

MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL CASES IN EAST ALGERIA

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ABSTRACT

Objective: The purpose of this study is to investigate the methicillin resistance gene, and some virulence factors in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates by polymerase chain reaction (PCR).

Methods: This study has included 12 MRSA isolates. All isolates were previously identified as *S. aureus* by a standard microbiological procedure, and a detection of methicillin resistance was realized by phenotypic methods. Following genomic DNA extraction, the presence of *gyrA*, *mecA*, *lukPV*, and *tst* genes was analyzed by duplex PCR. All retained *S. aureus* species have been found to contain *gyrA* gene.

Results: Ten stains have been found to harbor *mecA* gene indicating its responsibility for methicillin resistance in those strains. Among the 12 strains, six of which were found to be Panton-Valentine leukocidine positive while none of which has *tst* gene encoding the toxic shock syndrome toxin.

Conclusion: The pathogenesis of MRSA infections is related to the expression of a wide variety of virulence factors.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Panton-Valentine leukocidine, Toxic shock syndrome toxin 1, Multidrug resistance, *mecA*.

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INTRODUCTION

Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA), is one of the most prevalent human pathogens isolated from hospitalized patients worldwide and its importance in community settings continues to increase [1]. In 1950, the emergence of antibiotic-resistant strains provided a better understanding of epidemiology of *S. aureus* disease [2]. It often asymptotically colonizes the skin and mucous membranes of healthy individuals, in particular the anterior nares [3].

The clinical importance of MRSA is attributed to notable virulence factors, surface proteins, toxins, and enzymes as well as rapid development of drug resistance [4]. Some of these virulence factors are known to cause or to be associated with specific diseases, such as Panton-Valentine leukocidine (PVL) in necrotizing pneumonia and toxic shock syndrome toxin (TSST) in skin diseases [5].

The importance of assessing the presence and combination of virulence factors associated to MRSA infections has been recently emphasized by different authors [6] supporting the idea that the development of infections could be related to strain-specific pathogenic attributes.

This study was conducted so as to investigate the phenotypic and genotypic characterization of MRSA involved in east Algeria. The confirmation of the species *S. aureus* was performed by amplifying the gene *gyrA*. Resistance to methicillin was performed by detection of *mecA* gene and several virulence factors including toxin of the PVL coding gene as well as TSST coding gene were searched by polymerase chain reaction (PCR).

METHODS

Identification of *S. aureus* strains

Twelve strains of *S. aureus* resistant to methicillin from Annaba Health establishment were included in this study from clinical sources (pus,

urine, and vaginal samples). *S. aureus* strains were grown on blood agar at 37°C. After overnight incubation, strains were examined by Gram-stain and using catalase and coagulase tests as described previously [7]. Isolates were also tested using the Pastorex Staph Plus latex (Bio-Rad) for identification of *S. aureus*.

Antimicrobial susceptibility testing

Antibiotic resistance was determined by the disk diffusion method (Bio-Rad, France) in Mueller-Hinton agar (Bio-Rad) according to the recommendations outlined by the Comité de l'Antibiogramme de la Société Française de Microbiologie 2013.

Detection of methicillin resistance was realized by phenotypic methods as described previously [8].

The tested antibiotics were penicillin G (PG - 6 µg), oxacillin (Ox - 5 µg), cefoxitin (FOX - 30 µg), gentamicin (GM - 15 µg), tobramycin (TOB - 6 µg), kanamycin (K - 30 UI), tetracycline (TE - 30 µg), clindamycin (CM - 2 µg), erythromycin (E - 15 µg), pristinamycin (PR - 15 µg) chloramphenicol (C - 30 µg), ofloxacin (OFX - 5 µg), fusidic acid (FA - 10 µg), vancomycin (VA - 30 µg), teicoplanin (TEC - 30 µg), rifampicin (RA - 50 µg), and trimethoprim-sulfamethoxazole (cotrimoxazole) (SXT - 1.25/23.75 µg).

Genetic characterization

Bacterial DNA extraction

The extraction technique was simplified by directly suspending 5-10 colonies in 1 ml of water, centrifuged at 13,000 g for 10 minutes, discards the supernatant and recover the pellet, resuspended in 300 µl of water, and centrifuged the suspensions for 10 minutes at 100°C. The DNA was released by centrifugation at 13,000 g for 10 minutes in the supernatant. The collected DNA was visualized after electrophoresis on 1.5% agarose gels containing éthidium bromide.

Duplex PCR amplification of *gyrA* and *mecA* genes

PCR amplification of the *gyrA* gene in *S. aureus* acts as an internal control, allowing the quality of the DNA extraction and amplification for each sample [9]. The presence of the *mecA* gene was detected following a PCR procedure described elsewhere [10].

Duplex PCR amplification of *luk-PV* and *tst* genes

The 12 MRSA strains isolated were of object research of the gene encoding leukocidin Panton-Valentine toxin *Luk-PV* and the gene encoding the TSST *tst* using specific primers as described previously [11,12].

RESULTS

Precise identification of *S. aureus* is important for successful implementation of a staphylococcal infections control program. Therefore, according to the phenotypic, biochemical properties as well as by amplification of the *gyrA* gene, all of the isolates obtained in this study were identified as *S. aureus*.

Antibiotic resistance patterns of the MRSA strains isolated shown in Fig. 1.

Among the 12 strains of MRSA, 10 were *mecA* positive, indicating that *mecA* is responsible for methicillin resistance in those strains (Fig. 2).

Detection of PVL toxin by amplification of the gene *luk-PV* from extracted DNA of the strains revealed that five strains from 12 were positive for amplification of the 533 Pb fragment of *luk-PV* gene. While none strain had the gene *tst* encoding the TSST (Fig. 3).

DISCUSSION

The multidrug resistance of MRSA has become increasingly a major problem and responsible for most of the hospital-acquired infections. Many virulence factors are associated with MRSA that enable them to cause disease [13]. The aim of this study was to investigate the methicillin resistance gene and some virulence factors in MRSA isolates by PCR.

The obtained results showed that MRSA isolates exhibited low resistance to chloramphenicol and trimethoprim/sulfamethoxazole. This supports the potential utility of chloramphenicol and trimethoprim/sulfamethoxazole as empiric treatment agents for MRSA in Algeria.

However, almost of strains were resistant to gentamicin, tobramycin, tetracycline, and ofloxacin. Fortunately, none of this MRSA strains was resistant to vancomycin, teicoplanin, and pristinamycin. All strains showed also a high resistance to kanamycin.

Genotypic detection of *mecA* is widely used as a reference standard for identification of MRSA, whether used as a primary test or for confirmation [14]. In our study, 10 strains were harboring the gene *mecA* responsible for methicillin resistance. The two remaining strains were *mecA* negative must be methicillin resistant by implication of some other mechanism [14] and could thus be classified as producers of β -lactamase or often identified as borderline oxacillin-resistant *S. aureus* or, more rarely, moderately resistant *S. aureus* strains [15].

Many virulence factors are associated with MRSA that enable them to cause disease.

In our study, six MRSA strains were produced The PVL toxin which was investigated further by testing 12 strains of MRSA to lukPV PCR; two of them were *mecA* negative. In the previous studies on the general population in Algeria, rates of PVL+ strains were 67.2% in 2006 [16] down to 35.7% [9] and 29.7% [17]. Other studies were reported lower

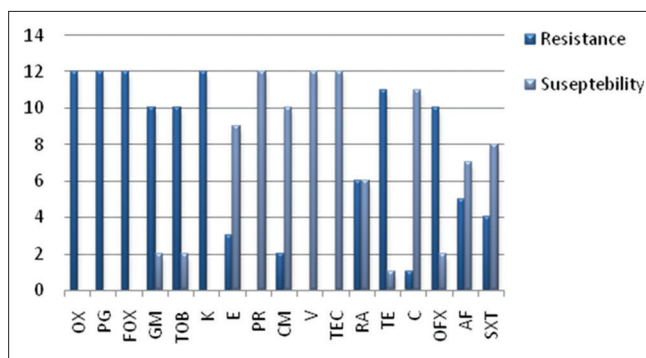


Fig. 1: Antibiotic-resistance profile of methicillin resistant *Staphylococcus aureus* strains

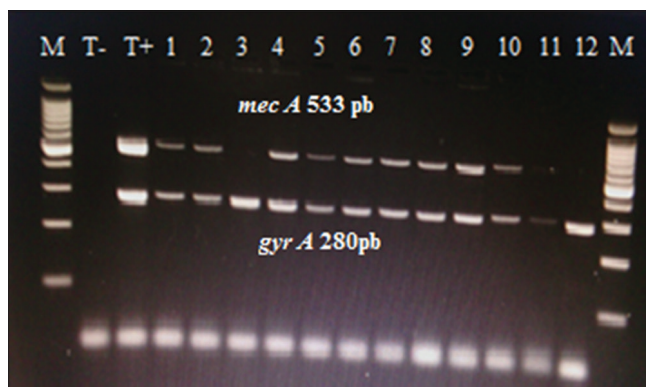


Fig. 2: Electrophoresis on agarose gel of *mecA* and *gyrA* genes. Lane 1-12: The polymerase chain reaction products of methicillin resistant *Staphylococcus aureus* studied DNA samples. T: Negative control. T+: Positive controls. M: 100 bp DNA marker

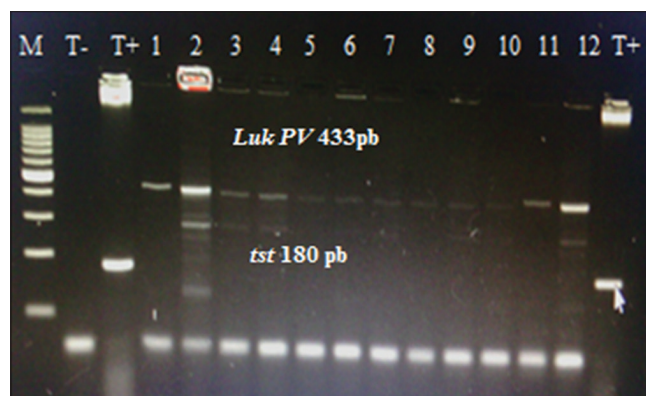


Fig. 3: Electrophoresis on agarose gel of *luk-PV* and *tst* genes. Lane 1-12: The polymerase chain reaction products of methicillin resistant *Staphylococcus aureus* studied DNA samples. T: Negative control. T+: Positive controls. M: 100 bp DNA marker

rates PVL positive MRSA such as 15% in the Netherlands [18] and 0.7% in Japan [19].

The *tst* PCR assay of MRSA strains showed that none of these strains generated positive results. Same results were reported in Nigerian study [20] and other in Tunisia [21].

However, El-Ghodban *et al.* in Libya reported a frequency of *tst* gene isolation, only 7.5% in 40 clinical isolates [22]. Teyhoo *et al.*, in Iran, detected 14.3% and 20% *tst* gene carriage in clinical isolates [23].

CONCLUSION

The pathogenesis of MRSA infections is related to the expression of a wide variety of virulence factors, including PVL and TSST toxins, which might be considered as potential threats especially that most of them have multidrug resistance, rendering it difficult to treat.

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