

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. aureus 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 ceftiofuran, mainly by testing a certain range of MICs. In recent years there are multiple published report suggest the use of ceftiofuran as surrogate marker for the detection of MRSA [5]. Same time the Clinical and Laboratory Standards Institute (CLSI) recommends usage of ceftiofuran instead of oxacillin when using the disk diffusion method to determine MRSA [19]. Ceftiofuran 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 ceftiofuran, 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), ceftiofuran DD test, and oxacillin DD test for detection of *mecA* gene.

2.2.1. Disk Diffusion

The oxacillin disc diffusion test:

The oxacillin disc (1 μ g) 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 \leq 10mm, as intermediate resistant when diameter was 11-12mm and as sensitive when the diameter was \geq 13mm.

The ceftiofuran disc diffusion test:

All the isolates were subjected to ceftiofuran disc diffusion test using a 30 μ g 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 \leq 21mm, and sensitive when the diameter was \geq 22mm.

The ceftiofuran 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 ceftioxin MICs, interpreted according to CLSI breakpoints (for oxacillin, susceptible with MICs of ≤ 2 $\mu\text{g/ml}$ and resistant with MICs of ≥ 4 $\mu\text{g/ml}$; for ceftioxin, susceptible with MICs of ≤ 4 $\mu\text{g/ml}$ and resistant with MICs of ≥ 8 $\mu\text{g/ml}$, in that if either oxacillin or ceftioxin 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 ceftioxin DD zones were distinct and easy to read. The BD Phoenix automated system detected three strains of *mecA*-positive which were detected resistant by ceftioxin 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 ceftioxin 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 ceftioxin and oxacillin DD methods for detection of MRSA comparison to the Phoenix system.

Method	The Phoenix system for <i>mecA</i> 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 ceftioxin 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 ceftioxin 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 ceftioxin 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 ceftioxin is superior to oxacillin as indicator of MRSA for the detection of methicillin resistance. All isolates were tested for methicillin resistance by ceftioxin and oxacillin DD test considering the BD Phoenix automated system as gold standard. Among the 359 *S. aureus* isolates 146 (40.7%) isolate were identified as MRSA by the BD Phoenix automated system (BD, Sparks, MD). Ceftioxin DD test showed 100% sensitivity and 100% specificity, while oxacillin DD test showed 97.5% sensitivity and 98.6% specificity. Ceftioxin 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 ceftioxin

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 strengthen 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 as it is easy to perform, do not require special technique, and finally more cost effective than other methods.

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References:

- [1]. **Iyer, A.P; Baghallab, I; Albaik, M., and Kumosani, T. (2014).** Nosocomial Infections in Saudi Arabia Caused by Methicillin Resistance *Staphylococcus aureus* (MRSA). *Clin Microbial* **3**: 146. doi:10.4172/2327-5073.1000146.
- [2]. **Al-Abdli, N.E., and Baiu, S.H. (2014).** Nasal Carriage of *Staphylococcus* in Health Care Workers in Benghazi Hospitals. *American Journal of Microbiological Research*, **2** (4):110-112.
- [3]. **Jain, A; Agarwal, A; and Verma, R. K. (2008).** Cefoxitin disc diffusion test for detection of methicillin-resistant *Staphylococci* *Journal of Medical Microbiology*; **57**: 957–961.
- [4]. **Diekema, D.J; Boots Miller, D.J; Vaughn, T.E; Woolson, R.F., and Yankey, J.W.(2004).** Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clin. Infect. Dis.* **38**:78-85.
- [5]. **Patil, N. R., and Ghorpade, M.V. (2014).** Comparison of Conventional Phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus*. *International Journal of Research and Development in Pharmacy and Life Sciences*, **5**(2): 20392044.
- [6]. **Velasco, D; Del Mar Tomas, M; Cartelle, M; Beceiro, A; Perez, A; Molina, F; Moure, R; Villanueva, R., and Bou, G. (2005).** Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. *J Antimicrob Chemother*; **55**: 379–382.
- [7]. **Mimica, M.J.; Carvalho, R.L.; Berezin, E.N.; Damaceno, N., and Caiaffa-Filho, H.H. (2012).** Comparison of five methods for oxacillin susceptibility testing of *Staphylococcus aureus* isolates from cystic fibrosis patients. *Rev. Inst. Med. Trop. Sao Paulo*, **54**(6): 305-6.
- [8]. **Jevons, M.P. (1961).** “Celbenin”-resistant staphylococci. *British Medical Journal* **i**, 124-5.
- [9]. **Knox, R. (1961).** “Celbenin”-resistant staphylococci. *British Medical Journal* **i**, 126.
- [10]. **Obasuyi, O. (2013).** Molecular Identification of methicillin-resistant *Staphylococcus aureus* in Benin-city Nigeria. *AFR. J. clin. exper. Microbiol* **14**(1): 1-4.
- [11]. **Karami, S; Rahbar, M., and Yousefi, J.V. (2011).** Evaluation of Five Phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). *Iranian Journal of Pathology*. **6** (1):27 – 31.
- [12]. **Sasirekha, B; Usha, M.S; Amruta, A.J; Ankit, S; Brinda, N., and Dviya, R. (2012).** Evaluation and Comparison of different phenotypic tests to detect Methicillin Resistant *Staphylococcus aureus* and their Biofilm Production. *Int J Pharm Tech Res.* **4**(2): 532- 41.
- [13]. **Mencacci, A; Montecarlo, I; Gonfia, F; Moretti, A; Cardaccia, A; Farinelli, S; Pagliochini, M.R; Giuliani, A; Basileo, M; Pasticci, M.B; and Bistoni, F. (2009).** Comparison of the BD Phoenix System with the Cefoxitin Disk Diffusion Test for Detection of Methicillin Resistance in *S. aureus* and Coagulase-Negative *Staphylococci*. *J. CLIN. MICROBIOL.* **7** (47):2288–2291.
- [14]. **Sapna, M.G; Kishore, I.V; Mayuri, B. P; and Rajaram, P. M. (2016).** Comparison of Four Diagnostic phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). *Int J Pharm Bio Sci.* **7**(2): 243 - 247.
- [15]. **Zhang, K; Sparling, J; Chow, B. L; Elsayed, S; Hussain, Z; Church, D. L; Gregson, D. B; Louie, T; and Conly, J. M. (2004).** New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *J. Clin. Microbiol.* **42**:4947–4955.
- [16]. **Fujita, S. Y; Senda, T; and Hashimoto, T. (2005).** Rapid identification of staphylococcal strains from positive-testing blood culture bottles by internal transcribed spacer PCR followed by microchip gel electrophoresis. *J. Clin. Microbiol.* **43**:1149– 1157.
- [17]. **Grisold, A. J; and Kessler, H. H. (2006).** Use of hybridization probes in a real-time PCR assay on the Light Cycler for the detection of methicillin resistant *Staphylococcus aureus*. *Methods Mol. Biol.* **345**:79–89.
- [18]. **Palka-Santini, M; Putzfeld, S; Cleven, B. E; Kronke, M; and Krut, O. (2007).** Rapid identification, virulence analysis and resistance profiling of *Staphylococcus aureus* by gene segment-based DNA microarrays: application to blood culture post-processing. *J. Microbiol. Methods* **68**:468–477.
- [19]. **Clinical and Laboratory Standards Institute. (2007).** Performance standards for antimicrobial susceptibility testing; 17th informational supplement. M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.

- [20]. **Cheesbrough, M. (2009).** District Laboratory Practice in Tropical Countries. Cambridge university, UK, **2 (2):** 65-67.
- [21]. **Clinical and Laboratory Standards Institute. (2012).** Performance Standards for Antimicrobial Susceptibility Testing, Twenty-second Informational Supplement. CLSI document M100-S22. Wayne. PA.
- [22]. **Bauer, A. W; Kirby, W. M; Sherris, J. C; and Turck, M, (1966).** Antibiotic susceptibility testing by a standard single disk method. *Am. J. Clin. Pathol.* **45:** 493-496.
- [23]. **Cekin, Y; Yazisiz, H; Kuskucu, M. A; Ongut, G; Baysan, B. O; Kilinckaya, H; Ogunc, D; Midilli, K; Ozen, N. S; and colak, D. (2014).** Evaluation of the BD Phoenix System for detection of Methicillin Resistance in *S. aureus* Isolates in comparison to BD Gene Ohm MRSA Assay. *Clin. Lab.* **5:** 1-5.
- [24]. **Anand, K. B; Agrawal, P; Kumar, S; and Kapila, K. (2009).** Comparison of Cefoxitin disc diffusion test, Oxacillin Screen agar, and PCR for *mecA* gene for Detection of MRSA. *Indian Journal of Medical Microbiology.* **27(1):** 27-29.
- [25]. **Skov, R; Smyth, R; Clausen, M; Larsen, A. R; Frimodt-Moller, N; and Olsson Liljequist, B. (2003).** Evaluation of a cefoxitin 30 µg disc on Iso-Sensitest agar for detection of methicillin resistant *Staphylococcus aureus*. *J Antimicrob Chemother.* **52:** 204-207.
- [26]. **Kapila, K; Kaushik, K. (2009).** Cefoxitin Disk Diffusion Test - Better Predictor of Methicillin Resistance in *Staphylococcus aureus*. *Indian Journal of Medical Microbiology.* **27(4):** 379-380.
- [27]. **Cauwelier, B; Gordts, B; Descheemaeker, P; and Van Landuyt, H. (2004).** Evaluation of a disk diffusion method with cefoxitin (30 mg) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* **23:** 389–392.
- [28]. **Felten, A; Grandry, B; Lagrange, P. H; and Casin, I. (2002).** Evaluation of three techniques for detection of low-level methicillin-resistant *S. aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA screen latex agglutination test. *J Clin Microbiol* **40:** 2766–2771.
- [29]. **Flayhart, D; Hindler, J. F; Bruckner, D. A; Hall, G; Shrestha, R. K; Vogel, S. A; Richter, S. S; Howard, W; Walther, R; and Carroll, K. C. (2005).** Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J Clin Microbiol* **43:** 5536–5540.
- [30]. **Swenson, J. M; and Tenover, F. C. (2005).** Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol* **43:** 3818–3823.
- [31]. **Broekema, N. M; Van, T. T; Monson, T. A; Marshall, S. A; and Warshauer, D. M. (2009).** Comparison of Cefoxitin and Oxacillin Disk Diffusion Methods for Detection of *mecA*-Mediated Resistance in *Staphylococcus aureus* in a Large-Scale Study. *J. Clin. Microbiol.* **47 (1):** 217–219.
- [32]. **Fernandes, C. J; Fernandes, L. A; and Collignon, P. (2005).** Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. *JAC.* **55:** 506–510.
- [33]. **El-Ahmady, M; and Hussain, A. W. (2011).** Evaluation of Different Techniques for Detection of *mecA*-Mediated Resistance in *Staphylococcus aureus*. *Egyptian Journal of Medical Microbiology,* **20 (3):** 131-137.
- [34]. **Boutiba-Ben Boubaker, I., Ben Abbas, R., Ben Abdallah, H., Mamlouk, K., Mahjoubi, F., Kammoun, A., Hammami, A; and Ben Redjeb, S. (2004).** Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* **10:** 762–765.
- [35]. **Swenson, J. M. D; Lonsway, S; McAllister, A; Thompson, L; Jevitt, W; Zhu; and Patel, J. B. (2007).** Detection of *mecA*-mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing borderline oxacillin MICs. *Diagn. Microbiol. Infect. Dis.* **58:** 33–39.