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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Fusidic acid resistance among staphylococci strains isolated from clinical specimens in a general hospital

Bir devlet hastanesindeki klinik örneklerden izole edilen stafilokok suşlarında fusidik asit direnci

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ABSTRACT

Objectives: The aim of this study was to investigate in vitro susceptibility of fusidic acid to clinic isolates of staphylococci.

Materials and methods: The forty-one coagulase negative staphylococci (CNS) and 18 Staphylococcus aureus strains isolated from various clinical specimens were included in this study. Staphylococci isolates were identified by conventional methods such as colony morphology onto medium, gram staining, catalase and coagulase tests. According to "Clinical and Laboratory Standards Institute (CLSI)" criteria, antimicrobial susceptibility testing of isolates was performed by Kirby-Bauer's disk diffusion method.

Results: The seventy-two percent of the isolated *S.aureus* were defined as methicillin sensitive-*S.aureus* (MSSA), 28% of the isolated *S.aureus* were defined as methicillin resistant-*S.aureus* (MRSA). The difference among fusidic acid susceptibility rates of MSSA and MRSA strains was not statistically significant (p=0.305). The twenty-nine percent of the isolated CNS were defined as methicillin sensitive-CNS (MS-CNS), 71% of the isolated CNS were defined as methicillin resistant-CNS (MR-CNS). There was no statistically significant difference between MS-CNS and MR-CNS strains for fusidic acid susceptibility rates (p=0.490). But the difference among fusidic acid susceptibility rates of CNS and *S.aureus* strains was statistically significant (p<0.001). CNS strains were found more resistance than *S.aureus* strains for fusidic acid.

Conclusion: In this study, the resistance rates were detected to increase for fusidic acid along with methicillin resistance. Among CNS isolates, fusidic acid resistance rates were significantly more elevated than that for *S.aureus*. Fusidic acid remains as an alternative in the treatment of infections due to staphylococci.

Key words: Staphylococcus aureus, fusidic acid, microbial sensitivity test

ÖZET

Amaç: Bu çalışmanın amacı, klinik örneklerden izole edilen stafilokok suşlarında fusidik asidin in vitro etkinliğinin araştırılmasıdır.

Gereç ve yöntem: Çalışmaya çeşitli klinik örneklerden izole edilen 41 koagülaz negatif stafilokok (KNS) izolatı ile 18 Staphylococcus aureus suşu dahil edildi. Stafilokok izolatları besiyeri yüzeyindeki koloni morfolojisi, gram boyama, katalaz ve koagülaz testleri gibi konvansiyonel yöntemlerle identifiye edildi. İzolatların antimikrobiyal duyarlılıkları "Clinical and Laboratory Standards Institute (CLSI)" önerileri doğrultusunda Kirby-Bauer disk difüzyon yöntemi kullanılarak çalışıldı.

Bulgular: İzole edilen *S.aureus* suşlarının % 72'si metisiline duyarlı (MSSA), % 28'i metisiline dirençli (MRSA) olarak tanımlandı. MSSA ve MRSA suşlarının fusidik asit duyarlılık oranları arasındaki fark istatistiksel olarak anlamlı bulunmadı (p=0.305). İzole edilen KNS'lerin % 29'u metisiline duyarlı (MS-KNS), % 71'i metisiline dirençli (MR-KNS) olarak tanımlandı. MR-KNS ve MS-KNS suşlarının fusidik asit duyarlılık oranları arasında istatistiksel olarak anlamlı fark yoktu (p=0.490). Ancak, KNS ve *S.aureus* suşlarının fusidik asit duyarlılık oranları arasındaki fark istatistiksel olarak anlamlıydı (p<0.001). KNS suşları fusidik aside *S.aureus* suşlarından daha fazla dirençli bulundu.

Sonuç: Bu çalışmada, metisilin direnci ile birlikte fusidik aside karşı da direnç gelişiminde artış olduğu gözlendi. KNS izolatları arasındaki fusidik aside direnç oranları *S.aureus* suşlarına göre önemli ölçüde artmıştır. Sonuç olarak, fusidik asit stafilokoklara bağlı enfeksiyonların tedavisinde hala bir alternatif olarak durmaktadır.

Anahtar kelimeler: Staphylococcus aureus, fusidik asit, mikrobiyal duyarlılık testi

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INTRODUCTION

Fusidic acid is an antimicrobial drug obtinated from Fusidium coccineum.[1] Since being made available for clinical use in 1960s, fusidic acid has been utilized in Europe and Australia for the treatment of staphylococcal infections. During the early development of this antimicrobial drug, resistance appeared to be selected easily in vivo and in vitro; but data from countries where fusidic acid was used in logical quantities showed that resistance rates stayed modest and that staphylococci showing elevated fusidic acid minimum inhibitory concentration (MIC) values hadn't emerged rapidly.[2]

Fusidic acid inhibits protein synthesis by blocking the elongation of the nascent polypeptide chain through binding to EFG on the ribosome and preventing the dissociation of EFG-GDP from the ribosome.[3,4] The rate of fusidic acid resistance isn't very high; but the existence of clinical staphylococcal species that are resistant to fusidic acid has been reported.[5]

In present study, in vitro susceptibilities of a variety of staphylococci strains isolated from clinical specimens to fusidic acid were investigated.

MATERIALS AND METHODS

Bacterial isolates

From April 2009 to August 2011, 41 coagulase negative staphylococci (CNS) and 18 coagulase positive *S.aureus* strains isolated from various clinical specimens that had been sent to microbiology laboratory of Kızıltepe General Hospital had been included in this study. *S.aureus* ATCC 29213 has consistently been used as a quality control strain. 5% sheep blood agar (Oxoid Ltd., Basingstoke, UK) medium was used for bacterial growth at 35±2°C with aeration for 18-24 hours. Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) medium was used for all determinations of Kirby-Bauer's disk diffusion method. All isolates were identified by conventional methods such as colony morphology onto medium, gram staining, catalase and coagulase reactions.

Antimicrobial susceptibility testing

Methicillin resistance was determined by incubation of oxacillin (1 μg) disk onto Mueller-Hinton agar medium aerobically at 35±2°C for 18-24 hours. Oxacillin inhibition zone diameter >13 mm were evaluated as susceptible, <10 mm were resistant. Antimicrobial susceptibility testing was performed by Kirby-Bauer's disk diffusion method in accordance with the recommendations of CLSI.[6]

For fusidic acid, where CLSI does not provide disk susceptibility breakpoints, the required diameters for sensitivity and resistance were ≥22 mm and <22 mm, respectively (10 μg fusidic acid disk) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints.[7] However, in this study fusidic acid susceptibility was detected according to the criteria of Comite de L'antibiogramme de la Societe Française de Microbiologie, and inhibition zone of ≥22 mm was considered as sensitive, 16-21 mm as intermediate, ≤15 mm as resistant.

Statistical analysis

Data of this study were analyzed by Epi InfoTM 7-Community Edition (Centers for Diseases Control and Prevention, Atlanta, GA, USA) statistical package program. Statistical evaluation of difference between MR-CNS and MS-CNS strains, between MSSA and MRSA strains, between CNS and *S.aureus* strains for fusidic acid susceptibility was performed with the Fisher's Exact test. The p value of <0.05 was selected for statistical significance.

RESULTS

The eighty-nine percent of *S.aureus* strains and 39% of CNS strains were found as sensitive to fusidic acid. Fusidic acid susceptibility rates of staphylococci strains were shown on Table 1. The difference among fusidic acid susceptibility rates of CNS and *S.aureus* strains was statistically significant ($p<0.001$). CNS strains were found more resistance than *S.aureus* strains for fusidic acid.

The seventy-two percent of the isolated *S.aureus* were defined as MSSA, 28% of the isolated *S.aureus* were defined as MRSA. Resistance to fusidic acid was observed in 20% (1 of 5) of MRSA isolates and 8% (1 of 13) of MSSA. The difference among fusidic acid susceptibility rates of MSSA and MRSA strains was not statistically significant ($p=0.305$) (Table 2).

The twenty-nine percent of the isolated CNS were defined as MS-CNS, 71% of the isolated CNS were defined as MR-CNS. While 45% of MR-CNS was resistant to fusidic acid, fusidic acid resistance was found to be 25% in MS-CNS. There was no statistically significant difference between MS-CNS and MR-CNS strains for fusidic acid susceptibility rates (p=0.490) (Table 3).

Table 1. Fusidic acid susceptibility rates of staphylococci strains.

Bacteria	S n (%)	R n (%)	p
S.aureus (n=18)	16 (89)	2 (11)	<0.001
CNS (n=41)	16 (39)	25 (61)	

S: sensitive; R: resistant

Table 2. Fusidic acid susceptibility rates of CNS strains.

Bacteria		S n (%)	R n (%)	p
CNS (n=41)	MS-CNS (n=12)	3 (25)	9 (75)	0.305
	MR-CNS (n=29)	13 (45)	16 (55)	

S: sensitive; R: resistant

Table 3. Fusidic acid susceptibility rates of *S.aureus* strains.

Bacteria		S n (%)	R n (%)	p
S.aureus (n=18)	MSSA (n=13)	12 (92)	1 (8)	0.490
	MRSA (n=5)	4 (80)	1 (20)	

S: sensitive; R: resistant

DISCUSSION

Fusidic acid is used in European Countries and Australia for a long time. It has also been used in other countries, except in the United States in recent years. Fusidic acid resistance has developed slowly, and the level of resistance and genetic mechanisms responsible generally reflect the time since introduction, indications for treatment, administration route, and prescribing practices widely throughout the world.[8]

Fusidic acid resistance has increased among *S.aureus* strains, including MRSA in the past twenty years. But, there are limited data concerning the relative importance in this process of the different staphylococcal determining factors that mediate resistance to fusidic acid. Moreover, the roles played by clonal dissemination of fusidic acid-resistant strains versus horizontal transmission of fusidic acid resistance determining factors have not been examined in detail.[9]

Previous studies related with fusidic acid resistance in strains isolated from clinical specimens have mainly focused on MSSA and other staphylococci.[5] Chen et al. recently reported that the prevalence of fusidic acid-resistance determinants was quite different between MRSA and MSSA groups.[10]

In spite of fusidic acid has been used on the world in recent ten years, has never been accepted in the United States. MRSA, with a long safety record has a great need for an oral MRSA antibiotic at the present time. In USA some drug companies worked to allow market exclusivity when this antibiotic is approved in the United States. A new dose arrangement that allowing fusidic acid to be used as monotherapy has been accepted, and it has been shown that fusidic acid resistance rates are reduced selectivity.[11] Fusidic acid resistance rates were lowest in the United States, where fusidic acid is not used routinely in clinical treatment. Also resistance rates were low in Australia and Canada, where fusidic acid has been used as drug for more than twenty years, the data were not especially elevated in Australia and Canada. This observation is in accordance with other reports that also noted that emergence of fusidic acid resistance hasn't been rapid, although its clinical use and show that this antimicrobial agent still provides a potentially useful treatment option for infections caused by multidrug-resistant gram-positive isolates (99.7% susceptibility among *S.aureus* strains), including MRSA strains. Fusidic acid resistance was viewed more frequently among MSSA isolates than among methicillin-resistant strains in the United States (0.6 and 0.1%, respectively). Conversely, fusidic acid resistance was higher among MR-CNS isolates than among MS-CNS isolates (9.2 and 5.2% for MR-CNS and MS-CNS, respectively). Fusidic acid resistance rate has not evaluated for Canada and Australia because of the decreased numbers of strains included in study. Moreover, 36.0% of the fusidic acid resistant *S.aureus* strains has got also methicillin resistance. Occurrence rates of fusidic acid resistance among *S.aureus* (0.3%) and CNS (7.2%) isolates were notably lower in the United States than in other two countries analyzed. *S.aureus* strains with elevated fusidic acid MIC values were slightly more

common in Australia than in Canada (7.0% for both countries), but the CNS resistance rates were different, with resistance being more common in Canada (20.0% versus 10.8% in Australia).[2]

In our country several studies have been done about fusidic acid resistance related to staphylococci (Table 4).

Table 4. Fusidic acid resistance rates of staphylococcal strains isolated in our country, in some studies.

	Year	MSSA	MRSA	MS-CNS	MR-CNS
Erdemoğlu et al.[15]	2000	3.2	7.7	10.8	14.9
Altun et al.[16]	2003	0	3	0	13
Şengöz et al.[17]	2004	1	9	21	33
Celen et al.[18]	2005	3	6	20	3
Nergiz et al.[19]	2007	-	-	-	28
Ekşi et al.[20]	2008	2.4	9.2	-	-
Mert Dinç et al.[21]	2009	-	1.4	-	-
Yaman et al.[22]	2010	4	6	-	-
Deveci et al.[23]	2011	4	1	14	8

MSSA: methicillin sensitive *S.aureus*, MRSA: methicillin resistant *S.aureus*

MS-CNS: methicillin sensitive coagulase negative staphylococcus

MR-CNS: methicillin resistant coagulase negative staphylococcus

Keşli et al.12 reported that 63% of *S.aureus* and 50 (66%) of CNS strains were methicillin resistant. Two (7%) of MRSA strains, 1 (6%) of MSSA strains, 16 (32%) of MR-CNS strains and 3 (12%) of MS-CNS strains were found to be resistant to fusidic acid. They indicated that fusidic has not to be excluded in preference of the antibiotic treatment of staphylococcal infections.

Kuzucu et al.13 investigated in vitro activity of fusidic acid in 112 MRSA and MR-CNS by disk diffusion and microdilution methods. 4% of MRSA and 27% MR-CNS were found to be resistant to fusidic acid.

In a study of Uluğ et al.14 resistance to fusidic acid has been found as 4.3% in MSSA strains, 16.7% in MRSA strains, 0% in MS-CNS, 36% in MR-CNS, however in none of the strains vancomycin, and teicoplanin resistance have been observed.

In conclusion, the present study is in accordance with other reports that also noted that emergence of fusidic acid resistance has not been rapid, despite its clinical use, and demonstrates that this antimicrobial agent still provides a potentially useful treatment option for infections caused by multidrug-resistant gram-positive isolates, including MRSA strains.

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