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Antibiotic Resistance Pattern of Uropathogenic Methicillin-resistant *Staphylococcus aureus* Isolated from Immunosuppressive Patients with Pyelonephritis

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S. aureus **Urinary Tract Infections had emergence of resistance against commonly used antibiotics and especially methicillin. The present investigation was done to study the prevalence rate and antibiotic resistance pattern of the MRSA strains isolated from immunosuppressive patients suffered from UTIs. One-hundred and twenty urine samples were collected and cultured. Those that were positive for S. aureus, were subjected to PCR and disk diffusion method. Of 120 urine sample studied, 10 samples (8.33%) were positive for *S. aureus*. Prevalence of MRSA among the bacterial isolates was 5.83%. MRSA strains had the highest levels of resistance against ampicillin (100%), penicillin G (100%), tetracycline (85.71%), ciprofloxacin (85.71%), amikacin (71.42%) and *trimethoprim-sulfamethoxazole* (71.42%). The lowest levels of resistance were seen against imipenem (14.28%) and clindamycin (28.57%). Considering the high prevalence of MRSA and its emergence for antibiotic resistance, rapid identification of infected immunosuppressive patients and their quick treatment with imipenem and clindamycin are recommended.**

Keywords: Methicillin resistant, Staphylococcus aureus, Antibiotic resistance, Immunosuppressive patients, Pyelonephritis.

Most urinary tract infections(UTIs) involve only the bladder and urethra. Pyelonephritis results when a UTI progresses to involve the upper urinary system including kidneys and ureters. Acute pyelonephritis is a potentially kidney and life-threatening infection that often leads to renal scarring. Acute pyelonephritis results from bacterial invasion of the renal parenchyma. Bacteria usually reach the kidney by ascending from the lower urinary tract. Bacteria may also reach the kidney via the bloodstream. Timely diagnosis and management of acute pyelonephritis has a significant impact on patient outcomes[1]. Bacteria are the most important cause of pyelonephritis.

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Among all bacterial agents causing UTIs, *Staphylococcus aureus* (*S. aureus*) is one of the most prevalent[2]. Highly distribution of this bacterium in the hospital environment, its high resistance against hard conditions and finally occurrence of high antibiotic resistance in some of its strains caused it to be one of the most important factors of pyelonephritis. It is a gram-positive and catalase-positive coccal bacterium that is a member of the Firmicutes which is responsible for various types on infections including respiratory, urinary, skin and soft tissue, burn and blood infections[2-4].

The ability of *S. aureus* to resist against wide range of antibiotics causing severe problems in treatment of hospital infections. Infections caused by *S. aureus* are treated mainly with methicillin but in recent years, increasing numbers of methicillin resistant *S. aureus* (MRSA) have been reported worldwide from patients with community-acquired infections[5-7]. According to the available

data near fifteen percent of *S. aureus* of hospital infections were MRSA [5-7]. Previously published data revealed that MRSA strains of various types of infections and especially UTIs had a considerable levels of resistance against various types of antimicrobial agents including quinolones, aminoglycosides, macrolides, cephalosporins, sulfonamides, fluoroquinolones and tetracycline [5-9].

To date, there were rare information about the epidemiology and prevalence of MRSA in the cases of UTIs in Iran. Therefore, the present investigation was carried out to study the prevalence and antibiotic resistance pattern of MRSA isolated from immunosuppressive patients suffered from pyelonephritis.

MATERIALS AND METHODS

Samples and Staphylococcus aureus isolation

From May 2014 to January 2015, a total of 120 urine samples were collected from immunosuppressive hospitalized patients of educational hospitals and health centers, Iran. All of these patients were suffered from pyelonephritis. Pyelonephritis was approved using the sonographic examination[10]. Midstream urine was collected in sterile condition to decrease potential bacterial, cellular and artifactual contamination. This procedure was done using the Suprapubic Aspiration (SPA)[11]. Urine samples were immediately transferred to the laboratory in a cooler with ice-packs.

All samples were directly cultured into 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 h. After incubation, suspicious colonies were examined by the use of morphologies compatible with *Staphylococcus* spp. (microscopical morphology, catalase and coagulase production). Studied colonies were cultured on Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany) and Tryptic Soy Agar (TSA) (Merck, Darmstadt, Germany). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol Salt Agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation test[12].

Identification of Methicillin-resistant Staphylococcus aureus

Identification of MRSA strains was done using the PCR-based method. Bacterial strains were sub-cultured in Tryptic Soy Broth (TSB, Merck, Germany) and further incubated for 48 h at 37 °C. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Fermentas, Germany) according to manufacturer's instruction. Those DNA samples which were simultaneously positive for *femA* and *mecA* genes were considered as MRSA. For this purpose, the PCR method which was introduced by Jonas et al. (2002) was used (13). The PCR reactions were performed in a total volume of 25 µL, including 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 µM dNTPs each (Fermentas, Germany), 2.5 µL PCR buffer (10X), 25 pmoL of each primer (*mecA1* (5'-GTAGAAATGACTGAACGTCCGATAA-3') and *mecA2* (5'-CCAATTCCACATTGTTTCGGTCTAA-3') (310 bp) and *femB1* (5'-TTACAGAGTTAACTGTTACC-3') (651 bp)), 1.5 U of Taq DNA polymerase (Fermentas, Germany) and 5 µL (40-260 ng/µL) of the extracted DNA template of the MRSA isolates. The PCR cycling conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of 45 s at 94°C, 45 s at 50°C, and 60 s at 72°C, with a final extension step at 72°C for 2 min.

Antimicrobial susceptibility test

Pattern of antimicrobial resistance of MRSA isolates of the cases of pyelonephritis was studied using the simple disk diffusion technique in the Mueller–Hinton agar (Merck, Germany) medium. Instruction of the Clinical and Laboratory Standards Institute guidelines (14) was used for this purpose. Susceptibility of MRSA isolates were studied against tetracycline (30 µg/disk), cotrimoxazole (30 µg/disk), gentamycin (10 µg/disk), amikacin (30 u/disk), ampicillin (10 u/disk), ciprofloxacin (5 µg/disk), imipenem (30 u/disk), clindamycin (2 µg/disk), penicillin G (10 u/disk), *trimethoprim-sulfamethoxazole* (25 *µg/disk*), oxacillin (1µg/disk), and erythromycin (15µg/disk)

antibiotic disks (Oxoid, UK). The plates containing the discs were allowed to stand for at least 30 min before incubated at 35°C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards (CLSI 2012) (14). *S. aureus* ATCC 43300 and *Escherichia coli* ATCC 11775 were used as quality control organism in antimicrobial susceptibility determination.

Statistical analysis

Statistical analysis was done using the SPSS/21.0 software (SPSS Inc., Chicago, IL) and chi-square and fisher exact tests were applied for analysis. Statistical significance was regarded at a P value < 0.05.

RESULTS

Table 1 represents the total Prevalence of *S. aureus* and MRSA in the urine samples taken from immunosuppressive patients suffered from pyelonephritis. Ten out of 120 urine samples (8.33%) taken from the immunosuppressive patients suffered from pyelonephritis were positive for *S. aureus*. Results of the gel electrophoresis for simultaneous detection of *femA* and *mecA* genes is shown in figure 1. We found that 7 out of 10 *S. aureus* (70%) strains had both *femA* and *mecA* genes which were known as MRSA. There was no significant difference between the prevalence of *S. aureus* and also MRSA (P > 0.05).

Table 2 indicated the antibiotic resistance pattern of MRSA isolated from the urine samples of immunosuppressive patients suffered from pyelonephritis. We found that MRSA strains of our study harbored the highest levels of resistance against ampicillin (100%), penicillin G (100%), tetracycline (85.71%), ciprofloxacin (85.71%), amikacin (71.42%) and *trimethoprim-sulfamethoxazole* (71.42%). Prevalence of resistance against imipenem (14.28%) and clindamycin (28.57%) were low. Statistically significant differences were seen between the prevalence of resistance against ampicillin and imipenem (P = 0.011), penicillin G and imipenem (P = 0.014), ampicillin and clindamycin (P = 0.021) and ampicillin and oxacillin (P = 0.025).

DISCUSSION

Diabetes, acquired immune deficiency syndrome (AIDS), some kinds of surgical operation and many other conditions cause suppression of human immunity. Suppression of immunity cause quickly occurrence of infectious diseases. Presence of immunosuppressive patients in the infected

Table 1. Prevalence of *S. aureus* and MRSA in the urine samples taken from immunosuppressive patients suffered from pyelonephritis

Types of samples	No. samples	Prevalence of *S. aureus* (%)	Prevalence of MRSA (%)
Urine	120	10 (8.33)	7 (70)

Table 2. Antibiotic resistance pattern of MRSA isolated from the urine samples of immunosuppressive patients suffered from pyelonephritis

Samples (No. positive)	Antibiotic resistance pattern (%)											
	Tet	Cot	Gen	Amk	Amp	Cip	Imp	Cln	Pen	Tr-Su	Ox	Ert
Urine (7)	6 (85.71)	4 (57.14)	4 (57.14)	5 (71.42)	7 (100)	6 (85.71)	1 (14.28)	2 (28.57)	7 (100)	5 (71.42)	4 (57.14)	5 (71.42)

Tet: tetracycline (30 µg/disk), cot: cotrimoxazole (30 µg/disk), gen: gentamycin (10 µg/disk), amk: amikacin (30 u/disk), amp: ampicillin (10 u/disk), cip: ciprofloxacin (5 µg/disk), imp: imipenem (30 u/disk), cln: clindamycin (2 µg/disk), pen: penicillin G (10 u/disk), tr-su: *trimethoprim-sulfamethoxazole* (25 *µg/disk*), ox: oxacillin (1µg/disk), ert: erythromycin (15µg/disk).

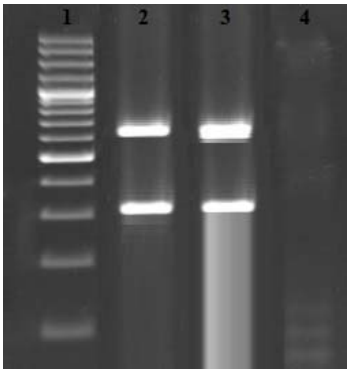

Fig. 1. Results of the gel electrophoresis for simultaneous detection of *femA* (651 bp) and *mecA* (310 bp) genes. 1: 100 bp marker (Fermentas, Germany), 2: Positive sample for *mecA* and *femA* genes, 3: Positive controls and 4: Negative control

environment of hospital facilitates occurrence of infectious diseases like UTIs. Dissemination of pathogenic agents which are mainly resistant against commonly used antibiotics to upper parts of the urinary system and especially kidney cause pyelonephritis which is a complex shape of UTIs.

Our results showed that 5.83% of urine samples taken from immunosuppressive patients suffered from pyelonephritis were infected with MRSA. High prevalence of resistance against various types of antibiotics including tetracycline, cotrimoxazole, gentamycin, ampicillin, ciprofloxacin penicillin G, *trimethoprim-sulfamethoxazole* and erythromycin is another important finding of our study. Indiscriminate and unauthorized prescription of antibiotics, inattention to the results obtained from the disk diffusion method, prescription of antibiotics based on the self-experience of medical practitioners, lack of proper disinfection of hospital environment and finally transmission of resistant pathogens from infected patients to hospital environment and also other patients are the most common reasons for the considerable prevalence of MRSA in our study. Onanuga et al. (2012)[15] revealed that the prevalence of *S. aureus in the urine specimens of patients suffered from UTIs in Nigeria was* 33.6% which was higher than our findings. They showed that all isolates were resistant to methicillin and the prevalence of resistance against tetracycline, chloramphenicol, cotrimoxazole, gentamycin, vancomycin, cefuroxime, nitrofurantoin, ofloxacin and ciprofloxacin were 97.8%, 80.4%, 73.9%, 69.6%, 54.3%, 39.1%, 34.8% and 32.6%, respectively. Prevalence of resistance against methicillin in Australia, Jamaica, France, Spain, and USA were 21.6%, 23%, 33.6%, 30.3%, and 75%, respectively[16-18]. Momtaz and Hafezi (2014) [19] reported that *S. aureus* strainsof human clinical infections had the highest resistance against penicillin (100%), cephalothin (100%), cefazoline (100%), ceftireaxon (100%), azitromycin (62.12%), tetracycline (57.57%) and erythromycin (54.54%) which was similar to our findings. Brown *et al*. (2007)[17] reported that the prevalence of MRSA strains was 23%. No MRSA was resistant to vancomycin and except for penicillin and to some extent co-trimoxazole (trimethoprim–sulfamethoxazole), most MSSA isolates were susceptible to nearly all antimicrobial agents.

CONCLUSIONS

In keeping with high prevalence of MRSA and also their considerable levels of antibiotic resistance, accurate, rapid and sensitive detection of MRSA is required especially in immunosuppressive patients which are more prone to get UTIs. Accurate application of simple disk diffusion method is another approach to reduce dissemination of MRSA. We recommended rapid identification of infected patients and their treatment with imipenem and clindamycin antibiotics.

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