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Investigation of Rapid Identification of Metisillin Resistance of Staphylococcus Aureus (MRSA) in Blood Culture Bottles at Three Different Pre-Incubation Periods

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BACKGROUND/AIMS
The aim of this study was to determine the early signal of methicillin-resistant Staphylococcus aureus (MRSA) growth in blood culture bottles.

MATERIALS and METHODS
The MRSA infections should be determined specially for the hospitalized patients to decrease the rates of mortality and morbidity. 19 MRSA isolates were included in the study. Strains were identified using the Phoenix 100 system (Becton-Dickinson Diagnostic Systems, Sparks, MD, USA). Three pre incubation grades were analysis: group I: 4°C, group II: 25°C, and group III: 37°C. Blood culture bottles were pre-incubated for 24 hours prior to the inoculation with MRSA. Contaminated bottles were incubated in the Bactec 3D Alert (Becton-Dickinson Diagnostic Systems, Sparks, MD, USA).

RESULTS
As indicated by the three different pre-incubation groups, the initial signals were detected at 37°C. The MRSA were grown for 12 hours in 37°C blood culture bottles.

CONCLUSION
Blood culture bottles which thermostatized at 37°C during 24 hours could give the early signal to determine the MRSA.

Keywords: Blood culture, incubation, storage

INTRODUCTION

Staphylococcus aureus is a commensal organism that lives on the skin, upper respiratory system, lower urogenital system, and digestive system in human (1). In 1930, methicillin was initially used for therapeutic treatment, and after 1 year, "methicillin-resistant *S. aureus* (MRSA)" were identified (1). Studies indicated that hospital infections due to MRSA had higher mortality rates than other hospital infections. Nowadays, most of the studies are based on this fact.

In blood infections, the identification of the isolated microorganisms and results of the antibiotic susceptibilities should be analyzed as soon as possible and results should be given to clinicians. Although new technology has been developed for the identification of the microorganisms associated with bacteremia and fungemia, the blood culture system remains the most reliable and practical method (2), as most of the hospitals and laboratories use blood culture systems.

The aim of the study to determine the time of detection (TTD) of an early signal of MRSA growth in blood culture bottles pre-incubated at different temperatures (4°C, 25°C, and 37°C) within 24 hours of inoculation with MRSA strains.

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MATERIALS/PATIENTS AND METHODS

19 MRSA strains were included in the study from the Microbiology Laboratory of the University Hospital using the Phoenix 100 (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) system. Three pre-incubation temperatures were assigned: group I: 4°C, group II: 25°C, and group III: 37°C. Blood culture bottles were pre-incubated 24 hours before inoculation with MRSA strains. Each group included the blood culture bottles inoculated with MRSA and one non-contaminated bottle as a negative control. The MRSA strains were resuspended in brain heart infusion broth according to the McFarland 0.5 turbidity. Ten milliliters of MRSA suspension was added in each blood culture bottle. Both inoculated and negative control bottle were incubated in the Bactec 3D Alert system (Becton–Dickinson Diagnostic Systems, Sparks, MD, USA). The values for the TTD rates were correlated with the pre-incubation time and storage temperature. Since this study was laboratory based study, we did not use any human materials. So ethic committee and informed consent aren't necessary. This study was performed in accordance with the principles of Declaration of Helsinki.

Statistical Analysis

The SPSS(Statistical Package for Social Sciences) software program version 3.0 (SPSS Inc.; Chicago, IL, USA) was used for statistical analyses. Variation analysis (ANOVA) was used for mean differences. Data were expressed as a mean value (standard deviation), minimum-maximum, and percentage where appropriate. A p value of <0.05 was considered statistically significant.

RESULTS

Depending on the three different pre-incubation groups, the early signals were seen at the 37°C (ANOVA, Tukey's multiple comparison test, F=8.927, dF=6.084; **p<0.05). No statistical relation was noted between pre-incubation at 4°C and 25°C (p>0.05) (Figure I)

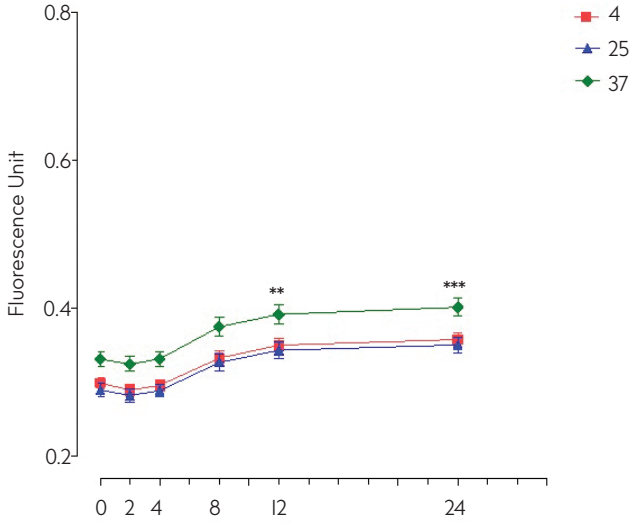

FIGURE I. The time of detection of three different pre-incubated blood culture bottles (ANOVA, Tukey's multiple comparison test, F=8.927, dF=6.084, p<0.001).

DISCUSSION

One of the main reasons for morbidity and mortality in hospitalized patients is blood stream infections (3). Various studies have shown that the mortality rates associated with bacteremia were 20%–50% (4). Therefore, the essential mission of the clinical microbiology laboratory is the correct interpretation and the earliest reporting of the blood culture results (3).

S. aureus is the most frequently isolated organism among the gram-positive bacteria in bacteremia (2). The most frequently isolated microorganisms in Turkey are *Escherichia coli* and the coagulase-negative Staphylococcus (CNS) (4). In a previous study performed in our laboratory (still in press), from a total of 454 blood cultures, 50 (11.01%) were culture positive. Among the positive cultures, 41 (82%) were gram-positive and 9 (18%) were gram-negative bacteria. The most frequently isolated gram-positive bacteria were *S. aureus*, (24.39%) and *S. hominis* (24.39%). oxacillin resistance was 82.93% in all *Staphylococcus* species.

Depending on the results from the three pre-incubation groups, TTD were seen at the 37°C blood culture bottles. MRSA growth were observed after 12 hours of incubation in bottles which pre-incubated at 37°C (ANOVA, Tukey's multiple comparison test, F=8.927, dF=6.084; **p<0.01, ***p<0.001; Figure I).

Koh et al. (5) indicated that the blood culture bottles that were pre-incubated at 37°C during the night ensure earlier final reports. Also, Lee et al. (6) reported that TTD was influenced by the pre-incubation temperature and duration rather than the colony-forming unit quality or bottle type. The prevalence of MRSA infections varies between countries. Scandinavia and the Netherlands have a low prevelance of MRSA infections, however in the USA, the prevelance of MRSA infections was 25% although Canada the prevelance rate was less than 5% (7). In Turkey, the prevalence of MRSA is >30% (8). Türk Dağı et al. (9) reported that the prevalence of MRSA infections was 42.5% in blood cultures. Earliest identification of MRSA are important for blood culture infections (3). The average reproduction time of the gram-positive and negative bacteria and yeasts is 18-19 hours, 15-19 hours, and 23-41 hours, respectively (10). Although, molecular techniques, such as nucleic acid probes and polymerase chain reaction (PCR), are used for rapid detection, the blood culture systems remain the most reliable and sensitive (3). Molecular tests that can be used include FISH, real-time PCR, MALDI-TOF and PNA-FISH (11).

Seegmüller et al. (12) reported that *Haemophilus influenzae, S. pneumoniae, Enterobacteriaceae, S. aureus, Enterococcus faecalis*, and *Candida glabrata* had similar sensitivity between pre-incubation of blood culture bottles at room temperature and at 36°C (12). Akan et al. (13) reported that if the blood culture processing step was delayed, the bottles may be stored at 22°C. According to the present study results, the blood culture bottles might be pre-incubated at 37°C for 12 hours to rapidly identify bacteremia. If we reduce the reproduction time of the MRSA detection, the clinician can be notified of the infection without delay. On the other hand, the storage of blood culture bottles recommended by the firm at 4°C. Further studies will be needed to focus on If the medium into the blood culture bottles will be affected at during 24 hours at 37°C."

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

Informed Consent: N/A

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