

## SHORT REPORT

# An outbreak of post-partum breast abscesses in Mumbai, India caused by ST22-MRSA-IV: genetic characteristics and epidemiological implications

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Received 21 December 2011; Final revision 8 March 2012; Accepted 9 March 2012;  
first published online 4 April 2012

## SUMMARY

A cluster of methicillin-resistant *Staphylococcus aureus* (MRSA) breast abscesses in women who had given birth at a hospital in Mumbai, India was investigated retrospectively. Nineteen of 20 cases were caused by a single clone: *pvl*-positive, *spa* type 648 (Ridom t852), *ccrB:dru* subtype 3:0, ST22-MRSA-IV. Despite the presence of *pvl* and SCC*mec* type IV, which are common genetic markers in community-associated MRSA, this outbreak was caused by a healthcare-associated, community-onset MRSA that was common in the hospital environment. Thus, infection control practices may have an important role in limiting the spread of this virulent clone.

**Key words:** Bacterial typing, methicillin-resistant *S. aureus* (MRSA), hospital-acquired (nosocomial) infections, outbreaks.

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported to be an increasingly common cause of post-partum mastitis [1, 2]. While 2–33% of breastfeeding women may develop some form of post-partum mastitis, in 3–11% of these cases the mastitis may progress to breast abscesses [3, 4]. In general, community-associated MRSA (CA-MRSA) infections have different risk factors and have been caused by different bacterial clones than traditional healthcare-associated MRSA (HA-MRSA) infections, although migration of CA-MRSA clones into healthcare facilities may erode these distinctions [5, 6]. While CA-MRSA have been reported to cause complicated post-partum mastitis [7, 8], much about

the epidemiology of these infections remains unknown.

Recently, D'Souza *et al.* [9] genetically characterized a mixed population of CA-MRSA and HA-MRSA collected from various tissue sources between October 2006 and June 2009 at Hinduja Hospital in north Mumbai, India, and elsewhere in the city. Their data highlighted the prevalence of the CA-MRSA clones, ST22-MRSA-IV and ST772-MRSA-V, and further indicated that these two clones may be replacing the HA-MRSA clone, ST239-MRSA-III, in this region [9]. A cluster of MRSA breast abscesses in women who had given birth at Breach Candy Hospital Trust, a tertiary care medical centre in south Mumbai, following the sampling period of D'Souza *et al.* [9], prompted this study of the infecting clone(s).

The sample included 50 non-duplicate MRSA isolates (four duplicate isolates were excluded) that were

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Table 1. Genetic characteristics of methicillin-resistant *Staphylococcus aureus* from a hospital in Mumbai, India

Isolate source	No. of isolates	<i>pvl</i>	MLST type	<i>spa</i> type*	SCC <i>mec</i> type	<i>ccrB:dru</i> subtype†
Nasal swabs from	1	+	ST22	113/t005	IV	3:0
healthcare workers	8	+	ST22	648/t852	IV	3:0
	1	+	ST772	692/t345	V	nt:11af
Post-partum breast abscesses	1	+	ST22	113/t005	IV	3:0
	19	+	ST22	648/t852	IV	3:0
Infections from other wards	2	+	ST22	113/t005	IV	3:0
	6	+	ST22	648/t852	IV	3:0
	1	+	ST22	1373/t9310	IV	3:0
	1	+	ST22	1375/t6717	IV	3:0
	1	+	ST30	43/t021	IV	7:10a
	2	–	ST239	351/t030	III	2:6q
	1	+	ST772	692/t345	IV	3:10a
	1	+	ST772	692/t345	V	nt:11a
	5	+	ST772	1080/t657	V	nt:10bk, nt:7z, nt:10ao, nt:12p

\* Nomenclature: eGenomics/Ridom.

† The *ccrB* gene is absent and thus non-typable (nt) for SCC*mec* type V.

collected between June 2009 and January 2010 from Breach Candy Hospital Trust. In this sample, 20 isolates were from breast abscesses of women who had delivered at the obstetrics and gynaecology (OBGYN) ward, 20 isolates were from various infections of patients of other wards (type and number of specimens: unspecified pus  $n=5$ , endotracheal secretion  $n=3$ , blood  $n=2$ , ear  $n=2$ , lip abscess  $n=2$ , conjunctiva  $n=1$ , perinephric abscess  $n=1$ , nasal abscess  $n=1$ , synovial fluid  $n=1$ , toe  $n=1$ , vagina  $n=1$ ), and 10 isolates were from nasal swabs of healthcare workers of the OBGYN ward, where the outbreak occurred, and the adjacent neonatal intensive care unit (NICU). The 40 infection isolates represented all of the non-duplicate MRSA isolates collected in this hospital during this time period.

The 20 breast abscess cases had previously come to the hospital for delivery. Length of initial stay was available for 14/20 of these cases and was 3–4 days for vaginal delivery and 5–6 days for caesarean section. These cases were healthy at discharge but subsequently returned as outpatients with MRSA breast abscesses (number of cases presenting by month: June  $n=1$ , July  $n=2$ , October  $n=2$ , November  $n=7$ , December  $n=4$ , January  $n=4$ ). Thus, a majority of these cases (17/20) presented in the second half of the sampling period, with a peak in November 2009. Incubation period from date of delivery to date of culture was available for 17/20 of these cases (range 17–165 days, median 36 days).

These cases meet an epidemiological definition of healthcare-associated, community-onset MRSA (HACO-MRSA) infections, as they were cultured  $\leq 48$  h after admission to the outpatient department and occurred in patients with healthcare-associated risk factors (recent delivery in a healthcare setting) [10].

To identify the genetic backgrounds of the MRSA isolates, multilocus sequence typing (MLST) and staphylococcal protein A (*spa*) typing was performed for all isolates [11]. Both eGenomics nomenclature ([www.egenomics.com](http://www.egenomics.com)) and Ridom nomenclature ([www.spaserver.ridom.de](http://www.spaserver.ridom.de)) was used to name *spa* types. Staphylococcal chromosomal cassette *mec* (SCC*mec*), which is a mobile genetic element that encodes the methicillin resistance determinant, was typed for all isolates using PCR assays that score the *mec* gene complex (classes A, B, C) and the *ccr* gene complex (*ccrAB1*, *ccrAB2*, *ccrAB3*, *ccrAB4*, *ccrC*) [11, 12]. In addition, sequence-based subtyping of SCC*mec* was done for all isolates using a fragment of the *ccrB* gene and the *mec*-associated direct repeat units (*dru*) [13, 14]. *lukS*-PV and *lukF*-PV genes, which encode the Pantone–Valentine leukocidin (PVL), were detected by PCR [15]. Example sequences of each unique multilocus sequence type were submitted to the MLST database ([www.mlst.net](http://www.mlst.net)), sequences of new *spa* and *dru* types were submitted to appropriate databases ([www.egenomics.com](http://www.egenomics.com) and [www.dru-typing.org](http://www.dru-typing.org)), and example sequences of each

unique *ccrB* allele were submitted to the GenBank database (accession numbers JQ746656–JQ746658).

Four *spa* types (eGenomics 648, 113, 1373, 1375; Ridom t852, t005, t9310, t6717) belonged to multi-locus sequence type (ST) ST22 and were represented by 39 isolates (Table 1). Two *spa* types (eGenomics 692, 1080; Ridom t345, t657) belonged to ST772 and were represented by eight isolates. Single *spa* types were found among the two isolates of ST239 (eGenomics 351, Ridom t030) and the single isolate of ST30 (eGenomics 43, Ridom t021). All of the ST22 isolates had *SCCmec* IV and *pvl* (Table 1). Moreover, all of the ST772 isolates had *SCCmec* V and *pvl*, with the exception of a single isolate that had *SCCmec* IV and *pvl*. The two isolates of ST239 had *SCCmec* III but lacked *pvl*, and the single isolate of ST30 had *SCCmec* IV and *pvl* (Table 1). These results confirmed the presence of these clones in this region as previously reported by D'Souza *et al.* [9].

While *spa* typing did not reveal substantial diversity differences within the ST22-MRSA-IV and ST772-MRSA-V backgrounds, subtyping of their *SCCmec* elements with *ccrB:dru* sequences revealed contrasting patterns of diversity. *SCCmec* IV from ST22 was exclusively of the *ccrB:dru* subtype 3:0, whereas *SCCmec* V from ST772 presented six *ccrB:dru* subtypes in the seven isolates and the single ST772 isolate with *SCCmec* IV also had a different *ccrB:dru* subtype (Table 1). These results suggested that ST22-MRSA-IV was a more genetically homogeneous background than ST772-MRSA-V in this hospital.

Nearly all breast abscess cases (19/20) were caused by a single clone: *pvl*-positive, *spa* type 648 (Ridom t852), *ccrB:dru* subtype 3:0, ST22-MRSA-IV (Table 1). The exception was one isolate with *spa* type 113 (Ridom t005), which differed by a single base pair from *spa* type 648. The MRSA isolates from breast abscesses were resistant to ciprofloxacin, half were resistant to gentamicin, and all but two isolates were susceptible to co-trimoxazole, erythromycin, and clindamycin. The outbreak clone was also the most frequent cause of infections in other wards (6/20) and was the most frequently carried clone by healthcare workers in the OBGYN and NICU wards (8/10) (Table 1). Thus, these results provided genetic confirmation of the outbreak and showed that the outbreak clone was common in the hospital environment.

A previous study from northern Italy reported a cluster of *S. aureus* post-partum mastitis, including six breast abscesses, and neonatal skin infections that

preceded additional infections in the community [16]. The genetically characterized isolates from subsequent infections were *pvl*-positive, *spa* type 113 (Ridom t005), ST22 methicillin-susceptible *S. aureus* (MSSA) [16]. The Indian cluster investigated here differed from the Italian cluster by the lack of concurrent neonatal infections and all isolates were MRSA. The ST22-MRSA-IV background is common in parts of Asia and Europe, but it exhibits variation in *spa* types and in carriage of *pvl* and it has been described from healthcare and community sources [9, 17–20]. Our results clearly indicated that the *pvl* and *SCCmec* type IV markers alone would not be accurate markers of a CA-MRSA infection source in India. The insufficiency of these two markers for classifying the infection source of MRSA has also been observed previously in Ireland [20]. Finally, isolates with the same genetic characteristics as the outbreak clone described here, *pvl*-positive, *spa* type 648 (Ridom t852), ST22-MRSA-IV, were recently reported to cause serious soft-tissue infections, including rare brain abscesses, in Bengaluru, southern India [19]. These observations indicate that this particular clone is highly virulent and has the capacity for wider geographical transmission in India. Given this clone's link to the hospital environment, infection control practices may have an important role in limiting its spread.

## ACKNOWLEDGEMENTS

This work was supported in part by NIH grant GM080602 (to D.A.R.).

## DECLARATION OF INTEREST

None.

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