ORIGINAL ARTICLE

Detection of ST772 Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* (Bengal Bay clone) and ST22 *S. aureus* isolates with a genetic variant of elastin binding protein in Nepal

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Abstract

Genetic characteristics were analysed for recent clinical isolates of methicillin-resistant and -susceptible *Staphylococcus aureus* (MRSA and MSSA respectively) in Kathmandu, Nepal. MRSA isolates harbouring Panton-Valentine leukocidin (PVL) genes were classified into ST1, ST22 and ST88 with SCC*mec*-IV and ST772 with SCC*mec*-V (Bengal Bay clone), while PVL-positive MSSA into ST22, ST30 and ST772. ST22 isolates (PVL-positive MRSA and MSSA, PVL-negative MRSA) possessed a variant of elastin binding protein gene (*ebpS*) with an internal deletion of 180 bp, which was similar to that reported for ST121 *S. aureus* previously outside Nepal. Phylogenetic analysis indicated that the *ebpS* variant in ST22 might have occurred independently of ST121 strains. This is the first report of ST772 PVL-positive MRSA in Nepal and detection of the deletion variant of *ebpS* in ST22 *S. aureus.*

Keywords: Bengal Bay clone, elastin binding protein, MRSA, Nepal, Panton-Valentine leukocidin (PVL), ST22, ST772
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as one of the most common pathogens of both nosocomial and community-acquired infections worldwide. As a feature distinct from methicillin-susceptible *S. aureus* (MSSA), MRSA has a transmissible genome element, staphylococcal cassette chromosome *mec* (SCC*mec*), inserted in a specific site of the chromosome. The SCC*mec* in MRSA has been differentiated into at least 12 genetic types (I–XII) [1,2], among which types I–III have been traditionally associated with hospital-acquired MRSA (HA-MRSA), while type IV and V have been commonly found in community-acquired MRSA (CA-MRSA) [3]. However, in recent years, CA-MRSA with the dominant SCC*mec* types (IV and V) has been brought to healthcare settings causing nosocomial infections [4–6], which makes distinction between HA- and CA-MRSA more difficult in terms of SCC*mec* type. The pathogenesis of many CA-MRSA strains have been attributed to the production of Panton-Valentine leukocidin (PVL), a two-component toxin encoded by two genes, *lukF-PV* and *luk-S-PV,* which are carried on lysogenic bacteriophages [7,8]. The PVL causes leukocyte lysis or apoptosis *via* pore formation [9]. Accordingly, PVL-positive *S. aureus* is associated with severe symptoms in a wide spectrum of infections including skin and soft tissue infections and necrotizing pneumonia [10,11]. Prevalence of CA-MRSA harbouring PVL genes has been increasing recently in hospitalized patients as well as healthy individuals in the community [12,13].

Distribution and spread of MRSA clones on a global scale have been revealed by genetic classifications with multilocus sequence typing and SCC*mec* typing [14,15]. Several HA-MRSA clones including ST5-MRSA-SCC*mec* II (ST5-II, NY/Japan clone) and ST22-IV (EMRSA-15) are known as pandemic clones

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TABLE 1. Frequency of staphylocoagulase (*coa*) genotypes and SCC*mec* types in *Staphylococcus aureus* isolates

	MRSA		MSSA	
	PVL(+)	PVL(−)	PVL(+)	PVL(−)
Genotype	(n = 25)	(n = 7)	(n = 48)	(n = 20)
coa				
IIa	1	1	2	12
IIIa	2	0	0	1
IVa	1	0	26	0
Va	0	1	5	3
VIa	16	3	6	0
VIc	0	0	0	1
VIIa	2	0	2	2
VIIb	0	0	0	1
Xa	0	0	1	0
XIa	3	2	6	0
SCC*mec*				
IV	1	1		
V	17	3		
NI[a]	7	3		

MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-susceptible *S. aureus;* PVL, Panton-Valentine leukocidin.
[a]Not identified. Ten SCC*mec*-NI strains: two isolates, mec class untypeable (mec-UT)/ccrA2B2; three isolates, mec-UT/ccrC1; three isolates, mec C2/ccr-UT; two isolates, mec-UT/ccr-UT.

predominating in East Asia/North America and Europe, respectively. In contrast, various clones have been documented for CA-MRSA which are distributed locally or predominate in a region, often associated with international spread. Globally predominant CA-MRSA includes five clones, i.e. ST1 (USA400 clone), ST8 (USA300 clone), ST30 (South West Pacific clone), ST59 (Taiwan clone) and ST80 (European clone), among which ST8 and ST30 are considered pandemic as a result of its distribution to every continent [15]. In Asia, two pandemic HA-MRSA clones with ST5 and ST239 are disseminating, whereas various CA-MRSA clones including those with ST8, ST30, ST59, ST72 and ST772 have been reported [16].

In Nepal, the prevalence of MRSA from clinical specimens in hospitals has been described to be 26–69% in several studies *via* antimicrobial susceptibility testing [17–21], although the rate varies depending on the types of infections or specimens examined. A recent study revealed a high prevalence of PVL genes in nosocomial isolates of MRSA and MSSA (26% and 52% respectively) [22]. However, in Nepal, there have been no studies conducted on genotypes (ST and SCC*mec* types) of clinical MRSA isolates, particularly PVL-positive isolates.

We analysed recent clinical isolates of MRSA and MSSA in hospitals in Nepal. We found high prevalence of PVL in MRSA and MSSA, as well as the presence of PVL-positive ST772 MRSA-V (Bengal Bay clone). A deletion variant of elastin binding protein gene was first identified in ST22 *S. aureus* isolates and its origin was analysed.

Materials and Methods

Bacterial isolates and initial genetic analysis

From August 2012 to October 2012, about 200 *S. aureus* isolates were collected from two general hospitals (approximately 100 isolates each) with more than 500 beds in Kathmandu, Nepal. These isolates were transported to Genesis Laboratory and Research and processed. Of these, only 100 isolates recovered were included in this study. The main specimen of the isolates was pus (*n* = 84), followed by urine (*n* = 12), sputum and blood (*n* = 2 each). A single isolate from each individual patient was subjected to study. Bacterial isolation and species identification were performed by

TABLE 2. Genetic characteristics and virulence factors in 17 *Staphylococcus aureus* isolates in Nepal

			Genotype							
*mec*A/PVL	Isolate no.	Isolate source	SCC*mec*	*coa*	*agr*	ST	Leukocidins, haemolysins[a]	Enterotoxins[b]	Adhesins[a,b,c]	Other[b]
mecA	NP154	Urine	V	IIa	III	ST1	*lukE-lukD, hla, hld, hlg2*	*sea, sec, seh, sei, sek, sel, seq*	*cna, ebpS, fnbA, fib, sdrD, sdrE*	*tst-1*
mecA	NP177	Pus	IV	XIa	I	ST22	*hla, hld*	*sec, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS-v, fnbA, sdrD, sdrE*	*chp, tst-1*
mecA	NP18	Urine	V	VIa	II	ST772	*hla, hld, hlg2*	*sea, sec, seg, sei, sel, sem, sen, seo*	*cna, ebpS, fnbA, fib, sdrD, sdrE*	
mecA/PVL	NP160	Pus	V	VIa	II	ST772	*lukE-lukD, hla, hlg2*	*sea, sec, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, fib, sdrD, sdrE*	
mecA/PVL	NP171	Blood	V	VIa	II	ST772	*lukE-lukD, hla, hlg2*	*sea, sec, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, fib, sdrD, sdrE*	
mecA/PVL	NP173	Pus	IV	XIa	I	ST22	*lukE-lukD, hla*	*sec, seg, seh, sei, sel, sem, sen, seo*	*cna, ebpS-v, fnbA, sdrD, sdrE*	*tst-1*
mecA/PVL	NP185	Pus	V	VIa	II	ST772	*lukE-lukD, hla, hlg2*	*sea, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, sdrD, sdrE*	
mecA/PVL	NP189	Urine	V	VIa	II	ST772	*lukE-lukD, hla, hlg2*	*sea, sec, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, sdrD, sdrE*	
mecA/PVL	NP190	Pus	V	VIIa	III	ST1	*lukE-lukD, hla, hld, hlg2*	*sea, seh, sek, sel, seq*	*cna, ebpS, fnbA, fib, sdrD, sdrE*	
mecA/PVL	NP27	Pus	V	IIIa	III	ST88	*lukE-lukD, hla, hld, hlg2*	*sed, sek, seq*	*ebpS, fnbA, fib, sdrD, sdrE*	
PVL	NP163	Pus		IVa	III	ST30	*lukE-lukD, hla*	*seg, seh, sem, sen, seo, sep*	*cna, ebpS, fnbA, bbp*	
PVL	NP169	Pus		IVa	III	ST30	*hla, hld*	*seg, sem, sen, seo*	*cna, ebpS, fnbA, fib, bbp*	
PVL	NP172	Pus		XIa	I	ST22	*lukE-lukD*	*seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS-v, fnbA, sdrD, sdrE*	
PVL	NP166	Pus		VIa	II	ST772	*lukE-lukD, hlg2*	*sea, sec, seg, seh, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, sdrD, sdrE*	
PVL	NP193	Pus		VIa	II	ST772	*lukE-lukD, hla, hlg2*	*sea, seg, sei, sel, sem, sen, seo, sep*	*cna, ebpS, fnbA, sdrD, sdrE*	
PVL	NP199	Urine		XIa	I	ST22	*lukE-lukD, hla*	*seg, sei, sem, sen, seo*	*cna, ebpS-v, sdrD, sdrE*	
—	NP195	Pus		IIa	I	ST672	*lukE-lukD, hla, hld, hlg2*	*seg, sei, sem, seo*	*ebpS, fnbA, fib, sdrD, sdrE*	

[a]The following genes were detected in all strains: *hlg, icaA, icaD, eno, fnbB, clfA, clfB, sdrC.*
[b]The following genes were not detected in any strain: *seb, see, sej, ser, ses, set, eta, etb, etd, edin-A, edin-B, lukM, bnp, sak, scn.*
[c]Elastin binding protein gene (*ebpS*) with internal deletion (180 bp).