

Author response to laboratory detection of MRSA. Comment on “Antibiotic susceptibility of vancomycin and nitrofurantoin in *Staphylococcus aureus* isolated from burnt patients in Sulaimaniyah, Iraqi Kurdistan”

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Question 1

The authors have used 2 McFarland turbidity to perform the Kirby Bauer disc diffusion which is four times the CLSI recommended turbidity of 0.5 McFarland.

Answer 1

This is a typing mistake; actually we used 0.5 McFarland (1.5×10^8 CFU/ml) tube, as a standard turbidity for Antibiotic susceptibility testing as it was recommended in CLSI (CLSI, 2007) and (Morello et al, 2003).

Question 2

The study was done between 2008 and 2011 and the authors used 2007 CLSI guidelines for interpretation which is outdated and the results based on it are not relevant.

Answer 2

The reason for using 2007 CLSI guidelines for interpretation of inhibition zone result of antibiotic discs is related to the fact that we had only that copyright of CLSI. However, we have used it after supporting and confirming all the diameters of antibiotic inhibition zones by other practical reference books (CLSI, 2007; Verhaegen et al, 2003).

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Question 3

The incubation temperature for oxacillin disc diffusion (DD) is 33°C-35°C & not 37°C. Testing at temperatures above 35°C may not detect MRSA. Oxacillin DD plates should be incubated for 24 hours before reading the zones of inhibition.

Answer 3

That is right; this is also a typing mistake. It was confused with growth temperature for bacterial isolation and identification. Whereas, in reality 35°C was used for testing all antibiotic discs according to these references (CLSI, 2007; Verhaegen et al, 2003); this temperature is ideal for optimal bacteria growth, and for preventing some antibiotic inactivation such as oxacillin. Whereas, for incubation time, as it is stated in materials and methods, we referred to (Verhaegen et al, 2003). According to (Verhaegen et al, 2003) reference during emergencies, a preliminary report may be made after 6 hours. However, this is not routinely recommended, and as it is usual the final report is always provided after conventional incubation time. In our study, for confirmation of test results we read the results after 18 and 24 hours. No variations have been recorded between the two time points (18 and 24 hours).

Question 4

Oxacillin DD has a sensitivity of only 91% and specificity of only 58.9% while cefoxitin DD has sensitivity and specificity of 97.8% & 100% re-

spectively. CLSI recommends the use of cefoxitin DD method for detection of MRSA.

Answer 4

As shown in Table 2 of the manuscript, *S. aureus* showed 88.9 % resistance and 11.1% sensitive to Oxacillin DD. Cefoxitin was never tested in our laboratory.

Question 5

The authors never made an effort to detect (erythromycin) inducible resistance to clindamycin which has got immense clinical significance.

Answer 5

That is correct! The detection of (erythromycin) inducible resistance to clindamycin is clinically important, but we have not done that at this time. As Dr Kumar knows, one paper cannot answer all questions raised by the scientific community. However, we will keep in mind this comment and we will address it in future works.

Question 6

The authors never mention beta-lactamases testing for their isolates. Nitrocefin disc test should have been used to test for beta-lactamases. Hyperproducers are resistant to oxacillin by disc diffusion resulting in false positive MRSA test results.

Answer 6

The reason why we are not testing beta-lactamase activity for these isolates is the following:

1. In this study we had more than one objective to be focused on, that is, which factors are associated with *Staphylococcus aureus* infection; which sites are more frequently infected by this pathogen, and the antibiotic resistance and its association with the site of infection, gender and age of patients;
2. DD method is useful and gives results according to CLSI, Table page 46.

TABLE

Disk Content	Zone diameter, nearest whole mm			Equivalent MIC Breakpoints ($\mu\text{g}/\text{mL}$)	
	R	I	S	R	S
1 μg oxacillin	≤ 17	-	≥ 18	≥ 0.5	≤ 0.25

Other tests are required only when intermediate result has been obtained. In this case you should to perform other tests such as the cefoxitin disk test, an oxacillin MIC test, or the oxacillin-salt agar screening test. Otherwise the result is acceptable without any problem.

Question 7

CLSI has done away with vancomycin DD and recommends only MIC testing. Therefore the data presented on vancomycin susceptibility based on DD is not valid.

Answer 7

Results of DD test for vancomycin are indicated for 1062 isolates; only 5 isolates showed resistance to vancomycin, which means that 1057 isolates are sensitive to vancomycin and inhibition zone is greater than 14 mm. Whereas, MIC testing is recommended when inhibition zone is equal or less than 14 mm. This fact is also mentioned at CLSI, page 44 (**All *Staphylococcal* isolates for which vancomycin zone diameters are 14 mm or less should be tested by reference MIC method**).

Question 8

The title of the article is highly misleading as the vancomycin DD is no longer recommended for susceptibility testing and nitrofurantoin can only be used for treating urinary tract infection which formed only 0.4% of the isolates in the study.

Answer 8

We disagree with Dr. Kumar on this point. The title was decided based on the results of our study, and we have to consider them.

REFERENCES

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