

# Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates; evaluation of colorimetric Quicolor ES agar and determination of breakpoint inhibition zone diameters of cefoxitin

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**Abstract** In this study, it was aimed to evaluate colorimetric Quicolor ES agar for the rapid detection of methicillin resistance and to determine susceptibility and resistance breakpoint zone diameters for cefoxitin by using 51 methicillin susceptible *Staphylococcus aureus* (MSSA) and 63 methicillin resistant *S. aureus* (MRSA) isolates. In the study, while oxacillin and cefoxitin results were obtained within 4–7 h (5.5 h in average) for MSSA isolates, the results of MRSA isolates were obtained within 5.5–9 h (6.6 h in average) for both antibiotics on QC ES agar. QC ES agar is an inexpensive medium for rapid detection (4–9 h) of methicillin resistance by disc diffusion method using oxacillin or cefoxitin. Additional studies for further evaluation of the efficiency of QC-ES agar in rapid determination of methicillin resistance in *S. aureus* may be beneficial.

**Keywords** Methicillin · Oxacillin · Cefoxitin · MRSA · MSSA · Quicolor ES agar

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important infectious agent related to both hospital and community-acquired infections and, it has a gradually increasing prevalence. Bacteremia and endocarditis are major life threatening infections caused by *S. aureus*. (Bennett and Sharp 2008; Carroll 2008; Libert et al. 2008; Shurland et al. 2007). Therefore, especially in bloodstream infections caused by *S. aureus*, early detection of oxacillin susceptibility has an important role for early initiation of appropriate treatment protocols (Bennett and Sharp 2008).

In routine clinical microbiology laboratories, disc diffusion method with oxacillin or cefoxitin, recommended by CLSI (2008), is used to determine methicillin resistance. In addition, CLSI also recommends MIC determination of both antibiotics by microdilution and agar screening test with oxacillin salt for susceptibility testing. However, CLSI recently recommends testing of cefoxitin instead of oxacillin because of problems encountered with oxacillin in these tests (CLSI 2005).

Phenotypic tests such as disc diffusion or microdilution are generally used in routine determination of MRSA. Unfortunately, susceptibility testing by these methods requires 24 h (CLSI 2008). Recently, colorimetric media which yield susceptibility testing in approximately 5–10 h were developed and started to use (Coban et al. 2006; Ercis et al. 2007). Quicolor agar (QC agar; Salubris Inc., Massachusetts; [www.salubris.com](http://www.salubris.com)), is one of these media which changes its color within 4–6 h due to metabolic activities of proliferating bacteria. QC agar has two types as QC ES and QC NF agar. QC ES agar is used for the antibiotic susceptibility testing of enterobacteriaceae and staphylococci. The original color of the medium is red which turns into yellow in the case of bacterial growth (Ercis et al. 2007).

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The first goal of this study was to determine the time required and accuracy of determination of methicillin resistance by QC ES. The second goal was to determine the breakpoint diameters of the inhibition zone of cefoxitin on QC ES agar that can distinguish MRSA from MSSA.

## Materials and methods

### Bacterial isolates

In the study, 51 methicillin susceptible and 63 methicillin resistant *S. aureus* strains isolated from various clinical specimens were tested. *S. aureus* ATCC 29213 (methicillin susceptible) and ATCC 43300 (methicillin resistant) strains were used as controls. It was previously determined by PCR that all MRSA isolates possessed *mec A*.

### Phenotypic determination of methicillin resistance

Phenotypically, methicillin resistance was determined by disc diffusion method according to CLSI (2008). Susceptibility of the strains to both oxacillin and cefoxitin was determined. Briefly, the suspension prepared from overnight fresh bacterial culture, having 0.5 McFarland turbidity was spread onto Mueller-Hinton agar medium and after 15 min, oxacillin (1 µg) and cefoxitin (30 µg) discs were placed. The plates were incubated at 35°C for 24 h.

### Determination of methicillin resistance by QC ES agar

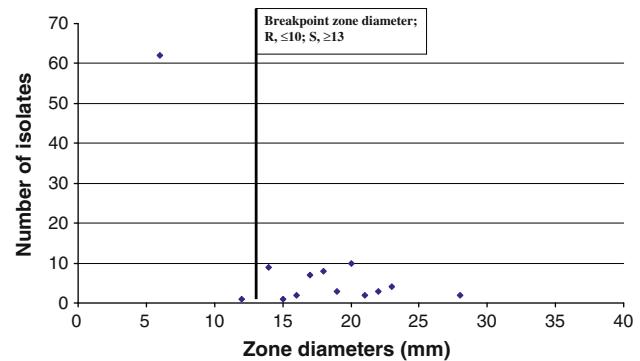
QC ES agars were obtained from Salubris A.S. (Istanbul, Turkey). Bacterial growth changes the red color of QC ES agar to yellow before visible bacterial growth is observed (Fig. 1). The application of the test was performed as instructed in the package insert. Bacteria to be tested were from fresh culture as recommended. Media were brought to room temperature before inoculation. Bacterial suspensions, prepared at approximately 1 McFarland turbidity (0.5–1) in sterile saline solution from fresh cultures, were spread thoroughly in three directions at 60° angle onto QC ES agar by the help of a swab. Then, oxacillin (1 µg) and

cefoxitin (30 µg) discs were placed. Plates were incubated at 35°C and controlled every 30 min beginning at the third hour. Test was terminated when color change was observed and the duration of time to detection, was recorded (Fig. 1).

## Results

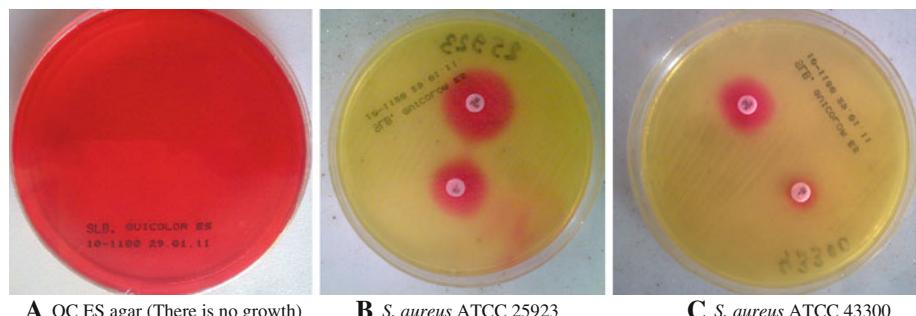
According to the phenotypic method defined in CLSI; oxacillin inhibition zone diameters of 51 MSSA isolates in which *mec A* gene has not been detected were between 14–28 mm (14 mm in 9 isolates; 15 mm in 1 isolate; 16 mm in 2 isolates; 17 mm in 7 isolates; 18 mm in 8 isolates; 19 mm in 3 isolates; 20 mm in 10 isolates; 21 mm in 2 isolates; 22 mm in 3 isolates; 23 mm in 4 isolates and 28 mm in 2 isolates) (Fig. 2). Cefoxitin inhibition zone diameters were between 22–35 mm (22 mm in 1 isolate; 24 mm in 2 isolates; 25 mm in 4 isolates; 27 mm in 6 isolates, 28 mm in 6 isolates; 29 mm in 1 isolate; 30 mm in 21 isolates, 31 mm in 1 isolate; 32 mm in 1 isolate; 33 mm in 4 isolates; 34 mm in 1 isolate and 35 mm in 3 isolates) (Fig. 3).

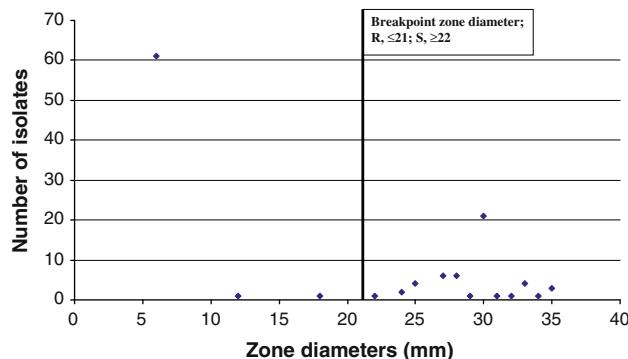
In 63 MRSA isolate, in which *mec A* gene was detected, oxacillin zone diameter was 6–12 mm; no zone diameter was observed in 62 isolates and 12 mm in 1 isolate (Fig. 2). Cefoxitin inhibition zone diameters were between



**Fig. 2** Distribution of oxacillin inhibition zone diameters of MSSA and MRSA isolates in MHA agar

**Fig. 1** QC ES agar (uninoculated) (a), oxacillin and cefoxitin susceptible (b) and resistant (c) standard strains on QC ES agar



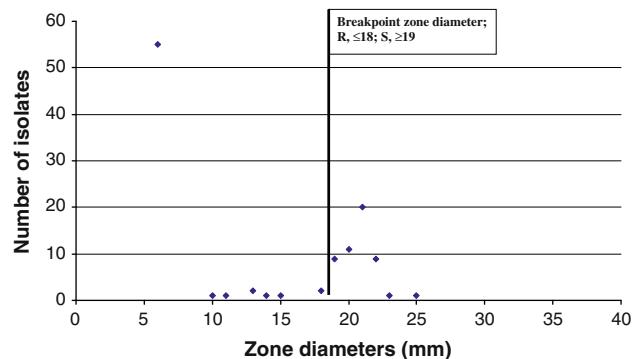


**Fig. 3** Distribution of cefoxitin inhibition zone diameters of MSSA and MRSA isolates in MHA agar

6–18 mm (there was no inhibition zone in 61 isolates, 12 mm in 1 isolate and 18 mm in 1 isolate) (Fig. 3).

Oxacillin inhibition zone diameters of 51 MSSA isolates lacking *mecA* gene were between 13–21 mm in QC ES agar (it was 13 mm in 11 isolates, 14 mm in 11 isolates, 15 mm in 20 isolates, 16 mm in 5 isolates, 17 mm in 3 isolates and 21 mm in 1 isolate) (Fig. 4). Cefoxitin inhibition zone diameters were between 19–25 mm (it was 19 mm in 9 isolates, 20 mm in 11 isolates, 21 mm in 20 isolates, 22 mm in 9 isolates, 23 mm in 1 isolate, 25 mm in 1 isolate) (Fig. 5). Oxacillin inhibition zone diameter is defined as susceptible by the manufacturer if is  $\geq 13$  mm. In our study, inhibition zone diameters of isolates that were detected to be susceptible to oxacillin in Mueller–Hinton agar were between 13–21 mm in QC ES agar and thus they were all determined to be methicillin susceptible in this medium.

Oxacillin inhibition zone diameters of 63 MRSA isolates with *mecA* gene were between 6–12 mm in QC ES agar (there was no zone in 60 isolates, 10 mm in 1 isolate and 12 mm in 2 isolates) (Fig. 4). Cefoxitin inhibition zone diameters were between 6–18 mm (there was no zone in 55 isolates, 10 mm in 1 isolate, 11 mm in 1 isolate, 13 mm in 2 isolates, 14 mm in 1 isolate, 15 mm in 1 isolate and

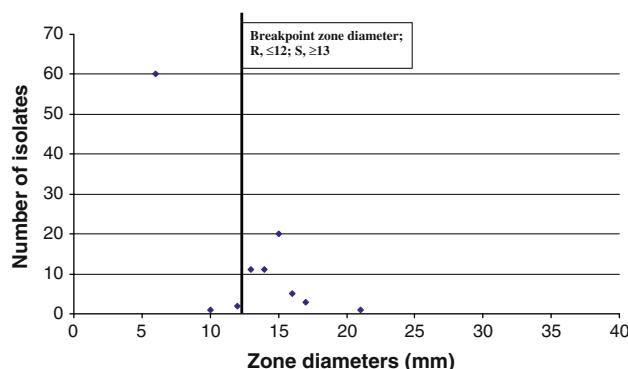


**Fig. 5** Distribution of cefoxitin inhibition zone diameters of MSSA and MRSA isolates in QC-ES colorimetric agar

18 mm in 2 isolates) (Fig. 5). Oxacillin inhibition zone diameters is defined as resistant by the manufacturer if is  $\leq 12$  mm. Inhibition zone diameters of 63 MRSA isolates were between 6–12 mm.

Determination of methicillin resistance by using cefoxitin disc in QC ES agar was defined by the manufacturer; however breakpoint inhibition zone diameters were not determined for cefoxitin previously. One of the goals of this study was to determine the breakpoint inhibition zone diameters for cefoxitin. According to disk diffusion results we have obtained, we have determined that if cefoxitin zone diameter of  $\leq 18$  mm was defined as resistant and if  $\geq 19$  mm, was defined as susceptible, than all the results would become 100% concordant with the results obtained by Mueller–Hinton medium.

In the study, while oxacillin and cefoxitin results for MSSA isolates were obtained in QC ES agar within 4–7 h (5.5 h in average), the results of MRSA isolates were obtained within 5.5–9 h for both antibiotics (6.6 h in average). The results of resistant strains were obtained approximately 1 h later compared to susceptible isolates. To summarize, methicillin resistance can be determined approximately in 4–9 h using QC ES by oxacillin and cefoxitin discs.



**Fig. 4** Distribution of oxacillin inhibition zone diameters of MSSA and MRSA isolates in QC-ES colorimetric agar

## Discussion

In recent years, automated systems are used in the determination of antibiotic susceptibilities, especially in developed countries. Vitek 2 (bioMe'rieux, Durham, NC) and BD Phoenix (BD Diagnostic Systems, Sparks, MD) are the most widely used systems. Susceptibility panels of these systems include oxacillin and cefoxitin. Nevertheless, they are expensive systems and have usage limitation due to economical impact in developing countries. Commercial molecular methods are also available for the rapid determination of methicillin resistance. Most commonly used

products are BD GeneOmh™ MRSA (BD Diagnostic, San Diego, CA), GeneXpert® MRSA (Cepheid, Sunnyvale, CA), Hyplex *Staphylo* Resist® (Biologische Analysensystem GmbH, Lich, Germany), BD GeneOmh™ StaphSR (SR; BD GeneOmh, San Diego, CA). In addition, latex agglutination test is also used for determination of PBP 2a (Carroll 2008; Murray et al. 2003). The high cost, requirement of advanced equipments and experienced staff for these commercial systems limit their usage in developing countries.

QC ES agar is a medium that yields antibiotic susceptibility results based on disc diffusion method within 4–6 h. Oxacillin inhibition zone diameters are defined as resistant by the manufacturer if it is  $\leq 12$  mm and susceptible if  $\geq 13$  mm. CLSI recommends breakpoint zone diameters of  $\leq 10$  mm for resistance and  $\geq 13$  mm for susceptibility on Mueller–Hinton agar. In this study, data obtained by using QC ES agar were evaluated as susceptible or resistant according to the values specified by the manufacturing company. All results were completely concordant for all MRSA and MSSA isolates. Oxacillin results for MSSA isolates on QC ES agar were obtained within 4–7 h (5.5 h on the average). Susceptibility profile for MRSA isolates became available in 5.5–9 h (6.6 h in average). Coban et al. (2006) developed two MIC-based colorimetric methods to determine oxacillin susceptibility (rezausurin microplate method and nitrate reductase test). Absolute agreement ratios of both methods were 96–100% and the results were obtained within 6 h. When Baker and Tenover (1996) had tested Alamar colorimetric broth microdilution susceptibility test methods and, absolute agreement had been reported to be 100% for oxacillin in their studies. However, susceptibility results by this method can only be obtained after 24 h.

The usage of cefoxitin for the determination of methicillin resistance is defined in 2005 by CLSI (2005). In this document, resistance was defined as  $\leq 19$  mm of inhibition zone diameter and, susceptibility was defined as  $\geq 20$  mm of inhibition zone diameter. CLSI changed these breakpoint inhibition zone diameters as  $\leq 21$  mm for resistance and  $\geq 22$  mm for susceptibility in 2008 (CLSI 2008). In this study, the results of the tested isolates were interpreted according to latest CLSI recommendation. Breakpoint inhibition zone diameters for cefoxitin were not defined for QC ES agar until this study. Cefoxitin breakpoint inhibition zone diameters determination was one of the goals of the study. When obtained data was evaluated according to *mecA* presence in the isolates, cefoxitin inhibition zone diameter was defined as resistant if it is  $\leq 18$  mm and

susceptible if it is  $\geq 19$  mm. While cefoxitin results were obtained within 4–7 h (5.5 h on the average) for MSSA isolates on QC ES agar, it was obtained within 5–9 h (6.6 h in average) for MRSA isolates. The results of resistant strains seem to be obtained in average 1 h later than the susceptible isolates. Considering all susceptible and resistant isolates, we can conclude that methicillin resistance may be determined between 4 and 9 h using QC ES agar by oxacillin and cefoxitin discs.

In conclusion, QC ES agar is an inexpensive rapid tool that is easy to apply and may be used for rapid determination of methicillin resistance. This is very important especially for critically ill patients with sepsis or bacteraemia. Multi-center studies may be advised for further evaluation of the efficiency of QC-ES agar for rapid determination of methicillin susceptibility of *S. aureus*.

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