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COMPARISON OF CEFOXITIN AND OXACILLIN DISKS DIFFUSION TEST FOR DETECTION OF MRSA STRAINS

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Abstract:-

It is very important for clinical microbiology laboratories to be able to detect MRSA strains accurately and reasonably fast, for the adjustment of antibiotic treatment, and for the implementation of infection control measures. Detection of the mecA gene or PBP2a is considered the gold standard for detecting MRSA. However, many laboratories throughout the world do not have the capacity to use molecular techniques to detect MRSA in routine clinical practice. This study evaluated the performance of the cefoxitin and oxacillin DD test in determining methicillin resistance in comparison to the BD Phoenix automated system (BD, Sparks, MD). A total of (359) S. aureus strains which were isolated from were included in this study. All isolates were tested for methicillin resistance by cefoxitin DD test and oxacillin DD test considering the BD Phoenix automated system as gold standard. Among the (359) S. auresus isolates 146 (40.7%) isolate were identified as MRSA by the BD Phoenix automated system (BD, Sparks, MD). Cefoxitin DD test showed 100% sensitivity and 100% specificity, while oxacillin DD test showed 97.5% sensitivity and 98.6% specificity. In a laboratory where it is not possible to carry out molecular method as a routine, cefoxitin disk diffusion test is a good surrogate marker for detecting methicillin resistance. It is far superior to most of the currently recommended phenotypic method. It is now an acceptable method for detection of MRSA by many reference groups including CLSI.

Keywords: - *MRSA; Oxacillin DD test; Cefoxitin DD test; BD Phoenix.*

INTRODUCTION:

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major causes of nosocomial infections in world [1, 2, 3,4]. Hence Rapid and accurate detection of MRSA is an important role of clinical microbiology laboratories to avoid treatment failure and to control the endemicity of MRSA [5]. Soon after the first reports of MRSA in 1961 the unusual behavior of the strains in susceptibility tests was noted [6, 7]. Early reports indicated that MRSA were heterogeneous in their expression of resistance to β-lactam agents [7, 8, 9, and 10]. Heterogeneous resistance to methicillin occurs due to variations in the expression of the *mecA* gene, which encodes penicillin binding protein 2a (PBP2a) [11, 12]. Detection of the *mecA* gene or PBP2a is considered the gold standard for detecting MRSA [13]. There are many traditional and commercial systems for detection of MRSA in clinical microbiology laboratories include the disk diffusion, E-test, broth microdilution, chromogenic agar medium, oxacillin agar medium, latex agglutination , and the detection of the *mecA* gene by PCR have evolved for rapid detection of MRSA, but the correct identification of MRSA using conventional methods is complex and some strains are difficult to classify, and can appear susceptible by one method and resistant by another method [5] Although detecting the *mecA* gene by molecular methods is the gold standard; however, all laboratories do not have molecular biology techniques in their routine clinical practice and generally limited to reference laboratories, especially in developing countries , performing this test not readily available and is relatively expensive [14,15, 16, 17,18]. Automated systems are widely used in clinical laboratories for species identification and susceptibility testing as well as detection of resistance mechanisms, and potential MRSA isolates are identified by showing their resistance to cefoxitin, mainly by testing a certain range of MICs. In recent years there are multiple published report suggest the use of cefoxitin as surrogate marker for the detection of MRSA [5]. Same time the Clinical and Laboratory Standards Institute (CLSI) recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine MRSA [19]. Cefoxitin results are easier to read in both transmitted and reflected light and are thus more sensitive for the detection of *mecA* mediated resistance than oxacillin results [5].

The aim of the present study was to evaluate the performance of the cefoxitin, and oxacillin DD test in determining methicillin resistance in comparison to the BD Phoenix automated system (BD, Sparks, MD) , which is considered as the gold standard.

2. Material and Methods

2.1. Collection of Samples and Isolation of Bacteria

This study was performed from April to August 2013 in ten hospitals of Benghazi, Libya. (Psychiatric Hospital, Benghazi Medical Center, 7 October Hospital, Benghazi childrens Hospital, Al- Gomhouria Hospital, Cardiac Center, Nephrology Center, Al-Jalaa Hospital, Urology and ENT Centers, Eye Hospital). A total of 591 Hospital Staff, and 395 hospital environment items were collected for detection of MRSA. Specimens were collected from the anterior nares with sterile dry cotton swabs (SPA Cultiplast, Melano-Italy), dipped in normal saline (0.9%). For the environment, surfaces of frequently handled items (beds, sinks, door handles, floors, and table surfaces). Surface swabs were collected at the different wards of the hospital (ICU, Medical units and HCWs rooms). All swabs were inoculated on blood agar (BA-HiMedia, India) and subsequently on Mannitol salt agar plates (MSA-HiMedia, India) and were incubated at 35°C for 24-48 hours.

2.2. identification of *S. aureus* strains

All isolates were identified by conventional methods (Gram-stained, catalase, slide and tube coagulase, and DNase) [20]. The biotype was determined by API-20 Staph (BioMérieux, France) for detection of *S. aureus*, and the Phoenix system (BD), cefoxitin DD test, and oxacillin DD test for detection of *mecA* gene.

2.2.1. Disk Diffusion

The oxacillin disc diffusion test:

The oxacillin disc (1μg) diffusion test (Oxoid Ltd., England, UK) was carried out on MullerHinton agar plates (MHA-HiMedia, India) which were supplemented with 2% NaCl to detect MRSA according to the CLSI guideline [21]. For each strain a bacterial suspension adjusted to 0.5 McFarland was used. The plates were incubated at 35°C and the results were recorded after 24 hrs. of incubation [22]. The isolates were considered as resistant when the diameter of inhibition was ≤ 10mm, as intermediate resistant when diameter was 11-12mm and as sensitive when the diameter was ≥ 13mm.

The cefoxitin disc diffusion test:

All the isolates were subjected to cefoxitin disc diffusion test using a 30μgm disc (Oxoid Ltd., England, and UK). Was carried out on Muller-Hinton agar plates (MHA- Hi Media, India). For each strain a bacterial suspension adjusted to 0.5 McFarland was used. The plates were incubated at 35°C and the results were recorded after 24 hrs. of incubation [22]. The isolates were considered as resistant when the diameter of inhibition was ≤ 21mm, and sensitive when the diameter was ≥ 22mm.

The cefoxitin and oxacillin disks were read using transmitted light as the CLSI document recommends. (CLSI, 2012). [21].

2.2.2. Phoenix panels (BD).
According to the manufacturer's instructions of PMIC/ID gram-positive Phoenix panels (BD) the detection of isolates was based on both oxacillin and cefoxitin MICs, interpreted according to CLSI breakpoints (for oxacillin, susceptible with MICs of ≤2 µg/ml and resistant with MICs of ≥4 µg/ml; for cefoxitin, susceptible with MICs of ≤4 µg/ml and resistant with MICs of ≥8µg/ml, in that if either oxacillin or cefoxitin MIC testing indicates that the isolate is resistant, the Phoenix final report is methicillin resistance.

3. RESULTS:
Among 359 *S. aureus* strains, 146(40.7%) were MRSA, *mecA* positive, and 213(59.33%) were MSSA, *mecA* negative. Comparing the results of the different methods used for detection of MRSA with the reference method *mecA* gene by the Phoenix automated system is shown in (Table.1).

Table. 1: Results of *S. aureus* isolates.

The Phoenix system detection (n=359)	FOX-DD		OX-DD	
	R	S	R	S
Positive (n=146)	146	0	143	3
Negative (n=213)	0	213	3	210

The cefoxitin DD zones were distinct and easy to read. The BD Phoenix automated system detected three strains of *mecA*-positive which were detected resistant by cefoxitin DD test but were not detected by oxacillin DD test, at the same time three strains of 213 *mecA*negative strains appeared to be resistant by oxacillin DD test.
The sensitivity and specificity of cefoxitin and oxacillin DD methods in comparison to the BD Phoenix automated system (gold standard), for the detection of MRSA, are summarized in (Table.2).

Table. 2: Sensitivity and specificity of cefoxitin and oxacillin DD methods for detection of MRSA comparison to the Phoenix system.

Method	The Phoenix system for *mecA* gene as a comparison test			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FOX-DD	100	100	100	100
OX-DD	97.5	98.6	97.9	98.6

PPV: Positive Predictive Values NPV:Negative Predictive Values

4. DISCUSSION
Detection of *mecA* gene or its product, penicillin binding proteins (PBP2a) is considered the gold standard for MRSA. Correct identification of MRSA using conventional methods is complex but the optimal method of detection remains controversial. Most of the methods are unable to detect methicillin resistance and species at the same time. [5] For these reasons, several molecular methods have been developed to detect the *mecA* gene in MRSA clinical isolates [5]. However, these molecular methods cannot be used in most clinical microbiology laboratories in libya due to their high costs and lack of required technical equipment. Many clinical laboratories use automated systems such as Phoenix for species identification and susceptibility testing as well as detection of resistance mechanisms. [23] Recent studies indicate that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA by many reference groups including CLSI. [24, 25].
This study evaluated the performance of the cefoxitin and oxacillin DD test in determining methicillin resistance in comparison to the BD Phoenix automated system (BD, Sparks, MD), which is considered as the gold standard. Numerous studies have informed that the results of the cefoxitin disc diffusion test correlates better with the presence of *mecA* compared with those of the oxacillin disc diffusion test [3, 6, 26,27, 28, 29,30,31,32,33]. Our study also strengthens the fact that cefoxitin is superior to oxacillin as indicator of MRSA for the detection of methicillin resistance. All isolates were tested for methicillin resistance by cefoxitin and oxacillin DD test considering the BD Phoenix automated system as gold standard. Among the 359 *S. auresus* isolates 146 (40.7%) isolate were identified as MRSA by the BD Phoenix automated system (BD, Sparks, MD). Cefoxitin DD test showed 100% sensitivity and 100% specificity, while oxacillin DD test showed 97.5% sensitivity and 98.6% specificity. Cefoxitin disk diffusion zones are much easier to read than those of oxacillin due to the frequent hazy oxacillin zones, which are commonly misinterpreted as evidence of oxacillin susceptibility. Oxacillin must also be read using transmitted light, unlike most other antimicrobials, including cefoxitin

to ensure correct interpretation. [5].The present study detected three strains of *mecA*-positive were detected resistant by cefoxitin DD test but were not detected by oxacillin DD test, at the same time three strains of 213 *mecA*negative strains appeared to be resistant by oxacillin DD test, these three isolates were β– lactamase positive.This rate of false susceptibility associated with the oxacillin disk diffusion test has been noted high in some studies [29, 31, 34, 35]. Numerous studies strengthens the fact that, these isolates could represent either false-positive resistance to the oxacillin disk test or it may be possible that some of these isolates are hyper β-lactam producers [29, 31, 33].
Our study strengthens the fact that oxacillin DD test for the detection of MRSA was less specific compared with the cefoxitin DD test.

5. CONCLUSION

It is concluded from the present study that cefoxitin disc diffusion method can be preferred in clinical microbiology ،it is easy to perform, do not require special technique, and finally more cost effective than other methods.

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