IN VITRO ACTIVITY OF QUINUPRISTIN-DALFOPRISTIN, METHICILLIN AND VANCOMYCIN AGAINST *STAPHYLOCOCCUS* STRAINS ISOLATED FROM CLINICAL SAMPLES

Klinik Örneklerden İzole Edilen *Staphylococcus* Suşlarına Karşı Kinupristin-Dalfopristin, Metisilin ve Vankomisinin in vitro Etkinliği

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Summary: *The aim of the study is to investigate susceptibility of staphylococci strains isolated from various clinical samples to quinupristin-dalfopristin. The ninety-eight strains of staphylococci [74 coagulase-negative staphylococci (CNSs) and 24 S. aureus] isolated from various clinical samples were included the study which had been sent to microbiology laboratory. Staphylococci strains were identified by using conventional methods. Methicillin and quinupristin-dalfopristin susceptibility of staphylococci strains were performed by Kirby-Bauer's disc diffusion method according to the Clinical and Laboratory Standards Institute criteria. Also, vancomycin susceptibility of strains was investigated by E-test method. Strain of S. aureus ATCC 25923 was used as the quality control strain. The fifty-three (72%) strains of the CNSs were defined as methicillin-resistant CNS (MR-CNS), three (13%) strains of S. aureus was defined as methicillin-resistant S. aureus (MRSA). The eight (15%) strains of MR-CNS were found resistant to quinupristin-dalfopristin, one (5%) strain of MS-CNS were found resistant to quinupristin-dalfopristin. None of MSSA or MRSA strains were resistant to quinupristin-dalfopristin. All of the strains were found as susceptible to vancomycin. Strains of staphylococci were found susceptible to quinupristin-dalfopristin at high rates. Consequently we think that quinupristin-dalfopristin combination may be an alternative option for treatment of resistant Gram-positive cocci infections like vancomycin.*

Keywords: *Quinupristin-dalfopristin, methicillin, staphylococcus, microbial susceptibility test, streptogramins.*

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Özet: *Bu çalışmada, çeşitli klinik örneklerden izole edilen stafilokok suşlarında kinupristin-dalfopristin duyarlılık oranlarının araştırılması amaçlanmıştır. Çalışmaya, mikrobiyoloji laboratuvarına gönderilen çeşitli klinik örneklerden izole edilen 98 stafilokok suşu [74'ü koagülaz negatif stafilokok (KNS) ve 24'ü S. aureus] dahil edildi. Stafilokok suşları konvansiyonel yöntemler ile tanımlandı. Stafilokok suşlarının metisilin ve kinupristin-dalfopristin duyarlılığı Clinical and Laboratory Standards Institute (CLSI) önerileri doğrultusunda Kirby-Bauer disk difüzyon yöntemiyle çalışıldı. Ayrıca suşların vankomisin duyarlılığı E-test yöntemi ile araştırıldı. Çalışmada kalite kontrol suşu olarak S. aureus ATCC 25923 kullanıldı. İzole edilen KNS'lerin 53(% 72)'ü metisiline dirençli KNS (MR-KNS) ve S. aureus'ların ise 3(%13)'ü metisiline dirençli (MRSA) olarak tanımlandı. MR-KNS'lerin 8(% 15)'i kinupristin-dalfopristine dirençli iken, metisiline duyarlı KNS'lerde (MS-KNS) kinupristin-dalfopristin direnci 1(%5) suşta tespit edildi. MSSA ve MRSA suşlarının hiçbirinde kinupristin-dalfopristin direnci tespit edilmedi. Suşların tamamının vankomisine duyarlı olduğu bulundu. Stafilokok suşlarının kinupristin-dalfopristine yüksek oranda duyarlı oldukları bulundu. Sonuç olarak vankomisin gibi kinupristin-dalfopristin kombinasyonun da özellikle dirençli Gram-pozitif kok enfeksiyonlarının tedavisinde alternatif olabileceğini düşünmekteyiz.*

Anahtar kelimeler: *Kinupristin-dalfopristin, metisilin, stafilokok, mikrobiyal duyarlılık testi, streptograminler.*

Resistance to antimicrobials has increasing become a problem from early 1970s. In the last four decades, treatment of infections caused by Gram-positive bacteria has been more problematic than previous. Nowadays, we have to cope with infections caused by multi-drug resistant microorganisms, especially methicillin-resistant staphylococci

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and vancomycin-resistant enterococci. There is an ongoing effort in pharmaceutical industry to develop new antimicrobial agents for therapy of resistant microorganisms. Linezolid, daptomycin, tigecycline, quinupristin-dalfopristin (Q-D), new glycopeptides (dalbavancin, telavancin and oritavancin), pristinamycin, iclaprim, ceftaroline and ceftobiprole are mainly therapeutic agents that are under use or clinical development (1).

Q-D is a new parenteral antimicrobial agent composed of two different streptogramin antibiotics (quinupristin and dalfopristin) that bind to different sites on the bacterial ribosome. Q-D have activity against a broad variety of multidrug resistant Gram -positive cocci, containing *S. aureus* and *S. epidermidis* (including methicillin-resistant strains), *Enterococcus faecium* (including vancomycin-resistant strains), *Streptococcus pneumoniae* (including penicillin-resistant strains) and other streptococci. This antimicrobial agent is remained as a good alternative in the therapy of infections caused by the resistant Gram-positive bacteria (2). Q-D combination which has not yet been utilized in Turkey, known as streptogramin, semisenthetic and the first antibiotic applied parenterally. By the Food and Drug Administration (FDA) in 1999, it was proposed for the treatment of vancomycin-resistant *E. faecium* (VREF) infections (3).

In this study, we aimed to investigate the efficacy of Q-D combination which is not in use in Turkey yet but probably in the near future will be one of the most often used antibiotics to infection diseases and to staphylococci.

MATERIALS AND METHODS

Bacterial isolates

In this prospective study, a total of 98 *Staphylococcus* strains (including 74 CNS and 24 coagulase positive *S. aureus*) were isolated from various clinical samples that had been sent to microbiology laboratory of Kiziltepe General Hospital between the date of February 2010 and April 2011. The clinical samples were inoculated onto 5% sheep blood agar (Oxoid Ltd., Basingstoke, UK) medium. The medium plates were incubated aerobically at 35 ± 2°C for 18-24 hours in the incubator. After incubation, identification of all isolates obtained from 5% sheep blood agar medium was performed by conventional methods such as colony morphology onto 5% sheep blood agar medium, gram staining, catalase and coagulase reactions. *S. aureus* ATCC 25923 was used as a standard strain of quality control.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *Staphylococcus* strains against methicillin and Q-D were performed by measuring of the inhibition zone diameter onto Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) medium aerobically at 35 ± 2°C for 18-24 hours using Kirby-Bauer's disc diffusion method accordance with CLSI recommendations (7). Methicillin susceptibility of strains was investigated with incubation of oxacillin (1 μg) and cefoxitin (30 μg) discs (Oxoid Ltd., Basingstoke, UK). Oxacillin inhibition zone diameter ≥ 13 mm was evaluated as sensitive, 11-12 mm was intermediate, ≤ 10 mm was resistant. Cefoxitin inhibition zone diameter ≥ 22 mm was evaluated as sensitive, ≤ 21 mm was resistant. Q-D susceptibility of strains was investigated with incubation of Q-D (15 μg) disc (Oxoid Ltd., Basingstoke, UK), and inhibition zone diameter ≥ 19 mm was considered as sensitive, 16-18 mm was intermediate, ≤ 15 mm was resistant. In addition, vancomycin susceptibility was investigated by determining the minimal inhibitory concentration (MIC) value using E-test method according to CLSI breakpoints (7). Vancomycin MIC value ≤ 2 μg/mL was evaluated as sensitive, 4-8 μg/mL was intermediate, ≥ 16 was resistant.

Statistical analysis

Statistical evaluation of difference between MR-CNS and MS-CNS strains, between CNS and *S. aureus* strains for Q-D susceptibility was performed with the *Fisher's Exact test*. The *p* value of < 0.05 was selected for statistical significance.

RESULTS

The twenty one (%28) strains of the CNSs were defined as methicillin-sensitive CNS (MS-CNS), 53(72%) strains of the CNSs were defined as methicillin-resistant CNS (MR-CNS), 21(84%) strains of *S. aureus* were defined as methicillin-sensitive *S. aureus* (MSSA), 3(16%) of *S. aureus* was defined as methicillin-resistant *S. aureus* (MRSA). The eight (15%) strains of MR-CNS were resistant to Q-D, one (5%) strain of MS-CNS were resistant to Q-D. The difference among Q-D susceptibility rates of MR-CNS strains and MS-CNS strains were not found statistically significant (p=0.430) (Table I). In MSSA and MRSA strains, resistance to Q-D was not detected (Table II). The difference among Q-D susceptibility rates of CNS strains and *S. aureus* were not found statistically significant (p = 0.107) (Table III). All the strains were found sensitive to vancomycin.

Table I. Distribution of Q-D susceptibility rates among CNS strains

Property	**S (Q-D) *n***	**R (Q-D) *n***	***p***
MS-CNS	20	1	0.430
MR-CNS	45	8	

MS-CNS: Methicillin-Sensitive Coagulase Negative Staphylococci
MR-CNS: Methicillin-Resistant Coagulase Negative Staphylococci
S (Q-D): Quinupristin-dalfopristin Sensitive
R (Q-D): Quinupristin-dalfopristin Resistant

Table II. Distribution of Q-D susceptibility rates among *S. aureus* strains

Property	**S (Q-D) *n***	**R (Q-D) *n***
MSSA	21	0
MRSA	3	0

MSSA: Methicillin-Sensitive *S. aureus*
MRSA: Methicillin-Resistant *S. aureus*
S (Q-D): Quinupristin-dalfopristin Sensitive
R (Q-D): Quinupristin-dalfopristin Resistant

Table III. Distribution of Q-D susceptibility rates among all *Staphylococcus* strains

Strains	**S (Q-D) *n***	**R (Q-D) *n***	***p***
CNS	65	9	0.107
S. aureus	24	0	

CNS: Coagulase Negative Staphylococci
S (Q-D): Quinupristin-dalfopristin Sensitive
R (Q-D): Quinupristin-dalfopristin Resistant

DISCUSSION

In recent years fluctations were observed in the antimicrobial resistance in the clinically important Gram-positive cocci, including staphylococci and enterococci (4). The proliferation of multi-drug resistant Gram-positive bacteria, including MRSA and VREF, has created an immediate need for effective alternative antibiotics. Q-D that has a selective antibacterial effectivity, primarily against Gram-positive aerobic bacteria, is a new combination of two different streptogramin derivates. It has been appreciated principally in emergency-use protocols, in hospitalized patients with skin and skin-structure infections, and in patients with VREF bacteremia (5). Quinupristin and dalfopristin are group B and A streptogramins, respectively, which act synergically: quinupristin blocks binding of aminoacyl-tRNA complexes to the ribosome whilst dalfopristin inhibits peptide bond formation and deforms the ribosome, promoting the binding of quinupristin (6).

Gram-positive pathogens, primarily *S. aureus,* CNSs, viridans group streptococci, and enterococci, are now the major reasons of infection in neutropenic haematology/oncology patients, but are frequently resistant to multiple antibiotic. From past to the present, glycopeptides have been a good alternative antimicrobial agent for the therapy of infections resulting from multi-drug resistant Gram -positive pathogens. Nevertheless, glycopeptides are not every time efficient and/or well tolerated, and have got nephrotoxic or ototoxic side effects. Q-D is a recently advertised streptogramin antibiotic that is active in vitro against most of the major Gram-positive pathogens causing infection in neutropenic patients. Q-D is active in vitro against the vast majority of recent isolates of relevant Gram-positive pathogens, including methicillin-resistant staphylococci, viridans group streptococci, and VREF, but except *Enterococcus faecalis*. Q/D is a potential alternative to glycopeptides in haematology or oncology patients with infection result from multi-drug resistant Gram-positive pathogens, particularly those who are unresponsive to, or intolerant of glycopeptides (7).

John et al. (8) reported that fifteen of 658 (2.3%) isolates were resistant to Q-D, but < 1% of the clinically most important isolates of *S. epidermidis, S. haemolyticus* and *S. hominis* demonstrated resistance to this agent. Hwang et al. (9) detected that all *S. aureus* including VISA, MRSA and MSSA were sensitive to Q/D. 96% of MR-CNS strains was sensitive to Q/D, 93% of MS-CNS strains was found also sensitive to Q/D.

Resistance to Q-D is very rare among staphylococci in the United States. Q-D demonstrates in vitro activity against a wide range of Gram-positive bacteria, including many isolates resistant to earlier antimicrobials. Surveys of isolates recovered through the year 2000 indicate that almost all strains of *S. aureus* (including MRSA) and CNS would be sensitive (10).

It has been reported that vancomycin and teikoplanin were detected most active antibiotic against Gram-positive cocci amongst other antimicrobial agents. Q-D combination might be as an alternative agent for treatment of infections due to resistant Gram-positive bacteria in the study of Doğanay et al. (11).

Doğruman-Al et al. aimed at determining the in vitro susceptibilities of 63 MRSA strains to Q/D and linezolid antibiotics and to determine the type B macrolide-lincosamide-streptogramine (MLSB) resistance. They obtained the bacterial isolates from various clinical samples of hospitalized patients in Gazi University Hospital. They found that all MRSA strains were sensitive to Q/D and linezolid in Gazi University Hospital (12).

Öksüz et al. investigated the in vitro susceptibility of 49 MRSA and 59 MR-CNS clinical strains to daptomycin, telithromycin, tigecycline, Q-D and linezolid. They have reported that all strains were found sensitive to daptomycin, Q-D and linezolid, thus, they suggested these antibiotics could be used as alternative to glycopeptides (13).

In conclusion, Q-D can be a reasonable alternative to *Staphylococcus* species infections in preventing the development of glycopeptides, and other antibiotics resistance that has been increased in recent

years. However, although this drug is not on market in Turkey, there is a need for rigid rules in selecting antibiotics because resistance to Q-D can develop in the future.

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