Microorganisms 2020, 8, 2052; doi:10.3390/microorganisms8122052 www.mdpi.com/journal/microorganisms

Communication

Molecular Characterization of *Staphylococcus aureus* Isolated from Chronic Infected Wounds in Rural Ghana

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Received: 2 December 2020; Accepted: 17 December 2020; Published: 21 December 2020

Abstract: Background: Globally, *Staphylococcus aureus* is an important bacterial pathogen causing a wide range of community and hospital acquired infections. In Ghana, resistance of *S. aureus* to locally available antibiotics is increasing but the molecular basis of resistance and the population structure of *S. aureus* in particular in chronic wounds are poorly described. However, this information is essential to understand the underlying mechanisms of resistance and spread of resistant clones. We therefore subjected 28 *S. aureus* isolates from chronic infected wounds in a rural area of Ghana to whole genome sequencing. Results: Overall, resistance of *S. aureus* to locally available antibiotics was high and 29% were Methicillin resistant *Staphylococcus aureus* (MRSA). The most abundant sequence type was ST88 (29%, 8/28) followed by ST152 (18%, 5/28). All ST88 carried the mecA gene, which was associated with this sequence type only. Chloramphenicol resistance gene fexB was exclusively associated with the methicillin-resistant ST88 strains. Panton-Valentine leukocidin (PVL) carriage was associated with ST121 and ST152. Other detected mechanisms of resistance included dfrG, conferring resistance to trimethoprim. Conclusions: This study provides valuable information for understanding the population structure and resistance mechanisms of *S. aureus* isolated from chronic wound infections in rural Ghana.

Keywords: rural Ghana; molecular epidemiology; chronic wounds; *Staphylococcus aureus*

1. Introduction

*Staphylococcus aureus* is an important bacterial pathogen in all parts of the world, causing both community and hospital acquired infections. In particular methicillin-resistant *S. aureus* (MRSA) has evolved as a global health threat due to its resistance to beta lactam and other classes of
In the last 20 years the prevalence of MRSA appears to be increasing in many African countries as suggested by data from the first decade of the present century [2]. More recent reviews indicate ongoing epidemiological relevance of this resistance type in Africa [3], with increased reporting of outbreak-association in Western African Ghana [4]. In Ghana, the abundance of MRSA in carriage studies or clinical samples demonstrated large geographical differences [5–7]. Moreover, resistance of S. aureus to a variety of other locally available oral antibiotics such as tetracyclines, trimethoprim/sulfamethoxazole and penicillins is frequently observed in Ghana [8,9]. However, the underlying mechanisms of resistance in this region are not well understood and to our knowledge has not been described for S. aureus from chronic wounds.

Effective surveillance of antimicrobial resistance in bacteria including S. aureus is essential for estimating the burden of resistance and molecular strain typing provides important information for understanding the spread of resistant clones. However, in Africa both surveillance and strain typing information are scarce due to the limited diagnostic microbiology infrastructure generally available in large parts of the continent. Molecular typing including spa-typing, multi-locus sequencing and also whole genome sequence typing has been applied in only a few studies in S. aureus in humans and livestock in Ghana [3,10–14], identifying strains of multiple clonal clusters [14]. In particular, the MRSA clone sequence type (ST)88-IV (2B) is not only abundant in Ghana, but also in other African counties: Angola, Cameroon, Gabon, Madagascar, Nigeria, as well as São Tomé e Príncipe [15]. In Ghana, reported rates range between 24.2–83.3% of all MRSA isolates [12].

In a previous study S. aureus was isolated from 14.0% (n = 28) of samples from patients with chronic wounds in Ghana [5]. In that study, a high frequency of methicillin-resistance (29%) was noted. Moreover, resistance to other commonly used antibiotics like penicillins, tetracyclines and trimethoprim/sulfamethoxazole was frequently observed. For Ghanaian S. aureus isolates from wounds, data on prevalent clones, resistance mechanisms and pathogenicity-associated genetic determinants is limited. To fill this gap in the global epidemiological picture, we have subjected the 28 S. aureus isolates from our previous study to whole genome sequencing (WGS), aiming at analyzing the underlying molecular basis of antimicrobial resistance and the population structure of this strain collection.

2. Materials and Methods

2.1. Sample Collection, Microbiology and Antibiotic Susceptibility Testing

S. aureus was isolated from female and male patients ≥15 years with an infected wound at the Outpatient Department of the Agogo Presbyterian Hospital, in the Asante Akim North District of Ghana from January to November 2016. Sample collection and microbiological investigations were reported previously [5]. Antibiotic susceptibility was tested by the disk diffusion method and interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines v.10.0 (http://www.eucast.org).

2.2. Whole Genome Sequencing and Data Analysis

Whole genome sequencing of the isolates was performed using the Illumina NextSeq platform. WGS data were analyzed using the Nullarbor pipeline (vers. 2.0.20181010; Seemann T, available at: https://github.com/tseemann/nullarbor). Reads were assembled with spades [16] (vers. 3.13.1) and annotated with Prokka [17] (vers. 1.13.3). The resistance and virulence gene profiles were determined with ABRicate (https://github.com/tseemann/abricate) (vers 0.9.9) employing NCBI AMR (7th October 2020; 5283 sequences), Resfinder [18] (3077 sequences) and VFDB [19] (2597 sequences) databases. SCCmec types were determined with the SCCmecFinder web tool (https://cge.cbs.dtu.dk/services/SCCmedFinder/10.1128/mSphere.00612-17).

The MLST sequence types were extracted from the WGS data using the MLST tool (vers. 2.16.1). Sequencing reads have been deposited in NCBI’s small reads archive (BioProject: PRJNA670821).
2.3. Ethical Considerations

The Committee on Human Research, Publications and Ethics, School of Medical Science, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana, approved this study (approval number CHRPE/AP/078/16) on 14th December, 2015.

3. Results

The identified MLST sequence types and selected associated resistance and virulence genes are summarized in Table 1; the complete MLST, virulence factor and resistance gene datasets (VFDB, Resfinder and NCBI AMR), as well as AST results are available in the supplemental material (Table S1). The most abundant sequence type was ST88 (8/28). All ST88 isolates were mecA positive, SCCmec type IV(2B), agr type 3 and negative for the Pantone–Valentine Leukocidin toxin (PVL) genes lukS-PV/lukF-PV. Seven of eight ST88 isolates carried the fexB gene, which confers resistance to chloramphenicol [20]. The five isolates of the second most abundant clone ST152 were all mecA negative and carried the PVL genes lukS-PV/lukF-PV.

| Sequence Type | n | lukS-PV/lukF-PV | mecA | fexB |
|---------------|---|----------------|------|------|
| ST88          | 8 | 0              | 8    | 7    |
| ST152         | 5 | 5              | 0    | 0    |
| ST15          | 3 | 0              | 0    | 0    |
| ST1           | 2 | 0              | 0    | 0    |
| ST5           | 2 | 0              | 0    | 0    |
| ST45          | 2 | 0              | 0    | 0    |
| ST2434        | 2 | 0              | 0    | 0    |
| ST72          | 1 | 0              | 0    | 0    |
| ST121         | 1 | 1              | 0    | 0    |
| ST3248        | 1 | 0              | 0    | 0    |
| ST3249        | 1 | 0              | 0    | 0    |
| Totals        | 28| 6              | 8    | 7    |

Highest rates of phenotypic antimicrobial resistance were detected for penicillin (100%, 28/28), tetracycline (57%, 16/28) and trimethoprim/sulfamethoxazole (39%, 11/28) (Table 2). All isolates were susceptible to linezolid, rifampicin, fosfomycin, tigecycline, ciprofloxacin, levofloxacin and daptomycin. WGS resistance gene profiling identified corresponding acquired resistance genes in 97% (65/67) of all phenotypically detected antimicrobial resistances (Table 2). In all but one penicillin-resistant isolate penicillinase-encoding blaZ was detected. The single penicillin resistant, blaZ negative strain carried mecA, reasonably explaining the observed betalactam-resistant phenotype. All oxacillin-resistant isolates were mecA positive and belonged to ST88. MecC was not detected in the strain collection. Two main mechanisms of resistance to tetracyclines have been described in S. aureus: active efflux, resulting from plasmid-located tetK and tetL genes and ribosomal protection mediated by tetM or tetO genes. In our collection tetracycline resistant isolates carried either tetK (7/16) alone or tetL (8/16) in combination with tetM (8/16). The tetL and tetM genes were exclusively detected in the ST88 MRSA isolates, while tetK was found in various clonal backgrounds. Beside mutation of the chromosomal dihydrofolate reductase (DHFR) gene, three acquired dihydrofolate reductase gene variants are known to confer resistance to trimethoprim in S. aureus of human origin: dfrA, dfrG and dfrK. In our collection all trimethoprim/sulfamethoxazole resistant isolates carried dfrG. Of the two erythromycin resistant isolates one carried msrA and one ermC. As expected, the latter isolate was also resistant to clindamycin. Gentamicin resistance is most commonly conferred by aminoglycoside-modifying enzymes. However, in the single case of a gentamicin-resistant isolate no corresponding resistance gene was found.
Table 2. Phenotypic antibiotic resistance and associated genetic resistance markers.

| Antibiotic                  | Phenotypic AST Resistant n (%) | WGS Resistance Gene or Mutation | Positive n (%) |
|-----------------------------|-------------------------------|---------------------------------|----------------|
| Penicillin                  | 28 (100)                      | blaZ                            | 27 (96)        |
|                             |                               | blaZ neg, mecA pos              | 1 (4)          |
| Tetracycline                | 16 (57)                       | tetK                            | 7 (44)         |
|                             |                               | tetL                            | 8 (50)         |
|                             |                               | tetM                            | 8 (50)         |
| Trimethoprim/Sulfamethoxazole | 11 (39)                      | dfrG                            | 11 (100)       |
| Oxacillin                   | 8 (29)                        | mecA                            | 8 (100)        |
| Erythromycin                | 2 (7)                         | msrA                            | 1 (50)         |
|                             |                               | ermC                            | 1 (50)         |
| Clindamycin                 | 1 (4)                         | not detected                    | none           |

4. Discussion

In this study we describe the molecular epidemiology of *S. aureus* isolated from chronic infected wounds in outpatients in a rural area of Ghana.

Overall, the frequency of MRSA (29%) was high, comparable to other clinical studies conducted in Ghana [6,21,22]. Nevertheless, the frequencies of MRSA seen in patients in Ghana seem subject to geographical variations [5–7]. Moreover, the previously reported high rates of resistance to orally available antibiotics including penicillin, tetracycline and cotrimoxazole in *S. aureus* [9] were confirmed by our study results. The particularly high frequencies of penicillin resistance might be attributed to the fact that penicillin-based antibiotics are amongst the most frequently prescribed drugs in Ghana and available over the counter without prescription [23]. High rates of resistance inevitably reduce effective antibiotic treatment in areas where resources are scarce. This favors the use of cleaning and disinfecting procedures for the management of wound infections whenever clinically possible.

All isolated MRSA belonged to ST88, described as the dominant clone in various African countries, including Ghana [15]. In addition, all but one MRSA strain also carried the *fexB* gene, conferring resistance to chloramphenicol, which was not found in any of the other sequence types. Previously, *fexB* has only been described in *S. aureus* strains from Ghanaian patients with Buruli ulcer [20], also a chronic wound. As previously described in 2007 [24], chloramphenicol has been extensively prescribed in Africa, although a low risk of 0.002% for chloramphenicol-induced aplastic anemia had been described in Nigeria as early as in 1993 with an associated recommendation for strict risk-benefit-assessments prior to its prescription [25]. To the authors best knowledge, little has changed in the meantime and the substance is still in broad use in Sub-Saharan Africa, as it is readily available and shows excellent penetration even in difficult to reach compartments including bradytroph tissue like bone [26]. It is therefore possible that that frequent application of chloramphenicol in patients that did not respond to beta-lactam antibiotics, due to *mecA*-carriage of their *S. aureus* strains, might have facilitated the selection of *fexB* carrying bacteria. Due to the lack of reliable clinical data on previous antibiotic treatment this hypothesis could not be confirmed. Other mechanisms of resistance detected included *dfrG*, conferring resistance to trimethoprim, frequently found in strains isolated from Ghana [27,28]. Earlier this was regarded as an infrequent cause of trimethoprim resistance in *S. aureus* isolated from patients but is now widespread in Africa and common in *S. aureus* from ill travelers returning to Europe [27].

Panton-Valentine Leukocidin (PVL), which has been proposed as an epidemiological marker for severe skin infections [29], was encoded in strains of the ST121 and ST152 clonal lineages. The ST152 clonal lineage, in particular, is both known to be associated with PVL expression [30] and wide distribution in Ghana [10,31].
5. Conclusions

This study provides insight into the molecular epidemiology of *S. aureus* sequence types found in chronic infected wounds in a rural area of Ghana.

However, the number of samples used was quite small, and they were taken from outpatients in one hospital, so they may not be representative of the community or the wider area. Moreover, we did not have reliable information about prior use of antibiotics in these patients.

Nevertheless, this study stipulates valuable information for understanding the spread of resistant clones found in patients visiting the study hospital, which is important for effective surveillance of antibiotic resistant *S. aureus* and vital for estimating the burden of resistance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2607/8/12/2052/s1, Table S1: Complete MLST, *agr* and SCCmec typing, virulence factor and resistance gene datasets (VFDB, Resfinder and NCBI AMR) and phenotypic AST results. VFDB, Resfinder and NCBI AMR: The numbers represent the percent identity in the alignment between the best matching resistance or virulence gene in ResFinder or VFDB and the corresponding sequence in the input genome. A perfect alignment is 100% and must also cover the entire length of the resistance gene in the database.

Author Contributions: M.W., D.D. and J.M. designed and coordinated this study. M.W., M.C., A.B. and H.R. performed data analyses analysis. H.F., D.D. and M.W. wrote the first draft of this manuscript. K.O. conducted and supervised fieldwork. C.W.A. conducted and supervised lab work. M.C. and H.R. supported writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by institutional funds of the Bernhard Nocht Institute for Tropical medicine (BNITM).

Acknowledgments: We are grateful to all patients, who participated in this study and to the personnel at the Agogo Presbyterian Hospital. Without their efforts, this research study would not have been possible.

Conflicts of Interest: The authors declare no conflict of interest.

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