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PCR-based identification of methicillin-resistant *Staphylococcus aureus* strains and their antibiotic resistance profiles

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable investigation in which authors identified clinical isolates of MRSA using two methods and determined their antimicrobial resistance patterns. The results propose that PCR assay for *mecA* gene is the best method for detecting true methicillin resistance in *S. aureus*.

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ABSTRACT

Objective: To evaluated the PCR for *mecA* gene compared with the conventional oxacillin disk diffusion method for methicillin-resistant *Staphylococcus aureus* (*S. aureus*) identification.

Methods: A total of 292 *S. aureus* strains were isolated from various clinical specimens obtained from hospitalized patients. Susceptibility test to several antimicrobial agents was performed by disk diffusion agar according to Clinical and Laboratory Standards Institute guidelines. The PCR amplification of the *mecA* gene was carried out in all the clinical isolates.

Results: Among antibiotics used in our study, penicillin showed the least anti-staphylococcal activity and vancomycin was the most effective. The rate of methicillin-resistant *S. aureus* prevalence determined by oxacillin disk diffusion method was 47.6%; whereas, 45.1% of *S. aureus* isolates were *mecA*- positive in the PCR assay.

Conclusions: This study is suggestive that the PCR for detection of *mecA* gene is a fast, accurate and valuable diagnostic tool, particularly in hospitals in areas where methicillin-resistant *S. aureus* is endemic.

KEYWORDS

Methicillin-resistant *Staphylococcus aureus*, Oxacillin disk diffusion, PCR, *mecA* gene

1. Introduction

Staphylococcus aureus (*S. aureus*) is perhaps the greatest concern of human pathogens because of its intrinsic virulence because of its ability to cause a diverse array of life-threatening infections and its capacity to adapt to different environmental conditions[1-3]. Nowadays, this organism is the leading overall cause of health-care associated infections globally and, as more patients are treated outside the hospital settings, is an increasing concern in the community[4,5]. There are many anti-staphylococcal drugs, including methicillin, tetracyclines, fluoroquinolones, linezolid and daptomycin, but they quickly loss their therapeutic value due to the ability of the bacterium to develop effective mechanisms to

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confront these agents[1,6].

Methicillin-resistant *S. aureus* (MRSA) strains have been recognized as serious nosocomaial infections and have spread worldwide that this in turn has an extensive impact on patient managing in health care settings and results in enormous increases in health care costs[7].

S. aureus acquires methicillin resistance by insertion of staphylococcal cassette chromosome (SCC*mec*), carrying the *mecA* gene, into chromosome. This gene encodes an altered penicillin-binding protein, PBP-2a, which is not inhibited by existing b-lactam antibiotics[8-10].

There are several antimicrobial susceptibility methods for detection of MRSA, including oxacillin screening test, oxacillin and/or cefoxitin disk diffusion method and oxacillin minimum inhibitory concentration test[11-13]. There are many reports that these conventional antimicrobial tests are associated with false negative and positive results for MRSA identification. Therefore, it is necessary to use more exact and specific methods, such as PCR that is considered as a DNA-based assay. As respects there is no *mecA* gene in methicillin-sensitive *S. aureus* (MSSA) strains, detection of this gene in any isolates of *S. aureus* is indicative of MRSA[14]. In this regard, we conduct the present investigation to compare oxacillin disk diffusion (ODD) method and PCR assay for identification of true methicillin-resistant strains of *S. aureus* from clinical specimens collected from three large hospitals in Tehran, Iran.

2. Material and methods

2.1. Clinical specimens and laboratory identification

In a period of 9 month, various clinical samples, including blood, urine, skin lesions, sputum, intratracheal tube, cerebrospinal fluid, synovial fluid and pus were obtained from patients of three large teaching hospitals of Tehran and then, transferred to the laboratory by brain-heart infusion broth medium. Each sample was cultured on mannitol salt agar (Merck Co., Germany) and incubated in 37 °C for 24 h. Then, all suspected *S. aureus* colonies were plated onto blood agar. Identification of *S. aureus* suspicious grown colonies was based on Gram staining and standard biochemical reactions, including catalase, coagulase, and novobiocin sensitivity tests.

2.2. Antimicrobial susceptibility testing

Susceptibility of clinical isolates to 10 antibiotics (Mast, Merseyside, UK), including penicillin (10 μg), oxacillin (1 μg), vancomycin (30 μg), cefalotin (30 μg), gentamicin (10 μg), tetracycline (30 μg), erythromycin (15 μg), clindamaycin (2 μg), ciprofloxacin (5 μg) and co-trimoxazole (25 μg) was evaluated by agar disk diffusion method on Mueller-Hinton agar plates, as recommended by Clinical and Laboratory Standards Institute (CLSI)[15]. *S. aureus* ATCC 29213 was used as control strain for disk susceptibility testing.

2.3. Detection of mecA gene by PCR technique

The standard PCR assay was performed using the DNA amplification instrument Mastercycler gradient (Eppendorf, Germany) to identify MRSA strains. Cellular DNA was obtained from *Staphylococci* colonies grown overnight on blood agar plates using DNA Extraction Kit (Bioneer Co., Korea) in accordance with manufacturer's instructions. The *mecA*- specific primer pairs used for amplification of 533 base pair (bp) fragment are Forward, 5′-AAAATCGATGGTAAAGGTTGGC-3′, and Reverse, 5′-AGTTCTGGAGTACCGGATTTGC-3′[16]. A volume of 1 μL of prepared DNA (0.5 μg) was added to a final volume of 25 μL PCR mixture containing 10 μL of 2× Master Mix (Ampliqon, Denmark), including 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, and 1.25 IU *Taq* DNA polymerase, (Ampliqon Co., Denmark), 0.7 μL of 0.8 μmol/L each primer and 12.6 μL of sterile distilled water. The thermal cycling protocol for PCR was comprised 95 °C for 3 min, followed by 33 cycles of 94 °C for 1 min, 53 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 6 min. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide.

3. Results

3.1. Bacterial isolates

Overall, 292 *S. aureus* strains were recovered from obtained clinical samples. The distribution analysis of the *S. aureus* isolates showed that the most isolates (29.0%) were recovered from the pus and the lowest (1.4%) found to be isolated from cerebrospinal fluid (Table 1). The prevalence of MRSA found using antibiotic sensitivity test (ODD), were 47.6% (133/279). The highest rate of oxacillin-resistant *S. aureus* was recovered from blood samples (49.2%).

3.2. Antibiotic resistance profiles

The antimicrobial susceptibility testing by agar disk diffusion method among *S. aureus* isolates determined that the percentage of resistance to penicillin, cefalotin, gentmicin, tetracycline, erythromycin, oxacillin, co-trimoxazole, clindamaycin, ciprofloxacin and vancomycin were 100%, 47.6%, 49.1%, 59.1%, 50%, 47.6%, 48.3%, 25%, 57.7% and 0.7%, respectively (Table 1). The highest rate of resistance among oxacillin-resistant *S. aureus* was related to penicillin with 100% frequency. Except for two strains, all MRSA isolates were susceptible to vancomycin.

3.3. PCR amplification of mecA gene

Identification of MRSA strains was performed by detection of *mecA* gene in all *S. aureus* strains using PCR assay. The results revealed that 45.1% (126/279) of *Staphylococci* isolates carried *mecA* gene. The PCR-amplified DNA products of this gene of five selected clinical isolates are shown in Figure 1. The antimicrobial resistance pattern of *mecA*-positive and negative strains is shown in Table 2.

Table 1

Antibiotic resistance profile of *S. aureus* isolates.

Origin sample	No. of *S. aureus* isolates [*n* (%)]	No. (%) of antimicrobial resistance									
		PEN	OXA	TET	GM	KF	ERY	CD	TS	CIP	VAN
Blood	65 (23.2)	65 (100)	32 (49.2)	41 (63.0)	37 (56.9)	30 (46.1)	38 (58.4)	21 (32.3)	29 (44.6)	38 (58.4)	1 (1.5)
Urine	46 (16.5)	46 (100)	17 (36.9)	28 (60.8)	30 (65.2)	19 (41.3)	18 (39.1)	8 (17.4)	23 (50.0)	20 (43.4)	0 (0.0)
Skin lesions	39 (13.9)	39 (100)	15 (38.4)	16 (41.0)	18 (46.1)	15 (38.4)	15 (38.4)	9 (23.0)	16 (41.0)	23 (58.9)	0 (0.0)
Sputum	14 (5.0)	14 (100)	2 (14.3)	8 (57.1)	6 (42.8)	7 (50.0)	8 (57.1)	5 (35.7)	8 (57.1)	8 (57.1)	0 (0.0)
Intratracheal tube	17 (6.0)	17 (100)	8 (47.0)	9 (53.0)	5 (29.4)	7 (41.1)	10 (70.5)	2 (11.7)	10 (58.8)	9 (52.9)	0 (0.0)
Cerebrospinal fluid	4 (1.4)	4 (100)	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)	3 (75.0)	2 (50.0)	0 (0.0)
Synovial fluid	12 (4.3)	12 (100)	2 (16.6)	6 (50.0)	3 (25.0)	6 (50.0)	4 (41.6)	4 (33.3)	5 (41.6)	6 (50.0)	0 (0.0)
Pus	82 (29.0)	82 (100)	37 (45.1)	56 (68.2)	36 (43.9)	48 (58.5)	45 (54.8)	20 (24.4)	41 (50.0)	55 (67.0)	1 (1.2)
Total	279 (100.0)	279 (100)	133 (47.6)	165 (59.1)	137 (49.1)	133 (47.6)	139 (50.0)	70 (25.0)	135 (48.3)	161 (57.7)	2 (0.7)

PEN: Penicillin, OXA: Oxacillin, TET: Tetracyclin, Gm: Gentamicin, KF: Cefalotin, ERY: Erythromycin, CD: Clindamaycin, TS: Co-trimoxazole, CIP: Ciprofloxacin, VAN: Vancomycin.

Table 2

Antibiotic resistance in *mecA*-positive and *mecA*-negative *S. aureus* isolates using PCR method.

S. aureus isolates	No. of antimicrobial resistance [*n* (%)]								
	PEN	TET	GM	KF	ERY	CD	TS	CIP	VAN
mecA-positive	126 (100)	73 (57.9)	81 (64.2)	70 (55.5)	79 (62.7)	35 (27.7)	71 (56.3)	80 (63.5)	2 (1.6)
mecA-negative	153 (100)	65 (42.4)	52 (33.9)	64 (41.8)	26 (17.6)	8 (5.2)	39 (30.9)	73 (47.7)	0 (0.0)

PEN: Penicillin, TET: Tetracyclin, GM: Gentamicin, KF: Cefalotin, ERY: Erythromycin, CD: Clindamaycin, TS: Co-trimoxazole, CIP: Ciprofloxacin, VAN: Vancomycin.

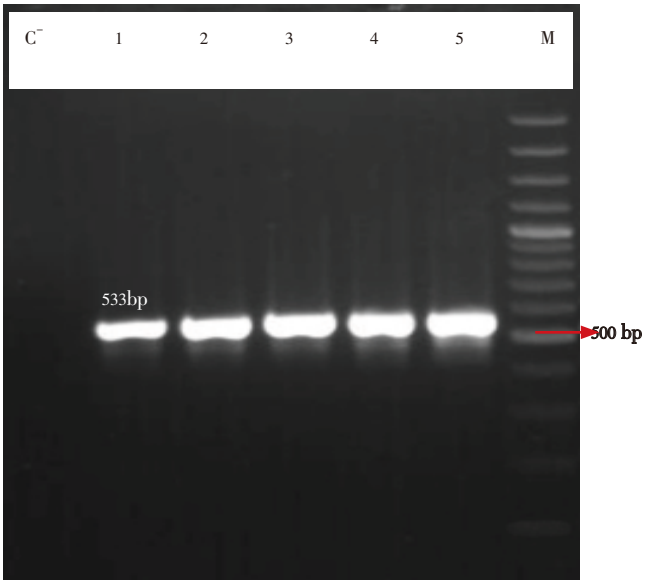

Figure 1. PCR amplification of *mecA* gene in five selected isolates of *S. aureus*.

Lane C⁻: *mecA* negative *S. aureus*; Lane 1-5: PCR product of *mecA* gene (533 bp); M: 100 bp DNA size marker.

4. Discussion

During the last decade, MRSA strains have emerged as serious nosocomial pathogens and spread in many regions of world because of its ability to acquiring resistance to antimicrobial chemotherapy[9,17]. Therefore, rapid recognition of these organisms and detection of methicillin resistance are essential for prompting effective therapy, preventing distribution of infection and reducing the risk of patient's mortality[18,19].

The susceptibility test profile obtained in the present study showed that a significant percentage of *S. aureus* isolates were resistant to most of the commonly used antibiotics, as similar to other studies[12,19-21]. All *Staphylococci* exhibited resistance to penicillin, while 99.3% of strains were sensitive to vancomycin and this the emergence of vancomycin-resistant *S. aureus*, although a few in number, is alarming. It should be noted that both vancomycin-resistant *S. aureus* isolates found in our study were belong to MRSA strains confirmed by PCR. This may be mainly due to the antibiotic abuse and selective pressure of vancomycin, as a main antibiotic available for the treatment of severe and life threatening infections caused by MRSA.

Our results indicated that the rate of antimicrobial resistance among *mecA*-positive compared with *mecA*-negative *Staphylococci* is more. These findings are consistent to the other studies[19-21], and support the fact that the MRSA isolates frequently carry resistance gene to other antibacterial agents. The rate of MRSA prevalence by ODD method was 47.6% (133/279); whereas, 45.1% of *S. aureus* isolates were identified as MRSA using PCR assay. The ODD method was performed according to CLSI guidelines using the *S. aureus* ATCC 29213 reference strain. However, 7 strains of all 133 *S. aureus* isolates that were found as MRSA by this method had no *mecA* gene in PCR assay. This finding has also been reported by Cekovska *et al.*[22] and Davoodi and coworkers[18]. Based on other studies, the ODD test usually shows false negative results and its sensitivity is low, especially for the strains with heterogeneous resistance[11,23-25]. Findings of our investigation indicate that the ODD method can also be associated with false-positive results, as consistent as other studies[19,25]. In general, accurate determination of methicillin resistance in *S. aureus* by conventional laboratory

tests is subject to variations, including inoculum size, diameter, pH and salt concentration of medium, incubation time, *etc*. In such circumstances, detection of *mecA* gene by molecular methods, including the gold standard PCR technique is very helpful and valuable. It seems that the ODD test-positive, but PCR-negative isolates would be penicillinase hyper producer that hydrolyze the penicillinase-resistant penicillins. Susceptibility tests to oxacillin in these strains, namely "borderline oxacillin-resistant *S. aureus*", show reduction or borderline in susceptibility. However, the borderline phenotypes have been attributed to other mechanisms: production of an inducible, plasmid-mediated methicillinase or different alterations in the penicillin-binding protein genes due to spontaneous amino acid substitutions in the transpeptidase domain[26,27]. Distinguish of these low level resistant bacteria by routine tests from true resistant strains that harbor *mecA* gene may be difficult. In the other hand, the clinical dilemma posed by borderline oxacillin-resistant *S. aureus* strains is that during beta-lactam chemotherapy, production of PBP-2a may be induced, converting them into oxacilline-resistant strains. So, detection of *mecA* gene is required for precise differentiation of MRSA and the PCR can be used as a useful method in clinical laboratories.

In conclusion, our study indicates that PCR assay is a easy and reliable tools for detection of MRSA from patients and carrier individuals. On the other hand, with respect to the emergence of multidrug resistant MRSA strains, rapid identification and timely treatment of their infections help to reduce the mortality and avoid the spread of these organisms.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Currently, methicillin-resistant *S. aureus* (MRSA) is considered as a notorious etiologic agent for a various infections and a leading cause of nosocomial infections worldwide. The even greater concern is that the spread of these bacteria is able to evolve resistance to all members of the β-lactam family. Therefore, rapid reporting of identification and antibiogram results can be conducted to good outcomes for patients infected by these pathogens.

Research frontiers

The increased incidence of MRSA and its antibiotic resistance are the major health problems in Iran. This research work was primarily done to compare the performance of MRSA-specific and rapid PCR assay with that of the conventional ODD method to identify true MRSA isolates. Moreover, the work is a epidemiological study regarding susceptibility patterns of these bacteria.

Related reports

The false positive results obtained by the conventional ODD test in the present study have also been reported by Cekovaska *et al*. (2005) in Republic of Macedonia.

Innovations and breakthroughs

This is a comprehensive survey on the antimicrobial susceptibility profiles of MRSA strains isolated from wide variety clinical specimens in three hospitals of Tehran. On the other hand, in Iran, like other countries, antimicrobial susceptibility methods are frequently used for MRSA identification which are associated with false positive and negative results. In the present study, authors indicated that PCR is the gold standard technique for detecting MRSA.

Applications

Rapid detection of clinical isolates of MRSA by easy and reliable tools such as PCR is of key importance in prevention and prognosis of infections caused by these recalcitrant bacteria. Beside this, it would be helpful to infectious specialist inform updated susceptibility data in *S. aureus* isolates for opting a proper antibiotics.

Peer review

This is a valuable investigation in which authors identified clinical isolates of MRSA using two methods and determined their antimicrobial resistance patterns. The results propose that PCR assay for *mecA* gene is the best method for detecting true methicillin resistance in *S. aureus*.

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