

## Full Length Research Paper

## Prevalence of *mecA*, PVL and *ica* genes in *Staphylococcus aureus* strains isolated from urinary tract infections patients

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The prevalence of *Staphylococcus aureus* among urinary tract infections (UTIs) patients has been increasing worldwide. The aim of this study was to determine the occurrence of the *mecA*, PVL, *ica* genes in a collection of MRSA urinary isolates by PCR. Methicillin resistance *S. aureus* (MRSA) is considered to have emerged from *S. aureus* through the acquisition of staphylococcal cassette chromosome (SCCmec), which carries the *mecA* gene for methicillin resistance. Panton-Valentine leukocidin (PVL)-producing strains of MRSA appear to be associated with increased risk of transmission, complications and hospitalization. *icaA* and *icaD* genes have been reported to play a significant role in biofilm formation in *S. aureus*. Out of 50 isolates of *S. aureus* from UTI patients, 36 (72%) were found to be MRSA by oxacillin screen agar. All these MRSA strains were found to be positive for *mecA* genes, 9 (25%) were found to be positive for PVL and 23 (64%) were positive for both *icaA* and *icaD* genes. MRSA isolated from UTI patients show the presence of *mecA*, PVL, *ica* genes, which may have consequences for the treatment of UTIs especially in catheter-associated and nosocomial infections.

**Key words:** Urinary tract infections (UTIs), *mecA* gene, PVL gene, *ica* gene, MRSA.

### INTRODUCTION

Urinary tract infections (UTIs) are the third common infections after respiratory and gastro-intestinal infections, and most common cause of both community-acquired and nosocomial infections (Najar et al., 2009; Hryniewicz et al., 2001). Although most UTIs are caused by Gram negative bacteria, other species such as *Staphylococcus* spp. are emerging (Bonadio et al., 2001; Farajnia et al., 2009). Several studies have reported the increasing prevalence of *Staphylococcus aureus* among UTI patients (Nwanze et al., 2007; Akortha and Ibadin, 2008). *S.*

*aureus* is an important human pathogen whose pathogenicity largely depends on producing a broad spectrum of extracellular and cell wall-associated virulence determinants. Among them, a wide variety of surface adhesins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) has been described (Sauer et al., 2008). MRSA is considered to have emerged from *S. aureus* through the acquisition of SCCmec, which carries the *mecA* gene for methicillin resistance (Takano et al., 2008). Methicillin resistance is

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clinically very important because a single genetic element confers resistance to the beta-lactam antibiotics, which include penicillins, cephalosporins and carbapenems (Grundmann et al., 2006). MRSA isolation frequency has increased in association with UTIs in Japan, USA and France (Shigemura et al., 2005; Baba-Moussa et al., 2008; Johnson et al., 2006). PVL is a cytotoxin, and a member of the bi-component family of staphylococcal leukocidins and one of the  $\beta$ -pore-forming toxins. The toxins subunits bind to leukocyte cell membrane inducing trans-membrane pore formation and subsequent cell lysis (Khosravi et al., 2012). PVL is a synergohymenotropic toxin (Lina et al., 1999) that can damage the membranes of human polymorphonuclear cells and macrophages by forming pores in the membranes of leukocytes, resulting in an increase in membrane permeability and cell lysis (Prévost et al., 2001). PVL is described as a key virulence factor because it can be found virtually in all community acquired (CA)-MRSA strains that cause soft-tissue infections (Vandenesch et al., 2003). PVL is carried by <5 % of isolates of *S. aureus*, both methicillin-sensitive *S. aureus* and MRSA (Dyer, 2007; Holmes et al., 2005). PVL-producing strains of CA-MRSA appear to be associated with increased risk of transmission, complications and hospitalization. Biofilm formation, especially on medical implants such as catheters, is another important virulence mechanism for *S. aureus*. Bacterial cells in a biofilm show much greater resistance to antibiotics than free living cells; biofilms also help microorganisms evade host immune responses. The inter-cellular adhesion (*ica*) locus consists of the genes *icaADBC*, and among the *ica* genes, *icaA* and *icaD* have been reported to play a significant role in biofilm formation in *S. aureus* and *Staphylococcus epidermidis* (Cramton et al., 1999). Co-expression of *icaA* and *icaD* genes leads to the full phenotypic expression of the capsular polysaccharide (Vasudevan et al., 2003). This study investigated the presence of *mecA* gene and various virulence factors (*pVL* and *ica* genes) of MRSA strains isolated from UTI patients in Khartoum State, Sudan.

## MATERIALS AND METHODS

The bacteria investigated comprised of *S. aureus* isolates collected from UTI patients from four main tertiary care hospitals in Khartoum (Khartoum Teaching Hospital, Sahiroon Hospital, Soba University Hospital, Ibrahim Malik Hospital) from 2011 to 2014 which were stored at  $-70^{\circ}\text{C}$  in Tryptic Soy broth with 20% glycerol at the microbiology laboratory (research lab) of Sudan University for Sciences and Technology (SUST). Identification of *S. aureus* was based on the colony morphology, Gram staining, catalase (Sigma), coagulase tests and latex slide agglutination Staphytest Plus test (Oxoid). *S. aureus* isolates were detected as MRSA (oxacillin-resistant) by inoculating the organism onto Oxacillin Screen Agar (Mueller-Hinton agar plates supplemented with 4% NaCl and oxacillin 6  $\mu\text{g}/\text{ml}$ ) according to NCCL (2001) guidelines, and confirmed by ceftioxin disc test (Oxoid). Any growth after incubation for 24 h at  $35^{\circ}\text{C}$  was interpreted as a positive MRSA (Louie et al., 2001). Reference strains MSSA ATCC 25923 and MRSA ATCC43300 were used as negative and positive controls, respectively.

The *mecA*, PVL, *icaA* and *icaD* genes of MRSA isolates were detected by PCR. Chromosomal DNA was isolated from overnight cultures grown on blood agar at  $37^{\circ}\text{C}$ . Genomic DNA was extracted by using microwave method (Ahmed et al., 2014) with some modification. Briefly, cell pellets were incubated for 30 min at  $65^{\circ}\text{C}$ , after washing with TE and addition of 50  $\mu\text{l}$  of 10% SDS (Sigma). The lysates were centrifuged and supernatants were removed. The micro-tubes were then placed in a microwave oven and heated three times for 1 min at 750 W. The pellets were dissolved in TE (Tris-EDTA, Sigma) buffer and were extracted with an equal volume of chloroform/isoamyl alcohol (24:1) for 15 min. The aqueous phase was recovered by centrifugation for 20 min and precipitated with ethanol. The primers used in this study are shown in Table 1. They were synthesized by IDT (Integrated DNA technologies, Interleucvenlaan, 12A,B 3001, Belgium). A 50  $\mu\text{l}$  PCR mixture containing 3  $\mu\text{l}$  of DNA template, 1  $\mu\text{l}$  (100 pmol) of each primer and a 25  $\mu\text{l}$  of Taq PCR Master Mix polymerase containing 100 mM Tris-HCl, 500 mM KCl at pH 8.3 at  $20^{\circ}\text{C}$ , 1.5 mM  $\text{MgCl}_2$ , 200 M of each of deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA) was prepared. Amplification of DNA was performed using Mastercycler PCR machine (Eppendorf Co, Germany). PCR thermocycling conditions for each reaction are shown in Table 2. During the PCR reaction for *icaA* and *icaD*, a further 1U of Taq DNA polymerase was added after the first 30 cycles. Ten microlitres of each PCR product was mixed with 2  $\mu\text{l}$  loading buffer and separated on a 2% agarose gel (Sigma) in TBE buffer. Amplified products were visualized under UVP BioDoct-It digital imaging system (UVP, Inc., Cambridge, UK) after staining with ethidium bromide (Sigma).

## RESULTS AND DISCUSSION

Out of 50 urinary *S. aureus* isolates, 36 (72%) were found to be positive MRSA by Oxacillin screening agar. All MRSA strains were positive for the *mecA* genes (Figure 1), 9 (25%) were positive for PVL (Figure 1) and 23 (64%) were positive for both *icaA* and *icaD* genes (Table 3). It was also found that 23 strains that were positive for *icaA* were also positive for *icaD* (Figures 2 and 3).

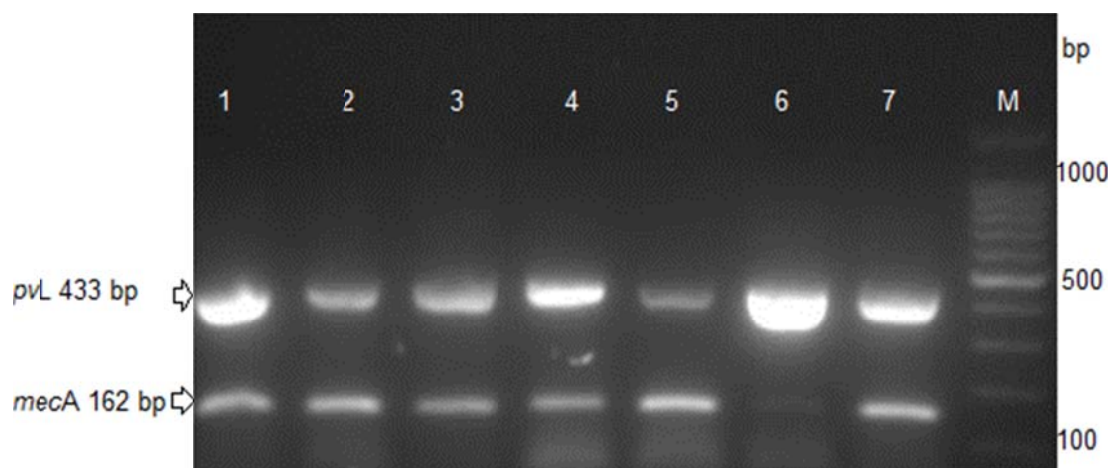
All MRSA strains isolated by oxacillin screening agar were found to be *mecA*-positive by PCR. Many studies have evaluated the sensitivity of different culture media and methods for the primary phenotypic detection of MRSA (Monsen et al., 2003; Davies and Zadik, 1997; NCCLS, 2001). In addition, the low-cost of such methods in comparison with PCR based kits make them likely to be used in low resource settings. The results of this study showed that 36 (72%) of *S. aureus* were *mecA*-positive. MRSA UTIs are increasing throughout the world. Similar studies from various countries (Onanuga and Awhowho, 2012; Araki et al., 2002; Martineau et al., 2000) reported different rates of MRSA among urinary isolates. CA-MRSA is more likely to produce PVL than HA-MRSA (Aires-de-Sousa et al., 2006). The results in this study demonstrated that 9 (25%) of MRSA were PVL-positive. All PVL-positive strains were community acquired strain. Although the PVL from *S. aureus* have rarely been described in cases of UTI (Park et al., 2008; Baba-Moussa et al., 2008), PVL carriage appears to be a possible virulent factor particularly among community-acquired strains. The ability of *S. aureus* to form biofilms

**Table 1.** Primers of genes used in the study.

Primer	Primer Sequence (5' _3')	Product size (bp)	Reference
<i>mecA</i> -P4	TCCAGATTACAACCTTCACCAGG	162	Milheiriç et al. (2007)
<i>mecA</i> -P7	CCACTTCATATCTTGTAACG		
<i>Luk-pvl</i> - F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	Lina et al . (1999)
<i>Luk-pvl</i> - R	GCATCAAGTGTATTGGATAGCAAAAAGC		
<i>icaA</i> -F	TCTCTTGCAGGAGCAATCAA	188	Cramton et al. (1999)
<i>icaA</i> -R	TCAGGCACTAACATCCAGCA		
<i>icaD</i> -F	ATGGTCAAGCCCAGACAGAG	198	Cramton et al. (1999)
<i>icaD</i> -R	CGTGTTTTCAACATTTAATGCAA		

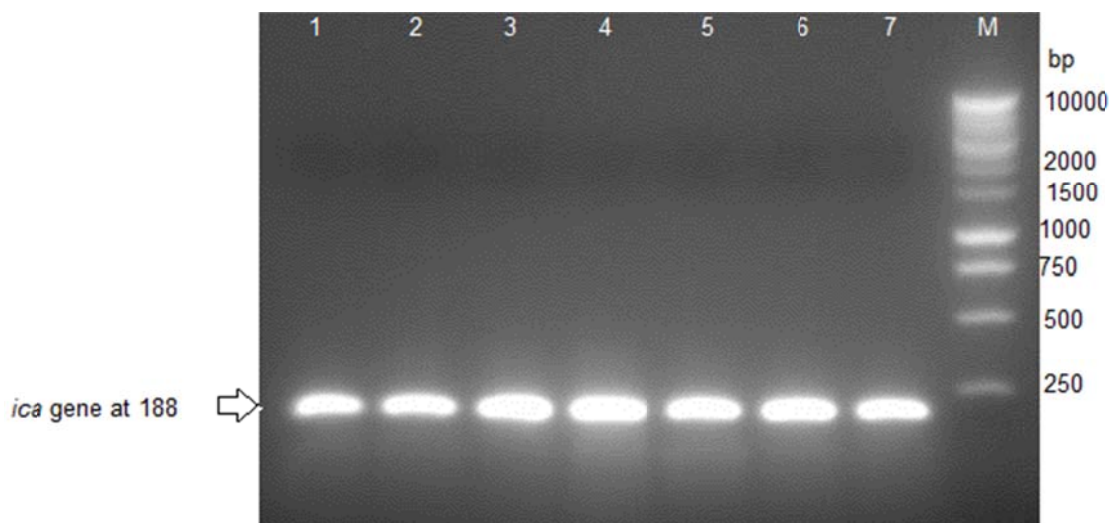
**Table 2.** PCR thermocycling conditions.

PCR	Temperature (°C)/Time					Cycle no.
	Initial denaturation	Cycling condition				
		Denaturation	Annealing	Extension	Final extension	
<i>Pvl</i>	94/5 min	94/40 sec	53/40 sec	72/1 min	72/10 min	35
<i>mecA</i>	94/5 min	94/40 sec	53/40 sec	72/1 min	72/10 min	35
<i>icaA</i>	94/5 min	94/30 sec	55.5/30 sec	72/30 sec	72/1 min	50
<i>icaD</i>	94/5 min	94/30 sec	55.5/30 sec	72/30 sec	72/1 min	50

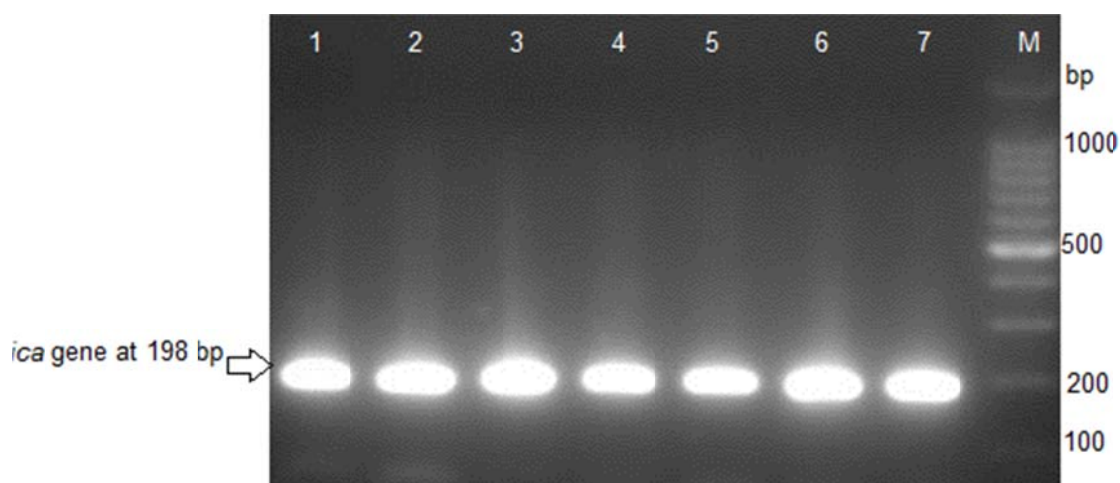
**Figure 1.** Multiplex PCR assay for *mecA* and PVL gene detection. Lane1; positive control, Lane 2, 3, 4, 5 and 7; 162-bp *mecA* and 433-pb PVL genes fragment, Lane 6; a 433-pb PVL with negative *mecA* gene, Lane M; 100-bp DNA ladder.**Table 3.** Frequency of *mecA*, *pvl*, *ica* genes among MRSA isolates.

Gene	Positive no. (%)	Negative no. (%)	Total (%)
<i>mecA</i>	36(100%)	0(0%)	
<i>pvl</i>	9(25%)	27(75%)	36(100%)
<i>icaA</i>	23(64%)	13(36%)	
<i>icaD</i>	23(64%)	13(36%)	

helps the bacterium to survive in hostile environments within the host and is considered to be responsible for persistent infections (Christensen et al., 1985; Bernardi et al., 2007). Synthesis of the capsular polysaccharide is, at least in parts, mediated by the *ica* operon. Upon the activation of this operon, a polysaccharide intracellular adhesion (PIA) is synthesized. In the present study, it was observed that 23 (64%) of the MRSA isolates



**Figure 2.** PCR assay for *icaA* gene detection. Lane 1; positive control, Lane 2, 3, 4, 5, 6 and 7; 188-bp *icaA*, Lane M; 1kb DNA ladder.



**Figure 3.** PCR assay for *icaD* gene detection. Lane 1; positive control, Lane 2, 3, 4, 5, 6 and 7; 198-bp *icaD*, Lane M; 100-bp DNA ladder.

harbored loci, *ica* and 9 (25%) harbored *pvL*. These results were higher than that of Park et al. (2008) who believed that the *icaA* genes may enhance the adherence of *S. aureus* to host cells of the urinary tract, and may play a pathogenic role in UTI patients. Several studies described the roles of regulatory elements associated with biofilm formation on the regulation of virulence (Yarwood et al., 2004; Caiazza and O'Toole, 2003). The formation of slime and biofilms by *S. aureus* strains causing catheter-associated and nosocomial infections have been shown to be associated with the presence of the *icaA* and *icaD* genes (Ziebuhr et al., 1997; Arciola et al., 2001). In conclusion, the results of this study suggest that MRSA isolated from UTI patients showed the

presence of *mecA*, PVL, *ica* genes, which may have consequences for the treatment of UTI especially in catheter-associated infections. Close surveillance of these strains is essential to monitor their spread among UTIs particularly among hospital inpatients.

#### Conflict of Interest

The author(s) have not declared any conflict of interests.

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