Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

A genomic infection control study for *Staphylococcus aureus* in two Ghanaian hospitals

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Eric S Donkor[1]
Dorota Jamrozy[2]
Richael O Mills[3,4]
Thomas Dankwah[3]
Philip K Amoo[5]
Beverly Egyir[6]
Ebenezer V Badoe[7]
Joana Twasam[8]
Stephen D Bentley[2]

[1]Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Accra, Ghana; [2]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; [3]Central Laboratory, Korle-Bu Teaching Hospital, Accra, Ghana; [4]Department of Biomedical Sciences, University of Cape Coast, Cape Coast Ghana; [5]Public Health Unit, Korle-Bu Teaching Hospital, Accra, Ghana; [6]Bacteriology Unit, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; [7]Department of Child Health, School of Medicine and Dentistry, University of Ghana, Accra, Ghana; [8]Laboratory Unit, Lekma Hospital, Accra, Ghana

Correspondence: Stephen D Bentley
Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK
Tel +44 122 383 4244
Email sdb@sanger.ac.uk

Background: Whole genome sequencing analysis (WGSA) provides the best resolution for typing of bacterial isolates and has the potential for identification of transmission pathways. The aim of the study was to apply WGSA to elucidate the possible transmission events involved in two suspected *Staphylococcus aureus* hospital outbreaks in Ghana and describe genomic features of the *S. aureus* isolates sampled in the outbreaks.

Methods: The study was carried out at Korle-Bu Teaching Hospital and Lekma Hospital where the suspected outbreaks occurred in 2012 and 2015, respectively. The *S. aureus* isolates collected from the two hospitals were from three sources including carriage, invasive disease, and the environment. Whole genome sequencing of the *S. aureus* isolates was performed and the sequence reads were mapped to the *S. aureus* reference genome of strain USA300_FPR3757. A maximum-likelihood phylogenetic tree was reconstructed. Multilocus sequence typing together with the analysis of antimicrobial resistance and virulence genes were performed by short read mapping using the SRST2.

Results: The *S. aureus* isolates belonged to diverse sequence types (STs) with ST15 and ST152 most common. All isolates carried the *blaZ* gene, with low prevalence of *tetK* and *dfrG* genes also observed. All isolates were *mecA* negative. The *pvl* genes were common and observed in distinct lineages that revealed diverse *Sa2int* phages. At Korle-Bu Teaching Hospital, the genomics data indicated several transmission events of *S. aureus* ST15 involving contamination of various surfaces in the pediatric emergency ward where the outbreak occurred.

Conclusion: The pattern of dissemination of the ST15 clone in the emergency ward of Korle-Bu Teaching Hospital highlights a basic problem with disinfection of environmental surfaces at the hospital. Diverse phage population rather than a single highly transmissible phage type likely mediates the high prevalence of *pvl* genes among the *S. aureus* isolates.

Keywords: *Staphylococcus aureus*, sequencing, transmission, outbreak, Ghana

Introduction

Staphylococcus aureus causes a range of serious infections including meningitis, septicemia, pneumonia, endocarditis, and osteomyelitis.[1] Methicillin-resistant *S. aureus* (MRSA) is of particular concern due to its extensive resistance to antibiotics and association with persistent outbreaks in hospital and community settings.[2,3]In addition to the public health significance of MRSA, methicillin-susceptible *S. aureus* (MSSA) is commonly implicated in bacteremia and skin and soft tissue infections (SSTI).[4] A striking feature of clinical MSSA isolates from Africa is the relatively high prevalence of *pvl* genes,[4–7] a pore-forming toxin that is associated with SSTI and severe necrotizing pneumonia.[8] This characteristic of African MSSA isolates is of interest since the highly successful community-associated MRSA clones are also characterized by frequent carriage of *pvl* genes.[9]

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/IDR.S167639

The field of microbial genomics is advancing at a fast rate with the introduction of next-generation sequencing technologies. Whole genome sequencing analysis (WGSA) is superior to bacterial typing methods as it provides better resolution of bacterial isolates[10] and is particularly suitable for highly clonal bacteria such as *S. aureus*.[11] In previous studies, WGSA was applied to *S. aureus* and used to describe the intercontinental and local transmission of MRSA,[12] investigate outbreaks,[13,14] predict antimicrobial resistance,[15] and type bacterial strains.[10] Generally, applications of WGSA to study *S. aureus* and other microbial pathogens have been carried out to a very limited extent in many countries in Africa, including Ghana.

Most of what is known about *S. aureus* emanates from the developed world, partly because that is where most outbreaks are detected, but also because in Africa, the focus of attention tends to be more toward bacterial pathogens with a greater burden of mortality, such as *Mycobacterium tuberculosis*. Though *S. aureus* is one of the most common causes of infections reported in hospitals in Ghana,[16] there are very limited surveillance data on the pathogen in the country. Recently in Ghana, several hospital-associated outbreaks of *S. aureus* infection have occurred, placing this pathogen high on the agenda of public health issues. In these outbreaks, extensive infection control investigations were carried out. However, characterization of the associated isolates was limited to basic phenotypic tests, leaving several important epidemiological questions unanswered, which require genomic analysis. To help address some of these concerns, we applied whole genome sequencing to retrospectively investigate carriage, environmental, and clinical isolates of *S. aureus* that were collected during two suspected hospital outbreaks in Accra, Ghana. The aim of the study was to generally describe genomic features of the *S. aureus* isolates, and the possible transmission events involved in the suspected outbreaks.

Methods

The study sites

The study sites were two hospitals located in Accra, the capital city of Ghana, namely, Korle-Bu Teaching Hospital (KBTH) and Lekma Hospital (LH). The KBTH is the largest hospital in Ghana and serves as a major referral center in Ghana, while LH is a district hospital. The KBTH has a bed capacity of 1,500 and has 17 departments,[17] while LH has a bed capacity of 100 (J Twasam, Lekma Hospital, personal communication, 2017). Both hospitals have an infection control unit, which supervises and coordinates hygienic practices to prevent and control outbreaks. The infection control unit has close links with the bacteriology laboratory of the hospital, where routine identification of bacteria from clinical specimens and susceptibility testing is carried out. Facilities available in the bacteriology laboratory of KBTH and LH permit only limited phenotypic characterization of bacteria, and the most common organisms reported in the laboratory are *Escherichia coli, S. aureus,* and *Pseudomonas aeruginosa*.[17]

The study isolates

The *S. aureus* isolates used in this study were collected during suspected *S. aureus* outbreaks at the pediatric wards of KBTH and LH in 2012 and 2015, respectively. The isolates were from three sources: disease cases of patients; nasal carriage of patients, health workers, and hospital visitors; and equipment and surfaces in the hospital wards. Unfortunately, isolates from the initial *S. aureus* cases that constituted the suspected outbreaks and therefore led to the study had lost viability and therefore could not be sequenced. Overall, there were 17 isolates included in the study. Thirteen of the isolates were from KBTH and were collected mainly from wards in the pediatric unit of the hospital, particularly the emergency ward. The other four isolates were from LH, and these were collected from mothers of babies who were resident in the wards of the hospital. An epidemiological map showing time of sampling, location of patients, and *S. aureus* isolate source at KBTH, where most of the isolates were collected, is shown in Figure 1.

Microbiological analysis

The study isolates were purified on blood agar and mannitol salt agar and confirmed to be *S. aureus* by the tube coagulase test.[18] The isolates were screened for methicillin resistance by cefoxitin disk diffusion test.[19]

DNA sequencing and analysis

DNA was extracted and prepared for sequencing with the use of a kit (Promega DNA Sample Prep Kit; Epicenter, Madison, WI, USA). Tagged DNA libraries were created according to the Illumina protocol. Whole genome sequencing was performed on the Illumina HiSeq 2000 platform with 100-cycle paired-end runs. Sequence data for all isolates have been submitted to the European Nucleotide Archive (www.ebi.ac.uk/ena), and the accession numbers are provided in Table 1. Annotated assemblies were produced as previously described.[20] Briefly, de novo assembly of whole genome sequences was performed using Velvet v1.2,[21] with Velvet Optimiser v2.2.5 (http://bioinformatics.net.au/software.velvetoptimiser.shtml). Contigs were scaffolded with SSPACE, and sequence gaps were closed using GapFiller.[22,23] The assembled contigs were annotated using Prokka v1.11 and an *S. aureus*-specific database from RefSeq.[24,25]

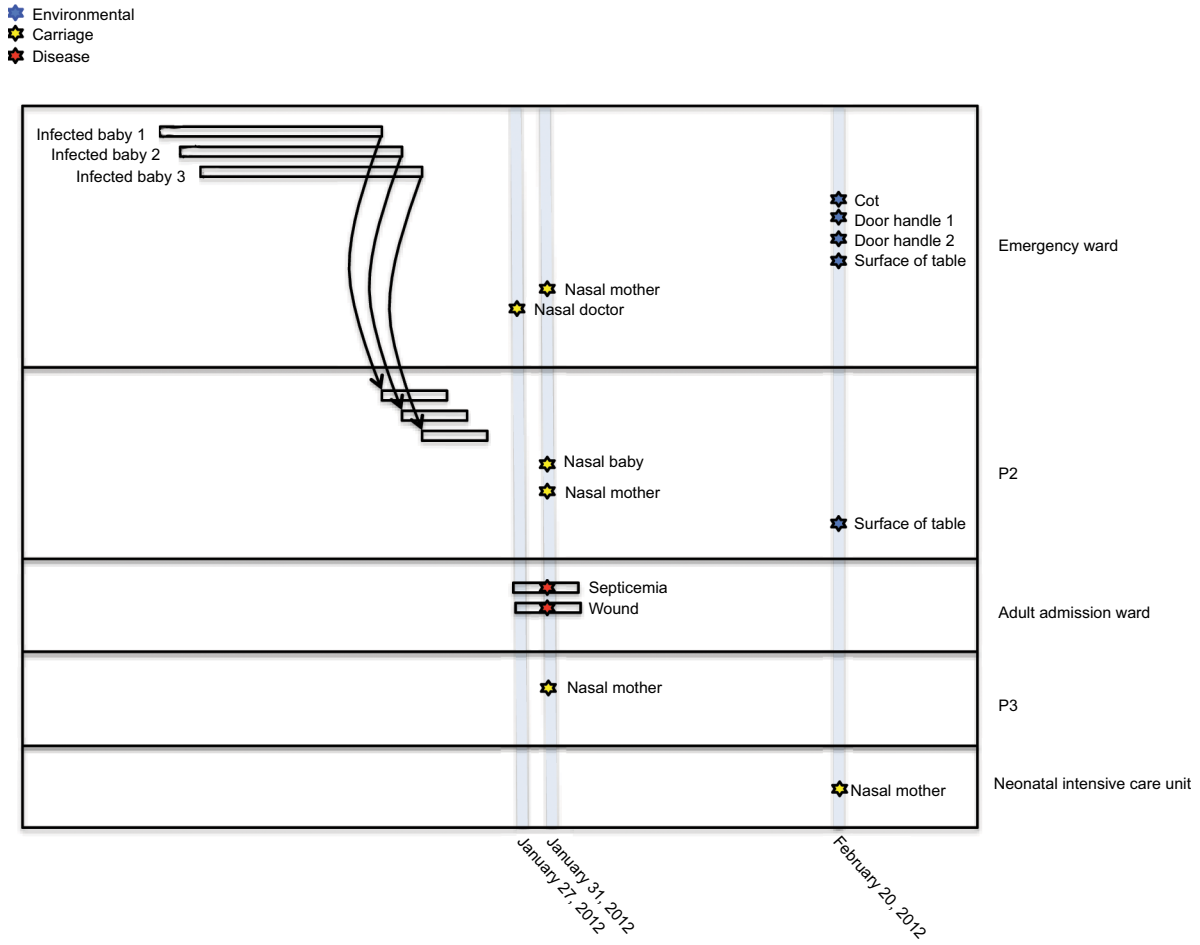

Figure 1 An epidemiological map showing time of sampling, location of patients and sources of *Staphylococcus aureus* isolates at Korle-Bu Teaching Hospital.
Notes: Three *S. aureus* cases (infected babies 1, 2, 3) at the emergency ward of Korle-Bu Teaching Hospital led to sampling at this hospital. Thirteen isolates were collected at various wards from a wide range of sources including disease cases, nasal carriage, equipment, and surfaces in the hospital wards. Background epidemiological data of the 13 *S. aureus* isolates have been reported in Table 1. Unfortunately, isolates from the initial *S. aureus* cases that led to the study, had lost viability and, therefore, could not be included in the study sample. P2, P3: different pediatric wards at Korle-Bu Teaching Hospital.

To query evolutionary relationships between isolates, single-nucleotide polymorphisms (SNPs) were detected by mapping sequence reads to the *S. aureus* reference genome of strain USA300_FPR3757,[26] using SMALT version 0.7.4 (http://www.sanger.ac.uk/science/tools/smalt-0). Mobile genetic elements (MGEs) were excluded from the whole genome alignment to create the core genome, which was used to reconstruct a maximum-likelihood phylogenetic tree using RAxML version 7.8.6.[27]

Multilocus sequence typing, together with the analysis of antimicrobial resistance and virulence gene carriage, was performed by short read mapping using the SRST2.[28] For *spa* typing, the *spa* gene X region was extracted from whole genome assemblies using in silico PCR and previously described primers.[29] The *spa*-type was then determined using an online *spa*-typer tool (http://spatyper.fortinbras.us/). Genome fragments representing MGEs were extracted from whole genome assemblies as described previously.[30] The identified contigs were screened for presence of the *pvl* gene and antimicrobial resistance genes identified with SRST2. The distribution of the identified *pvl*-carrying Sa2*int* phage variants as well as resistance-associated MGEs was performed based on short read mapping with SRST2, further verified by assembly alignment with the use of MUMmer.[31]

Ethical considerations

For this study, the Ethical and Protocol Review Committee of the study hospitals waived ethical approval as it was regarded as part of routine surveillance measures for infection control. Additionally, as the samples used in the study were archived isolates, we could not obtain patients' consent for use of their clinical data. However, all patients' data and isolates were de-identified to ensure anonymity.

Results

Description of *S. aureus* disease cases

A suspected outbreak occurred at the Child Health Department of KBTH in January 2012 with three *S. aureus* cases.

Table 1 Epidemiological information and genotypes of the analyzed *Staphylococcus aureus* isolates

No.	Accession	Date	Hospital	Ward	Source	Site	Sequence type	*spa*	AMR genes	*pvl*
1	ERS760539	February 20, 2012	Korle-Bu	Emergency ward	Environmental	Baby's cot	15	t084	*blaZ, dfrG, tetK*	–
2	ERS760543	February 20, 2012	Korle-Bu	Emergency ward	Environmental	Door handle of ward	15	t084	*blaZ, dfrG, tetK*	–
3	ERS760544	February 20, 2012	Korle-Bu	Emergency ward	Environmental	Door handle of ward	15	t084	*blaZ*	–
4	ERS760548	January 31, 2012	Korle-Bu	Adult admission ward	Clinical	Patient with septicemia	15	t084	*blaZ, dfrG, tetK*	+
5	ERS760550	February 20, 2012	Korle-Bu	Emergency ward	Environmental	Surface of table	15	t084	*blaZ*	–
6	ERS760533	February 20, 2012	Korle-Bu	NICU	Carriage	Nasal specimen of baby's mother	152	t355	*blaZ*	+
7	ERS760534	January 31, 2012	Korle-Bu	Emergency ward	Carriage	Nasal specimen of baby's mother	152	t355	*blaZ*	+
8	ERS760549	January 31, 2012	Korle-Bu	Adult admission ward	Clinical	Patient with wound infection	152	t355	*blaZ*	+
9	ERS760545	January 27, 2012	Korle-Bu	Emergency ward	Carriage	Nasal specimen of doctor	5	t071	*blaZ*	+
10	ERS760540	January 31, 2012	Korle-Bu	P2	Carriage	Nasal specimen of baby	45	t939	*blaZ*	–
11	ERS760551	January 31, 2012	Korle-Bu	P3	Carriage	Nasal specimen of baby's mother	707	NT	*blaZ*	–
12	ERS760542	January 31, 2012	Korle-Bu	P2	Carriage	Nasal specimen of baby's mother	121	t314	*blaZ, dfrG*	+
13	ERS760536	February 20, 2012	Korle-Bu	P2	Environmental	Surface of table in ward	508	t1510	*blaZ*	–
14	ERS760508	February 10, 2015	Lekma	PD	Carriage	Nasal specimen of baby's mother	72	t537	*blaZ*	–
15	ERS760505	February 10, 2015	Lekma	PD	Carriage	Nasal specimen of baby's mother	6	t701	*blaZ*	–
16	ERS760501	February 10, 2015	Lekma	PD	Carriage	Nasal specimen of baby's mother	3,248	t127	*blaZ*	–
17	ERS760506	February 10, 2015	Lekma	PD	Carriage	Nasal specimen of baby's mother	508	t095	*blaZ, aacA-aphD, aadD, dfrC, lnuA, tetK, qacA*	–

Note: P2, P3: different pediatric wards at Korle-Bu Teaching Hospital.
Abbreviations: AMR, antimicrobial resistance; PD, pediatric ward; NT, non-typeable/unknown; NICU, neonatal intensive care unit.

In the first case, *S. aureus* was isolated from the blood of a 4-month old baby girl (Figure 1; Baby 1) who had been admitted to the emergency ward. She had been at the emergency ward for 5 days and was later transferred to another ward (designated P2), where she died. The second case was reported 4 days after the first case and was isolated from cerebrospinal fluid of a 5-day old baby boy (Figure 1; Baby 2), who had been admitted to the emergency ward. The child was later transferred to the P2 ward. Two days after the second case, *S. aureus* was isolated from the blood of a 4-month old baby girl (Figure 1; Baby 3). Like the first two children, this baby had been admitted to the emergency ward and was later transferred to the P2 ward.

The Infection Prevention and Control Unit of KBTH requested an investigation into the situation following the death of the baby involved in the first case. It was identified that the babies from which *S. aureus* were obtained shared the same respiratory equipment. Babies shared the same cot and were connected by tubes (lines of giving set) to one oxygen cylinder. The clinical staff confirmed that they resort to this practice due to inadequate respiratory apparatus.

Following this development, KBTH took steps to disinfect materials (cot and bedding) of the deceased baby. Blood specimens for culture were obtained from babies who were in close proximity and shared tubes with the deceased baby, but the specimens grew no bacteria. A survey on nasal carriage of *S. aureus* was carried out among babies, their mothers, and health care staff in the unit as well as environmental screening. Nasal carriage of *S. aureus* and MRSA were 49.7% (88/147) and 4.8% (7/147), respectively. Environmental specimens collected from hospital equipment and surfaces in the wards yielded *S. aureus* and MRSA prevalence of 27.5% (39/142) and 11.3% (16/142), respectively. During this period, the bacteriology laboratory of KBTH maintained surveillance of *S. aureus* isolates recovered from all patients who visited the hospital; an average of five clinical isolates of *S. aureus* are reported in this laboratory daily.

At the LH, there was a suspected outbreak of *S. aureus* septicemia in the pediatric unit in May 2015. Following this, a survey on nasal carriage of *S. aureus* was carried out among babies, their mothers, and health care staff at the unit. Nasal carriage of *S. aureus* and MRSA were 25.2% (26/103) and 4.8% (9.7/103), respectively.

Genomic investigations

Multilocus sequence and *spa* typing

We sequenced 17 *S. aureus* isolates from a variety of sources contemporary to the infections at the two hospitals (Table 1). Multilocus sequence typing revealed a total of eleven distinct sequence types (STs) (Table 1). The highest relative prevalence was observed for ST15, identified only among the isolates from KBTH. The ST15 isolates were isolated from various environmental surfaces of the pediatric emergency ward and also from the blood of a patient on admission to an adult ward (Table 1). The second most common lineage was ST152, also identified at KBTH only. Isolates belonging to ST152 were isolated from anterior nares of two mothers of babies in different pediatric wards (NICU, P2) and also from the wound of a patient on admission to an adult surgical ward (Table 1). Other lineages detected at KBTH included ST5, ST45, ST121, ST508, and ST707. ST45 and its single locus variant ST508 were isolated from anterior nares of a baby and the surface of a table in the pediatric P2 ward. The other STs were isolated from anterior nares of hospital staff or mothers of babies in different pediatric wards, though each was represented by a single isolate only. The isolates of *S. aureus* from LH yielded diverse and unrelated STs (ST6, ST72, ST508, and ST3248, the latter a single locus variant of ST1).

Isolates represented eleven distinct *spa* types, which included a single isolate with novel *spa* type (Table 1). Isolates that belonged to the same ST also revealed identical *spa* types, except for ST508. As such, all ST15 isolates were *spa* type t084 whereas all ST152 isolates belonged to *spa* type t355.

Antimicrobial resistance genes

Analysis of antimicrobial resistance gene carriage revealed that all isolates were *mecA* negative (Table 1). We screened for presence of other resistance-associated genes, which revealed that all isolates contained the penicillin resistance gene *blaZ*. Less common were the tetracycline resistance gene *tetK* and the trimethoprim resistance determinant *dfrG*. Both genes were observed mostly in ST15 isolates. A single isolate from LH representing ST508 displayed a unique composition of multiple antimicrobial resistance genes, which in addition to the *tetK* gene also included the aminoglycoside resistance determinants *aacA-aphD* and *addD*, the trimethoprim resistance gene *dfrC*, the lincosamide resistance gene *lnuA,* and the antiseptic resistance gene *qacA*. We interrogated the genetic environment of the identified resistance genes to check for association with MGEs. Carriage of the *blaZ* gene was associated with four distinct MGEs, although the majority of isolates carried a plasmid that closely resembled the 21 kb pSaa6159, identified previously in *S. aureus* ST93 (accession: CP002115). Less prevalent was the Tn*552*-like element found in three isolates, with a single isolate carrying a plasmid that matched part of the pLUH02 element from *S. aureus* (accession: FR714929). In most of the *tetK*

positive isolates, the gene was carried on a plasmid resembling the previously reported SAP095B plasmid (accession: GQ900445). In a single isolate representing ST508, the *tetK* gene was carried on a plasmid fragment, which also contained the *aacA-aphD* gene and that shared a high level of sequence identity with a large, multiple resistance plasmid pT33G-1 derived from *Staphylococcus lugdunensis* (accession: KU882683). This isolate revealed a second plasmid fragment, which also matched the pT33G-1 and contained *addD*, *lnuA,* and *qacA* genes. Finally, the *dfrG* gene was associated with a chromosomally integrated insertion sequence as described previously,[32] which was nearly identical to the *dfrG* accessory element found in MRSA ST772 strain DAR4145.[33,34]

pvl gene

Genes encoding *pvl* were detected in six isolates representing four distinct STs (Table 1). Carriage of the *pvl* genes was associated with the acquisition of Sa2*int*-type phages, and three distinct Sa2*int* phages were identified. All of the ST152 isolates carried a very closely related phage element of 46.6 kb in size, which showed high sequence identity (99%) with Sa2*int* found in *S. aureus* ST152 reference genome BB155 (accession: LN854556). In addition, it shared 91% of its sequence with the Sa*2int* derived from the MRSA USA300_FPR3757 strain.[26] The single *pvl*-positive ST15 isolate carried a distinct 42.7 kb Sa2*int* phage that shared only 17% of its sequence with the ST152 *pvl* phage and was most closely related to a phage found in MRSA ST239 strain XN108.[35] Also different was the Sa2*int* found in the *pvl* positive ST5 isolate, which was most closely related to the phiSa119 (84% sequence coverage), found in MRSA ST772.[36] Finally, the single *pvl* positive *S. aureus* ST121 isolate contained a short, truncated *pvl*-associated phage sequence, which matched most closely (99% sequence identity) Sa2*int* carried by MRSA ST80 reference genomes such as GR2 and 11819–97.[37,38]

Phylogenetic analysis

A phylogenetic tree based on the core-genome alignment was reconstructed to analyze evolutionary relationships between the isolates (Figure 2). The ST152 isolates revealed high divergence; the three isolates were non-clonal showing pairwise SNP distances of 77, 94, and 95. The ST15 isolates from KBTH formed two clusters. One cluster of the ST15 isolates from KBTH comprised three isolates from a baby's cot in the emergency ward, door handle of the emergency ward, and a patient with septicemia (Table 1: isolates 1, 2, and 4). Isolates from the door handle (isolate 2) and baby's cot (isolate 1) in the emergency ward were isolated on the same day and had a pairwise SNP distance of 1, suggesting a possible transmission. The clinical isolate from the septicemia patient (isolate 4) was isolated a few weeks before the isolation of isolates 1 and 2; the SNP distance between isolate 4 and the other two isolates was 97 for isolate 1 and 95 for isolate 2. The other cluster of ST15 isolates from KBTH comprised two isolates, which were isolated from the door handle of the emergency ward and the surface of a table in the ward (Table 1: isolates 3 and 5). The two isolates were isolated the same day and had a pairwise SNP distance of 0 suggesting a hand contamination problem.

Discussion

This study is the first in Ghana and one of the few in sub-Saharan Africa to employ whole genome sequencing to study *S. aureus* isolates. The isolates were collected during suspected *S. aureus* outbreaks at a teaching and a district hospital in Ghana. We describe our investigation of the outbreaks as indirect, as the initial disease *S. aureus* isolates

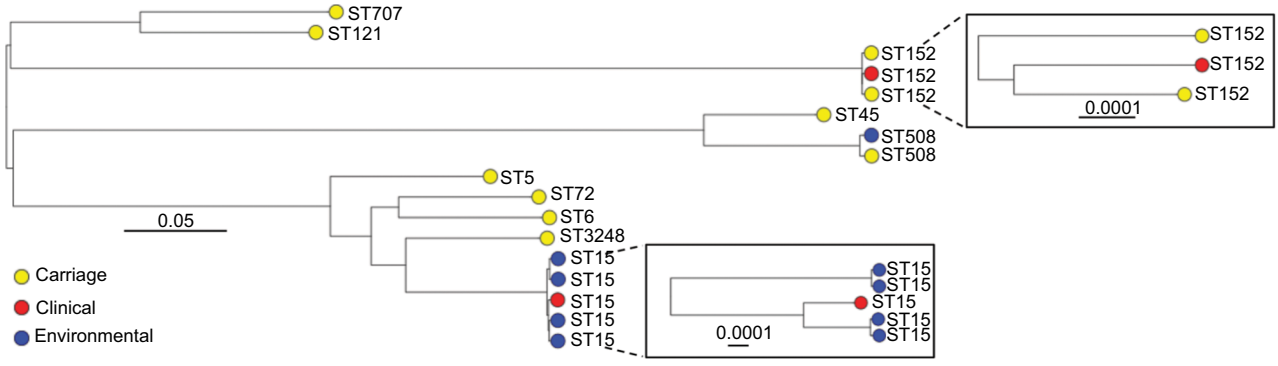

Figure 2 Mid-point rooted maximum likelihood phylogenetic tree of all *Staphylococcus aureus* isolates analyzed in this study.
Notes: The tree was reconstructed based on core-genome alignment, generated after mapping short reads to the *S. aureus* USA300_FPR3757 reference genome. The tips are labeled with the isolate's sequence type (ST) and color coded to show the sample source. Values above scale bars describe number of nucleotide substitutions per site.

that led to the investigations were not available for sequencing. At KBTH, the data showed that there was circulation of an *S. aureus* ST15 clone in the pediatric emergency ward (location of the outbreak) during the period of the outbreak. The data showed that there were two pathways of transmission of the ST15 clone in the pediatric emergency ward of KBTH, both of which could be linked to the door handle of the ward. This concurs with several studies that have shown that door handles could be an important source of *S. aureus* contamination in health care settings.[39,40] Within our data, it is difficult to identify the source of the *S. aureus* ST15 clone in the pediatric emergency ward of KBTH. However, since *S. aureus* ST15 represents lineage commonly carried in the anterior nares,[41,42] it may have been transferred from this source to the door handle of the emergency ward through contamination of the hand of a health care worker or visitor to the ward. *S. aureus* is known to survive for as long as 7 months on dry surfaces.[43] This survival feature of the organism, coupled with overcrowding at the pediatric emergency ward of KBTH and the fact that equipment is shared among patients, could facilitate its rapid transmission among patients. The ST15 clone of *S. aureus* appears to be widely disseminated at KBTH as it was isolated from patients in adult wards, and the genomic data showed that these isolates were unrelated to the transmission events at the pediatric ward. Generally, ST15 appears to be one of the common clones of *S. aureus* in Ghana, and it has been isolated from both diseased and asymptomatic carriers.[44] The pattern of dissemination of the ST15 clone in the emergency ward does provide us with some insights about transmission events in this ward, and highlights a basic problem with disinfection of environmental surfaces at this health care facility. Generally, *S. aureus* and other bacterial pathogens, such as *Clostridium difficile* and *P. aeruginosa*, are common contaminants of environmental surfaces and may be resistant to sanitation procedures due to biofilm formation.[39,40,45,46] The situation at LH is unlikely to be a true outbreak, as *S. aureus* strains from this hospital were all of unrelated STs. The *S. aureus* septicemia situation at LH thus appears to be an isolated one and not related to any transmission events at the hospital. Since the *S. aureus* outbreak at KBTH in 2012, there has been a heightened awareness of this pathogen in Ghana, and isolated invasive cases like the one in LH have attracted attention and been misinterpreted as outbreaks.

Like this study, a number of studies have reported that *S. aureus* isolates from West and Central Africa show a relatively high prevalence of *pvl* genes.[4–7] Based on genomic analysis in the current study, we can now report that high frequency of these genes is likely mediated by diverse phage population rather than a single highly transmissible phage type. Furthermore, the full sequence of each identified Sa2*int* variant revealed close similarity with *pvl*-carrying phages identified in globally disseminated MRSA lineages, providing a link between the *pvl* positive MSSA populations from Africa and the pandemic MRSA clones. An *S. aureus* lineage that is known to be associated with *pvl* is ST152,[47,48] which is also evident from our data as all the ST152 isolates were *pvl* positive. As previously observed,[49] the ST152 isolates analyzed in this work revealed high divergence.

Analysis of genetic context behind carriage of the identified resistance genes revealed that the majority was associated with MGEs that shared high sequence identity with previously described plasmids, transposons, or other elements. It is important to note the uniquely wide range of resistance genes harbored by the single ST508 strain, which was identified to be MSSA. In a previous community nasal carriage study in Ghana, the only two MRSA strains isolated were ST508.[5] ST508 is a single locus variant of ST45, which was also identified among our isolates and represents a lineage that includes the Berlin epidemic clone.[50] *S. aureus* ST508 thus appears to be an interesting and important carriage clone in Ghana, and further studies are needed to elucidate its epidemiological significance.

The main limitation of the study is the limited number of isolates used in the investigations. This resulted from loss of isolates during their storage while funding was being sought for the genomic investigations. Despite this limitation, we have carried out extensive genomic analyses on the isolates, adding to the few studies that have undertaken whole genome analysis of *S. aureus* isolates in Africa.

Conclusion

The *S. aureus* isolates studied belonged to diverse STs with ST15 and ST152 being relatively more common. There was a high prevalence of *pvl* genes among the isolates, which is likely mediated by diverse phage population rather than a single, highly transmissible phage type. Though we were not able to identify the specific strain of *S. aureus* implicated in the outbreak at the pediatric emergency ward of KBTH, we are confident that its transmission is similar to that of the ST15 clone, which we have elucidated through whole genome sequencing. Contamination of various surfaces in the emergency ward by strains of ST15 highlights the need for more rigorous disinfection of environmental surfaces at KBTH. This investigation also highlights the need for proper storage of bacterial isolates in laboratories in the developing world where facilities for molecular investigations are limited.

Acknowledgments

The study was funded through a grant from the Alborada Research Fund and Wellcome Trust Sanger Institute core funding (Wellcome Trust grant 098051). The authors acknowledge the contributions of the technical staff of the Department of Microbiology, Korle-Bu Teaching Hospital.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Todar K. *Todar's Online Textbook of Bacteriology*. Madison, WI: University of Wisconsin–Madison; 2006.
2. Hall GS. MRSA: lab detection, epidemiology, and infection control. *Microbiol Front*. 2003;3:1–6.
3. Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev*. 1997;10(4):781–791.
4. David MZ, Boyle-Vavra S, Zychowski DL, Daum RS. Methicillin-susceptible *Staphylococcus aureus* as a predominantly healthcare-associated pathogen: a possible reversal of roles? *PLoS One*. 2011;6(4):e18217.
5. Egyir B, Guardabassi L, Esson J, et al. Insights into nasal carriage of *Staphylococcus aureus* in an urban and a rural community in Ghana. *PLoS One*. 2014;9(4):e96119.
6. Breurec S, Fall C, Pouillot R, et al. Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. *Clin Microbiol Infect*. 2011;17(4):633–639.
7. Schaumburg F, Köck R, Friedrich AW, et al. Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. *PLoS Negl Trop Dis*. 2011;5(5):e1150.
8. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29(5):1128–1132.
9. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9(8):978–984.
10. Salipante SJ, Sengupta DJ, Cummings LA, Land TA, Hoogestraat DR, Cookson BT. Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. *J Clin Microbiol*. 2015;53(4):1072–1079.
11. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol*. 2004;186(5):1518–1530.
12. Harris SR, Feil EJ, Holden MT, et al. Evolution of MRSA during hospital transmission and intercontinental spread. *Science*. 2010;327(5964):469–474.
13. Harris SR, Cartwright EJ, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis*. 2013;13(2):130–136.
14. Kong Z, Zhao P, Liu H, et al. Whole-genome sequencing for the investigation of a hospital outbreak of MRSA in China. *PLoS One*. 2016;11(3):e0149844.
15. Gordon NC, Price JR, Cole K, et al. Prediction of *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. *J Clin Microbiol*. 2014;52(4):1182–1191.
16. Newman MJ, Frimpong E, Donkor ES, Opintan JA, Asamoah-Adu A. Resistance to antimicrobial drugs in Ghana. *Infect Drug Resist*. 2011;4:215–220.
17. Annual Report of the Korle-Bu Teaching Hospital. 2010. Accra, Ghana: Korle-Bu Teaching Hospital.
18. Bannerman TL. Staphylococcus, Micrococcus and other catalase-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, YolkenRH, editors. *Manual of Clinical Microbiology, 8th ed, vol 1*. Washington DC: American Society for Microbiology; 2003.
19. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S23*. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
20. Page AJ, de Silva N, Hunt M, et al. Robust high-throughput prokaryote *de novo* assembly and improvement pipeline for Illumina data. *Microb Genom*. 2016;2(8):e000083.
21. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res*. 2008;18(5):821–829.
22. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics*. 2011;27(4):578–579.
23. Boetzer M, Pirovano W. Toward almost closed genomes with GapFiller. *Genome Biol*. 2012;13(6):R56.
24. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068–2069.
25. Pruitt KD, Tatusova T, Brown GR, Maglott DR. NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res*. 2012;40:D130–135.
26. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. *Lancet*. 2006;367(9512):731–739.
27. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9):1312–1313.
28. Inouye M, Dashnow H, Raven LA, et al. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med*. 2014;6(11):90.
29. Kahl BC, Mellmann A, Deiwick S, Peters G, Harmsen D. Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of genetic change in *Staphylococcus aureus*. *J Clin Microbiol*. 2005;43(1):502–505.
30. Harris SR, Robinson C, Steward KF, et al. Genome specialization and decay of the strangles pathogen, *Streptococcus equi*, is driven by persistent infection. *Genome Res*. 2015;25(9):1360–1371.
31. Kurtz S, Phillippy A, Delcher AL, et al. Versatile and open software for comparing large genomes. *Genome Biol*. 2004;5(2):R12.
32. Jensen SO, Lyon BR. Genetics of antimicrobial resistance in *Staphylococcus aureus*. *Future Microbiol*. 2009;4(5):565–582.
33. Steinig EJ, Andersson P, Harris SR, et al. Single-molecule sequencing reveals the molecular basis of multidrug-resistance in ST772 methicillin-resistant *Staphylococcus aureus*. *BMC Genomics*. 2015;16:388.
34. Manoharan A, Zhang L, Poojary A, Bhandarkar L, Koppikar G, Robinson DA. An outbreak of post-partum breast abscesses in Mumbai, India caused by ST22-MRSA-IV: genetic characteristics and epidemiological implications. *Epidemiol Infect*. 2012;140(10):1809–1812.
35. Zhang X, Hu Q, Yuan W, et al. First report of a sequence type 239 vancomycin-intermediate *Staphylococcus aureus* isolate in Mainland China. *Diagn Microbiol Infect Dis*. 2013;77(1):64–68.
36. Sanchini A, del Grosso M, Villa L, et al. Typing of Panton-Valentine leukocidin-encoding phages carried by methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* from Italy. *Clin Microbiol Infect*. 2014;20(11):O840–O846.
37. Sabat AJ, Pournaras S, Akkerboom V, Tsakris A, Grundmann H, Friedrich AW. Whole-genome analysis of an oxacillin-susceptible CC80 mecA-positive *Staphylococcus aureus* clinical isolate: insights into the mechanisms of cryptic methicillin resistance. *J Antimicrob Chemother*. 2015;70(11):2956–2964.
38. Stegger M, Price LB, Larsen AR, et al. Genome sequence of *Staphylococcus aureus* strain 11819-97, an ST80-IV European community-acquired methicillin-resistant isolate. *J Bacteriol*. 2012;194(6):1625–1626.
39. Oie S, Hosokawa I, Kamiya A. Contamination of room door handles by methicillin-sensitive/methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*. 2002;51(2):140–143.

40. Visalachy S, Palraj KK, Kopula SS, Sekar U. Carriage of multidrug resistant bacteria on frequently contacted surfaces and hands of health care workers. *J Clin Diagn Res*. 2016;10(5):18–20.
41. Schaumburg F, Ngoa UA, Kösters K, et al. Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. *Clin Microbiol Infect*. 2011;17(10):1507–1513.
42. Schaumburg F, Alabi AS, Peters G, Becker K. New epidemiology of *Staphylococcus aureus* infection in Africa. *Clin Microbiol Infect*. 2014;20(7):589–596.
43. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis*. 2006;6:130.
44. Egyir B, Guardabassi L, Sørum M, et al. Molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. *PLoS One*. 2014;9(2):e89716.
45. Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib NE. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. *Arch Microbiol*. 2014;196(7):453–472.
46. Hammond EN, Donkor ES, Brown CA. Biofilm formation of Clostridium difficile and susceptibility to Manuka honey. *BMC Complement Altern Med*. 2014;14:329.
47. Ruimy R, Maiga A, Armand-Lefevre L, et al. The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. *J Bacteriol*. 2008;190(11):3962–3968.
48. Monecke S, Berger-Bächi B, Coombs G, et al. Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton-Valentine leukocidin. *Clin Microbiol Infect*. 2007;13(3):236–249.
49. Ruimy R, Maiga A, Armand-Lefevre L, et al. The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. *J Bacteriol*. 2008;190(11):3962–3968.
50. Wannet WJ, Spalburg E, Heck ME, et al. Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in the Netherlands. *J Clin Microbiol*. 2005;43(7):3341–3345.