ORIGINAL ARTICLE

Possible healthcare-associated transmission as a cause of secondary infection and population structure of *Staphylococcus aureus* isolates from two wound treatment centres in Ghana

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Abstract

We have previously shown that secondary infections of Buruli ulcer wounds were frequently caused by *Staphylococcus aureus.* To gain understanding into possible routes of secondary infection, we characterized *S. aureus* isolates from patient lesions and surrounding environments across two Ghanaian health centres. One hundred and one *S. aureus* isolates were isolated from wounds ($n = 93$, 92.1%) and the hospital environment ($n = 8$, 7.9%) and characterized by the *spa* gene, *mecA* and the Panton–Valentine leucocidin toxin followed by *spa* sequencing and whole genome sequencing of a subset of 49 isolates. *Spa* typing and sequencing of the *spa* gene from 91 isolates identified 29 different *spa* types with t355 (ST152), t186 (ST88), and t346 dominating. Although many distinct strains were isolated from both health centres, genotype clustering was identified within centres. In addition, we identified a cluster consisting of isolates from a healthcare worker, patients dressed that same day and forceps used for dressing, pointing to possible healthcare-associated transmission. These clusters were confirmed by phylogenomic analysis. Twenty-four (22.8%) isolates were identified as methicillin-resistant *S. aureus* and *lukFS* genes encoding Panton–Valentine leucocidin were identified in 67 (63.8%) of the isolates. Phenotype screening showed widespread resistance to tetracycline, erythromycin, rifampicin, amikacin and streptomycin. Genomics confirmed the widespread presence of antibiotic resistance genes to β-lactams, chloramphenicol, trimethoprim, quinolone, streptomycin and tetracycline. Our findings indicate that the healthcare environment probably contributes to the superinfection of Buruli ulcer wounds and calls for improved training in wound management and infection control techniques.

Keywords: Buruli ulcer, health care-associated, infection, spa typing, *Staphylococcus aureus*, whole genome sequencing
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Introduction

Microbial contamination and colonization of wounds is common to all wounds healing by secondary intention and has been proposed as a precondition to the formation of granulation tissue and stimulation of wound healing [1]. Wounds can be infected through three main sources; the surrounding skin, endogenous sources such as the nasal mucosa, gastrointestinal tract and genitourinary tract and the wider environment. Within a healthcare facility, the sources of contamination and subsequent infection of a wound may include healthcare workers (HCW), patients and the inanimate environment. Direct contact of a patient with an infected HCW during general care or medical treatment can result in the transmission

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of microorganisms. Indirectly, an infected patient or HCW could touch and contaminate an object, instrument or surface and subsequent contact between the item and a second patient is likely to contaminate that patient leading to infection. Wound microflora is usually polymicrobial [2] comprising organisms such as staphylococci, enterococci, streptococci, facultative Gram-negative bacteria and anaerobic bacteria [3].

Staphylococcus aureus is a notorious opportunistic nosocomial pathogen, and one of the main organisms involved in the infection of chronic wounds [4]. It may be carried asymptomatically by a carrier and transferred from patient to patient. It is estimated that about 25% of the normal population may be carriers, with higher carriage rates around 50% in patients with insulin-dependent diabetes, intravenous drug users and patients on dialysis [5].

In a previous study, we identified infection of Buruli ulcer (BU) wounds by bacterial pathogens as a possible cause of wound healing delay among study participants [6]. Several bacterial species were isolated with *S. aureus* and *Pseudomonas aeruginosa* dominating. Despite the finding that most of the organisms identified were nosocomial pathogens, the possible routes of infection of the lesions were not examined in this study. In seeking to have a better understanding of the possible routes of infection of the lesions, we initiated the current study to characterize *S. aureus* isolated from different sources in two health facilities in Ghana treating BU.

Materials and methods

Study sites, participants and sample collection

The study was carried out at the two main BU treatment centres in the Ga West and Ga South districts of the Greater Accra Region of Ghana, designated facility A and facility B, respectively. The study involved the analysis of *S. aureus* isolates from wounds of microbiologically confirmed BU (BU) and non-BU (NBU) patients (initially suspected BU cases that were not confirmed by any of the three confirmation methods—Ziehl–Neelson microscopy, IS2404 PCR and culture) who received treatment either as inpatients on admission or outpatients at one of the two health centres from October 2010 to February 2014. Lesions of patients were swabbed and the samples were analysed at the Bacteriology Department of the Noguchi Memorial Institute for Medical Research. A total of 173 samples were collected from 162 patients and *S. aureus* was isolated from 88 samples originating from 76 patients, 61 of whom were BU patients and 15 were NBU patients. Of the 61 BU patients, 56 were outpatients and five were inpatients at the time of sampling, whereas all the NBU patients were outpatients. The five inpatients had been on admission for 4 weeks or more and therefore isolates from these patients could be classified as potentially hospital acquired. To elucidate the sources of infection, between August 2013 and February 2014 the hands of HCW, dressing rooms and wards of the health centres, surfaces of dressing tables, door handles, instruments and equipment as well as dressing solutions and materials were sampled for microbiological analysis. Swab samples were also taken from the palms of nurses and their gloved hands in between dressing of patients and the lesions of the patients they dressed before and after dressing. Swab samples were transported in phosphate buffered saline at 4°C to the Noguchi Memorial Institute for Medical Research. Eighty-six samples were collected and analysed and *S. aureus* was isolated from 13 of them (see Supplementary material, Fig. S1).

Microbiological methods

The samples were processed and inoculated on blood and mannitol salt agars and incubated for 18–24 h at 37°C, after which they were examined. Identification of *Staphylococcus* species was by colony and microscopic morphology, catalase reaction and the coagulase biochemical test (BD, Franklin Lakes, NJ, USA). The Staphylase Kit, BD BBL™ Staphyloslide Latex Test (BD, Franklin Lakes, NJ, USA) was used to differentiate the catalase-positive Gram-positive bacteria *S. aureus* from other *Staphylococcus* species.

Antibiogram of isolated bacteria

Antibiotic susceptibility testing was determined by the Kirby–Bauer disc diffusion method according to CLSI guidelines [7]. Susceptibility was determined for the antibiotics; amikacin (30 µg), sulphamethoxazole-trimethoprim (23.75 µg/1.25 µg), ampicillin (10 µg), tetracycline (30 µg), gentamicin (10 µg), erythromycin (15 µg), cefuroxime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), cefoxitin (30 µg), rifampicin (5 µg), streptomycin (10 µg), vancomycin (30 µg), clindamycin (2 µg) and cefotaxime (30 µg). Cartridges of antibiotics were obtained from Oxoid (Wade Road, Basingstoke, Hampshire, United Kingdom) and BD. *Staphylococcus aureus* ATCC 25923 was used as reference strain.

Genotyping—traditional molecular typing

Crude DNA extracts were prepared by boiling and used as template in the PCR. Genetic relatedness of the isolates was determined through amplification of the polymorphic X region of the protein A (*spa*) gene using the primer pairs spa-1113F and spa-1514R (see Supplementary material, Table S1) [8] and DNA from *S. aureus* ATCC® 25923 as positive control. A total of 95 isolates out of the 101 were typed using these primers. A number of suspected *S. aureus* strains could not be typed by these primers and were further typed using another set of primers, spaT3-F and spa-1517R (see Supplementary material, Table S1). These primers