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Antibiotic Resistant Patterns in MRSA Isolates from Patients Admitted in ICU and Infectious Ward

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ABSTRACT

Background: *Methicillin- Resistant Staphylococcus Aureus (MRSA) has become one of the highest – ranking hospital acquired pathogens throughout the world, capable of causing a wide range of hospital infections. Staphylococcus aureus is a major nosocomial pathogen that causes a range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome, food poisoning, carbuncles, and boils.*

Materials and Methods*: One hundred S.aureus isolates recovered from patients in Loghman Hakim hospital were included in this study. Minimum inhibitory concentration (MIC) of strains for methicillin was determined by broth macrodilution method as recommended by NCCLS. Antibiotic susceptibility was tested by using the "disk diffusion technique on Mueller-Hinton Agar". Nineteen antibiotics were tested including Ampicillin, Penicillin, Cephalexin, Cefepime, Gentamicin, Doxycycline, Erythromycin, Chloramphenicol, Tetracycline, Nitrofurantoin, Kanamycin, Amikacine, Cefotaxime, Clindamycin, Cefazolin, Amoxicillin, Sulfamethoxazole-trimethoprim, Vancomycin, and Ciprofloxacin.*

Results: *The MIC range for methicillin was from 1µg/ml to 1024µg/ml. Ninety percent of the isolated strains had methicillin MIC≥ 16µg/ml and were designated as resistant. Vancomycin and Chloramphenicol were the most effective antibiotics and only 7% and 14% of the isolates were resistant respectively. Forty-four percent hospital acquired MRSA strains were resistant to Co-trimoxazole. The high antibiotic resistance among MRSA strains could be originated due to widespread use of antibiotics.*

Conclusion: *Out of 90 MRSA isolates characterized in this study, approximately half of them displayed resistance to one or more antimicrobial agents, including Penicillin, Cephalosporins, Tetracycline and aminoglycosides. These data are in accord with previous study suggesting use of these drugs was important in the emergence of antimicrobial resistance in MRSA. In addition, 66% of MRSA isolates were sensitive to Trimethoprim-Sulfamethoxazole (Co-Trimoxazole). Since this drug combination is recommended for treating a range of human infections, S.aureus isolates should be monitored for further emergence of Co-Trimoxazole resistance.* ***(Tanaffos 2004;3(11): 37-44)***

Key words: *Staphylococcus aureus, MRSA, (Methicillin Resistant Staphylococcus Aureus), Antibiotic Resistant*

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INTRODUCTION

Staphylococcus aureus is a major nosocomial pathogen that causes a range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome, food poisoning, carbuncles and boils (1). In the early 1950s acquisition and spread of beta – lactamase producing plasmids thwarted the effectiveness of penicillin for treating *S.aureus* infections. In the year 1959, methicillin, a synthetic penicillin was introduced.

Since first reported by Jevons in 1961, methicilin-resistant staphylococcus aureus (MRSA) has been implicated as a pathogen in hospital-acquired infections causing endemic and epidemic infections in health care centers world wide (2). The proportion of nosocomial infections caused by MRSA increased substantially, and MRSA is now a leading cause of such infections (3, 4).

Recent studies suggest that the epidemiology of MRSA may be changing, as isolation of MRSA is no longer limited to hospitalized persons or persons with predisposing risk factors. MRSA infections as emerging pathogens are responsible for substantial diseases and death (5, 6). While no satisfactory explanation exists for the recent proliferation of MRSA, expanded use of antimicrobial drugs in outside the hospitals has been suggested as a major contributor in emerging resistance in community (7).

Health care workers and infection control personnel depend on the laboratory for the reliable detection of MRSA in clinical specimens. This has implications for treatment, invasive infections, perioperative prophylaxis, and infection control procedures. Surveillance of MRSA locally, nationally and globally depends on accurate laboratory reporting. Nosocomial MRSA strains in the community including nursing homes and other care facilities, may be transmitted by discharged patients and health care workers.

MRSA has emerged as a significant cause of both nosocomial and community acquired infections in Iran now. In a recent study in Shiraz, Iran 37.7% of the isolates were methicillin – resistant and resistance to vancomycin or rifampin was not seen (8). The aim of this study was to determine methicillin – resistant phenotype in isolated *S.aureus* and also to ascertain the susceptibility pattern of isolates to different antibiotics.

MATERIALS AND METHODS

The Staphylococcal infection was confirmed by clinical and paraclinical conditions. All strains were isolated from patients in whom *S.aureus* was the sole or predominant causative infectious agent. Skin, wounds, sputum, and external ear were the potential sites for contamination; therefore, only the isolates from these sites were accepted where the *S.aureus* was the dominant pathogen. The isolates were from different parts of body. The number and percentage of isolated strains from different sites of body was shown in Table 1.

Isolated strains were identified by standard biochemical test. In brief, gram-positive cocci that were catalase and coagulase positive were identified as *S.aureus*.

Antibiotic susceptibility was tested by using the disk diffusion technique on Mueller-Hinton agar, according to the procedures established by the "National Committee for Clinical Laboratory Standards" (NCCLS). Plates were incubated at 37ºc for 18h for antibiotics (9). The antibiotics tested were Ampicillin, Penicillin, Cephalexin, Cefepime, Gentamicin, Doxycycline, Erythromycin, Chloramphenicol, Tetracycline, Nitrofurantoin, Kanamycin, Amikacine, Cefotaxime, Clindamycin, Cefazolin, Amoxicillin, Sulfamethoxazole–Trimethoprim, Vancomycin, and Ciprofloxacin (B.BL, Becton Dickenson Microbiology system).

Mueller-Hinton agar supplemented with 2% NaCl and Methicillin (5µg/ml) (Sigma Co.St.Louis, USA) was used for screening of MRSA. In this method, the inoculum's suspension was prepared by microdilution method and inoculated with 10^4 cfu/ml of *S.aureus* strains. After 24 hours incubation at 35ºc the test plates were examined for any evidence of growth. Isolates were defined as resistant or sensitive according to detecting "growth" or "no growth" on these plates respectively.

Minimum inhibitory concentration (MIC) of strains for methicillin was determined by broth macrodilution method as recommended by NCCLS. *S.aureus* ATCC 29213 was used as the control strain with Methicillin MIC 2 µg/ml (9).

RESULTS

The isolates were from different parts of body. The number and percentage of isolated strains from different sites of body was shown in Table 1.

Table 1. The number and percentage of isolated strains from different sites of body.

Body sites	No (%)
Sputum	41(41)
Sinus	2 (2)
Ear	2 (2)
Eye	2 (2)
Abscess	10 (10)
Blood	9 (9)
Urine	4 (4)
Wound	17 (17)
Others	13 (13)

The MIC range for methicillin was from 1µg/ml to 1024µg/ml. The MIC range was shown in table 2.

Nearly 90% of the isolated strains had methicillin MIC≥ 16µg/ml and were designated as resistant. Ten percent of strains were methicillin–sensitive *S.aureus* (MSSA).

The MIC at which 50% of isolates are inhibited, The MIC_{50} and MIC_{90} were 256 µg/ml and 16 µg/ml respectively. Otherwise, results obtained from plate method demonstrated 65 (72%) strains that had no growth on methicillin plate. Thus, disk diffusion method alone without MIC is not reliable.

The antibiotic susceptibility patterns of MSSA and MRSA to nineteen antibiotics tested are shown in figure-1 and figure 2, respectively. Analyzing the antibiotic susceptibilities to the nineteen antibiotics tested with the 90 isolates of MRSA showed 100% resistant to penicillin, 92% to ampicillin, and 93% to cefotaxime. Comparison of antimicribial resistance frequencies for *S.aureus* is shown in figure 2. Vancomycin and Chloramphenicol were the most effective antibiotics and only 7% and 14% of isolates were resistant respectively. Nitrofurantoin, gentamycin, amikacine, ciprofloxacin and other cephalosporins like cefepim and cefazolin were at the second row. These antibiotics represented the second most effective agents. Our study showed that 44% of hospital acquired MRSA strains were resistant to co-trimoxazole.

Table 2. The MIC range of methicillin – among isolated strains of methicillin resistant S.aureus. MRSA had MIC 16≥ µg/ml.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
µg/mL	1024	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-
Number	28	20	17	7	11	4	3	2	4	1	1	1	1	

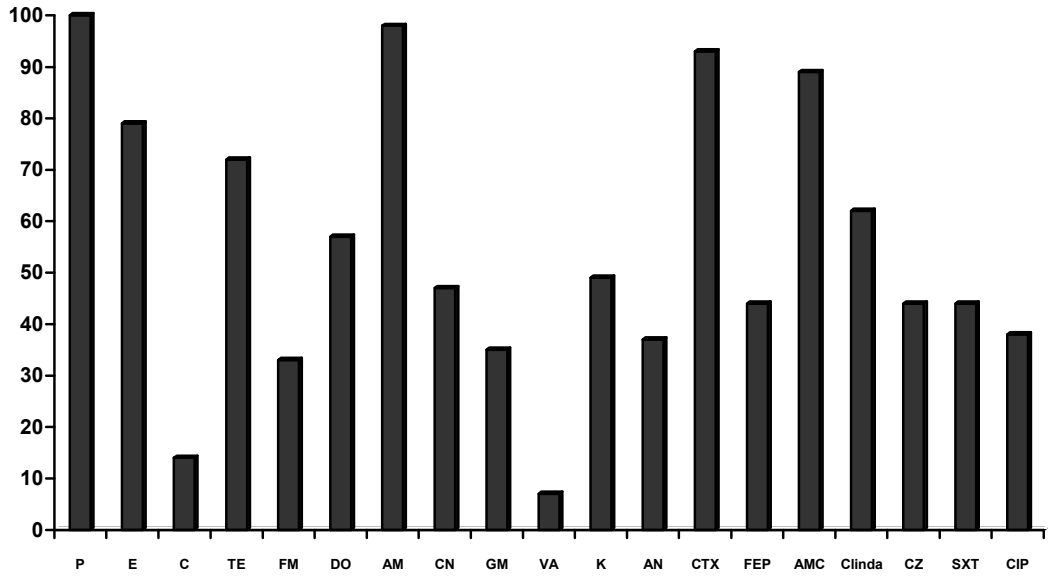

Figure 1. Comparison of antimicrobial resistance frequencies for S. aureus.

P: Penicillin, E: Erythromycin, C: Chloramphenicol, Te: Tetracycline, FM: Nitrofurantoin, DO: Doxycycline, AM: Ampicillin, CN: Cephalexin, GM: Gentamicin, Va: Vancomycin, K: Kanamycin, AN: Amikacine, CTX: Cefotaxime, FEP: Cefepime, AMC: Amoxicillin, Clinda: Clindamycin, CZ: Cefazolin, SXT: Sulfamethoxazole-Trimethoprim, CIP: Ciprofloxacin

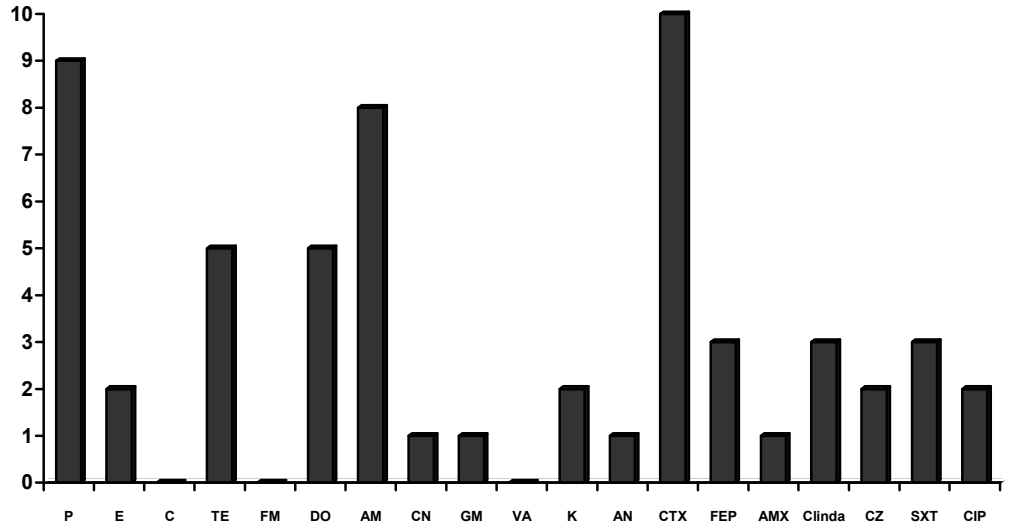

Figure 2. Comparison of antimicrobial resistance frequencies for Methicillin Resistant S. aureus.

P: Penicillin , E: Erythromycin, C: Chloramphenicol, Te: Tetracycline, FM: Nitrofurantoin, DO: Doxycycline, AM: Ampicillin, CN: Cephalexin, GM: Gentamicin, Va: Vancomycin, K: Kanamycin, AN: Amikacine, CTX: Cefotaxime, FEP: Cefepime, AMC: Amoxicillin, Clinda: Clindamycin, CZ: Cefazolin, SXT: Sulfamethoxazole-Trimethoprim, CIP: Ciprofloxacin

DISCUSSION

During the past few years, news on MRSA have usually been discouraging and clinicians and infection control practitioners appear to have lost confidence in their capability to control the hospital acquired spread of this pathogen. The number of papers focusing on the over whelming spread of MRSA is increasing, whereas those addressing successful efforts of control or stating that hospital acquired spread of MRSA can and should be controlled are few (1, 10, 11). A number of researchers debating the control of MRSA have questioned whether controlling this microorganism is reasonable, feasible or justified and whether the tracing of colonized people are justified or not. There is a report from a university hospital and a medical-district-wide control policy for MRSA on the elimination of MRSA after the outbreak (12).

From the 90 MRSA isolates characterized in this study, approximately half of them displayed resistance to one or more antimicrobial agents, including penicillin, cephalosporins, tetracycline and aminoglycosides. These data are in accord with previous study suggesting use of these drugs has been a key factor in the emergence of antimicrobial resistance in MRSA (8). In addition, 66% of MRSA isolates were sensitive to trimethoprim-sulfamethoxazole (co-trimoxazole). Since this drug combination is recommended for treating a range of human infections, *S.aureus* isolates should be monitored for further dissemination of co-trimoxazole resistance.

Eventually, our data may favor the use of Co-Trimoxazole as a potentially cost effective antimicrobial drug for treating MRSA infections. Co-trimoxazole has been shown to be effective against MRSA both invitro and invivo in mice, as well as in clinical reports on meningitis, septicemia and endocarditis (13, 14).

In a controlled comparative trial of intravenous co-trimoxazole versus intravenous vancomycin in 101 cases of severe *S.aureus* infection, the authors reported 100% cure rates for either drug in MRSA infections, including bacteremia (15). More recently, Stein et al. showed varying degrees of success in treating with co-trimoxazole orthopedic implant infections caused by *S.aureus.* Unfortunately, this study did not distinguish MRSA from MSSA strains (16).

As we can see in this study, there is a significant usefulness of chloramphenicol against MRSA. However, it seems that by passing time and introduction of new drugs, this antibiotic is forgotten. Soon after chloramphenicol was released in the United States in 1949, reports linked this highly effective agent with aplastic anemia, and it quickly fell into disfavor. The increased awareness of the pathogenicity of anaerobic organisms and the development of ampicillin-resistant *H. influenzae* accounted for a brief resurgence. However, the availability of other agents has dramatically reduced the need for this antibiotic. Since it is effective, readily available (often over the counter), and inexpensive, it is still used as first-line therapy for enteric fever and other infections in many parts of the world. In the United States and other developed nations, chloramphenicol remains as a useful antibiotic, but only as alternative therapy in seriously ill patients or for patients infected with highly antibiotic-resistant organisms. But unfortunately there is not any perfect and suitable study in this regard, and from this matter, we can conceive that MRSA must be resistant to chloramphenicol as well. But it needs more evaluations.

In this study, we report infections due to MRSA strains with reduced susceptibility to vancomycin. In a study conducted in Shiraz, 100 percent of isolates were sensitive to vancomycin (8). In our study 7% of isolates were resistant to vancomycin. This report is an early warning that *S.aureus* strains with full

resistance to vancomycin might emerge in future. MRSA strains with reduced susceptibility to vancomycin in Japan and in the United States were isolated after prolonged exposure to antibiotics. These strains were isolated from a patient treated with vancomycin for 75 days with teicoplanin for 3 days and/or the strains were isolated after 14 days of vancomycin therapy (17, 18). In some cases, the strains might have been transmitted to the patient from another patient who had undergone treatment for MRSA infections. Since in our study, the patient does not have any history of antibiotic treatment with vancomycin or related antibiotics, these strains might have been transmitted from other patients who were infected with vancomycin – resistant MRSA strains. The high antibiotic resistance among MRSA strains could be originated due to widespread use of antibiotics.

Further phenotypic and genotypic studies are needed to establish and clarify the genetic mechanism behind reduced susceptibilities to antibiotics.

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