Comparison of a Seven Conventional Phenotypic Methods with Polymerase Chain Reaction for Detection of Methicillin-Resistant *Staphylococcus aureus*

Receved :4/12/2014 Accepted :2/2/2015

Ali M. Al-Mohana[1], Azhar N. Hussein[2] and Mee'aad K. Al-Kudheiri[3*]
[1]Department of Microbiology, College of Medicine, Kufa University.
E. Mail: alialmohana@yahoo.com.
[2]Department of Microbiology, College of Education, Qadisiya University
[3]Department of Community Health, Kufa Technical Institute.
E. Mail: miaad44@yahoo.com.

Abstract

Early detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is critical for both the management of infected patients, and the timely institution of appropriate infection control measures. Although detection of the *mecA* gene by polymerase chain reaction (PCR) remains the gold standard, this technology is inaccessible for many laboratories. Therefore, this study sought to evaluate seven phenotypic methods and compare them with PCR. A total of 135 *S. aureus* were collected from 343 clinical samples between August 2012 and January 2013 from several clinical sources that were randomly selected from patients in three main hospitals in Al-Najaf city. MRSA isolates were identified using PCR with primers specific for the *mecA* gene. PCR was used as the reference method, thus, the prevalence of MRSA in Najaf hospitals was 64 (47.4%) and the remaining 71 (52.6%) isolates were *mecA*-negative methicillin sensitive *S. aureus* (MSSA), and all MRSA isolates were tested for comparison using cefoxitin and oxacillin disc diffusion, oxacillin and methicillin HiComb E-test, oxacillin screen agar, BBL™ CHROMagar™ MRSA, HiCromeMeReSa agar. The findings of this study revealed that FOXDD had a high accuracy (97.8%) comparative to other phenotypic methods tested for detection of MRSA followed by OX (E-test), CHROMagar, HiCrome agar (97%), OXDD (96.3%). The OXSA (93.3%) and MET E-test (88.9%) had the lowest concordance with PCR results. Finally, FOXDD method may be preferred in this study and in other clinical laboratories because it is highly accuracy, easy to perform, low cost, and does not require special equipment.

Zoology **Classification QL 700-739.8**

Key words: *Staphylococcus aureus*, MRSA, MSSA.

* The research is a part of on PhD dissertation in the case of the third researcher.

2013 from several clinical sources including the urine, skin, burn, wounds, sputum, diabetic foot ulcer, nares and vagina that were randomly selected from patients in Al-Zaharaa, Al-Hakeem and Al-Sader hospitals in Al-Najaf city. Isolates were identified depending on the morphological features on culture medium and biochemical tests according to the classification of Bergey's manual [6] and MacFaddin [7]. PCR amplification of the *coa* gene was performed for all isolates as confirmatory detection by molecular method.

Detection of MRSA isolates

1. phenotypic methods

A. Cefoxitin Disc Diffusion Test (FOXDD)

All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc (Himedia, India). A 0.5 McFarland standard suspension (Fluka, Switzerland) of the isolate was made and lawn culture done on Muller-Hinton agar (MHA) plate (Himedia, India). Plates were incubated at 35˚C for 18 hr and zone diameters were measured. Positive result was considered as *mecA* meditated oxacillin resistance [8, 9].

B. Oxacillin Disc Diffusion Test (OXDD)

A direct colony suspension of each *S. aureus* isolate was prepared to a 0.5 McFarland standard and plated on MHA containing (4%) NaCl (BDH, England). An oxacillin 1 µg disc (Himedia, India) was placed on the surface and incubated at 35˚C for 24 hr [9].

C. Oxacillin Screen Test (OXA)

Oxacillin salt agar medium [NaCl (4%) and oxacillin (0.6 mg, Alembic, India) was added to MHA] was inoculated from a direct colony suspension equivalent to a 0.5 McFarland standard by using a swab, expressing excess fluid as for the disc diffusion test and spotting an area at 15 mm in diameter. The plate was incubated at 35˚C for 24 hr [8, 9].

Introduction

From the time when the first detection of MRSA in 1961[1], MRSA has spread worldwide and causes serious problems in clinical settings. MRSA is a major nosocomial pathogen causing significant morbidity and mortality [2]. Since MRSA strains are resistant to all β-lactam antibiotics and a wide range of other antimicrobial agents as well as the treatment options are limited significantly [3]. The overall frequency of health care facility associated infections caused by MRSA has increased during the last decade in Iraq, where the recent frequency of MRSA among patients is estimated to be 37.8% in Najaf [4]. Therefore, detection of MRSA is important for patient care and use of appropriate antimicrobial therapy. Rapid, accurate identification of patients with MRSA is important in preventing its transmission and enabling early therapeutic decisions. Phenotypic methods as disc diffusion and microdilution techniques are employed in few Iraqi laboratories for the detection of methicillin resistance. However, these methods are often not entirely reliable at detecting some strains that harbor the *mecA* gene [5]. In recent years, PCR detection of the *mecA* gene has become the gold standard for MRSA detection but this is an expensive method, and use of this assay is restricted to reference centers and is not routinely carried out in vast majority of Iraqi clinical laboratories. The present study was undertaken to compare seven phenotypic methods for the detection of MRSA. This investigation would give us a definitive idea or would recommend the most appropriate phenotypic tests for diagnosing MRSA in a routine diagnostic laboratory with utmost accuracy, which is very much needed to avoid false positives and false negatives, as well as for early diagnosis. This in turn helps to prevent the spread of MRSA in Iraqi hospitals.

Materials and Methods

Bacterial isolates

A total of 135 *S. aureus* isolates were isolated between August 2012 and January

aerobically at 35°C for 48 hr. Colonies of MRSA were appeared bluish to green on the HiCrome agar medium.

2. Molecular Method

Polymerase chain reaction (PCR) protocol

The DNA was extracted directly from overnight colonies according to the manufacturer's instruction of DNA extraction kits (Promega, USA, Geneaid, USA). The DNA extract of *S. aureus* isolates were subjected (*coa* and *mecA*) genes listed in Table (1) by monoplex PCR. The protocol used depends on manufacturer's instruction. All PCR components assembled in PCR tube and mixed on ice bag under sterile condition as shown in Table (2). PCR mixture was circulated in thermal cycler instrument that was programmed as shown in Table (3). All genomic DNA and all amplified PCR products were visualized following electrophoresis in 1% agarose gels run at 70 V with ethidium bromide staining (USA, Sigma), and comparison to standard positive control (Monterial, Alpha). *coa* gene and *mecA* gene positive strains yielded an amplify ication product of shining band with (100 bp) DNA marker (ladder) (Promega, USA and Bioneer, Koria).

D. Methicillin and Oxacillin HiComb E-test (MET, OX E-test)

The inoculum was standardized to 0.5 McFarland turbidity and plated on MHA supplemented with (2%) NaCl. HiComb™ MIC strips for methicillin and oxacillin (Himedia, India) were applied on the MHA surface with MIC scale facing down wards. Plates incubated at 35°C and examined after 24 hr.

E. Chromogenic Screen Test

The samples were inoculated onto a BBL™CHROMagar™MRSA (CHROMagar, Becton and Dickinson, France) plate before antimicrobial agents had been administered, and streaked for isolation, or sub cultured on MHA and incubated overnight. A colony suspension in brain heart infusion (BHI) broth with a turbidity equivalent to a 0.5 McFarland standard was made from the pure subculture on MHA. Plates were incubated at 35°C for 24 hr in an inverted position in dark incubator because light may reduce recovery and/or coloration of isolates. Colonies of MRSA will appear pink to mauve on the CHROMagar medium [10]. While for testing on HiCromeMeReSa Agar (HiCrome agar, Himedia, India), all of the clinical samples were directly streaked. Plates were incubated

Table 1. Primers for Detection of *S. aureus* and MRSA (Bioneer, Koria)

Primer		Oligonucleotide Sequence (5′-3′)	Size (bp)	Reference
Coa	F	CGAGACCAAGATTCAACAAG	810	(11)
	R	AAAGAAAACCACTCACATCAGT		
mecA	F	AAAATCGATGGTAAAGGTTGGC	533	(12)
	R	AGTTCTGCAGTACCGGATTTTGC		

F, forward; R, reverse; A, amplicon; bp, base pair.

Table 2. Protocols of monoplex PCR reaction mixture volumes

PCR reaction mixture	(Promega, USA) protocol (Final volume 25µl)	(Bioneer, Koria) protocol (Final volume 20µl)
Master mix 2X	12.5 µl	10 µl
Primer forward (10 µM)	2.5 µl	1 µl
Primer reverse (10 µM)	2.5 µl	1 µl
DNA template	5 µl	5 µl
PCR grade water	2.5 µl	3 µl

µl, microliter; µM, micromole.

Table 3. Programs of PCR thermocycling conditions for detection of monoplex genes

Gene	Temperature ˚C / Time					Cycle no.
	Initial Denaturation	Cycling Condition			Final Extension	
		Denaturation	Annealing	Extension		
Coa	95/2 min	95/30 sec	55/30 sec	72/90 sec.	72/5 min	35
mecA	95 /5 min	95/1 min	63/1 min	72/1 min	72/5 min	30

˚C, celsius; sec, second; min, minute.

tested and divided by total number of positives and negatives results of both (*mecA* positive and *mecA* negative).

Results and Discussion

Polymerase chain reaction amplification of the *coa* gene was performed for all 135 isolates. All the isolates expressed *S. aureus* specific band in their PCR products of 810 bp size, which confirmed the assumption that all these isolates were *S. aureus* (Figure 1), and all of them were detected by assessed for the presence of *mecA* by PCR. The *mecA* specific PCR product of 533 bp was seen in 64 isolates (MRSA). Thus, the prevalence of MRSA in Najaf hospitals was 47.4%. The remaining 71 (52.6%) isolates were *mecA*-negative (MSSA), (Figure 2).

Statistical Analysis

Sensitivity of a certain method was calculated as the number of resistant *S. aureus* isolates determined using this method, divided by the sum of *mecA*-positive strains. Specificity was calculated as the number of MSSA strains determined by this method, divided by the sum of *mecA*-negative strains. To calculate the positive predictive value (PPV), the number of true positives (*mecA* positive) was divided by the number of positive results by the other tests; and to calculate negative predictive value (NPV), the number of true negatives (*mecA* negative) was divided by the number of negative results by the other tests. While the accuracy (ACC) was calculated by the sum of true positive (*mecA* positive) and true negative (*mecA* negative) results which were

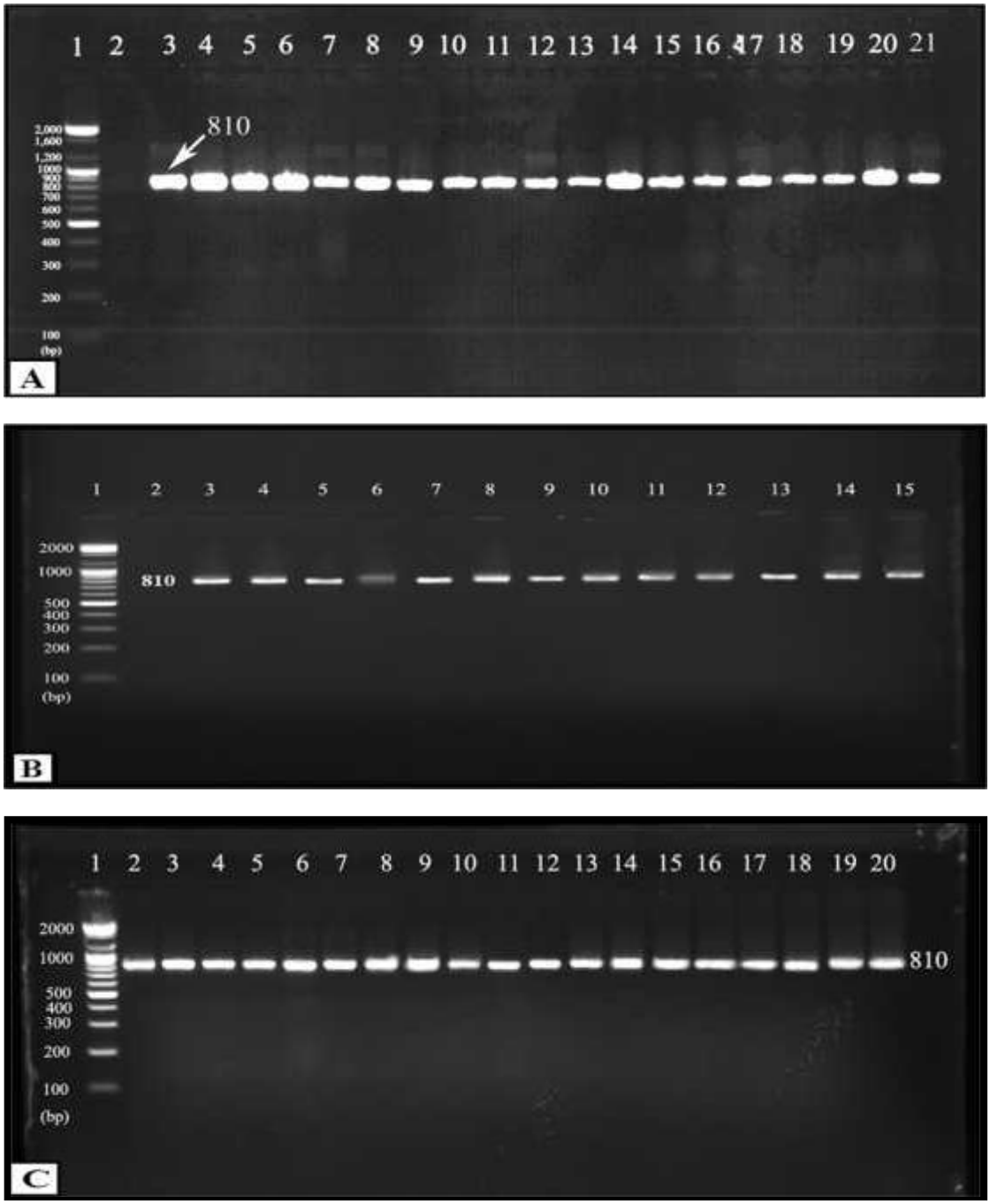


Figure 1. An ethidium bromide-stained gel demonstrating the typical banding patterns observed with the monoplex PCR assay, PCR amplification of the *coa* gene from *S. aureus*, with the amplicon size 810 bp. DNA amplification products were separated by electrophoresis in an (2%) agarose gel. The electrophoresis was performed at 70 volt for 1.5 hr. (A) Lane 1, marker DNA ladder (100) bp, lane 2, negative *coa* gene, lane: 3-21 *S. aureus* carrying *coa* gene; (B) Lane 1, marker DNA ladder (100) bp, lane 2 negative *coa* gene, Lane 3-15 positive *coa* gene; (C) Lane 1, DNA ladder (100) bp, lane 2-20 positive *coa* gene.

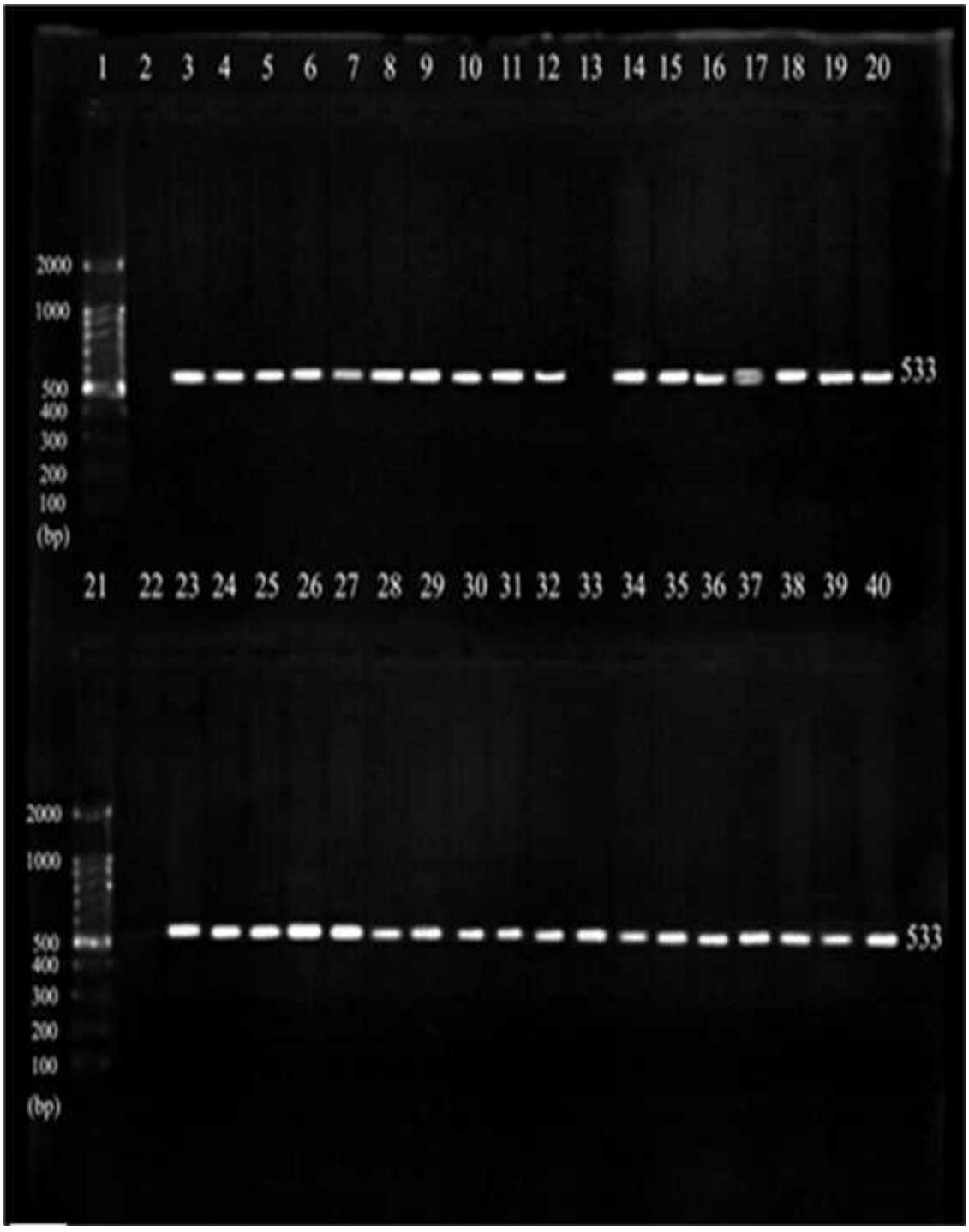


Figure 2. An ethidium bromide-stained gel demonstrating the typical banding patterns observed with the monoplex PCR assay. PCR amplification of the *mecA* gene from *S. aureus*, with the amplicon size 533 bp. DNA amplification products were separated by electrophoresis in an 2% agarose gel. The electrophoresis was performed at 70 volt for 1.5 hr; Lane 1 and 21, marker DNA ladder (100) bp, lane 2, 13 and 22: negative *mecA* gene, Lane 2-12, 14-20 and 23-40, *S. aureus* carrying *mecA* gene.

(2.2%) gave false positive. The present study revealed that FOXDD was found to be highly sensitive (100%) and specific (95.8%), (Table 4). The results showed that FOXDD is a better method of MRSA detection compared with other methods tested the current result in accordance with those of several studies [15, 16]. This means that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods and is now an accepted method for the detection of MRSA by many reference groups and various workers [9, 15, 17]. This higher sensitivity to cefoxitin can be explained by the increased expression of the *mecA*-encoded protein PBP2a, cefoxitin being an inducer of the *mecA* gene leading to more accurate detection of the heteroresistant MRSA [5].

Table (4) shows the results of the comparison of conventional phenotypic testing methods for MRSA using the *mecA* gene detection test as a reference standard. The sensitivity and specificity of each test were determined in an attempt to find out the most suitable method for detection of MRSA in a routine diagnostic laboratory in Najaf hospitals.

Cefoxitin, a cephamycin, is a more potent inducer of the *mecA* regulatory system than are the penicillins [13]. Several groups of investigators have reported that the results of cefoxitin disc diffusion (FOXDD) test correlate better with the presence of *mecA* than do the results of disc diffusion tests using oxacillin, OXDD [9,14,15]. FOXDD testing showed that 64 (47.4%) isolates were MRSA, 68 (50.4%) isolates were MSSA and 3

Table 4. Evaluation of seven phenotypic tests for detection of methicillin resistance as compared with PCR in 135 *S. aureus* isolates

Phenotypic test	*mecA* +ve n= 64		*mecA* -ve n= 71		TPR	SPC	PP	NPV	ACC
	T +ve	F –ve	T –ve	F +ve					
FOXDD test	64	0	68	3	100	95.8	95.5	100	97.8
OXDD test	64	0	66	5	100	93.0	92.8	100	96.3
OX (E-test)	64	0	67	4	100	94.4	94.1	100	97.0
MET (E-test)	62	2	58	13	96.9	81.7	82.7	96.7	88.9
OXSA test	57	7	69	2	89.1	97.2	97	90.8	93.3
CHROMagar	61	3	70	1	95.3	98.6	98.4	95.9	97.0
HiCrome agar	60	4	69	2	96.9	97.2	96.9	97.2	97.0

FOXDD, cefoxitin disc diffusion; OXDD, oxacillin disc diffusion; OX (E-test), oxacillin (HiComb E-test); MET (E-test), methicillin (HiComb E-test); OXSA, oxacillin screen agar; CHROMagar, BBL ™CHROMagar ™ MRSA; HiCrome agar, HiCromeMeReSa Agar; T, true; F, false; +ve, positive; -ve, negative; TPR, sensitivity or true positive rate; SPC, specificity or true negative rate; PPV, positive predictive value; NPP, negative predictive value; ACC, accuracy.

respectively. The results emphasized 5 (3.7%) non-MRSA isolates were misidentified as MRSA (false positives).

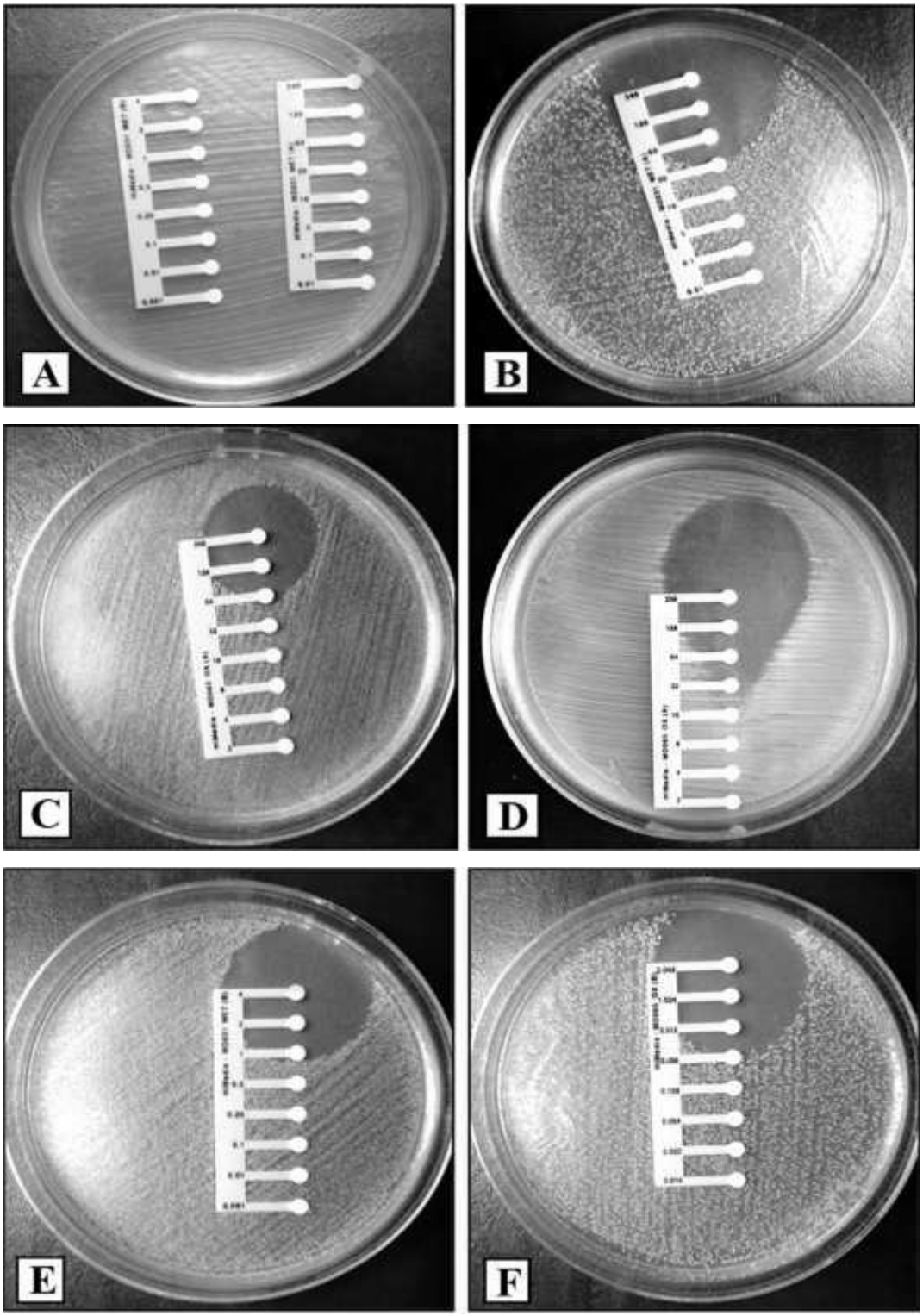
Of the 135 *S. aureus* isolates, 64 (47.4%) and 66 (48.9%) were confirmed using OXDD test as MRSA and MSSA,

comparison with *mecA*-based PCR method are given in Table (4).

The reason for these two to seven MRSA isolates detected as MSSA (false negative) by the MET (E-test), OXSA CHROMagar and HiCrome agar test may be attributed to the fact that accurate determination of MRSA by conventional tests is subject to variations in inoculum size, incubation time, medium pH, medium salt concentration and type of agar used can also influence the above findings [21]. These factors emphasize the need for a rapid, standardized, accurate, and sensitive method for detection of MRSA, which is not dependent on growth conditions. Studies have reported that the heterogeneous nature of methicillin resistance in *S. aureus* limits the accuracy and reliability of phenotypic methods such as disc diffusion, broth, and agar dilution tests. Swenson *et al.* [22] noted that sensitivity decreased when heterogeneous resistant strains were tested and specificity decreased with strains having borderline MIC. Another important reason for these few MRSA isolates being detected phenotypically as MSSA is the over expression of *mecR* and *mecI* genes which are co-repressors of *mecA* gene [23]. Other authors [24] reported that false-negative results might occur with MRSA isolates with low oxacillin MICs (4 or 8 μg/ml) due to production of smaller amounts of PBP2a or the failure to express the gene phenotypically. Additionally, some of these methods require oxacillin powder and skilled laboratory staff for its implementation and as, it cannot easily be implemented in routine laboratories. The present study shown that false negative susceptibility results may lead to treatment failure and the spread of MRSA, especially appropriate infection control measures are not applied in Najaf hospitals.

The sensitivity and specificity was found to be 100% and 93%, respectively. This finding is similar to those of many studies conducted in different parts of the world [18], while Jain *et al.* [19] found the sensitivity equal to (100%), but the specificity is low (58.3%) as compared to the PCR for *mecA* gene. Many labs still prefer using oxacillin for detection of MRSA, because oxacillin maintains its activity during storage better than methicillin, and more likely to detect heteroresistant strains [14]. Oxacillin is less resistant to hydrolysis by staphylococcal β-lactamases so problems with hyper producers of penicillinase are reduced with methicillin and this may explain why present study get 5 isolates gave false positive results with oxacillin. The oxacillin disc diffusion test has previously been found to be less reliable, with high numbers of both false-susceptible and false-resistant results [20]. However, in this study, specificity of FOXDD test was found to be better than OXDD, this relatively implies that the FOXDD test is an available alternative to the OXDD for routine antibiotic susceptibility testing in hospitals.

All these *S. aureus* isolates were also subjected to MRSA detection by another five phenotypic methods, which resulted in detection of 64 isolates as MRSA (47.4%) by oxacillin HiComb E-test [OX (E-test)], while 62 MRSA (45.9%) were identified by methicillin HiComb E-test [MET (E-test)], (Figure 3). Oxacillin screen agar (OXSA) test gave lower result compared with other tests, detecting 57 (42.2%) MRSA isolates. However, CHROMagar and HiCrome agar (Figure 4) methods showed 61 (45.2%) and 60 (44.4%) MRSA isolates. Sensitivity, specificity, PPV, NPV and ACC of the five phenotypic tests in



Figur 3. Detection MIC of methicillin and oxacillin against MRSA isolates by HiComb E-test. (A) Positive result, isolate NJ-99 [(MET)-MIC ≥ 240 µg/ml]; (B) Positive result, isolate NJ-88 [(MET)-MIC 30 µg/ml]; (C) Positive result, isolate NJ-78 [(OX)-MIC 64 µg/ml]; (D) Positive result, isolate NJ-79 [(OX)-MIC 32 µg/ml]; (E) Negative result, isolate N-5a [(MET)-MIC 1 µg/ml]; (F) Negative result, isolate N-16a [(OX)-MIC 0.256 µg/ml].

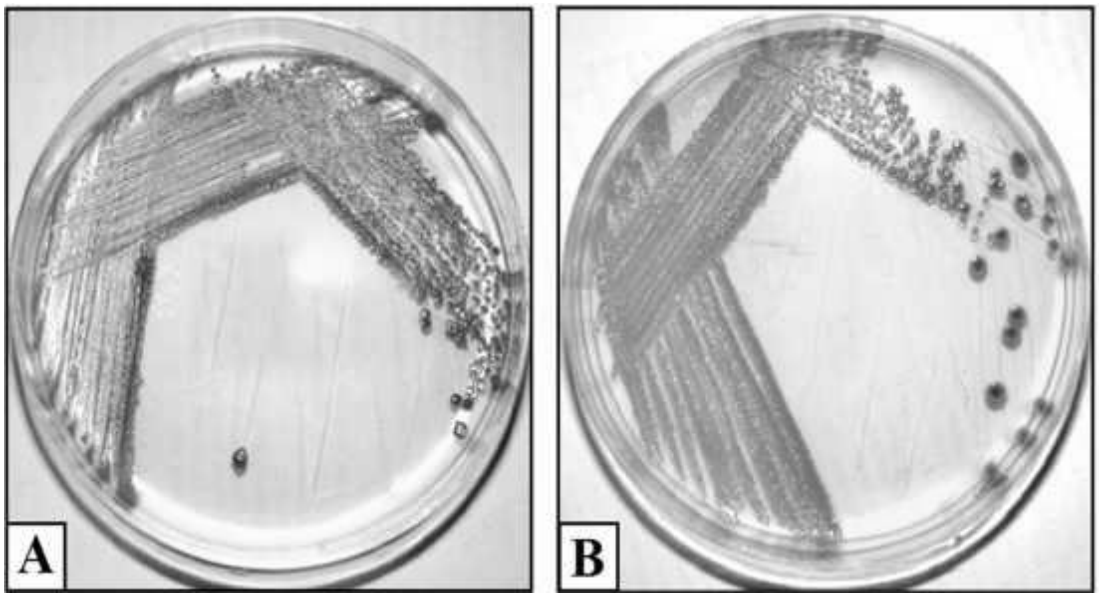


Figure 4. The appearance and color of the MRSA colonies in the chromogenic media. (A), MRSA colonies of NJ-47 isolate appear bluish green on HiCrome agar; (B), MRSA colonies of NJ-18 isolate appear mauve to pink on CHROMagar.

resistance at the borderline of the inhibition zone and were thus termed "moderately resistant *S. aureus*" (MODSA) as described by Pillai *et al.* [21]. Under some test conditions, resistance may also be seen in isolates which produce large amounts of β-lactamases, and these isolates have been referred to as "borderline oxacillin-resistant *S. aureus*" (BORSA), [25], and these can be difficult to distinguish from resistant isolates that carry the *mecA* gene by routine tests (Figure 5).

It is noted that some of the 71 *mecA*-negative isolates (such as NJ-A23, Figure 5) were resistant to methicillin, cefoxitin, and/ or oxacillin (false positive). The study found that false positive rates for FOXDD, OXDD, OX (E-Test), MET (E-Test), OXSA, CHROMagar and HiCrome agar were 3, 5, 4, 13, 2, 1, 2 isolates, respectively (Table 4). The reason for this phenomenon may be due to heterogeneous expression of methicillin resistance in these isolates. Most of these isolates expressed

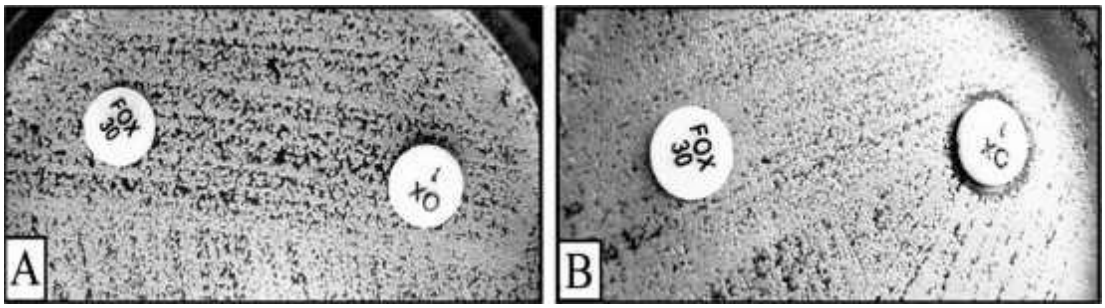


Figure 5. Identical phenotypic expression in both MRSA and borderline oxacillin-resistant *S. aureus* (BORSA) isolates. (A), Represent MRSA phenotypic expression by NJ-A23 isolate according to CLSI [9]; (B), Represent BORSA phenotypic expression by NJ-A23 isolate MSSA (NJ-A20).

Conclusion

detection of MRSA followed by OX (E-test), OXDD, OXSA and MET (E-test). The study create that FOXDD method can be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, its cost effective and finally it can be used as an alternative to the technically demanding PCR.

Finally, there was no significant difference ($p<0.05$) in the detection rate of the MRSA isolates among molecular technique and phenotypic methods. In Najaf province where molecular techniques are not feasible as a routine, the findings of this study revealed that FOXDD had a high accuracy comparative to other phenotypic methods tested for

(MRSA). Indian J. Med. Microbiol. **27**(1): 27-29.

9- Clinical and laboratory standards institute. (CLSI) (2012). Performance standards for antimicrobial susceptibility testing. M100-S22. Clinical and laboratory standards institute, Wayne, PA.

10- Flayhart, D., Hindler, J.F., Bruckner, D.A. and Hall, G. (2005). Multicenter evaluation of BBL CHROMagar methicillin-resistant *Staphylococcus aureus* (MRSA) medium for direct detection of from MRSA surveillance cultures of the anterior nares. J. Clin. Microbiol. **43**: 5536-5540.

11- Tiwari, H.K. and Sapkota, D. and Sen, M.R. (2008). Evaluation of different tests for detection of *Staphylococcus aureus* using coagulase (*coa*) gene polymerase chain reaction as the gold standard. Nepal. Med. Coll. J. **10**(2): 129-131.

12- Moon, J.S., Lee, A.R., Kang, H.M., Lee, E.S., Kim, M.N., and Koo, H.C. (2007). Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy. Sci. **90**: 1176-1185.

13- McKinney, T. K., Sharma, V. K., Craig, W. A. and Archer, G. L. (2001). Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus* (*mecA*) is corepressed but not coinduced by cognate *mecA* and β-lactamase regulators. J. Bacteriol. **183**: 6862-6868.

14- Centers for disease control and prevention (CDC) (2005). Healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA).

15- Farahani, A., Mohajeri, P., Gholamine, B., Rezaei, M. and

References

1- Jevons, M. P. (1961). 'Celbenin'-resistant staphylococci. Br. Med. J. 124-125.

2- Anand, K., Agrawal, P., Kumar, S. and Kapila, K. (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and polymerase chain reaction for *mecA* gene for detection of methicillin resistant *Staphylococcus aureus*. Indian. J. Med. Microbiol. **21**: 27-29.

3- Skrupky, L.P., Micek, S.T. and Kollef, M.H., (2009). Bench-to-bedside review: understanding the impact of resistance and virulence factors on methicillin-resistant *Staphylococcus aureus* infections. Crit. Care. **13**: 222.

4- Al-Borqaawi, E.C.A. (2013). The Role of *Staphylococcus aureus* superantigens in the pathogenesis of psoriasis vulgaris. Thesis, Phd. University of Kufa, Iraq.

5- Velasco, D., Del-Mar-Tomas, M., Cartelle, M., Beceiro, A, Perez, A., Molina, F. (2005). Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. J. Antimicrob. Chemother. **55**: 379-382.

6- Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T. and Williams, S.T. (1994). Bergey's manual of determinative bacterial. 9the dn. Baltimore, MD: Williams.

7- MacFaddin, J.F. (2000). Biochemical tests for identification of medical bacteria 3rd ed. Lippincott Williams and Wilkins, USA.

8- Agrawal, A. P. and Kapila, S.K. (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar and polymerase agar for *mecA* gene for detection of methicillin resistant *Staphylococcus aureus*

Abbasi, H. (2013). Comparison of different phenotypic and genotypic methods for the detection of methicillin-resistant *Staphylococcus aureus*. North Am. J. Med. Sci. **5**: 637-640.

16- Al-Zaidi, J.R. and Al-Sulami, A.A. (2013). Comparison of chromogenic agar medium and diffusion disk test for detection of hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) from patients and hospital environment in Nasiriyah city, Iraq. Afr. J. Microbiol. Res. **7**(19): 1888-1895.

17- Sasirekha, B., Usha, M.S. and Amruta, (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): 532-541.

18- Rostami, S., Moosavian, M., Shoja1, S., Torabipour, M. and Farshadzadeh, Z. (2013). Comparison of *mecA* gene-based polymerase chain reaction with clinical and laboratory standards institute cefoxitin and oxacillin disc diffusion methods for detecting methicillin resistance in *Staphylococcus aureus* clinical isolates. Afr. J. Microbiol. Res. **7**(21): 2438-2441.

19- Jain, A., Agarwal, A. and Verma, R.K. (2008). Cefoxitin disc diffusion test for detection of meticillin-resistant staphylococci. J. Med. Microbiol. **57**: 957-961.

20- Abu Hujier, N.S. and Sharif, F.A. (2006). Detection of methicillin resistant *Staphylococcus aureus* in nosocomial infections in Gaza Strip. Afr. J. Microbiol. Res. **2**: 235-241.

21- Pillai, S.K., Sakoulas, G., and Wennersten, C. (2001). Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. J. Clinic. Microbiol. **30**: 1685-1691.

22- Swenson, J. M., Williams, P. P., Killgore, G., O'Hara, C. M. and Tenover, F. C. (2001). Performance of eight methods, including two new rapid methods, for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organisms. J. Clin. Microbiol. **39**: 3785-3788.

23- Khan, A.U., Sultan, A., Tyagi, A., Zahoor, S., Akram, M. and Kaur, S. (2007). Amplification of *mecA* gene in multi-drug resistant *Staphylococcus aureus* strains from hospital personnel. J. Infect. Dev. Ctries. **3**: 289-295.

24- Louie, L., S. O. Matsumura, E. Choi, M. Louie, and A. E. Simor. (2000). Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **38**: 2170-2173.

25- Mohanasoundaram, K.M. and Lalitha, M.K. (2008). Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. Indian. J. Med. Res. **127**: 78-84

.

المقارنة بين سبعة طرائق مظهرية مع تقنية التفاعلات المتسلسلة لإنزيم البلمرة لتشخيص المكورات العنقودية الذهبية المقاومة للمثيسيلين

علي محسن المحنة[1] ، ازهار نوري حسين[2]، ميعاد كاظم الخضيري[3*]
[1]قسم الاحياء المجهرية، كلية الطب، جامعة الكوفة
البريد الالكتروني: alialmohana@yahoo.com
[2]قسم الاحياء المجهرية، كلية التربية، جامعة القادسية
[3]قسم صحة المجتمع، المعهد التقني، كوفة
البريد الالكتروني: miaad44@yahoo.com

الخلاصـــــــــــة

ان التشخيص المبكر للإصابة بالمكورات العنقودية الذهبية المقاومة للمثيسيلين يُمكن المؤسسات الصحية من السيطرة على العدوى لدى الأشخاص المصابين، بالإضافة إلى تمكينها من وضع معايير مناسبة للسيطرة على العدوى، ولذلك فان عملية التشخيص لهذه البكتريا بواسطة استعمال تقنية التفاعلات المتسلسلة لإنزيم البلمرة لازالت الطريقة المثلى والقياسية إلا انها غير متاحة لجميع المختبرات، ويقتصر استعمالها على بعض المختبرات، لذا فان هذه الدراسة اقترحت عملية تقييم أو اختبار لسبعة من الطرائق المظهرية، ومقارنتها مع تقنية التفاعلات المتسلسلة لإنزيم البلمرة، وقد تم تشخيص 135 عزلة من المكورات العنقودية الذهبية من مجموع 343 عينة سريرية تم جمعها من مصادر مختلفة ومتنوعة وبشكل عشوائي من المرضى للفترة من شهر آب للعام 2012 إلى شهر كانون الثاني للعام 2013، وكانت عملية جمع العزلات السريرية من ثلاث مستشفيات رئيسية تابعة لمدينة النجف، ونتيجة لاستعمال مورثة *mecA* الخاصة للكشف عن العزلات المقاومة للمثيسلين في تقنية التفاعلات المتسلسلة لإنزيم البلمرة، فقد وجد ان عدد عزلات المكورات العنقودية الذهبية المقاومة للمثيسيلين هو 64 عزلة وبنسبة (47.4%)، وعدد العزلات الحساسة للمثيسيلين هو 71 وبنسبة (52.6%)، كما أخضعت جميع العزلات المقاومة للمثيسيلين لغرض المقارنة للاختبارات السبعة التالية (اختبار انتشار القرص باستعمال قرص السيفوكستين والاكزاسيلين، واختبار فحص الحساسية للاوكزاسيلين والمثيسيلين باستعمال الأشرطة المشطية المشبعة بالمضادات الحيوية، وطريقة التخطيط على وسط الاوكزاسيلين الملحي بالإضافة إلى التخطيط على وسط الكروم الملون بنوعيه الوردي والأزرق)، ونتيجة لاستعمال الطرائق السابقة فقد وجد ان طريقة انتشار القرص للمضاد الحيوي (السيفوكستين) ذات دقة عالية (97.8%) مقارنة بالطرائق المظهرية الأخرى، تتبعها طريقة الشريط المشطي المشبع بالاوكزاسيلين، وكذلك طريقة التخطيط على وسط الكروم الوردي والأزرق وبنسبة (97%)، أما طريقة الشريط المشطي المشبع بالمثيسيلين، وكانت طريقة التخطيط على وسط الاوكزاسيلين الملحي اقل دقة مقارنة بالطرائق المظهرية سابقة الذكر وبنسبة (93.3%)، ويمكن الاستنتاج بان طريقة انتشار قرص السيفوكستين هي الأفضل لكونها عالية الدقة، وسهلة الإعداد، ومنخفضة الكلفة، ولا تتطلب أجهزة خاصة لغرض الإعداد.

الكلمات المفتاحية: المكورات العنقودية الذهبية، المكورات العنقودية المقاومة للمثيسيلين، المكورات العنقودية الحساسة للمثيسيلين.

* البحث مستل من اطروحة دكتوراه للباحث الثالث.