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METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS STRAINS ISOLATED IN ISTANBUL

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ÖZET

İstanbul'da izole edilen Staphylococcus aureus suşlarında metisilin direnci.

İstanbul'da izole edilen 300 *S.aureus* suşunda metisilin direnci bazı diğer antibiyotiklerle mukayeseli olarak incelenmiştir.

Metisilin direnci litresine 20 g NaCl, 50 mg $CaCl_2$ ve 25 mg $MgCl_2$ ilave edilmiş Mueller-Hinton buyyonunda dilüsyon yöntemi ile incelenmiştir. MIC değeri 16 µg/ml veya daha yüksek olan suşlar dirençli olarak kabul edilmiştir. Vankomisin direnci ilavesiz Müller-Hinton buyyonunda dilüsyon yöntemi ile, diğer antibiyotiklere direnç disk-diffüsyon yöntemi ile araştırılmıştır.

Metisiline dirençli suşlar % 31.7 olarak saptanmıştır. Vankomisine dirençli suşa rastlanmayan çalışmada metisiline dirençli suşlar arasında sefalotin, ampisilin+sulbaktam, amoksisilin+klavulanik asit, rifampisin, kloramfenikol, gentamisin, kanamisin, streptomisin, amikasin ve tobramisine dirençli suş oranları, suşların tümü veya metisiline duyarlı olanları ile mukayese edildiğinde, çok daha yüksek bulunmuştur. Örneğin, suşların tümünde % 22.3, metisiline duyarlı suşlarda % 7.8 olan sefalotin direnci, metisiline dirençli suşlarda % 53.7 olmuştur. Metisiline dirençli suşlarda netilmisin direnci değişmemiş, füsidik asit direnci ise daha düşük bulunmuştur. Bu bulgulardan bekleneceği gibi, füsidik asit ve netilmisin dışında, diğer antibiyotiklere dirençli suşlarda da metisiline direnç oranı ortalama metisilin direncinden (% 31.7) daha yüksek bulunmuştur.

SUMMARY

Methicillin resistance in 300 *S.aureus* strains isolated in Istanbul was investigated in comparison with some other antibiotics.

Methicillin resistance was determined by dilution method in Mueller-Hinton broth supplemented with 20 NaCl, 50 mg $CaCl_2$ and 25 mg $MgCl_2$ per liter. The strains

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with MIC≥16 μg/ml were considered as resistant. Susceptibility to vancomycin was determined by dilution method in unsupplemented Mueller-Hinton broth and to other antibiotics by disk-diffusion method.

The ratio of strains resistant to methicillin was found to be 31.7 %. There was no resistant strain to vancomycin. The resistance to cephalothin, ampicillin+sulbactam, amoxicillin+clavulanic acid, rifampicin, chloramphenicol, gentamicin, kanamycin, streptomycin, amikacin and tobramycin was more frequent among methicillin resistant strains when compared with methicillin sensitive strains or with overall resistance. For example, 22.3 % overall resistance or 7.8 % resistance of methicillin sensitive strains to cephalothin leaped to 53.7 % in methicillin resistant strains. On the contrary, fusidic acid resistance was lower and netilmicin resistance did not change among methicillin resistant strains. As expected from these findings, the resistance to methicillin among strains resistant to other antibiotics was also found higher than average methicillin resistance (31.7 %) except for fusidic acid and netilmisin.

INTRODUCTION

Because of their increased resistance to the antibiotics most appropriate for the treatment of staphylococcal infections, the methicillin-resistant *Staphylococcus aureus* (MRSA) strains cause an important problem in chemotherapy, especially in hospital environment.

Outbreaks of hospital infections due to MRSA strains were reported in early 1960s following the introduction of methicillin in 1959 (3). The rate of MRSA strains among clinical isolates increased until 1970. For example, approximately 18 % of staphylococcal isolates were found to be methicillin resistant in Denmark between 1968 and 1970 (12), and 17.3 % of staphylococcal infections were reported to be due to MRSA strains in Zürich in 1968 (4). The same situation was found to be true in almost all parts of the World from where reliable results were reported. In the first half of the last decade, a general decline in the incidence of MRSA strains were experienced due to reasons not completely understood (6, 18). Unfortunately, the MRSA strains and the problems they create emerged again in the latter of the 1970s (6). The etiological agents were reported to be MRSA strains in 49 % of wound infections and 40 % in bacteremias in 1980 in a US center (22). One of the main features of the new MRSA strains was their increased resistance to gentamicin (6, 7).

In this presentation, the incidence of MRSA strains among staphylococcal isolates from clinical specimens in a Medical School Hospital in Istanbul is reported. The differences in the resistance percentages of the methicillin resistant and sensitive strains to some other antibiotics are also given.

MATERIALS AND METHODS

Three hundred *Staphylococcus aureus* strains isolated from various clinical specimens were investigated.

For methicillin sensitivity testing, tube dilution method was used in Mueller-Hinton broth supplemented with 20 g NaCl, 50 mg $CaCl_2$, 25 mg $MgCl_2$ per litre

(pH7). Suspensions of the strains adjusted to the turbidity of Mc Farland tube no. 0.5 from overnight broth cultures were used for inoculation. Results were evaluated following 24 h incubation at 35 °C. A MIC value equal to or greater than 16 µg/ml was considered as the sign of resistance (13).

Sensitivity to vancomycin was also determined by the same method in unsupplemented Mueller-Hinton broth. Sensitivity to other antibiotics was determined by the disk-diffusion method on Mueller-Hinton agar.

RESULTS

MIC values of methicillin for 300 *S.aureus* strains are shown in table 1. Fifteen of the strains were inhibited by 16 µg/ml and 80 strains by 32 µg/ml of methicillin. So, 95 (31.7 %) of 300 strains were considered as MRSA.

Table 1. MIC values of methicillin for 300 S.aureus strains.

MIC value (µg/ml)	< 2	2	4	8	16	32
Number of strains	5	22	117	61	15	80

All the strains were found to be sensitive to vancomycin. The MIC of vancomycin was 2 µg/ml for 19 strains and less than 2 µg/ml for the rest.

The numbers, and percentages of the strains resistant to other antibiotics and their distribution among methicillin resistant and sensitive strains are shown in table 2.

Table 2. Resistance of methicillin resistant and sensitive *S.aureus* strains to some antibiotics.

Antibiotics	In all strains (n= 300)		In MRSA strains (n= 95)		In MSSA strains (n= 205)	
	n	(%)	n	(%)	n	(%)
Methicillin	95	(31.7)				
Cephalothin	67	(22.3)	51	(53.7)	16	(7.8)
Ampicillin+sulbactam	42	(14)	28	(29.5)	14	(6.8)
Amoxicillin+ clavulanic acid	76	(25.3)	56	(58.9)	20	(9.8)
Vancomycin	0	(0)	0	(0)	0	(0)
Fusidic acid	58	(19)	11	(11.6)	47	(22.9)
Rifampicin	142	(47.3)	56	(58.9)	86	(42)
Chloramphenicol	69	(23)	37	(38.9)	32	(15.6)
Gentamicin	92	(30.7)	69	(72.6)	23	(11.2)
Kanamycin	108	(36)	56	(58.9)	52	(25.4)
Streptomycin	105	(35)	56	(58.9)	49	(23.9)
Amikacin	63	(21)	34	(35.8)	29	(14.1)
Netilmicin	74	(24.7)	23	(24.2)	51	(24.9)
Tobramycin	90	(30)	43	(45.3)	47	(22.9)

MRSA= methicillin resistant S.aureus; MSSA= methicillin sensitive S.aureus.

As it is seen in table 2, the percentages of the strains resistant to any antibiotic except vancomycin, fusidic acid and netilmisin were found to be considerably higher among MRSA strains than those among sensitive strains. The 7.8 % cephalothin resistance for methicillin sensitive strains rised to 53.7 % for methicilin resistant strains. The percentages of resistant strains among methicillin sensitive and resistant *S.aureus* strains were found to be 6.8 % and 29.5 % for ampicillin+sulbactam combination, 9.8 % and 58.9 % for amoxicillin+ clavulanic acid combination, 42 % and 58.9 % for rifampicin, 15.6 % and 38.9 % for chloramphenicol, 11.2 % and 72.6 % for gentamicin, 25.4 % and 58.9 % for kanamycin, 23.9 % and 58.9 % for streptomycin, 14.1 % and 35.8 % for amikacin, 22.9 % and 45.3 % for tobramycin.

There was no difference in vancomycin sensitivity among the two groups of strains as they were homogenously sensitive. The percentages of netilmicin resistance among methicillin resistant and sensitive strains were virtually the same. On the contrary to above mentioned results, fusidic acid resistance was found to be considerably lower among methicillin resistant strains than that among methicillin sensitive strains (11.6% and 22.9 %).

As it was expected from above given results, methicillin resistant strains among strains resistant to other antibiotics were also found to be more frequent except fusidic acid and netilmicin (Table 3).

Table 3. Methicillin resistance in S.aureus strains resistant or sensitive to other antibiotics.

Antibiotic	Methicillin resistance in*	
	Resistant strains %	Sensitive strains %
Cephalothin	76.1	18.9
Ampicillin+sulbactam	66.7	26
Amoxicillin+clavulanic acid	73.7	17.4
Vancomycin	0	0
Fusidic acid	19	34.7
Rifampicin	39.4	24.7
Chloramphenicol	53.6	25.1
Gentamicin	75	12.5
Kanamycin	51.9	20.3
Streptomycin	53.3	20
Amikacin	54	25.7
Netilmicin	31.1	31.9
Tobramycin	47.8	24.8

* Methicillin resistance in all strains is 31.7 %.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* strains are going to create a serious problem as in 1960s and 1970s, in spite of the availability of some new antibiotics effective on staphylococci (21). For example, an outbreak of hospital infection with MRSA lasted four years from 1982 to 1986, and affected more than 500 patiens (10).

The mechanism by which the methicillin resistance appears in staphylococci is a complex one. One of the proposed mechanisms is the production of an alternative penicillin binding protein called PBP 2 or PBP 2a by resistant strains, either coagulase positive or negative (11, 13, 24). This protein has a decreased affinity for beta-lactam antibiotics and is expressed in various levels by bacterial cells in a culture. Because of this heterogenecity in expression, only a portion of the cells in a culture of a MRSA strain are able to grow in the presence of methicillin. This is the reason of the term "heteroresistance" for methicillin resistance in staphylococci. To provoke the expression of this altered PBP, the sensitivity test should be performed in media supplemented with NaCl, $CaCl_2$ and $MgCl_2$, with a dense inoculum and the result should be recorded after a prolonged incubation (11, 13, 24). Otherwise, the methicillin-resistance could go unnoticed. Since the resistance of this kind is not due to drug inactivation, it is also called intrinsic resistance (17).

While plasmids play a more important role in staphylococci as far as the resistance to chemotherapeutics is concerned, the methicillin resistance is believed to be coded exclusively by the chromosome (14, 20). In an investigation, an additional chromosomal DNA was detected in methicillin resistant strains (14). A fragment of this additional DNA was cloned into a plasmid in *E.coli*, and only the methicillin resistant strains were shown to have complementary sequences for this fragment in hybridization experiments.

More recently, another mechanism was proposed for the diminished methicillin susceptibility of some *S.aureus* strains for which the term "borderline" or "acquired resistance" were used (16). These strains produce an excessive amount of beta-lactamases which show hydrolysis on beta-lactamase-resistant penicillins. The over-production of beta-lactamases is plasmid mediated and can be partly neutralized by beta-lactamase inhibitors, like sulbactam and clavulanic acid. The clinical importance of such strains have not been completely evaluated yet (13, 16).

Whatever the mechanism for methicillin resistance is, the incidence of MRSA strains has reached to a high level among hospital isolates in many parts of the World. The percentage of MRSA among staphylococcal isolates was reported to be as high as 49 % (22). We had found the percentage of MRSA strains to be 11 % ten years ago (23), and it was found to be 31.7 % in this study. In another study from Ankara, Turkey, the ratio of methicillin resistant staphylococci, coagulase positive or negative, was reported to be 48 % (1).

An important feature of MRSA strains is their increased level of resistance for many other chemotherapeutics (25). Especially the resistance to other beta-lactam antibiotics, including cephalosporins, is very frequent. Some MRSA strains are found to be susceptible to cephalosporins in disk-diffusion test, as in the case of this study, but this may be due to the testing method. In a study, all of 40 MRSA strains were found sensitive to cephalothin at 35°C while 37 of these strains were found resistant at 30°C (5). The infections with such apparently cephalosporin sensitive MRSA strains cannot generally be treated with cephalosporins. For this reason, many laboratories report methicillin resistant staphylococci as resistant to all beta-lactams, irrespective of test results with the individual drugs (13). In this study, cephalothin resistance was found as

53.7 % in MRSA strains while it was only 7.8 % in sensitive strains. In a study from Ankara, all of methicillin resistant staphylococcal isolates were found to be cephalothin resistant (1). We also found many folds of increase in the levels of ampicillin+sulbactam and amoxicillin+clavulanic acid resistance in MRSA strains. The high level of resistance of MRSA strains to beta-lactam antibiotics potentiated by sulbactam and clavulanic acid was also reported by others (2).

Chloramphenicol resistance is also more prevalent among MRSA strains. Our MRSA strains showed more than two fold increase for chloramphenicol resistance when compared with their sensitive counterparts.

The first gentamicin resistance among MRSA strains was reported in 1976 (19). Such strains increased in time (7). In this study, 69 of 300 strains were found to be methicillin and gentamicin resistant (23 %). The 11.2 % gentamicin resistance among methicillin sensitive strains was found to be 72.6 % in MRSA strains. We also found at least two fold increase in the resistance for other aminoglycosides among MRSA strains, as it is shown in table 2, except for netilmicin for which the percentage of resistance was the same in both groups of *S.aureus* strains. The increased aminoglycoside resistance was reported by many investigators (7, 8, 9, 15).

Our findings that MRSA strains are totally sensitive to vancomycin and mostly sensitive to fusidic acid is in accordance with other reports (6, 9, 25).

As a consequence of higher resistant rates to other antibiotics among methicillin resistant strains, methicillin resistance among strains resistant to other antibiotics was found to be higher, except fusidic acid and netilmicin, as it is shown in table 3.

REFERENCES

1. Akalın H E, Çelik E, Baykal M, Kardeş T: Metisiline dirençli Staphylococcus'ların bazı antibiyotiklere in vitro duyarlılıkları, *ANKEM Derg 1:* 122 (1987).
2. Aldridge K E, Sanders C V, Marier R L: Variation in the potentiation of beta-lactam antibiotic activity by clavulanic acid and sulbactam against multiply antibiotic resistant bacteria, *J Antimicrobial Chemother 17:* 463 (1986).
3. Barber M: Methicillin-resistant staphylococci, *J Clin Pathol 14:* 385 (1986).
4. Benner E J, Kayser F H: Growing clinical significance of methicillin-resistant Staphylococcus aureus, *Lancet 2:* 741 (1968).
5. Canawati H N, Witte J L, Sapico F L: Temperature effect on the susceptibility of methicillin-resistant Staphylococcus aureus to four different cephalosporins, *Antimicrob Agents Chemother 21:* 173 (1982).
6. Casewell M W: Epidemiology and control of the "modern" methicillin-resistant Staphylococcus aureus, *J Hosp Infect 7 (Suppl A):* 1 (1986).
7. Coleman D C, Cafferkey M, Keane C T, Baxter L, Pomeroy H, Foster T J, Hone R, Mulvey M, Arbuthnott J P: Mechanisms of pathogenicity of multi-resistant Staphylococcus aureus, *J Hosp Infect 7 (Suppl A):* 29 (1986).
8. Crossley K, Loesch D, Landesman B, Mead K, Chern M, Strate R: An outbreak of infections caused by strains of Staphylococcus aureus resistant to methicillin and aminoglycosides, *J Infect Dis 139:* 273 (1979).

9. Dixson S, Brumfitt W, Hamilton-Miller J M T: In vitro activity of combinations of antibiotics against Staphylococcus aureus resistant to gentamicin and methicillin, *Infection 13:* 35 (1985).

10. Duckwirth G J, Lothian J L E, Williams J D: Methicillin-resistant Staphylococcus aureus: report of an outbreak in a London teaching hospital, *J Hosp Infect 11:* 1 (1988).

11. Hartman B J, Tomasz A: Expression of methicillin resistance in heterogeneous strains of Staphylococcus aureus, *Antimicrob Agents Chemother 29:* 85 (1986).

12. Jebsen O B: The demise of the "old" methicillin-resistant Staphylococcus aureus, *J Hosp Infect 7 (Suppl A):* 13 (1986).

13. Jorgensen J H: Laboratory and epidemiologic experience with methicillin-resistant Staphylococcus aureus in the USA, *Eur J Clin Microbiol 5:* 693 (1986).

14. Kayser F H, Berger-Bachi B, Beck W D: Genetics of multiply-resistant Staphylococcus aureus, *J Hosp Infect 7 (Suppl A):* 19 (1986).

15. Lacey R W, Barr K W, Barr V E, Inglis T J: Properties of methicillin-resistant Staphylococcus aureus colonizing patients in a burns unit, *J Hosp Infect 7:* 137 (1986).

16. McDovgal L K, Thornsberry C: The role of beta-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins, *J Clin Microbiol 23:* 832 (1986).

17. Sabath L D: Mechanisms of resistance to beta-lactam antibiotics in strains of Staphylococcus aureus, *Ann Intern Med 97:* 339 (1982).

18. Shanson D C: Antibiotic-resistant Staphylococcus aureus, *J Hosp Infect 2:* 11 (1981).

19. Shanson D C, Kensit J G, Duke R: Outbreak of hospital infection with a strain of Staphylococcus aureus resistant to gentamicin and methicillin, *Lancet 2:* 1347 (1976).

20. Sjöström J, Löfdahl S, Philipson L: Transformation reveals a chromosomal locus of the gene(s) for methicillin resistance in Staphylococcus aureus, *J Bacteriol 123:* 905 (1975).

21. Stamm W E, Weinstein R A, Dixon R E: Comparison of endemic and epidemic nosocomial infections, *Amer J Med 70:* 393 (1981).

22. Thompson R L, Cabezudo I, Wenzel R P: Epidemiology of nosocomial infections caused by methicillin-resistant Staphylococcus aureus, *Ann Intern Med 97:* 309 (1982).

23. Töreci K, Çetin E T, Anğ Ö: Antibiotic susceptibility of 7.726 bacterial strains isolated from clinical specimens, Curr Chemother (Ed W Siegethaler, R Lüthy), *Proceedings of the 10th International of Chemotherapy,* Vol 1, p.553, Amer Soc Microbiol, Washington (1978).

24. Utsui Y, Yokots T: Role of an altered penicillin binding protein in methicillin and cephem-resistant Staphylococcus aureus, *Antimicrob Agents Chemother 28:* 397 (1985).

25. Waldvogel F A: Treatment of infections due to methicillin-resistant Staphylococcus aureus, *J Hosp Infect 7 (Suppl A):* 37 (1986).