THE RELATIONSHIP BETWEEN COAGULASE PRODUCING TIME AND METHICILLIN SUSCEPTIBILITY IN *STAPHYLOCOCCUS AUREUS*

STAPHYLOCOCCUS AUREUS SUŞLARINDA METİSİLİN DUYARLILIĞI VE KOAGULAZ ÜRETİMİ ARASINDAKİ İLİŞKİ

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ÖZ

AMAÇ: *Koagulaz testi* ***Staphylococcus aureus*** *suşlarının tanımlanmasında ve ayrımında kullanılır. Bu şuşlarda metisilin direnci mortalite ve morbidite ile ilişkili bulunmuştur. Bu çalışmada* ***S. aureus*** *suşlarında metisilin duyarlılığı ve koagulaz üretimi arasındaki ilişki araştırılmıştır.*

GEREÇ VE YÖNTEMLER: *Çalışmaya 108* ***S. aureus*** *suşu alınmıştır. Suşların 43 tanesi metisilin dirençli ve 65 tanesi metisilin duyarlı idi. Koagulaz üretiminin saptanmasında tüp koagulaz testi kullanılmıştır. Tüpler birinci, ikinci, üçüncü, dördüncü ve 24. saatin sonunda pıhtı oluşumuna göre değerlendirilmiştir.*

BULGULAR: *Sonuçlar üç grupta değerlendirilmiştir. Grup 1: Pıhtı oluşturmayan grup, Grup 2: Zayıf pıhtı oluşturan grup, Grup 3: Güçlü pıhtı oluşturan grup. Birinci ve ikinci saatin sonunda metisilin duyarlılığında grup 2 ve 3 arasında anlamlı bir sonuç bulunamamıştır. Fakat grup2 ve 3 arasında üçüncü saatin sonunda anlamlı bir ilişki saptanmıştır (p=0,036). Grup 3' teki suşların çoğunun metisiline duyarlı olduğu görülmüştür.*

SONUÇ: *Çalışmanın sonuçlarına göre üçüncü saatin sonunda güçlü pıhtı oluşumu,* ***S. aureus*** *suşlarında metisilin duyarlılığı açısından yol gösterici olabilir.*

Anahtar kelimeler: Staphylococcus aureus, *fenotipik test, koagulaz test, metisilin duyarlılığı*

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ABSTRACT

OBJECTIVE: *Coagulase test is used for diagnosing and differentiating* ***Staphylococcus aureus*** *strains. Methicillin resistance is associated with significant mortality and morbidity for* ***S. aureus*** *infections. This study aimed to evaluate the relationship between coagulase producing time and methicillin susceptibility in* ***S. aureus.***

MATERIAL AND METHODS: *One hundred and eight* ***S. aureus*** *strains were included in the study. Fourty three strains were methicillin resistant and sixty five were methicillin susceptible. The tube coagulase test was used for detecting coagulase production. The tubes were examined for clot formation at the end of the first, second, third, fourth and twenty fourth hour.*

RESULTS: *Results were evaluated in three groups. Group 1: No clot formation, Group 2: Weak clot formation, Group 3: Strong clot formation. At the end of the first and second hour, there was no significant difference in methicillin susceptibility between group 2 and group 3. But there was a significant difference in methicillin susceptibility between group 2 and group 3 at the end of the third hour (p=0,036). Most of the bacteria in group 3 were susceptible to methicillin.*

CONCLUSION: *According to the results of our study, strong clot formation at the end of the third hour may be indicative for methicillin susceptibility of* ***S. aureus.***

Keywords: *Staphylococcus aureus, phenotypic test, coagulase test, methicillin susceptibility*

INTRODUCTION

Staphylococcus aureus is a bacterium which can be found on human skins and anterior nares and may cause serious infections. In developing countries, phenotypic tests are essential for the diagnosis of staphylococcal infections and also coagulase tests are usually confirmatory for *S. aureus* identification. Coagulase production is used as a diagnostic test, which differentiates *S. aureus* isolates from other staphylococci (1).

S. aureus produces two forms of coagulase: bound and free. Bound coagulase, or clumping factor, is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. Free coagulase, an extracellular protein enzyme that causes the formation of a clot when *S. aureus* colonies are incubated with plasma. Coagulase testing can be performed by using the slide coagulase (SCT) or the tube coagulase (TCT) tests. Bound coagulase is detected by the SCT, whereas free coagulase is detected by the TCT. The results of SCT and TCT are compatible (2). However conducted studies showed that coagulase production can not be detected by SCT in especially methicillin resistance *S. aureus* (MRSA) strains due to lack of clumping factor (3,4).

In this study, methicillin sensitive *S. aureus* (MSSA) and MRSA strains were evaluated by TCT. We attempted to establish a relationship between weak or strong coagulase producing time and methicillin susceptibility in *S. aureus.*

MATERIAL AND METHODS

In this study 108 *S. aureus* strains isolated from different clinical specimens were included. The strains were identified by VITEK 2 (bioMerieux, St. Louis, Missouri, USA) automated system. Also, strains were tested with DNase agar (Scharlau, Barcelona, Spain) and growth on mannitol salt agar (MSA) (Oxoid, Cambridge, UK).

Fourty-three strains were resistant and 65 were susceptible to methicillin. Coagulase test was performed according to the manufacturer's instructions. Each test was carried out in duplicate. Results were interpreted as follows:

Negative: No clot formation.

1+ positive: little, disorganised clot formation

2+ positive: the clot fills less than half the total volume occupied by the broth

3+ positive: the clot fills more than half but less than the total volume occupied by the broth

4+ positive: the clot fills the complate volume occupied by the broth

The tubes were examined for clot formation at the end of first, second, third, fourth and twenty fourth hours and the results were scored. The strains were divided into three groups according to their scores.

First group (group 1): negative

Second group (group 2): 1+ and 2+ positive results (weak coagulase producing group)

Third group (group 3): 3+ and 4+ positive results (strong coagulase producing group)

In this study, S.aureus ATCC 25923 strain was used for positive control and S. epidermidis ATCC 12228 strain was used for negative control.

Chi-square test was used for comparing groups.

RESULTS

All the strains grown in MSA (growth of yellow colonies on MSA) and were DNase positive. (DNase agar clear zones around the bacterial colonies indicated DNase positive colonies.)

All the strains produced clot formation within 24 hours. 106 bacteria had produced clot formation in the first four hours. Only two bacteria produced clot formation at the end of 24 hours.

Clot structure was lost at the end of 24 hours in 28 of clot-forming bacteria in the first four hours.

At the end of first and second hours, 24 and 90 bacteria produced clot formation respectively. There were no significant difference between groups according to methicillin susceptibility at first and second hours. (p=0,360 and p=0,627 respectively) Similarly there were no significant difference at fourth and 24th hours (p=0,287 and p=0,469 respectively) (Table 1).

Table 1: The coagulase producing time and methicillin susceptibility of *S. aureus*

Time	Group 1		Group 2		Group 3		Total	
	MR n (%)	MS n (%)	MR n (%)	MS n (%)	MR n (%)	MS n (%)	MR n (%)	MS n (%)
First hour	32 (75)	52 (80)	10 (23)	10 (15)	1 (2)	3 (5)	43 (100)	65 (100)
Second hour	9 (20)	9 (13)	14 (33)	26 (40)	20 (47)	30 (47)	43 (100)	65 (100)
Third hour	5 (11)	-	11 (26)	8 (12)	27 (63)*	57 (88)*	43 (100)	65 (100)
Fourth hour	3 (7)	-	8 (19)	8 (12)	32 (74)	57 (88)	43 (100)	65 (100)
Twenty-fourth hour	3 (7)	25 (38)	11 (26)	14 (22)	29 (67)	26 (40)	43 (100)	65 (100)

MR: Methicillin resistant, MS: Methicillin susceptible
**p<0.05*

However at the end of third hour, 8 of the 19 strains (42.11 %) at group 2 were susceptible to methicillin and 57 of 84 strains (67.86 %) at group 3 were susceptible to methicillin. The difference between groups were significant (p=0,036) (Table 1).

S. aureus ATCC 25923 strain was negative at the end of first hour, 3+ at the end of second and third hour, 4+ at the end of fourth hour and 2+ at the

end of 24th hour for clot formation.
S. epidermidis ATCC 12228 strain was negative for clot formation during test period.

DISCUSSION

S. aureus is distinguished clinically from other strains of staphylococci by the coagulase test (1). This test, first described in 1903, has been studied with great interest for more than a century. The genes that produce this effect are important virulence factors during the pathogenesis of *S. aureus* infections, enabling the formation of abscesses for staphylococcal replication and the depletion of clotting factors from blood (5).

Coagulase testing is the single most reliable method for identifying *S. aureus*. Coagulase production can be detected using either SCT or TCT (2). The TCT test remains a simple, cheap, rapid, and reliable method available for all diagnostic laboratories (6). The TCT with diluted rabbit plasma is the basic confirmation of an identification of *S. aureus.* Rabbit plasma diluted 1: 5 is mixed with an equal volume of broth culture or growth from colonies on agar and subsequently incubated at 37° C and examined for clot formation at the end of first, second, third, fourth and 24th hours (4).

Methicillin resistant *S. aureus* was first discovered in 1961, and outbreaks of MRSA have been reported since the 1970s (7-9). MRSA infection is associated with significant mortality and morbidity (10). Lally and Woolfrey found that some strains of MRSA may be particularly deficient in clumping factor (11). So coagulase production may not be detected by SCT in these strains.

To the best of our knowledge there is no data regarding evaluation of these strains by TCT. In this study, the relationship between methicillin susceptibility and weak/strong clot formation time in *S. aureus* strains was evaluated by TCT.

In this study at the end of third hour, MSSA strains produced stronger clot formation than MRSA strains and the difference was statistically significant.

As a conclusion, according to the results of our study, TCT especially at the end of the third hour is not absolutely definitive but may be directive for methicillin susceptibility of *S. aureus.* Coagulase test which is used for diagnosis of staphylococci strains may also be used for methicillin susceptibility.

Resistance to methicillin and coagulase production have different pathways in *S.aureus.* For this reason that more comprehensive studies are needed for supporting this result.

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