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ORIGINAL ARTICLE

High Prevalence of Exfoliative Toxins Among Carrier Isolates of *Staphylococcus aureus* from Healthy Individuals from Various Communities in Chennai, South India

Nagarajan Abimanyu · Saravanan Murugesan · Padma Krishnan

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Abstract *Staphylococcus aureus* causes infections both in community and hospital settings, nasal carriage is the important source of these infections. A total of 103 carrier isolates of *S. aureus* from 352 asymptomatic individuals were screened for methicillin-resistant *S. aureus* (MRSA) and exfoliative toxins (A, B and D) by two sets of multiplex PCRs. The overall nasal carriage of MRSA was found to be 13/352 (3.7 %), of which 4 were found to be positive for Panton valentine leucocidin (PVL). Twelve (11.65 %) strains were found to carry exfoliative toxins and belonged to one of the following spa types t159, t209 and t1515. High prevalence of exfoliative toxins, *pvl* and MRSA pose a major threat to public health, since the isolates were from the healthy in various community settings.

Keywords CA-MRSA · *pvl* · Exfoliative toxins · t159 · t209 · SCC*mec*

Staphylococcus aureus is a ubiquitous pathogen which causes infections both in healthy individuals as well as in hospitalized patients, the clinical spectrum of which varies from mild skin infections to life threatening manifestations such as invasive pyogenic infections, pneumonia and septicemia. The type and severity of the infections by *S. aureus* depends on the presence or absence of virulence factors carried on the mobile genetic elements (MGEs). Exfoliative toxins (ETs) are exotoxins of *S. aureus,* associated with staphylococcal scalded skin syndrome (SSSS) or Ritter disease and bullous impetigo [1]. The mechanism of action of ET is by intra-epidermal splitting by specific cleavage of desmoglein-1, a desmosomal cadherin protein that mediates cell–cell adhesion of keratinocytes [1]. There are four serotypes of ETs- A, B, C and D of which ETA and ETB were associated with human infections. These toxins are carried by about 1–2 % of *S. aureus* isolates causing human infections [1, 2]. ETD was also associated with human infections, but is less common than the above two. ETD producing isolates are mainly isolated from furuncles or cutaneous abscesses, and not from SSSS. ETC is associated with animal infections. ETs encoding genes *eta*, *etb* and *etd* are carried on MGEs (phage, plasmid and pathogenicity island, respectively) and thus can potentially be transmitted to other isolates. In this study, a high prevalence of ET producing *S. aureus* were detected among asymptomatic individuals from various closed communities (orphanages, old age homes and sports teams) while screening for an array of toxin genes by PCR assays.

A total of 352 nasal swabs were collected from asymptomatic individuals from old-age homes (n = 100), orphanages (n = 77) and sports teams (n = 175) in Chennai, South India between the period of March and May 2011. Demographic data including age, sex and history of hospitalization along with underlying disease were recorded. Individuals with hospital associations such as currently employed, previous hospitalization within a period of 1 year were not included to provide community background to the study. The study population included both male (n = 216) and female (n = 136) with the age ranging from 6 to 72 years. The study was approved by the Institutional review committee and an informed consent was obtained from study participants. Nasal swabs were collected from the anterior nares and inoculated into 7.5 % NaCl nutrient broth for enrichment [3]. Initial isolation of staphylococci was done on 5 % sheep blood agar and

N. Abimanyu · S. Murugesan · P. Krishnan (✉)
Department of Microbiology, University of Madras, Chennai 600113, India
e-mail: padma.abpkn@gmail.com

S. aureus identity was confirmed by standard phenotypic methods. Screening of MRSA was done by cefoxitin disc diffusion method and interpreted as per CLSI guidelines. DNA was extracted by using QIAGEN DNAeasy extraction kit (Qiagen, Germany) as per the manufacturer's protocol for gram positive bacteria. Multiplex PCR method targeting *mecA*, 16S-rDNA and Panton-Valentine leucocidin (PVL) was done [4]. The three serotypes of ETs-A, B and D associated with human infections were amplified by multiplex PCR as described earlier [5]. *S. aureus* (ATCC25923, ATCC43300 and RN4850) were used as positive controls for detection of *pvl*, *mec*A and ETs respectively. Further, the ET positive isolates were typed by accessory gene regulator (*agr*) typing [6] and *spa* typing. The data was stored and analysed using MS excel.

The overall nasal carriage of *S. aureus* among asymptomatic carriers from various communities was found to be 103/352 (29.3 %), of which 13 (3.7 %) were found to be MRSA by *mec*A gene PCR. Of the 103 *S. aureus* isolates, 25 (24.3 %) were found to be positive for PVL toxin, of which 4 were MRSA. Of the 103 isolates, 13 (12.62 %) were positive for ET, of which 11.65 % of isolates carried ETA and 8.7 % of isolates carried ETB. About 7.7 % of isolates carried both ETA and ETB. None of the isolates was found to carry ETD. The distribution of MRSA, *pvl* and exfoliative toxins between different groups in this study were compared (Table 1). The *agr* typing of ET producing isolates showed that isolates carrying both *eta* and *etb* genes and isolates carrying *etb* gene alone, belonged to *agr*IV, those carrying the *eta* gene alone belonged to *agr*II. *S. aureus* isolates with ETA alone belonged to t209 and isolates carrying ETB alone belonged to t1515, whereas isolates with both ETA and ETB belonged to t159 by *spa* typing (Table 2).

Table 1 Prevalence of nasal carriage of *S. aureus,* MRSA, PVL, PVL MRSA and exfoliative toxins among various healthy communities

	Old age home [100] N (%)	Orphanage [77] N (%)	Sports persons [175] N (%)	Overall [352] N (%)
Age in years (range)	62–83	6–40	17–21	4–83
Nasal carriage of *S. aureus*	24 (24)	28 (36.4)	51 (29.1)	103 (29.3)
Prevalence of MRSA	3 (3)	5 (6.5)	5 (2.9)	13 (3.7)
Prevalence of PVL	6 (6)	7 (9.1)	12 (6.8)	25 (7.1)
Prevalence of PVL MRSA	1 (1)	2 (2.6)	1 (0.6)	4 (1.1)
Exfoliative toxins				
Eta	4 (4)	4 (5.2)	4 (2.3)	12 (3.4)
Etb	4 (4)	4 (5.2)	1 (0.6)	9 (2.5)
Etd	0	0	0	0

Number in parenthesis indicates percentage

Table 2 Exfoliative toxin producing carrier isolates of *S. aureus*

Strain	Exfoliative toxins		Agr type	*spa* type	Community	Age/ sex
	ETA	ETB				
SA502	+	–	agrII	t209	Sports	20/M
SA512	+	–	agrII	t209	Sports	14/M
SA520	+	–	agrII	t209	Sports	18/M
SA531	–	+	agrIV	t1515	Sports	13/M
SA537	+	–	agrII	t209	Sports	12/F
SA552	+	+	agrIV	t159	Old age home	69/M
SA561	+	+	agrIV	t159	Old age home	84/M
SA563	+	+	agrIV	t159	Old age home	67/F
SA566	+	+	agrIV	t159	Old age home	62/F
SA568	+	+	agrIV	t159	Orphanage	13/M
SA569	+	+	agrIV	t159	Orphanage	10/F
SA571	+	+	agrIV	t159	Orphanage	40/M
SA588	+	+	agrIV	t159	Orphanage	17/F

The nasal carriage of *S. aureus* in this study was found to be 29.3 % which is within the normal range (25–30 %), but MRSA (12.6 %) among these carrier isolates was found to be higher than previously reported in India [3]. About 25 % of carrier isolates in this study was found to be *pvl* positive. More than 12 % of carrier isolates from asymptomatic carriers in this study were found to carry the ET. The results of *agr* typing were in concordance with the previous study [7]. In both the studies, the isolates producing ETB alone or ETA and ETB belonged to the *agr*IV, while isolates with ETA alone were associated with *agr*II.

Several studies showed that nasal carriage of *S. aureus* plays an important role in both hospital and community acquired infections [8], but data on carriage of ET producing *S. aureus* among high risk communities is generally lacking. Our study reports high prevalence (12.6 %) of ET among carrier isolates when compared to that reported from Germany [9]. ET producing isolates belong to same *spa* types (*eta*–t209, *etb*–t1515 and *eta/etb*–t159), it points out that there are some restrictions in the spread of exfoliative toxin among *S. aureus* clones.There have been reports of skin and soft tissue infections by ET producing CA-MRSA isolates in China, Japan and Switzerland [10–12]. Fortunately, none of the ET producing isolates in

the present study was found to be MRSA. Since ETs and methicillin resistance genes are present on MGEs, there is a possiblility of transfer of these toxins to rapidly spreading CA-MRSA or vice versa, resulting in emergence of ET producing CA-MRSA.

We found a high prevalence of *pvl*, MRSA and exfoliative toxins among carrier isolates of *S. aureus* from various communities who are at high risk for CA-MRSA infections. The present study highlights the need to screen *pvl*, MRSA and ETs among carrier isolates of *S. aureus* from asymptomatic individuals in closed communities to break the transmission chain and thereby preventing the possible emergence of ET producing CA-MRSA infections.

Conflict of interest None.

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