Antibiotic Resistance in Clinical Methicillin-resistant *Staphylococcus aureus* Isolates of Three Different Hospitals in Ankara

Ankara'daki Üç Farklı Hastaneden Toplanan Metisilin Dirençli *Staphylococcus aureus* İzolatlarının Antibiyotik Direnci

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Research Article

ABSTRACT

In this study, we assessed the antimicrobial resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from three different hospitals in Ankara, detected the occurrence of multidrug-resistance among MRSA using agar disc diffusion test. The greatest resistance was observed against ampicillin, amoxicillin-clavulanic acid, penicillin and tetracycline (fully resistant), followed by ciprofloxacin (98%), gentamicin and rifampicin (96%), clindamycin and eritromycin (72%). The least resistance was observed against trimethoprim-sulfamethoxazole (4%), fusidic acid (2%), whereas vancomycin showed no resistance. For MRSA isolates, multidrug-resistance was common and only few antibiotics were active against these isolates. Therefore, successive surveillance on antibiotic susceptibility of MRSA is necessary for the determination of emerging trends and the development of appropriate therapeutic strategies.

Key Words
MRSA, antimicrobial resistance, multidrug-resistance

ÖZET

Bu çalışmada Ankara'daki üç farklı hastaneden toplanan metisilin dirençli Staphylococcus aureus (MRSA) izolatlarının antibiyotik direnç paternleri incelendi ve agar disk difüzyon testi kullanılarak MRSA izolatları arasındaki çoklu antibiyotik direnci araştırıldı. En yüksek direnç oranı ampisilin, amoksisilin-klavulanik asit, penisilin ve tetrasiklin (tümüyle direnç) antibiyotiklerine karşı gözlendi. Bu direnç oranını takiben siprofloksasin (% 98), gentamisin ve rifampisin (% 96), klindamisin and eritromisin (% 72) antibiyotiklerine karşı da yüksek oranda direnç gözlendi. En düşük direnç trimetoprim-sülfametoksazol (% 4), fusidik asit (% 2) antibiyotiklere karşı gözlenirken vankomisine karşı direnç gözlenmemiştir. MRSA izolatlarında ortak olarak çoklu antibiyotik direnci bulunmuş ve bu izolatlara karşı az sayıda antibiyotiğin etkili olduğu tespit edilmiştir. Böylece gelişmekte olan direnç durumlarının belirlenmesi ve uygun teröpatik stratejilerin geliştirilmesi için MRSA'ların antibiyotik duyarlılıkları üzerinde başarılı sürveyans çalışmalarının gerekliliği bir ortaya konulmuştur.

Anahtar Kelimeler
MRSA, antimikrobiyal direnç, çoklu antibiyotik direnci

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INTRODUCTION

Staphylococcus aureus is one of the most important pathogens causing nosocomial infections and methicillin resistant *Staphylococcus aureus* (MRSA) has become an important clinical problem [1]. The emergence of methicillin resistance along with the resistance to some other antibiotics limited the therapeutic options and made the control of staphylococcal infections difficult [2].

In the early 1940s, medical treatment of the staphylococcal infections became successful with the discovery and introduction of penicillin. In the late 1940s, penicillinase-producing strains dominated and the resistance of penicillin has increased gradually. In 1960s, methicillin, a derivative of penicillin, was introduced to overcome staphylococcal infections. In the 1980s the methicillin resistance has been identified, MRSA has gradually disseminated and became endemic in many hospitals [3]. Meanwhile MRSA has become resistant not only to β-lactam antibiotics, but also to various antibiotics such as quinolones, chloramphenicol, clindamycin, tetracycline and aminoglycosides by the occurence of multi-drug resistance [3,4]. As a result, vancomycin, which belongs to the glycopeptides class of antibiotics, has been considered as the only available effective antibacterial agent against MRSA infections until its therapeutic failure [5-7]. Therefore, MRSA have been recognized as an important health problem due to involving in hospital-acquired infections [4].

In this study, the antibiotic resistance of MRSA was determined using agar disc diffusion test. We aimed to detect the occurrence of multidrug-resistance among MRSA isolates and to generate antibiotic susceptibility profiles for MRSA from three different hospitals in Ankara.

MATERIALS AND METHODS

MRSA isolates

A total of 50 MRSA isolates (20 from Hospital 1, 20 from Hospital 2 and 10 from Hospital 3) were collected between March 2006 and December 2006. MRSA isolates, which belong to patients who were admitted to 3 different hospitals, were derived from variety of sample sources including wounds (16 isolates), catheter (3 isolates), bile (1 isolate), blood (3 isolates), sputum (3 isolates), pus (5 isolates), operation material (1 isolate), tracheal aspirate (12 isolates), bronchus alveolar irrigation (1 isolate), urine (2 isolates), prostate (1 isolate), others (2 isolates). These MRSA isolates were cultivated in brain heart infusion (BHI) broth with 10% glycerol for the examination and maintained at +4°C.

Phenotypic identification of MRSA

MRSA isolates were identified as *S.aureus* according to β-hemolysis, pigmentation, colonial morphology, gram staining, fermentation of mannitol, positive catalase, coagulase and DNase.

Agar disc diffusion test and antibiotyping

Agar disc diffusion test was applied to determine the resistance of *S.aureus* isolates to the methicillin and to several other antibiotics. Clinical isolates were cultured in BHI broth and incubated at 37°C for the antibiogram test. The cultures were adjusted to an optical density matching 0.5 McFarland turbidity. 0.5 McFarland Standardized overnight cultures were inoculated to Mueller Hilton Agar (MHA) plates, on which antibiotic discs were placed, and incubated at 37°C for 18–24 h [8]. After incubation the inhibition zone diameters were measured and compared to Clinical and Laboratory Standards Institute (CLSI) guidelines and criteria recommended by Antibiogram Committee of the French Society for Microbiology for fusidic acid susceptibility [9-11].

Table 1. Antibiotic Resistance Profiles of MRSA isolates from Hospitals 1, 2 and 3.

	Antibiotic Resistance Profile												Ratio (%)
	AM	AMC	CC	CIP	E	FA	GM	P	RA	SXT	TE	VA	
H1	R	R	S	R	S	S	R	R	R	S	R	S	45
	R	R	R	R	R	S	R	R	R	S	R	S	40
	R	R	R	R	R	I	R	R	R	S	R	S	15
	R	R	R	R	R	S	R	R	R	S	R	H	85
H 2	R	R	S	R	S	S	S	R	R	S	R	H	5
	R	R	I	R	I	S	R	R	S	S	R	H	5
	R	R	R	S	R	S	S	R	S	R	R	H	5
H 3	R	R	S	R	S	S	R	R	R	S	R	H	30
	R	R	R	R	R	S	R	R	R	S	R	H	40
	R	R	R	R	R	R	R	R	R	S	R	H	10
	R	R	S	R	S	S	R	R	R	R	R	H	10
	R	R	R	R	R	S	R	R	R	R	R	H	10

AM=Amphicilin; AMC=Amoxicillin-clavulanic acid; CC=Clindamycin; CIP=Ciprofloxacin; E=Eritromycin; FA=Fusidic Acid; GM=Gentamicin; P=Penicilin; RA=Rifampicin; SXT=Trimethoprim-sulfamethoxazole; TE=Tetracycline; VA=Vancomycin; H 1=Hospital 1; H 2=Hospital 2; H 3=Hospital 3, R=Resistant, I=Intermediate; S=Susceptible

The antimicrobial agents were selected according to the tests conducted in clinical laboratories. The tested antimicrobial agents included ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), ciprofloxacin (5 μg), clindamycin (2 μg), eritromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), oxacillin (1 μg), penicillin (10 unit), rifampicin (5 μg), trimethoprim-sulfamethoxazole (1.25 μg), tetracycline (30 μg), vancomycin (30 μg).

RESULTS AND DISCUSSION

Hospital-acquired infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) continue to be a major problem in the world. In recent years, as a result of the integration of transpozenes that carries various resistance determinants, multi resistant *S.aureus* strains have emerged. Antimicrobial resistance among MRSA isolates causes serious difficulties in clinical settings and increases the treatment costs. Therefore, it is important to recognize antimicrobial resistance rates of MRSA in hospitals [12].

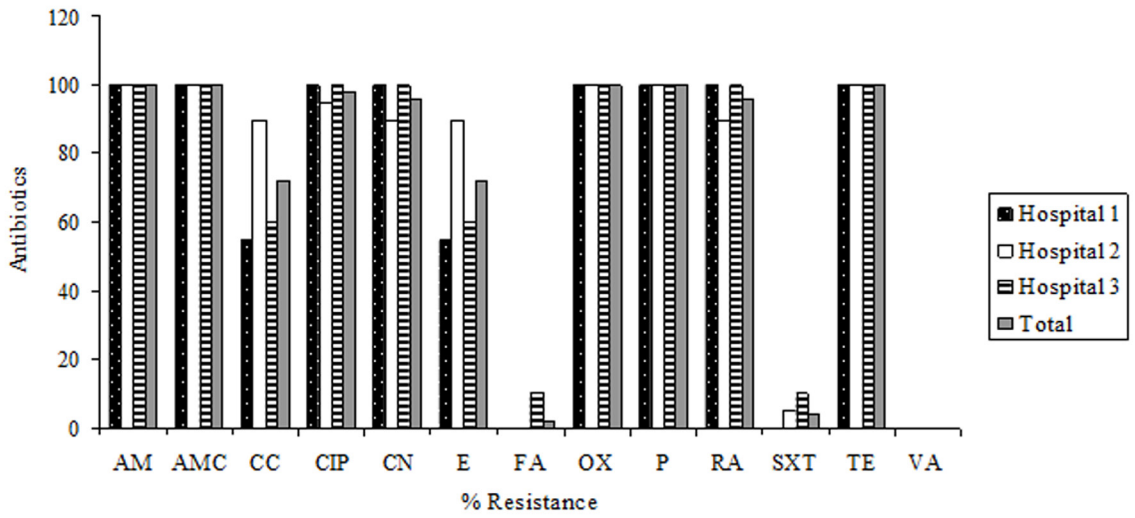
Methicillin resistance in *Staphylococcus* species gradually increases in Turkey like many other countries. It is significant to identify MRSA strains, detect appropriate treatment strategies for MRSA infections and use antibiotics against MRSA [13].

In this study, antibiotic susceptibility patterns of MRSA isolates were determined in three different hospitals in Ankara, Turkey. MRSA isolates were classified according to their resistance patterns to 12 antibiotics (Table 1).

The prevalence of multi-drug resistance in MRSA isolates is shown in Table 2. In this study, multi-drug resistance was described as resistance to seven or more of the antibiotics tested, 96% of the MRSA isolates exhibited multi-drug resistance and none was fully sensitive to all the tested antibiotics (Table 2).

Table 2. Prevalence of multiple-drug resistance among MRSA isolates.

Parameter	Frequency of multi-drug resistance in MRSA	
	Number of isolates	Ratio (%)
Fully sensitive	0	0
Resistant to 1 agent	0	0
Resistant to 2 agents	0	0
Resistant to 3 agents	0	0
Resistant to 4 agents	0	0
Resistant to 5 agents	0	0
Resistant to 6 agents	2	4
Resistant to 7 agents	13	26
Resistant to 8 agents	1	2
Resistant to 9 agents	32	64
Resistant to 10 agents	2	4



AM=Amphicilin; AMC=Amoxicillin-clavulanic acid; CC=Clindamycin; CIP=Ciprofloxacin; E=Eritromycin; FA=Fusidic Acid; GM=Gentamicin; P=Penicilin; RA=Rifampicin; SXT=Trimethoprim-sulfamethoxazole; TE=Tetracycline; VA=Vancomycin.

Figure 1. Antibiotic resistance rates of MRSA.

It is becoming clinically important to determine the antimicrobial resistance rates of MRSA isolates for the eradication of infections. The result of antibiotic resistance of MRSA isolates to various clinical antimicrobial agents is shown in Figure 1.

The greatest resistance was observed against ampicillin, amoxicillin-clavulanic acid, penicillin and tetracycline (fully resistant), followed by ciprofloxacin (98%), gentamicin and rifampicin (96%), clindamycin and eritromycin (72%). The least resistance was observed against trimethoprim-sulfamethoxazole (4%), fusidic acid (2%) and none against vancomycin.

Since the emergence of MRSA, vancomycin has been the only effective treatment choice and widely used for MRSA infections. Widespread use of vancomycin leads to the emergence of *S. aureus* with reduced susceptibility to these antibiotics [12]. Reduced susceptibility to vancomycin has been first described in Japan, and then in USA, Europe and Korea [13-15]. Reports describing vancomycin resistance and the therapeutic failure of vancomycin treatment of MRSA infections aroused substantial concern associated with the controlled use of vancomycin and give point to the need for new antibiotics.

Results of this study revealed that all tested MRSA strains were sensitive to vancomycin and no isolates had reduced susceptibilities. The observed full susceptibility to vancomycin in this study supports some previous reports [16-18].

MRSA refers to bacteria of the *Staphylococcus aureus* species that are resistant to all currently available β-lactam antibiotics and also tetracycline [4, 19]. The results for the all antibiotics tested illustrate that MRSA isolates resistant to penicillin, ampicillin, amoxicillin-clavulanic acid and tetracycline showed the highest resistance (fully resistant). Our findings exhibit that these antibiotics were the least effective antimicrobial agents for MRSA infections and are parallel with other previous studies [16, 17].

The other antibiotic tested in this study was rifampicin among MRSA isolates. MRSA isolates, resistant to rifampicin predominated with a high incidence of MRSA, representing more than 95% of the total MRSA isolates. By comparison with the previous studies, it has been seen that rifampicin resistance gradually increased [11,17]. This observation could suggest that, rifampicin should not be used alone for the treatment of MRSA infections due to the rapid development of resistance.

It is generally accepted that MRSA is resistant to aminoglycoside antibiotics [20]. In the present study, the resistance rates of MRSA to gentamicin exceeded 90% in Hospital 1, Hospital 2 and Hospital 3. The frequency of occurrence of gentamicin resistance was 71.4% in 1999 [21], 77.1% in 2003 [22], 95.0% in 2004 [16], 96.7% in 2006 [17] in previous studies. The results of these studies are similar to our findings and these indications argue that there is a significant increase in the resistance to gentamicin.

The gentamicin resistance has been frequently seen along with the existence of quinolone resistance [23]. In this study, ciprofloxacin, member of quinolone group of antibiotics, was investigated and results show significantly high incidence of ciprofloxacin resistance among MRSA isolates. There have been a few studies, documenting high incidence of ciprofloxacin-resistance among MRSA isolates in Turkey [8,11,24,25]. The result of widespread ciprofloxacin resistance indicates that it is hard to use this antibiotic as a treatment choice of MRSA infections.

Majority of Hospital 2 strains were resistant to eritromycin and clindamycin, while 55% and 60% of Hospital 1 and Hospital 3 MRSA strains, respectively were resistant to those antibiotics. These varied rates among MRSA isolates from different hospitals in the same city may be due to differences in patient populations and/or control of infection. The result by evaluation of three hospitals together was similar to other previous studies [17].

Fusidic acid is an effective treatment for *S. aureus* infections. It seems to be a good choice for multi-resistant staphylococcal infections due to lack of any side effects, usefulness for oral forms, and low treatment costs. Widespread use of fusidic acid for the treatment of these infections leads to the consumption of glycopeptides and contributes to the prevention of glycopeptide resistant strains in the community and hospitals [27].

Fusidic acid appears to be the most effective antibiotic with the 2% resistance ratio when all tested strains are evaluated according to their susceptibility profile. Another research, done in South Africa in 2006, exhibits that all tested strains are susceptible to fusidic acid. In a national research on MRSA strains, the fusidic acid resistance rate was detected as 2.6% [4]. Taking these figures into account, it can be concluded that there has been no increase in the resistance of fusidic acid since 2000. The researches on fusidic acid resistance of MRSA showed that especially fusidic acid is not only a good alternative choice but also a good antimicrobial agent that shouldn't be ignored for MRSA infections.

The resistance ratio for trimethoprim-sulfamethoxazole among tested antibiotics in our study varies between 0-10%. This ratio is parallel with the results those of previous national studies 6.5%, 8.8% and the study in Korea 8.9% [16,24, 28]. If we regard the development of the resistance against glycopeptides with multi-drug resistance, we may consider trimethoprim-sulfamethoxazole as an effective antibiotic for MRSA infections when cases without the use of glycopeptides exist.

The present study has shown a steady increase in the resistance rates of MRSA strains. Because of the widespread and random use of antibiotics, the resistance profile of microorganisms is gradually changing. Further, we noted that MRSA isolates exhibited various multi-resistant phenotypes and resistance rates of these isolates to a variety of antimicrobials were significantly high. None of the beta lactam antibiotics are effective in MRSA infections. In addition, resistance against the antibiotics excluding beta lactam group has been increasingly seen in the course of time. MRSA isolates, susceptible to vancomycin, trimethoprim-sulfamethoxazole and fusidic acid, are predominated in three hospitals in Ankara with a high incidence. Therapeutic options in MRSA infections for empiric therapy vancomycin, trimethoprim-sulfamethoxazole and fusidic acid may be recommended.

Antibiotic use, prolonged hospitalisation, serious underlying illness, skin disease, frequent staff-patient contact and the use of invasive devices predispose to invasive MRSA infections. If we regard the most predominant and common resistance phenotype in three hospitals in Ankara, it appears that our results is similar to data from national and international researches on antibiotic susceptibilities of MRSA as mentioned above.

MRSA isolates, resistant to beta lactam antibiotics, ciprofloxacin, clindamycin, eritromycin, gentamicin, rifampicin, tetracycline, generate the most common phenotype in our country and all over the world. As a result of this study, only few antibiotics are available to treat MRSA infections. As an indicator of multi-resistance, most of MRSA isolates were resistant to nine antibiotics and the resistance against these antibiotics has increased within years. Therefore, life threatening MRSA infections have to be immediately eradicated. Eventually, it is essential that local surveillance of common nosocomial microorganisms and their antibiotic susceptibilities be implemented to advise the proper use of antimicrobial agents.

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