High prevalence of methicillin resistant Staphylococcus aureus in the surgical units of Mulago hospital in Kampala, Uganda

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Abstract

Background: There is limited data on Methicillin resistant Staphylococcus aureus (MRSA) in Uganda where, as in most low income countries, the routine use of chromogenic agar for MRSA detection is not affordable. We aimed to determine MRSA prevalence among patients, healthcare workers (HCW) and the environment in the burns units at Mulago hospital, and compare the performance of CHROMagar with oxacillin for detection of MRSA.

Results: One hundred samples (from 25 patients; 36 HCW; and 39 from the environment, one sample per person/item) were cultured for the isolation of Staphylococcus aureus. Forty one S. aureus isolates were recovered from 13 patients, 13 HCW and 15 from the environment, all of which were oxacillin resistant and meca/femA/nuc-positive. MRSA prevalence was 46% (41/89) among patients, HCW and the environment, and 100% (41/41) among the isolates. For CHROMagar, MRSA prevalence was 29% (26/89) among patients, HCW and the environment, and 63% (26/41) among the isolates. There was high prevalence of multidrug resistant isolates, which concomitantly possessed virulence and antimicrobial resistance determinants, notably biofilms, hemolysins, toxin and ica genes. One isolate positive for all determinants possessed the bhp homologue which encodes the biofilm associated protein (BAP), a rare finding in human isolates. SCCmec type I was the most common at 54% prevalence (22/41), followed by SCCmec type V (15%, 6/41) and SCCmec type IV (7%, 3/41). SCCmec types II and III were not detected and 10 isolates (24%) were non-typeable.

Conclusions: Hyper-virulent methicillin resistant Staphylococcus aureus is prevalent in the burns unit of Mulago hospital.
of Health Sciences. Informed consent was obtained from patients and HCW. Patients and HCW enrolled had stayed in the hospital units for at least 72 hours. HCW included doctors, nurses and nursing aids that had direct involvement with patients. For the environment, surfaces of frequently handled items (door handles, sinks, surgical trays, beds and table surfaces) were swabbed, as well as air samples on settle plates (with blood agar). For patients, wounds or nostrils were swabbed while for HCW, hands or nostrils were swabbed. One hundred samples (one sample per person or item) from 25 patients, 36 HCW and 39 items (including air settle plates) were obtained using sterile swabs and transported to the laboratory for culture in tubes containing Amies transport medium (Biolab, Budapest, Hungary).

**Cultures and drug susceptibility testing (DST)**
Swabs were cultured on blood agar and Mannitol salt agar at 37°C for 24 hours, ensuring growth of distinct colonies. The predominant colony type per sample was selected, and S. aureus was identified to species level microbiologically as previously described [6]. Drug susceptibility testing (erythromycin, 15 μg; vancomycin, 30 μg; gentamicin, 10 μg; oxacillin, 1 μg; tetracycline, 30 μg; chloramphenicol, 30 μg; penicillin, 10 units; and sulphamethoxazole-trimethoprim, 1.25/23.15 μg) was performed with the disc diffusion method (Biolab inc, Budapest, Hungary). Isolates resistant to erythromycin were screened for the macrolide-lincosamide and streptogramin B (MLSβ) phenotype. MRSA was identified based on oxacillin resistance following standard procedures [7], and confirmed by PCR detection of the "mecA" gene. For comparison, oxacillin resistant-"mecA" positive isolates were further screened for growth on CHROMagar (BD diagnostics, Sparks, USA).

**SCCmec genotyping**
Staphylococcus Cassette Chromosomal mec (SCCmec) genotyping was performed in a multiplex PCR protocol described by Boye et al, 2007 (see additional file 1 for primers and PCR conditions) [8]. The SCCmec types were determined based on the banding patterns upon agarose gel electrophoresis of the amplicons [8]. Isolates with no visible bands were classified as non-typeable [8].

**Detection of virulence determinants**

**Biofilms**
Biofilms were detected with the microtiter plate method [9] and the biofilm unit calculated according to Amaral et al. [10]. Briefly, assays were performed in triplicates in tryptic soya broth (TSB) with 1% glucose in 96-well polystyrene flat-bottom tissue culture plates. Isolates were incubated at 37°C overnight with gentle shaking and standardized to OD₅₆₀ = 0.005 with normal saline. Then, 50 μl of standardized cells mixed with 150 μl TSB/1% glucose were incubated at 37°C for 17 hr. After washing three times with sterile water and staining with crystal violet for 15 minutes, cells were washed again and incubated at room temperature for 1 hr in 95% ethanol. Then, biofilms were measured with a spectrophotometer at OD₅₇₀. The biofilm unit (BU) was calculated using negative control values with the formula A₁/A₂, where A₁ is the test value while A₂ is the negative control value. Isolates with BU > 2× the negative control value were considered biofilm producers and were classified as: weak, 0.182 < BU < 0.364; moderate, 0.364 < BU < 0.728; strong, BU > 0.728 [10]. The biofilm forming S. epidermidis RP62A and its non-biofilm forming variant (ATCC 12228) were used as controls.

**Virulence and antimicrobial resistance genes**
Molecular assays for the detection of virulence associated genes, ica (intercellular adhesion); cna (collagen adhesion [11]); hemolysins (hla, hlb, hld, hlg [12]); sdrE (serine-aspartate repeat protein E [11]); PVL (Panton-Valentine leukocidin [13]); and S. aureus super-antigenic toxins (tst, toxic shock syndrome toxin [12,14] and sea, staphylococcal enterotoxin A [12,14]) were performed with primers and conditions described in literature (also see additional file 1). To detect genes encoding aminoglycoside-modifying enzymes (AMEs) [15], PCR on aac(6’)-Ie-aph(2’)-Ia (bifunctional aminoglycoside-6-N-acetyltransferase/2”-O-phosphoryltransferase), aph(3’)-IIIa (aminoglycoside-3’-O-phosphoryltransferase III) and ant(4’)-Ia (aminoglycoside-4’-O-nucleotidyltransferase I) was performed. Presence of mecA (the molecular determinant of methicillin resistance), vanA/vanB1 (encode vancomycin resistance variants) and blaZ (encodes β-lactamase) was also determined by PCR. For mecA genotyping, methicillin resistant S. aureus (MRSA-252) and methicillin sensitive S. aureus (MSSA, ATCC 29213) were used as positive and negative controls, respectively. For analysis, amplicons were electrophoresed on 1% agarose gels in TBE (Tris borate and EDTA) and representative samples sequence-confirmed. The data was analyzed with GraphPad Prism 5 software and presented graphically. Primers and PCR conditions are described in additional file 1. To minimize cross-contamination, DNA extraction and PCR-amplifications were performed in molecular laboratories that are separate from the clinical microbiology laboratory where cultures were grown. The PCR laboratory has designated sections for pre-amplification, amplification and post-amplification.

**Results**
Gram positive isolates grew from 62 samples but 11 were lost (five from HCW, four from patients and two from the environment), leaving 51 samples that were completely
processed. From these, *S. aureus* grew from 41 samples: 14 from patients (67%, 14/21; 4 from nostrils and 10 from wounds), 13 from HCW (42%, 13/31; 5 from nostrils and 8 from finger swabs), and 14 from the environment (38%, 14/37, see Table 1). Each isolate grew from a distinct sample per person or item. Thus, the prevalence of *S. aureus* in the burns unit was 46% (41/89), (Table 1).

All the 41 isolates were sensitive to vancomycin; conversely, all the isolates were oxacillin resistant and mecA/nuc/femA-positive (implying they were *S. aureus*), revealing an MRSA prevalence of 46% (41/89) in the burns unit (i.e., among patients, HCW and environment) and 100% (41/41) among isolates (see Table 1). SCCmec type I was the most common at 54% prevalence (22/41), followed by SCCmec type V (15%, 6/41) and SCCmec type IV (7%, 3/41). SCCmec types II and III were not detected and 10 isolates (24%) were non-typeable (see table 1). For CHROMagar, only 26 isolates grew with the characteristic mauve color indicative of MRSA, revealing a prevalence of 29% in the unit (i.e., among patients, HCW and environment) and 63% (26/41) among isolates.

Overall, 26 six isolates (63%, 26/41) MDR with the commonest pattern being resistance to β-lactams, sulphonmethoxazole-trimethoprim (SXT) and tetracycline. Indeed, resistance to penicillin, tetracycline and SXT was high (93%, 38/41; 68%, 28/41 and 66%, 27/41 prevalence, respectively); resistance to penicillin correlated with the high prevalence of the genes encoding hemolysins: *ila*, 100% (41/41); *ild*, 100% (41/41); and *hlg*, 61% (25/41). Many isolates possessed additional virulence genes: *sea*, 85% (35/41); *sdrE*, 83% (34/41); and *PVL*, 73% (30/41), while *cna* and *tsst* were less prevalent (29%, 12/41 and 10%, 4/41 respectively). Thirty four (83%, 34/41) isolates were *ica/sdrE*-positive; 33 (80%, 33/41) *ica/sea*-positive; 28 (68%, 28/41) *ica/PVL*-positive; 23 (56%, 23/41) *ica/hlg*-positive and three (7%, 3/41) *ica/tsst*-positive. All the 12 (29%, 12/41) cna-positive isolates were also *ica*-positive. Interestingly, an isolate positive for all the determinants studied possessed the homologue of the *bhp* gene, which encodes the biofilm associated protein (BAP); this is rarely detected in human isolates but frequently detected in staphylococci causing bovine mastitis [18]. This *bhp*-positive isolate was MDR and negative for only three determinants, * tst*, *aph(3’)-Ila* and *ant(4’)-la*, conforming to its susceptibility to erythromycin/gentamicin. Detailed analyses for the virulence and antimicrobial resistance determinants are provided in additional file 2.

**Discussion**

In this study, high prevalence of MDR-MRSA was found in the burns unit of Mulago hospital, predisposing patients to infection with intractable isolates and underscoring the need for improved infection control practices in this setting. Ojulong et al 2009, reported a relatively lower prevalence (31.6%) from the general surgery ward, possibly because this earlier study determined MRSA infections in only patients with post-operative surgical wound infections [5]. Although data are still limited, there are emerging reports of prevalent MRSA infections in sub-Saharan Africa [19].

CHROMagar, generally considered more efficient at detecting MRSA than oxacillin discs [20] was inefficient

| Sample source (n = 89) | *S. aureus* | meca | Oxacillin disc | CHROMagar | SCCmec types |
|------------------------|------------|------|----------------|------------|---------------|
|                        | Oxacillin disc | MSSA | MRSA | MSSA | MRSA | I | II | III | IV | V | NT |
| Patients (n = 21)       | 14 | 14 | ND | 14 | 4 | 10 | 8 | ND | ND | 3 | 1 | 2 |
| HCW (n = 31)            | 13 | 13 | ND | 13 | 4 | 9 | 8 | ND | ND | 3 | 2 | |
| Environment (n = 37)    | 14 | 14 | ND | 14 | 7 | 7 | 6 | ND | ND | 2 | 6 | |
| Prevalence              | 46 | 100 | 46 | 17 | 29 | 54 | - | - | 7 | 15 | 24 | |
| (41/89)                 | (41/41) | (41/89) | (15/89) | (26/89) | (22/41) | (3/41) | (6/41) | (10/41) |
in this setting (i.e. 46% vs. 29% prevalence, respectively). Since all isolates were oxacillin resistant, mecA-positive (mecA encodes the penicillin binding protein 2a, the molecular determinant for methicillin resistance [21]), nuc- and femA-positive, they were confirmed as MRSA. Prior to use, the CHROMagar batch passed quality control screening with known MRSA and MSSA strains; yet only 26 isolates grew mauve colonies (which is indicative of MRSA); the use CHROMagar in this setting may need further investigations. A high prevalence of biofilm/ica-positive isolates correlated with that of MDR isolates. Although biofilms/ica genes are debatable as virulence markers [22], in this study, the biofilm/ica-positive isolates were concomitantly positive for other virulence genes. Notably was the absolute prevalence for staphylococcal hemolysins: hla and hld. The hla gene encodes a dermanecrotic and neutrotoxic toxin that is also responsible for abscess formation; hld producing S. aureus can cause severe enteritis, while hld lyses mammalian red blood cells and together with tst-1 (toxic shock syndrome toxin-1), can be involved in the pathogenesis of toxic shock syndrome (TSS) [15]. PVL, also prevalent in this study, causes severe disease in children and young adults with no known exposure to healthcare establishment, and is used as a stable marker for community acquired MRSA [15]. Furthermore, the staphylococcal super-antigenic toxins, sea and tst-1 were also detected (85% and 10% prevalence respectively). sea producing strains are responsible for staphylococcal food intoxications, while tst strains produce antigens that are responsible for TSS.

Conclusion
Hypervirulent methicillin resistant S. aureus is prevalent in the burns unit of Mulago hospital.

Additional material

Additional file 1: Primers and PCR conditions
Additional file 2: Speciation, drug susceptibility testing and detection of virulence and antimicrobial resistance genes/determinants
Abbreviations
SCCmec: Staphylococcus Cassette Chromosomal mec; oxa: Oxacillin; penG: Penicillin G; SXT: Sulphamethoxazole-trimethoprim; MDR: Multi Drug Resistant
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2. Mugisa DB: Complications Following Laparotomy in Mulago Hospital.
Veterinary Medicine, Makerere University, Kampala, Uganda.

3. Olaro C: Risk factors for Postoperative Complication following abdominal
Global prevalence of meticillin-resistant

1. Wertheim HFL, Verbrugh HA: Global prevalence of meticillin-resistant
Staphylococcus aureus. The Lancet 2006, 368(9550):1866.

2. Mugisa DB: Complications Following Laparotomy in Mulago Hospital.
Master of Medicine (Surgery) Dissertation. Makerere University, Department of Surgery; 1988.

3. Oloro C: Risk factors for Postoperative Complication following abdominal
Surgery in Mulago hospital. Master of Medicine (Surgery) Dissertation. Makerere University, Department of Surgery. 1999.

4. Ojikan-Odeke: Hand Infections in Uganda. Master of Medicine (Medical Microbiology) Dissertation. Makerere University, Department of Medical Microbiology. 1978.

5. Ojulong JMT, Joloba M, Bwanga F, Kaddu-Mulindwa DH: Relative
prevalence of methicillin resistant Staphylococcus aureus and its
susceptibility pattern in mulago hospital, Kampala, Uganda. Tanzanian Journal of Health Research 2009, 11(3):149-153.

6. Kateete D, Kimani C, Katafazi F, Okeng A, Okoe M, Nanteza A, Joloba M, Najuka F: Identification of Staphylococcus aureus: DNase and Mannitol
sact agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials. 2010, 9(1):23.

7. Guideline for isolation precautions in hospitals: Part II. Recommendations for isolation precautions in hospitals. Hospital Infection Control Practices Advisory Committee. Am J Infect Control, 1996, 24(1):32-52.

8. Boye K, Bertels MD, Andersen IS, Mattar JA, Westh H: A new multiplex PCR
for easy screening of methicillin-resistant Staphylococcus aureus
SCCmec types I-V. Clinical Microbiology and Infection 2007, 13(7):725-727.

9. Klingenberg C, Araag E, Ronnestad A, Sollid JE, Abrahamsen TG, Kjeldsen G, Nastouli E: Coagulase-negative staphylococcal sepsis in neonates.
Association between antibiotic resistance, biofilm formation and the
host inflammatory response. Pediatr Infect Dis J 2005, 24(9):817-822.

10. Amaral MM, Coelho LR, Flores RP, Souza RR, Silva-Carvalho MC, Teixeira LA, Ferreira-Cavalho BT, Figueiredo AM: The predominant variant of the
Brazilian epidemic clonal complex of methicillin-resistant Staphylococcus aureus has an enhanced ability to produce biofilm and to adhere to
and invade airway epithelial cells. J Infect Dis 2005, 192(5):801-810.

11. Kumar JD, Negi YK, Gaur A, Khanna D: Detection of virulence genes in
Staphylococcus aureus isolated from paper currency. International Journal of Infectious Diseases 2009, 13(6):e453-e455.

12. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J, Vandenesch F: Relationships between
Staphylococcus aureus Genetic Background, Virulence Factors, agr Groups (Alleles), and Human Disease. Infect Immun 2002, 70(2):631-641.

13. McClure J-A, Conly JA, Lau V, Elsayed S, Louie T, Hutchins W, Zhang K: Novel Multiplex PCR Assay for Detection of the Staphylococcal Virulence
Marker Panton-Valentine Leukocidin Genes and Simultaneous Discrimination of Methicillin-Susceptible from -Resistant Staphylococci. J Clin Microbiol 2006, 44(3):1141-1144.

14. Stotts SN: Virulence and Antibiotic Resistance Gene Combinations
among Staphylococcus aureus Isolates from Coastal Waters of Oahu, Hawaii. The Journal of Young Investigators. 2005, 12(4).

15. Cunha MLRS, Caloiari RA: Toxicity of Staphylococcus aureus and
Coagulase-Negative Staphylococci: Epidemiological and Molecular
Aspects. Microbiology Insights 2008, 2008(MBI-1-Cunha-et-al).

16. Montanaro L, Campoccia D, Pirini V, Reauro S, Otto M, Arciola CR: Antibiotic multiresistance strictly associated with IS256 and ica genes in
Staphylococcus epidermidis strains from implant orthopedic infections. J Bone Joint Res 2007, 83(3):813-818.

17. Verhoef J, Beaujean D, Blok H, Baas A, Meyler A, van der Werken C, Weersink A: A Dutch approach to methicillin-resistant Staphylococcus
aureus. Eur J Clin Microbiol Infect Dis 1999, 18(7):461-466.

18. Vauter E, Abadie G, Pont A, Thiery R: Evaluation of the presence of the
bap gene in Staphylococcus aureus isolates recovered from human and
animals species. Vet Microbiol 2008, 127(3-4):407-411.

19. Shitu AO, Okon K, Adesida S, Oyedara O, Wette W, Stommenger B, Layer F, Nuel U: Antibiotic resistance and molecular epidemiology of
Staphylococcus aureus in Nigeria. BMC Microbiol 2011, 11:92.

20. Perry JD, Davies A, Buttenworth LA, Hopley AJ, Nichollson A, Gould FK: Development and Evaluation of a Chromogenic Agar Medium for
Methicillin-Resistant Staphylococcus aureus. J Clin Microbial 2004, 42(10):4519-4523.

21. Malloqui-Fernández G, Marrero A, García-Piqué S, García-Castellanos R, Cornis-Ruth F: Staphylococcal methicillin resistance: fine focus on folds
and functions. FEMS Microbiology Letters 2004, 235(1):1-8.

22. Frank KL, Hanssen AD, Patel R: icaA Is Not a Useful Diagnostic Marker for
Prosthetic Joint Infection. J Clin Microbiol 2004, 42(10):4846-4849.

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