present study the prevalence of UTI was found to be 21.6%. This finding was similar to the study performed by the other authors Ahmad S et al and Suhail A. et al., where the prevalence was found to be 20.54% and 32% respectively [30,31] .

In the current study the maximum number of isolates were from the Females 79 (60.7%) as compared to that of the males 51 (39.2%). This study was similar to the study by Suhail A. et al, and Martin Odoki et al., in 2019 where the ratio of females was more as compared to the males [31] [32].

It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. It was observed that the maximum number of isolates were from the *E.coli* 60 (46.1%) followed by *Klebsiella pneumonia* 21 (16.1%) and least for *Proteus vulgaris, Acinetobacter baumannii* with 1 (0.76%). Among the gram positive isolates the *S.aureus* (7.6%) was observed to be the maximum with 3 (30%) isolates resistant for MRSA. Similar study was performed by the other research workers where among 206 bacterial isolates obtained from 417 urine samples, majority of the isolates (99%) were Gram negative bacteria which included *Escherichia coli* (56.79%), *Klebsiella sps* (19.9%), *Pseudomonas sps* (6.3%), *Proteus sps* (5.8%), *Enterobacter sps* (3.8%), *Citrobacter sps* (1.4%), *Enterococcus sps* (0.9%), and other *NFGNB* (4.8%) [33].

In the present study it was found that antimicrobial resistance was seen both in Gram-positive and Gram-negative bacteria. Multiple resistances were high among the isolated urinary pathogens.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

One important human pathogen that causes the majority of nosocomial and hospital-acquired illnesses is Staphylococcus aureus. Numerous illnesses, such as UTIs, respiratory and soft tissue infections, endocarditis, osteomyelitis, and endocarditis, are brought on by it. Severe antibiotic resistance has emerged in the bacterium. Based on clinical observations, approximately half of the *S. aureus* isolates exhibited total resistance to the penicillin and cephalosporin antibiotic groups; these strains were referred to be methicillin-resistant *S. aureus* (MRSA). Due to hospitalisation and treatment costs, MRSA strains produced complex disorders over longer periods of time and at a higher cost [34].

In the present study it was observed that the rate of MRSA among the UTI cases were 2.3%. This study was in support with the study performed by the other author Khaleel R et al., [35] where 7.7% of the urine specimens of hospitalized patients who suffered from UTIs were positive for the MRSA strains. MRSA isolates displayed a boost resistance rate toward erythromycin, ceftaroline, penicillin, gentamicin, and ciprofloxacin antimicrobial agents. Additionally, MRSA isolates harbored a boost distribution of beta lactamase gene MecA antimicrobial resistance-encoding genes. It seems that the antimicrobial-resistant MRSA isolates may be an emerging cause of UTIs in Uttar Pradesh. Similarly, Lunacek et al [36] labelled that the MRSA prevalence amongst urine specimens in Austria was 4.06%. They disclosed that MRSA isolates were resistant toward cephalosporin, aminopenicillin, penicillin G, carbapenem, and β-lactamase antimicrobial agents. They also presented that catheter utilization is the most critical risk factor for MRSA occurrence in UTIs. An Irish survey [37] described that the prevalence of MRSA strains was 27.9%. Besides, MRSA isolates of the urine specimens displayed the uppermost resistance rate toward flucloxacillin (100%), co-amoxiclav (100%), and ciprofloxacin (98%).

Our study showed a very high rate of resistance (>70%) among *E. coli* isolates to piperacillin. Among *Klebsiella* isolates, no resistance was found for meropenem and low resistance was found for ciprofloxacin (9.52%), norfloxacin(9.52%) , and cefotaxime(23.80%) but high for nitrofurantoin (95.23%) and trimethoprim/sulfamethoxazole (42.85%) . This resistance is most likely due to the massive use of third-generation cephalosporins and fluoroquinolone antibiotics in UTIs patients. The high resistance in trimethoprim/sulfamethoxazole susceptibility pattern may be due to non-judicious use and over-the-counter selling of this antibiotic [31].

It is well known that the antibiotic susceptibility of uropathogenic bacteria varies over time and between geographical locations [38]. The effectiveness of the top antimicrobials against the uropathogens in this study that have a low resistance rate (overall resistance%) is discussed here. The best antibiotics for Gram-negative organisms were cefepime, gentamicin, amikacin, tobramycin, and meropenem. Antimicrobials with a moderate resistance risk included ceftazidime, cefpodoxime, piperacillin/tazobactam, cefuroxime, ciprofloxacin, cefotaxime, cefoxitin, and norfloxacin. Notably, a high rate of resistance was discovered to be present

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

against ampicillin, amoxicillin/clavulanic acid piperacillin, nitrofurantoin, trimethoprim/sulfamethoxazole, and cefuroxime.

The current finding is similar to other reports which suggest that gram negative bacteria, particularly *E. coli* was the commonest pathogens isolated from patients with UTI [39,40]. The incidence of *E. coli* in our study was higher when compared with the Nigerian studies reporting 42.10% [41] and 51% [42]. Most of the studies conducted in Africa and Arab countries showed less than 50% isolation of *E coli* from the UTI patients but reported a higher percentage (29%) of *S aureus* as second most frequently isolated bacteria from UTI cases. Reports from other developing or developed countries were the isolation of Gram positive bacteria as uropathogen is very low <10% [43,44].

In contrast, the antimicrobial sensitivity pattern of antimicrobials for Gram-positive organisms shows linezolid , teicoplanin , vancomycin , cefalotin screen , moxifloxacin , nitrofurantoin , and levofloxacin were sensitive however there were 33.33% resistance observed for cefoxitin.

The overall proportion of MRSA among isolated *S. aureus* in this study was elevated from previous studies conducted in Iran (25.8%) [45] Nepal (30.8%) [46] Uganda (33.3%) [47] and Nigeria (13%) [48].This increased proportion of MRSA might be due to differences in geographic area, MRSA becoming a global nosocomial pathogen with rapid spread to health care as well as community and urinary tract infection associated factors may also play an important role in increasing the prevalence of MRSA in the community

Both the aetiology of UTI-causing bacteria and their resistance to antibiotics are changing over time and amongst various nations [49].

Correct identification of the causal microorganism is essential for the successful treatment of UTI patients. Failing to do so will not only cause the patient to experience problems and extend the course of the illness, but it will also encourage the unfavourable effects of bacterial resistance as a result of the careless use of unnecessary antibiotics.

Continuous monitoring of the etiology of infections and antibiotic resistance pattern is essential not only for selecting appropriate antibiotics for empirical therapy but also for reducing the overuse/misuse of antibiotics.

CONCLUSION

The abuse of antibiotics has led to the emergence of drug resistance in bacteria, which is a global health concern. Therefore, in order to guarantee proper treatments, it is necessary to be aware of the changes in the spectrum of drug resistance.

The high prevalence of *S. aureus* and MRSA infections should be decreased by using the effective infection prevention and control techniques. To determine and elucidate the genetic mechanism underlying antibiotic resistance and prevalence, further phenotypic and genotypic research is required. Nonetheless, more investigations ought to be conducted to evaluate additional MRSA epidemiology characteristics in UTIs.

ISSN: 0975-3583, 0976-2833 VOL12, ISSUE 7, 2021

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