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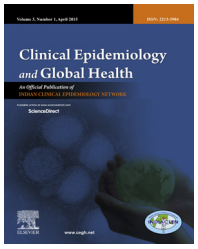

Original Article

Prevalence of antibiotic resistance and virulence factors encoding genes in clinical *Staphylococcus aureus* isolates in Saudi Arabia

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ABSTRACT

Background: The present work intended to investigate the carriage of antibiotic resistance and virulence genes in multidrug-resistant *Staphylococcus aureus* isolated from various clinical specimens.

Methods: A total of fifty *S. aureus* isolates from blood cultures, wound swabs, urine sample, nasal swabs, and sputum sample were examined for their antibiotic resistance against 20 different antibiotics by means of E-test, M.I.C Evaluator Strips, and disk diffusion methods. Detection of resistance and virulence-encoding genes (*mecA*, *van*, fnBPA, and Panton-Valentine Leucocidin (PVL)-encoding genes) was performed by PCR.

Results: In the current study, low number of MRSA isolates has been detected from five different clinical samples (22%, $n = 50$). In this study, we observed multiple drug resistance in *S. aureus* isolates from wound swabs; nasal swabs, blood cultures, and urine sample. No vancomycin-resistant genes were detected in all 50 isolates; similarly, no PVL and *van* genes were detected, while *mecA* and FnBP genes were detected in low number of isolates.

Conclusion: Although the number of MRSA and fnBPA-positive *S. aureus* reported in this study is generally low, and despite the absence of PVL and *van*-encoding genes, the results reported in this study may continue to shed some light on the prevalence of MRSA in Makkah, Saudi Arabia. Further epidemiological surveys are required together with additional infection control measures to limit the spread of MRSA particularly multidrug-resistant strains.

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1. Introduction

Staphylococcus aureus is one of the most common human pathogens and is capable of causing a wide range of infections such as cellulitis, impetigo, and soft tissue abscesses. The ability of *S. aureus* to cause a multitude of infections is probably due to the expression of a wide range of virulence factors, as the following: adhesion proteins, toxins and enzymes.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently the most prevalent antibiotic-resistant pathogen causing nosocomial infections in hospitals (HA-MRSA) in many parts of the world with increasing prevalence in various community populations (CA-MRSA).[2,3]

S. aureus expresses Fibronectin-Binding Protein A (fnBPA), a cell wall-anchored protein, which mediates the adhesion of the cells to fibrinogen, elastin, and fibronectin.[4] The fnBPA protein is believed to be indispensable for adhesion to and internalization by nonprofessional phagocytic cells upon ingestion by inflammatory macrophages. FnBPA have been shown to be important in *in vitro* and *in vivo* infections by *S. aureus*; furthermore, the cooperation between fnBPA and fnBPB is essential for the induction of severe infections resulting in septic death.[5–7] The Panton-Valentine Leucocidin (PVL) is a bicomponent leucotoxin composed of S-related and F-related proteins that are secreted separately but act synergistically; the cytotoxin is found to cause leukocyte destruction and tissue necrosis.[8]

Methicillin resistance in *S. aureus* is conferred by the *mecA* gene, which codes for penicillin-binding protein 2a (PBP2a) causing decreased binding affinity for the β-lactam antibiotics, including the penicillinase-resistant penicillin. The *mecA* gene resides on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec). The *mecA* gene complex contains insertion sites for other mobile genetic elements (*e.g.* plasmids and transposons) that facilitate the acquisition of resistance genes to other antibiotics.[1] Epidemiological studies have shown that hospital- and community-acquired MRSA infections are increasing in many parts of the world.[2]

Vancomycin (glycopeptides) has become an approved and highly recommended antibiotic worldwide for the treatment of *S. aureus* infections, particularly MRSA.[9] Although vancomycin-resistant *S. aureus* is rare, the emergence of strains with decreased susceptibility and/or vancomycin intermediate resistance has been reported.[10–12]

In Saudi Arabia, the incidence of community- and hospital-acquired MRSA infections is well documented.[13–17] However, few published accounts have reported the prevalence of PVL-positive *S. aureus*,[18,19] with no available reports regarding the fnBPA-positive *S. aureus* as well as vancomycin resistant and/or vancomycin intermediate *S. aureus* in Saudi Arabia. Therefore, this dearth of information about the incidence of *S. aureus* with PVL, fnBPA, and *van*-encoding genes in Saudi Arabia has led us to investigate the carriage of these genes in addition to *mecA* gene in a collection of *S. aureus* isolates from various clinical specimens in western Saudi Arabia.

2. Materials and methods

2.1. Isolates

A total of 50 *S. aureus* isolates were thankfully obtained from clinical laboratories in Makkah, western Saudi Arabia. These isolates were recovered from wound swabs (17), nasal swabs (24), urine (1), sputum (3), and blood cultures (5). The isolates were transported to the laboratory on blood agar plates, in an icebox.

2.2. Biochemical confirmation of the isolates

Once the isolates were received in the laboratory, they were given a code number, and each isolate was subcultured on blood agar and DNase agar plates (Oxoid, Basingstoke, UK); incubation was at 34 °C for 24 h for further identification and confirmation. All *S. aureus* isolates growing on blood agar plates were observed for their type of hemolysis, and were observed for their ability to produce deoxyribonuclease on DNase agar.[20] All isolates were examined for their ability to

Table 1 – Primers used for detection of mecA, Van, PVL, and FnBPA-encoding genes by PCR.

Gene	Primer sequence	Product size (bp)	Reference
mecA	F 5′-AAAATCGATGGTAAAGGTTGGC-3′ R 5′-AGTTCTGGAGTACCGGATTTGC-3′	533	27
vanA/vanA1	F 5′-ATGAATAGAATAAAAGTTGCAATAC R 5′-CCCCTTTAACGCTAATACGAT	1029	28
vanB/vanB1	F 5′-CCCGAATTTCAAATGATTGAAAA R 5′-CGCCATCCTCCTGCAAAA	457	28
vanC/vanC1	F 5′-GCTGAAATATGAAGTAATGACCA R 5′-CGGCATGGTGTTGATTTCGTT	811	28
vanS/vanS1	F 5′-AACGACTATTCCAAACTAGAAC R 5-GCTGGAAGCTCTACCCTAAA	1094	28
vanY/vanY1	F 5′-ACTTAGGTTATGACTACGTTAAT R 5′-CCTCCTTGAATTAGTATGTGTT	866	28
PVL	LukS-PV 5′-AGTGAACTTATCTTTCTATTGAAAAACACTC-3′ LukS-PV 5′-GCATCAASTGTATTGGATAGCAAAAGC-3′	433	29
fnBPA	F 5′-CACAACCAGCAAATATAG-3′ R 5′-CTGTGTGGTAATCAATGTC-3′	1362	29

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