Original Article

Prevalence of virulence determinants in *Staphylococcus epidermidis* from ICU patients in Kampala, Uganda

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Abstract

Introduction: *Staphylococcus epidermidis* is often considered a non-pathogenic organism but it causes nosocomial infections. To distinguish invasive strains, comparative studies of patient and community isolates may offer some clues. We investigated the distribution of virulence determinants in patient isolates from Uganda.

Methodology: *S. epidermidis* isolates were identified with the Staph API ID 32 kit. Antimicrobial susceptibility, biofilm formation and hemolysis were detected with standard procedures. Genes associated with virulence (*aap*, *atlE*, *bhp*, *hla*, *hld*, *ica*, *IS*256, *sdrE*, *sea*, *tsst*) and antimicrobial resistance (*aac(6')-Ie-aph(2'')-Ia, aph(3')-IIIa, ant(4')-Ia, blaZ, mecA, vanA/vanB1*) were detected by PCR.

Results: *S. epidermidis* grew in 30 (30/50, 60%) ICU samples and 20 (20/60, 33%) community samples (one isolate per sample per patient/person). All ICU isolates (30/30, 100%) were *IS*256 and *hld* positive, 22 (22/30, 73%) were biofilm/*ica* positive, 21 (21/30, 70%) were hemolytic on blood agar, nine (9/30, 30%) contained *atlE* gene, six (6/30, 20%) *hla* gene, five (5/30, 17%) *aap* gene, and three (3/30, 10%) *bhp* gene. A gene encoding an aminoglycoside-modifying enzyme, *aph(3')-IIIa*, was highly prevalent (28/30, 93%), while *blaZ* (2/30, 7%), *mecA* (3/30, 10%), *vanA* (3/30, 10%) and *vanB1* (3/30, 10%) were less prevalent. Of the community isolates, one (1/20, 5%) was *ica* positive, two (2/20, 10%) formed biofilms, and three (3/20, 15%) possessed the *atlE* gene. *bhp*, *aap*, *IS*256, *hld* and antimicrobial resistance genes were not detected in community isolates.

Conclusions: *S. epidermidis* from ICU patients in Mulago Hospital is potentially virulent and could be a reservoir for antimicrobial resistant genes.

Key words: *Staphylococcus epidermidis*; virulence determinants; antimicrobial resistance genes; intensive care unit; Mulago Hospital; Uganda

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Introduction

Staphylococcus epidermidis, a common normal flora, frequently causes infections in hospitalized patients with indwelling support devices [1,2]. Unlike *Staphylococcus aureus*, *S. epidermidis* lacks obvious virulence determinants and is often regarded an accidental pathogen [3]. Distinguishing invasive from commensal strains is challenging since virulence factors can occur in both; the sudden transition of the organism to a pathogenic state is the subject of intense investigations [1,2,4,5]. Studies aiming at distinguishing invasive from commensal strains are needed [3].

While there is limited data on the molecular epidemiology of *S. epidermidis* infections in sub-Saharan Africa, elsewhere many investigators elucidating the pathogenicity of the organism mainly focus on detection of biofilms and intercellular adhesion *(ica*) genes. The usefulness of these as virulence markers has been debated widely [6,7]. Recently, it was demonstrated that the insertion sequence *IS*256 correlates highly with aminoglycoside resistance in *S. epidermidis* [1,2], and its superior to *ica* gene detection in distinguishing clinically relevant isolates [1]. Additionally, more genes implicated in biofilm production (*i.e.*, bifunctional autolysin E, *atlE;* accumulation-associated protein, *aap;* and biofilm associated protein, *bhp*) have been elucidated [2,3,8]. While they were demonstrated as useful in the detection of clinical strains [2], their prevalence has not been widely investigated. Since patients in

intensive care units (ICU) are easily colonized/infected with nosocomial pathogens, particularly those associated with the frequent use of support devices, we aimed to determine the prevalence of a collection of virulence and antimicrobial resistance determinants in *S. epidermidis* from ICU patients at Mulago Hospital in Kampala, Uganda. Biofilms as well as genes encoding the staphylococcal hemolysins (*hla, hld*), superantigenic toxins (*tsst, sea*), putative adhesin serine aspartate repeat protein (*sdrE*), and antimicrobial resistance genes (*mecA, vanA/vanB1*, *blaZ* and the aminoglycoside modifying enzymes, *aac(6')-Ie-aph(2'')-Ia, aph(3')-IIIa* and *ant(4')-Ia*), which frequently occur in invasive isolates, were studied.

Methodology

Study setting and sampling

Approval was obtained from the institution review board of Mulago Hospital. Written informed consent was obtained from the participants. This cross-sectional study was conducted from December 2007 to June 2008, on 50 patients in the Mulago Hospital ICU and 60 healthy participants from Makerere University. Duplicate samples from ICU patients included catheter tips (24/50, 48%), blood (16/50, 32%), swabs (7/50, 14%) and aspirates (3/50, 6%, two pleural and one bronchial). Catheter tips were aseptically excised and transported to the laboratory in culture bottles with 10 ml tryptic soy broth. After incubating overnight at 37°C, samples were vortexed and aliquots streaked on blood agar, and incubated overnight at 37°C. For blood and bronchial samples, 2 ml each was injected into blood culture bottles and incubated at 37°C in an automated blood culture system (BACTEC 9120). Wounds (skin, ears and eyes) were sampled by aspirating pus with a sterile syringe or using cotton swabs with Amies transport medium and incubated at 37°C overnight on blood agar. Nasal swabs from randomly selected healthy subjects were similarly processed. After standard microbiological procedures, *S. epidermidis* was identified with the Staph API ID 32 system (Biomerieux, Lyon, France), and confirmed by PCR [9]. Antimicrobial susceptibility testing (penicillin 10U, oxacillin 5µg, clindamycin 2µg, erythromycin 15µg, tetracycline 30µg, ciprofloxacin 5µg, trimethoprim/sulfamethoxazole 1.25/23.75µg, chloramphenicol 30µg, rifampicin 5µg, gentamicin 10µg and vancomycin 30µg) was performed with the disc diffusion method following standard guidelines [10].

Detection of virulence determinants

Biofilms and hemolysis: Biofilms were detected with the microtiter plate method [11] and the biofilm unit calculated according to Amaral *et al*. [12]. Briefly, assays were performed in triplicate in TSB/1% glucose in 96-well polystyrene flat-bottom tissue culture plates. Isolates were incubated at 37ºC overnight with gentle shaking and standardized to OD_{600} = 0.005 with normal saline. Then 50 µl of standardized cells mixed with 150 µl TSB/1% glucose were incubated at 37ºC for 17 hours. After washing three times with sterile water and staining with crystal violet for 15 minutes, cells were washed again with sterile water and incubated at room temperature for one hour in 95% ethanol, and the biofilms were measured with a spectrophotometer at OD_{570}. The biofilm forming *S. epidermidis* RP62A and its non-biofilm forming variant (ATCC 12228) were used as controls. Hemolysis was determined on blood agar plates supplemented with 5% sheep blood.

Virulence and antimicrobial resistance genes: Since virulence and antimicrobial resistance tend to co-exist in invasive strains [1], molecular assays to detect genes encoding/associated with these phenotypes were performed. DNA was extracted with the MasterPure purification kit (Epicenter, Madison, USA). *ica*, *IS*256, *hla*, *hld*, *tsst* and *sea* genes, which frequently occur in invasive strains, as well as *bhp*, *aap* and *atlE*, were detected by PCR. Each PCR sample contained 20 pmoles each of forward (fwd) and reverse (rev) primers, 1.5U *Taq* polymerase (Thermo Scientific, Surry, UK), Custom PCR-Master Mix (Thermo Scientific, Surry, UK), template DNA and nuclease-free water, in 10µl reaction volume. Details of primer sequences are shown in Table 1. Positive (*S. epidermidis* DNA template) and Negative controls (water, none-*S. epidermidis* DNA template) were always included in the reactions depending on the amplification target. icaSe1 and icaSe2 primers amplified a 639bp product containing *icaA*, *icaD* and *icaB* genes when amplified under the following conditions: 94°C, 5 minutes; (94^{o}C, 1 minute, 60^{o}C, 1 minute and 72°C, 1 minute) x 30 cycles; 72°C, 10 minutes. To detect genes encoding aminoglycoside-modifying enzymes (AME) [3[3], PCR of *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*

Table 1. Primers and PCR conditions

Name	Sequence (5' → 3')	Target (bp)	Reference/ Conditions[1]
Se705-1	ATCAAAAAGTTGGCGAACCTTTTC	(124)	[9]
Se705-2	CAAAAGAGCGTGGAGAAAAGTATC		
Detection of virulence genes			
icaSeF	GAAAGGTGGCTATGCTAC (fwd)	*ica* (639)	This study[2]
icaSeR	GACGTCGTGTGCTTTAAGCCATTG (rev)		
IS256F-P5	AAGATGTTGGCTGTGATTAC (fwd)	*IS256* (762)	[13]
IS256R-P3	CAACAAGTTGAAGGCATATC (rev)		
Hla1	CTGATTACTATCCAAGAAATTCGATTG (fwd)	*hla* (209)	[14]
Hla2	CTTTCCAGCCTACTTTTTTATCAGT (rev)		
Hlb1	GTGCACTTACTGACAATAGTGC (fwd)	*hlb* (309)	[14]
Hlb2	GTGCACTTACTGACAATAGTGC (rev)		
Hld1	AAGAATTTTTATCTTAATTAAGGAAGGAGTG (fwd)	*hld* (111)	[14]
Hld2	TTAGTGAATTTGTTCACTGTGTCGA (rev)		
hlgF	GCCAATCCGTTATTAGAAAATGC (fwd)	*hlg* (937)	[15]
hlgR	CCATAGACGTAGCAACGGAT (rev)		
tstF	ATGGCAGCATCAGCTTGATA (fwd)	*tst1* (350)	[16]
tstR	TTTCCAATAACCACCCGTTT (rev)		
seaF	GGATATTGTTGATAAATATAAAGGGAAAAAAG (fwd)	*seA* (439)	[16]
Sear	GTTAATCGTTTTATTATCTCTATATATTCTTAATAGT		
sdrE1	AGTAAAATGTGTCAAAAGA (fwd)	*sdrE* (767)	[15]
sdrE2	TTGACTACCAGGCTATAT (rev)		
bhp1	CCCTATATCGAAGGTGTAGAATTGCAC (fwd)	*Bap* (970)	[17]
bhp2	GCTGTTGAAGTTAATACTGTACCTGC (rev)		
atlE-F	GCTAAGGCACCAGTAAAAAGT (fwd)	*atlE* (480)	[4]
atlE-R	GACCTCATCTTGTTTTACCCA (rev)		
aap-F	CAACGAAGGCAGAAGAAGGA (fwd)	*aap* (719)	[4]
aap-R	CATCCCCATCTTTCTTGCTG (rev)		
Detection of antimicrobial resistance genes			
mecA1	GTAGAAATGACTGAACGTCCGATAA (fwd)	*mecA* (391)	[18]
mecA2	CCAATTCCACATTGTTTCGGTCTAA (rev)		
486F	GTTGCGAACTCTTGAATAGG (fwd)	*blaZ* (674)	[19]
486R	GGAGAATAAGCAACTATATCATC (rev)		
aac1	CAGAGCCTTGGGAAGATGAAG (fwd)	*aac*[3] (348)	[14]
aac2	CCTCGTGTAATTCATGTTCTGGC (rev)		
ant1	CAAACTGCTAAATCGGTAGAAGCC (fwd)	*ant*[4] (294)	[14]
ant2	GGAAAGTTGACCAGACATTACGAACT (rev)		
aph1	AAGAATTTTTATCTTAATTAAGGAAGGAGTG (fwd)	*aph*[5] (523)	[14]
aph2	TTAGTGAATTTGTTCACTGTGTCGA (rev)		
vanA1	GTT GCA ATA CTG TTT GGG GG (fwd)	*vanA* (1,014)	[20]
vanA2	CCC CTT TAA CGC TAA TAC GAT CAA (rev)		
vanB1F	GTGACAAACCGGAGGCGAGGA	*vanB* (433)	[20]
vanB1R	CCGCCATCCTCCTGCAAAAAA		

(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 beta-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. PCR amplicons were analyzed by agarose gel electrophoresis and representative samples sequence confirmed. The data was analyzed with GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, USA).

Results

Of the 50 patients (26 females, 52%; 24 males, 48%; mean age, 50 years, with an average stay in ICU of 17 days), 10 (10/50, 20%) suffered from respiratory tract infections, nine (9/50, 18%) from bacteraemia and six (6/50, 12%) from urinary tract infections. Others suffered from surgical wound infections (6/50, 12%), cardiovascular disorders (6/50, 12%), ocular infections (4/50, 8%), peritonitis (3/50, 6%), pneumonia (2/50, 4%), otitis media (1/50, 2%), pneumothorax (1/50, 2%) and burns (1/50, 2%).

Staphylococcus epidermidis was isolated from 30 (60%, 30/50) ICU samples (one isolate per sample per patient). *S. epidermidis* grew in nine of the 16 blood cultures (56%, 9/16) and these were considered clinically relevant; from patient records, eight of the patients with blood cultures had bacteraemia while one had peritonitis. Isolates from the other samples were considered colonizers since they were difficult to correlate with disease. Other organisms were isolated but not investigated due to low prevalence: *Staphylococcus aureus* (8/50, 16%), *Staphylococcus waneri* (1/50, 2%), *Streptococcus pneumoniae* (1/50, 2%), *Haemophilus influenzae* (1/50, 2%), *Enterococcus fecalis* (3/50, 6%), *Escherichia coli* (2/50, 4%), *Klebsiella pneumoniae* (2/50, 4%) and *Pseudomonas aeruginosa* (2/60, 3%, which were also detected in previously hospitalized healthy participants). For community samples, *S. epidermidis* was isolated from 20 (33%, 20/60).

Prevalence of virulence determinants

Of the *S. epidermidis* ICU isolates, all (30/30, 100%) contained the *IS*256 and *hld* genes, 22 (73%, 22/30) were biofilm/*ica* positive, 21 (21/30, 70%) were hemolytic on blood agar, nine (9/30, 30%) possessed the *atlE* gene, six (6/30, 20%) the *hla* gene, three (3/30, 10%) the *bhp* gene and five (5/30, 17%) the *aap* gene (see Figure.1). The *sdrE* (2/30, 7%), *tsst1* (2/30, 7%) and *sea* (1/30, 3%) genes were less prevalent. In contrast, one community isolate was *ica* positive (1/20, 5%), two formed biofilms (2/20, 10%), and three (3/20, 15%) possessed the *atlE* gene. Overall, biofilms, *ica* and *atlE* were the only virulence determinants detected in community isolates; *aap, bhp, hla, hld, IS*256, *sea, sdrE* and *tsst1* were not detected.

Prevalence of antimicrobial resistance genes

S. epidermidis ICU isolates contained genes encoding the aminoglycoside-modifying enzymes, with *aph(3')-IIIa* being the most prevalent (28/30, 93%). The *aac(6')-Ie-aph(2'')-Ia* and *ant(4')-Ia* genes were detected in eight (8/30, 27%) and three (3/30, 10%) isolates, respectively. *blaZ* was detected in two (2/30, 7%) while *vanA*/*vanB1* and *mecA* were detected in three (3/30, 10%) isolates. None of the antimicrobial resistance genes were detected in community isolates (Figure 1). The three *mecA*-positive isolates were phenotypically resistant to oxacillin and other antibiotics, and were considered methicillin resistant *S. epidermidis* (MRSE). The strong biofilm-producing ICU isolates were also multidrug resistant. Of the community isolates, only the *atlE* positives produced biofilms and exhibited antimicrobial resistance (see Table 2).

Discussion

In this study, we report a high prevalence of virulence/antimicrobial resistance determinants in *S. epidermidis* from the Mulago Hospital ICU. Since catheter-related staphylococcal infections are common in this setting [21], ICU patients could be at risk of infection with intractable pathogens.

Genes involved in biofilm production (a major virulence determinant relevant for colonization of surfaces/biomaterials) have been suggested as potential markers for clinically relevant strains [11]. Consequently, the majority of the ICU isolates were biofilm/*ica* positive, in agreement with previous reports [1,2]; however, the usefulness of *ica* and biofilms is curtailed by their concomitant presence in commensal isolates [7]. Furthermore, all ICU isolates were *IS256* positive while 73% were biofilm/*ica*/*IS256* positive, supporting the recent association of these factors with clinically relevant strains [1]. Nine ICU isolates were biofilm/*atlE*/*ica* positive and five were biofilm/*aap*/*atlE*/*ica* positive.

Table 2. Hemolysis, biofilm production and antimicrobial susceptibility patterns

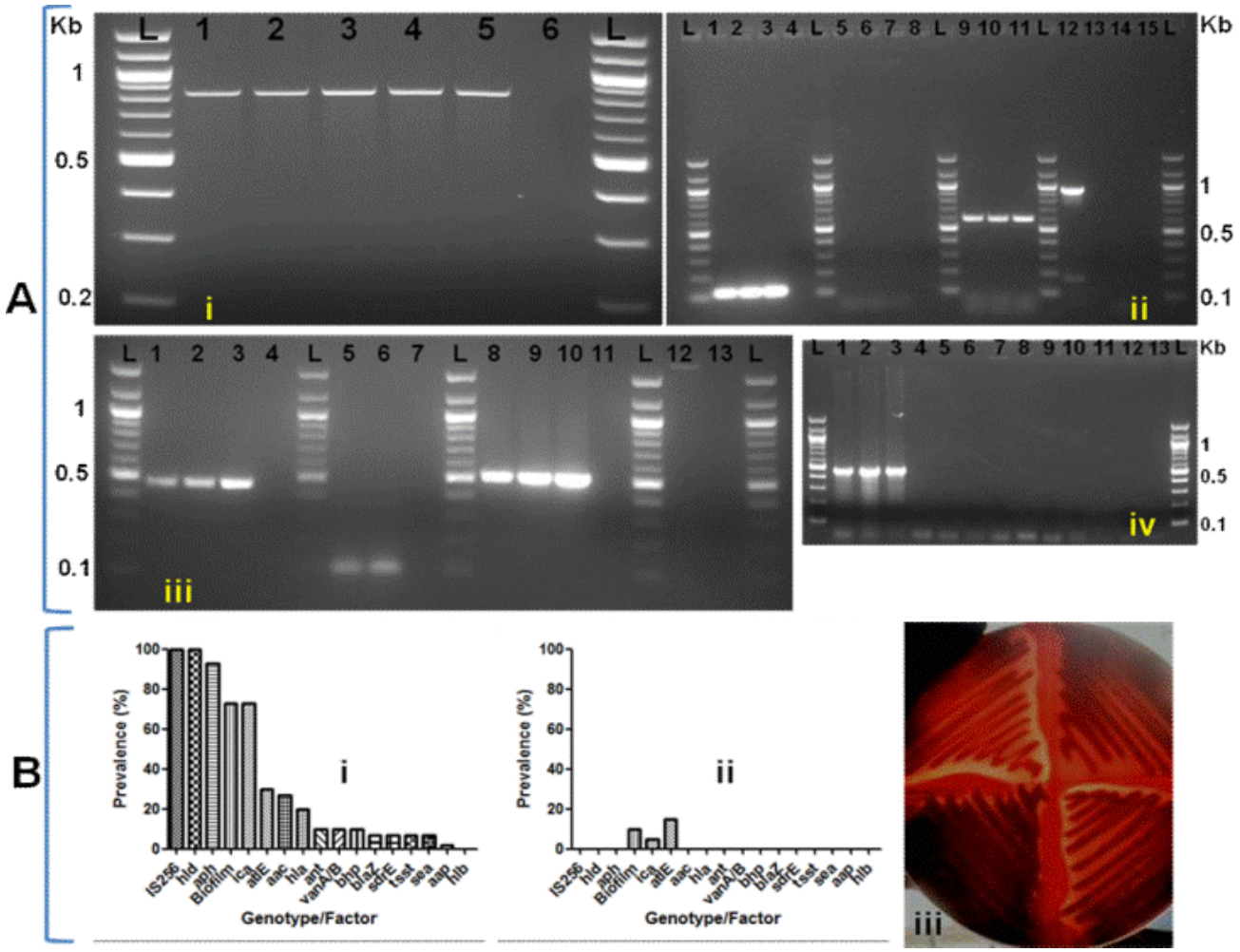
Sample ID	Source	*atlE*	*hld*	*hlb*	*hla*	Hemolysis	*bhp*	*ica*	CRA	BU	BP	Antimicrobial resistance pattern
M1	Catheter	ND	+	ND	ND	+	ND	+	+	0.19	Weak	ERY, GEN, PEN
M2	Blood	ND	+	ND	ND	+	ND	ND	ND	0.088	None	ERY, GEN, PEN, TET
M3	Blood	ND	+	ND	ND	+	ND	+	+	0.432	Moderate	ERY, PEN, SXT, TET
M4	Blood	ND	+	ND	ND	+	ND	ND	ND	0.174	None	ERY, GEN, PEN
M5	Blood	ND	+	ND	ND	+	ND	ND	ND	0.152	None	PEN, TET
M6	Catheter	ND	+	ND	ND	ND	ND	+	+	0.198	Weak	PEN, SXT
M7	Catheter	ND	+	ND	+	+	ND	+	+	0.187	Weak	ERY, PEN, TET
M8 (MRSE)	Catheter	ND	+	ND	+	+	ND	+	+	0.352	Weak	CIP, ERY, GEN, OXA, PEN, TET, SXT
M9	Wound	ND	+	ND	+	+	ND	ND	ND	0.111	None	ERY, GEN, PEN, TET
M10	Catheter	ND	+	ND	ND	ND	ND	ND	ND	0.15	None	CIP, GEN, PEN, SXT, TET
M11 (MRSE)	Wound	ND	+	ND	ND	ND	ND	+	+	0.321	Weak	CIP, ERY, GEN, OXA, PEN, TET, SXT
M12	Blood	+	+	ND	ND	ND	ND	+	+	1.353	Strong	CIP, ERY, GEN, OXA, PEN, TET, SXT
M13	Catheter	+	+	ND	ND	+	ND	+	+	0.7	Moderate	CIP, CHL, ERY, GEN, PEN, SXT
M14	Blood	ND	+	ND	ND	ND	ND	ND	ND	0.1	None	GEN, PEN, SXT, TET
M15	Catheter	+	+	ND	ND	+	ND	+	+	0.572	Moderate	CIP, GEN, PEN
M16	Blood	ND	+	ND	+	+	ND	+	+	0.234	Weak	GEN, OXA, PEN
M17	Blood	ND	+	ND	ND	ND	ND	+	+	0.25	Weak	CIP, GEN, PEN, SXT
M18	Pus	ND	+	ND	ND	+	ND	+	+	0.221	Weak	CIP, GEN, PEN, SXT
M19	P. aspirate	ND	+	ND	ND	+	ND	+	+	0.278	Weak	GEN, PEN, SXT
M20	Catheter	+	+	ND	ND	+	ND	+	+	0.669	Moderate	CIP, CHL, ERY, GEN, OXA, PEN, SXT
M21	Catheter	ND	+	ND	ND	ND	+	ND	ND	0.175	None	CIP, GEN, PEN, SXT
M22	Catheter	+	+	ND	ND	ND	ND	+	+	0.547	Moderate	CIP, CHL, ERY, GEN
M23	B. aspirate	+	+	ND	ND	+	ND	+	+	0.589	Moderate	PEN, SXT
M24	Wound	ND	+	ND	ND	+	ND	+	+	0.7	Moderate	CHL, ERY, GEN, PEN
M25	Pus	+	+	ND	ND	+	ND	+	+	0.615	Moderate	ERY, GEN, PEN, TET
M26	Catheter	ND	+	ND	ND	+	ND	ND	ND	0.122	None	CIP, ERY, GEN, , PEN, SXT
M27	Catheter	+	+	ND	ND	ND	+	+	+	1.676	Strong	CIP, CHL, ERY, GEN, PEN, SXT
M28	Catheter	ND	+	ND	+	+	+	+	+	0.544	Moderate	CIP, ERY, GEN, PEN
M29	Blood	+	+	ND	ND	+	ND	+	+	0.55	Moderate	ERY, GEN, PEN

Table 2. Hemolysis, biofilm production and antimicrobial susceptibility patterns (continued.)

M30 (MRSE)	Catheter	ND	+	ND	ND	+	ND	+	+	0.302	Weak	CIP, ERY, GEN, OXA, PEN, TET, SXT
M31	Nose	+	ND	ND	ND	ND	ND	ND	ND	0.102	None	PEN, TET
M32	Nose	+	ND	ND	ND	ND	ND	+	+	0.676	Moderate	ERY, GEN, PEN, TET
M33	Nose	+	ND	ND	ND	ND	ND	ND	+	0.69	Moderate	CIP, ERY, GEN, PEN, TET
M34	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.174	None	ND
M35	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.152	None	ND
M36	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.178	None	ND
M37	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.187	None	ND
M38	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.105	None	ND
M39	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.1	None	ND
M40	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.098	None	ND
M41	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.021	None	ND
M42	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.057	None	ND
M43	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.076	None	ND
M44	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.1	None	ND
M45	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.152	None	ND
M46	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.12	None	ND
M47	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.125	None	ND
M48	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.174	None	ND
M49	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.166	None	ND
M50	Nose	ND	ND	ND	ND	ND	ND	ND	ND	0.119	None	ND

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 > 2x the negative control value were considered biofilm producers and were classified as follows: weak, 0.182 < BU < 0.364; moderate, 0.364 < BU < 0.728; strong, BU > 0.728 [12]. Isolates M1 to M30 were from the ICU, while M31 to M50 were from the community. MRSE, Methicillin resistant *Staphylococcus epidermidis*; BP, biofilm production; +, positive; ND, not detected; CIP, ciprofloxacin; CHL, chloramphenicol; ERY, erythromycin; GEN, gentamicin;

Figure 1. Prevalence of virulence/antimicrobial resistance determinants in *S. epidermidis*



Panel A shows representative images for gene detection in ICU (i, ii, iii) and community (iv) isolates. i, *IS*256: lanes 1 to 5, test isolates; 6, negative control (water). ii, PCR-confirmation of *S. epidermidis* with Se705 primers: lanes 1, positive control RP62A; 2 & 3, test isolates; 4, 5 to 8, negative controls (water, *S. aureus*, *S. waneri*, *E. coli*, and *Streptococcus pneumoniae*, respectively). Lanes 9 to 11, *ica*; 12, *bhp* (upper band) and *tsst* (lower band) in a multiplex PCR. iii, lanes 1 to 3, *atlE* genes in test isolates; 5 and 6, *hld*; 8 to 10, *aph(3')-IIIa*; 12, *vanA*; 4, 7, 11 and 13, negative controls (water). iv, *atlE*-positive community isolates negative for *IS*256 (lanes 4 to 6), *ica* (7 to 9), and *aph(3')-IIIa* (10 to 12); 13 negative control (water). L, 100bp DNA ladder (for all images). *aac*, (*aac(6')-Ie-aph(2")-Ia*); *aph*, *(aph(3')-IIIa*); ant, *(ant(4')-Ia*). Panel B, graphical presentation of prevalence for the different virulence determinants, i) ICU-; ii) community isolates; iii), hemolysis by ICU-isolates on blood agar plates.

de Araujo *et al*. reported a concomitant presence of *ica*, *atlE* and *aap* genes as being strongly associated with biofilms [7]. Two of the three *bhp* positive ICU isolates were concomitantly positive for *ica*, *aap* and *atlE* genes, while one was *aap*/*atlE* negative but biofilm/*ica* positive. Three community isolates were *atlE* positive, of which one was biofilm/*atlE*/*ica* positive and another biofilm/*atlE* positive. Although previously detected at high prevalence in commensal strains [7], *atlE* and *aap* genes were less common in community isolates.

Biofilms are formed in two steps: an initial adherence of bacteria to inert surfaces (involving the AtlE protein [7]) and biofilm accumulation. In the second phase, bacteria connected to the polymer surface produce and accumulate the biofilm, which is thought to be the main mechanism for bacterial adherence to plastic surfaces and of auto-aggregation. In *S. epidermidis*, the *ica* operon encodes enzymes for the biosynthesis of polysaccharide intercellular adhesin (PIA), which, together with an additional protein, AAP, appear necessary for biofilm accumulation [7]. Furthermore, an alternative pathway involving BAP protein is responsible for biofilm production in *ica*-negative isolates [8]. Although BAP occurs in animal *S. aureus*, a BAP homolog, BHP, exists in human *S. epidermidis* and can induce biofilms in absence of PIA [8].

S. aureus toxin-encoding genes were prevalent in ICU isolates, with absolute prevalence for the *hld*

gene. Six isolates exhibiting near complete hemolysis on blood agar concomitantly contained *hld* and *hla*, while one contained the staphylococcal enterotoxin a (*sea*), *hla* and *hld* genes. The *S. aureus hld* is similar to that of *S. epidermidis*; it is thermostable, damages membranes of mammalian cells, and possibly causes severe enteritis [22]. The *hla* gene encodes a dermanecrotic, neurotoxic toxin that is also responsible for abscess formation. Although prevalent in ICU isolates, the enterotoxigenicity of coagulase negative staphylococci is still debatable. Nevertheless, expression of toxin genes was demonstrated in *S. epidermidis* [22]. While *S. epidermidis* is considered a reservoir of antimicrobial resistance genes for *S. aureus*, the presence of homologues of *S. aureus* toxin genes in *S. epidermidis* may contribute to a repertoire of virulence determinants yet to be elucidated [22].

All the three AME encoding genes [20] were detected in ICU isolates with the most prevalent being *aph(3')-IIIa* (only two isolates tested negative), while *aac(6')-Ie-aph(2'')-Ia* and *ant(4')-Ia* were less prevalent. AME are highly associated with the *IS*256 element, a component of *Tn4001* that mediates gentamicin resistance by the product of the *aac(6')-Ie-aph(2'')-Ia* gene [1]. Arciola *et al*. reported full association between the presence of *IS*256 and resistance to gentamicin [1]. While *aac(6')-Ie-aph(2'')-Ia* was detected in all *IS*256-positive isolates in a previous study [2], it was detected in only 27% of the ICU isolates. In staphylococci, aminogylcoside resistance highly correlates with methicillin resistance, due to genetic linkage between resistance determinants [20]. However, in this study, *mecA* was detected in only three ICU isolates, contrasting with the high prevalence of AME. Probably we did not succeed in detecting *mecA* in the majority of the isolates.

In conclusion, *S. epidermidis* from the Mulago Hospital ICU is potentially virulent and could be a reservoir of antimicrobial resistance genes. This sub-Saharan African study supports recent reports from industrialized settings that virulence/antimicrobial resistant determinants are co-present in clinical *S. epidermidis*, and confer selective advantage for colonization/survival in hospital settings [1]. Conclusive comparison requires similar sample types but this was not possible due to difficulty in obtaining consent; furthermore, such a comparison assumes that control subjects are healthy. Due to financial constraints, robust tools such as pulse field gel electrophoresis or multilocus sequence typing, which determine isolate relatedness, were not utilized. We hope future studies will take these omissions into consideration.

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