Original Article

Predominance of methicillin resistant *Staphylococcus aureus* -ST88 and new ST1797 causing wound infection and abscesses

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Abstract

Introduction: Although there has been a worldwide emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA), little is known about the molecular epidemiology of MRSA in Tanzania.

Methodology: In this study, we characterized MRSA strains isolated from clinical specimens at the Bugando Medical Centre, Tanzania, between January and December 2008. Of 160 *S. aureus* isolates from 600 clinical specimens, 24 (15%) were found to be MRSA. Besides molecular screening for the Panton Valentine leukocidin (PVL) genes by PCR, MRSA strains were further characterized by Multi-Locus Sequence Typing (MLST) and *spa* typing.

Results: Despite considerable genetic diversity, the *spa* types t690 (29.1%) and t7231 (41.6%), as well as the sequence types (ST) 88 (54.2%) and 1797 (29.1%), were dominant among clinical isolates. The PVL genes were detected in 4 isolates; of these, 3 were found in ST 88 and one in ST1820. Resistance to erythromycin, clindamicin, gentamicin, tetracycline and co-trimoxazole was found in 45.8%, 62.5%, 41.6%, 45.8% and 50% of the strains, respectively.

Conclusion: We present the first thorough typing of MRSA at a Tanzanian hospital. Despite considerable genetic diversity, ST88 was dominant among clinical isolates at the Bugando Medical Centre. Active and standardized surveillance of nosocomial MRSA infection should be conducted in the future to analyse the infection and transmission rates and implement effective control measures.

Key Words: MRSA; ST88; ST1797; Tanzania

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Introduction

Infections caused by *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), are emerging as a major public health problem. MRSA has been associated with various diseases, such as skin and soft tissue infections, endovascular infections, pneumonia, endocarditis and septic shock [1]. In hospitals with well-implemented surveillance systems MRSA has been found to be a leading cause of nosocomial infections [1,2]. The emergence and spread of both health-care and community-associated MRSA challenges both infection control interventions and treatment [1].

Molecular epidemiological studies of MRSA isolates are crucial in controlling the spread of these isolates; pulsed-field gel electrophoresis (PFGE) has been the gold standard for strain typing, but it lacks an inter-laboratory reproducibility [3]. The sequence-based methods, such as staphylococcal protein A-encoding gene (*spa*)-typing and multi-locus sequence typing (MLST), offer excellent intra- and inter-laboratory reproducibility, and the opportunity to compare results internationally [4]. *Spa* typing has also been validated for long-term nationwide surveillance studies [5]. In the few studies which have been conducted to characterize ST and *spa* types in Africa, the majority of MRSA isolates has been assigned to ST8 and ST88 [6,7]. In a multicentre study ST88 was detected in five major African towns in Morocco, Senegal, Niger, and Cameroon [6]. ST 88 has recently been reported in Nigeria [8], as well as in children from Western Africa who had undergone surgery in Switzerland and who had later been hospitalized in their home

countries [9]. In South Africa, 67% of MRSA isolates were assigned to ST 8 [7]. Other STs reported in Africa include ST239, ST30, ST72, ST1289 and ST243 [6-9].

Despite its importance, many hospital laboratories in developing countries do not routinely perform MRSA testing. Moreover, there is no published study on molecular epidemiology of MRSA in Tanzania. This study aimed at a molecular characterization of clinical MRSA strains isolated from nasal swabs, wounds and abscesses over a 12–months period at the Bugando Medical Centre, Northern Tanzania, by *spa* typing and MLST. We here present baseline data from a large tertiary hospital, thus enabling comparisons with other African countries.

Methodology

Isolates

A total of 24 MRSA isolates out of 160 *Staphylococcus aureus* strains recovered from wound swabs (428), pus (108), and nasal swabs (64) over a period of 12 months in various wards of the Bugando Medical Centre were analyzed. Isolates were identified using colony morphology on sheep blood agar plates (creamish to golden yellow color, beta-hemolytic), followed by DNase testing [10]. Slide and tube coagulase tests were performed as described previously [10]. In the case of discrepant coagulase test results, the staphylase Kit was used (OXOID, Hampshire, United Kingdom) per the manufacturer's instructions. In all tests the *S. aureus* strain ATCC 25923 from the National Medical research Institute Tanzania was used for quality control.

Susceptibility testing

Standard disc diffusion techniques as recommended by the Clinical Laboratory Standards Institute (CLSI) were performed for susceptibility testing of cefoxitin, erythromycin, clindamycin, gentamicin, fusidic acid, co-trimoxazole, moxifloxacin, oxacillin and tetracycline [11]. Screening for methicillin resistance was done using cefoxitin and oxacillin discs on Mueller Hinton agar plates (OXOID, Hampshire, United Kingdom) [11,12]. Bacterial colonies were re-suspended in normal saline to a turbidity standard of 0.5 McFarland and inoculated on a Mueller Hinton agar plate. The plates were incubated at 35°C for screening with cefoxitin, and at 30°C for screening with oxacillin [10,11]. All isolates resistant to cefoxitin and oxacillin were presumptively considered MRSA. In all MRSA isolates, susceptibility testing was also performed using the microdilution broth break point method using the automated VITEK2 system (BioMérieux, Marcy-L'Etoile, France).

Amplification of PVL and mecA *genes*

The PVL genes were amplified as described previously [13,14]; briefly, bacterial colonies were resuspended in 500 µL of distilled water and boiled for 15 minutes. After centrifugation at 13,000 rpm for 5 minutes, 5 µL of the supernatant was used as template in the PCR reaction to make a final volume of 50 µL [14]. Primers *luk*SF-forward (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3') and *luk*SF-reverse (5'-GCATCAAGTGTATTGGATAGCAAAAGC-3') directed against the PVL S and F precursor genes (*luk*S/F-PV) were used. The known PVL-positive MRSA isolate VA17763 (*spa* type t008) was used as a positive control. After initial denaturation for 10 minutes at 95°C, 30 cycles were run with 95°for 30s, 55° for 30s and 72°C for 30s each, followed by a final extension step for 7 min at 72°C [14].

The *mecA* gene was amplified as described previously [15] using primers *mec*A1 (5'GTAGAAATGACTGAACGTCCGATAA-3') and *mec*A2 (5'-CCAATTCCACATTGTTTCGGTCTAA-3'). The following PCR conditions were used: 94°C for 5 minutes, followed by 34 cycles of 94°C for 1 minute, annealing at 54°C for 1.5 minutes, and extension at 72°C for 1 minute, followed by a final extension for 10 minutes at 72°C.

MLST and spa *typing*

Genomic DNA was extracted from overnight grown cultures using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). *Spa* typing was performed according to the method described previously [16] using the primers *1095*F (5'-AGACGATCCTTCGGTGA-3') and *1517*R (5'-GCTTTTGCAATGTCATTTACTG-3'). DNA sequences were obtained with an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA). Isolates were assigned to particular *spa* types using the spa server (http://www.spaserver.ridom.de, Ridom GmbH, Wuerzburg, Germany). MLST was performed according to a protocol described previously [17] and isolates were assigned to a sequence type (ST) according to the MLST website http://www.mlst.net.

Table 1. Molecular characteristics of MRSA isolates recovered in BMC wards

ID NO	spa type	mec PCR	PVL PCR	ST	CN	E	FA	CN	MOX	TET	STX	WARD
13	t064	pos	neg	8	R	R	S	S	S	S	R	E9
14	t064	pos	neg	8	R	R	S	S	S	R	R	E9
5	t104	pos	neg	8	R	R	S	R	S	R	R	C7
20	t1855	pos	pos	88	S	S	S	S	S	S	I	C5
1	t186	pos	pos	88	R	R	S	S	S	S	I	C9
10	t667	pos	neg	88	R	R	S	R	S	S	R	C9
12	t667	pos	neg	88	S	S	S	R	S	S	R	C6
2	t690	pos	neg	88	R	R	S	S	S	S	I	E8
6	t690	pos	neg	88	R	R	S	S	S	R	R	C4
9	t690	pos	pos	88	R	R	S	S	S	R	I	E8
16	t690	pos	neg	88	S	S	S	S	S	S	I	E8
7	t690	pos	neg	88	S	S	S	S	S	S	I	C5
23	t690	pos	neg	88	S	S	S	S	S	S	I	E8
18	t7237	pos	neg	88	S	S	S	S	S	S	I	C5
19	t7237	pos	neg	88	S	S	S	S	S	S	I	ICU
25	t7237	pos	neg	88	S	S	S	S	S	S	I	E6
3	t7231	pos	neg	1797	R	I	S	R	S	R	R	E6
11	t7231	pos	neg	1797	R	I	S	R	S	R	R	E9
7	t7231	pos	neg	1797	R	I	S	R	S	R	R	E9
8	t7231	pos	neg	1797	R	I	S	R	S	R	R	E9
15	t7231	pos	neg	1797	R	R	S	R	S	R	R	C6
22	t7231	pos	neg	1797	R	R	S	R	S	R	S	C6
24	t7231	pos	neg	1797	R	R	S	R	S	R	R	C6
21	t690	pos	pos	1820	S	S	S	S	S	S	I	E9

ST: Sequence type, CN: gentamicin, E: Erythromycin, FA: Fusidic Acid, MOX: Moxifloxacin, TET: Tetracycline, SXT: sulphamethaxazole/trimethoprim, R: Resistant, S: Sensitive

Results

Out of 160 *S. aureus* isolates, 24 (15%) were found to be methicillin resistant by cefoxitin and oxacillin screening. All 24 isolates were found to harbor the *mec*A gene and 4 of these (16.7%) also the PVL gene. Resistance to erythromycin, clindamycin, gentamicin, tetracycline and co-trimoxazole was found in 45.8%, 62.5%, 41.6%, 45.8% and 50%, respectively, and 45% of isolates were intermediate resistant to co-trimoxazole (Table 1). All 24 isolates were found to be sensitive to vancomycin, fusidic acid and moxifloxacin.

The 24 MRSA isolates could be assigned to four sequence types with ST88 being the most common sequence type detected, found in 13 (54.2%) of the isolates. This was followed by a new ST1797 which was found in seven (29.2%). Of the four isolates positive for the PVL gene, three were ST88 and one was ST1820, which is a single locus variant of ST88 (Table 1). Using seven housekeeping gene loci, these isolates were divided in the two clonal complexes (CC) CC88 and CC8 (Table 1). Eight *spa* types were observed among the 24 MRSA isolates, and the most common *spa* types were the new t7231 detected in 7 (29.2%) of the MRSA isolates and t690 also detected in 7 (29.2%) of isolates. These *spa* types were found in three surgical wards and were isolated at different times with overlapping hospital stays in ward E9 (Table 2). No peculiarities of the PVL expressing strains and the diseases they caused were observed.

Discussion

Resistance to oxacillin and cefoxitin which signify MRSA was observed in 15% of the *S. aureus* isolates. This is relatively low compared to the findings in developed countries, where more than 25% of the *S. aureus* strains have been found to be MRSA [18]. Similar rates were observed in South Africa, where the prevalence of MRSA ranged from 4-74% in various hospitals, with an overall prevalence of 45%. Prevalence varied significantly between different regions, suggesting evolution of resistant clones [18,19]. Interestingly, the findings of this study resemble data from a study conducted at

Table 2. Distribution of the spa t7231 and spa t690 across BMC wards

Strain NO	ST	*Spa* type	Ward	Date of sampling	Diagnosis	Specimen
3	1797	t7231	E6	05-12-2008	Diabetic foot	Wound swab
4	1797	t7231	E9	14-03-2008	Burns (Trunk)	Wound swab
7	1797	t7231	E9	17-04-2008	SSI	Wound swab
8	1797	t7231	E9	04-02-2008	Burns (Trunk)	Wound swab
15	1797	t7231	C6	24-07-2008	Screening	Nasal swab
22	1797	t7231	C6	31-03-2008	SSI(Abdomen)	Wound swab
24	1797	t7231	C6	29-04-2008	Diabetic foot	Wound swab
2	ST88	t690	E8	22-4-2008	Infected pin	Wound swab
6	ST88	t690	C4	8-4-2008	SSI	Wound swab
9	ST88	t690	E8	29-5-2008	Leg wound	Wound swab
16	ST88	t690	E8	13-05-2008	Leg wound	Wound swab
17	ST88	t690	C5	20-05-2008	Screening	Nasal swab
23	ST88	t690	E8	8-4-2008	Leg wound	Wound swab

E9, C5; Pediatric wards, E9, E6, E8, C6, C4, C9: surgical wards, , C7: Medical ward. SSI; Surgical site infections

the Tanzania National Hospital-Muhimbili, in which 12% of hospital-isolated *S. aureus* were found to be resistant to cloxacillin [20]. As described in other studies [18-20], our MRSA isolates were also resistant to commonly used antibiotics, especially to co-trimoxazole. In our local setting, all MRSA isolates were sensitive to moxifloxacin and vancomycin. As these drugs are expensive and most of the time not available in our local pharmaceutical market, treatment options for infections caused by MRSA are limited.

In the present study the PVL genes were detected in four (16.7%) of the MRSA isolates. Of these, two were associated with surgical site infections and one with burn wound infection and the other was from a nasal swab. There has been much debate about the role of PVL, a beta-pore-forming toxin that causes lysis and apoptosis of leukocytes in the virulence of MRSA infections. The presence of the PVL genes has been associated with community acquired-MRSA [18]. Severe infections have been reported caused by PVL-positive *S. aureus* strains, such as furuncles, abscesses, and cases of necrotizing pneumonia [21]. All four PVL-positive strains belonged to CC88 in this study. One of these genotypes, ST88 (spa t186), was identified in another study from Africa [6].

Twenty-four isolates were grouped into four STs and two CCs. As in our study, the majority of isolates described in a few African surveys [6,8,9] were also grouped in ST88. These findings highlight the ability of this particular clone to spread over a large geographical area encompassing Central, East and West Africa, and the potential role of the African continent as a reservoir for this clone. ST1797 was first detected in this study. In the MLST database (http: //www.mlst.net), the only single locus variant of this clone is ST1173, which was identified in an Australian isolate. ST1173 was also shown to occur in South Africa [22]. It will be of interest to determine the geographic distribution of ST1797 and the closely related ST1173 in Africa, as well as the question of whether the two STs have emerged from each other recently. ST1797 was also associated with a newly identified *spa*-type, supporting the need to characterize the epidemiology of this new clone. In the present study 29.2% of the isolates were grouped to the new ST1797 and all of them were found to be *spa* type t7231. ST1797/t7231 is therefore another predominant clone in our hospital, and possibly in Tanzania. This clone was detected in three surgical wards and these isolates were detected at different times, indicating clonal spread. These findings reveal

the necessity to improve surveillance systems, staff screening, and personal hygiene routines in our hospital.

Conclusion

To the best of our knowledge this is the first molecular characterization of MRSA at a Tanzanian hospital. The finding of the new MRSA clone ST1797/t7231 was intriguing. As in other parts of Africa, MRSA ST88 is a predominant cause of infection at the Bugando Medical Centre, a large tertiary hospital in Mwanza, Tanzania. To quantify nosocomial transmission and infection rates, state-of-the-art strain typing tools now need to be supplemented by active and standardized surveillance of MRSA infection to provide the data for the implementation of effective hospital hygiene intervention strategies.

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