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MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM A HOSPITAL IN GHANA

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INTRODUCTION

- *Staphylococcus aureus* cause a variety of infections.
- From simple infections such as boils (furuncles) and styes to more severe ones like meningitis, pneumonia, endocarditis and osteomyelitis.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of hospital- and community-acquired infection.
- Both hospital and community strains harbour genes associated with increased virulence and are resistant to multiple drug classes (Diekema et al., 2019).

INTRODUCTION

- MRSA strains are produced when the *mecA* gene is acquired by methicillin susceptible *S. aureus* (MSSA) (Moellering Jr, 2011)
- The *mecA* gene is borne on the mobile element referred to as the staphylococcal cassette chromosome (SCC) *mec* (Kawamura et al., 2019)
- Other mechanisms of resistance include changes in affinity of penicillin-binding proteins for oxacillin (Becker et al., 2018).
  - *mecC, mecB, FemA and FemB*

INTRODUCTION

- There is considerable variability in the epidemiology of MRSA within and between countries (Breurec et al., 2011).
- Limited data on the antibiotic susceptibility patterns and molecular epidemiology of MRSA in Africa.
- Several African countries report a MRSA prevalence ranging from 4.8% to 20.0% (Breurec et al., 2011, Shittu et al., 2011, Kateete et al., 2011).

RATIONALE AND AIMS

- There exists limited data on antibiotic susceptibility patterns and molecular epidemiology of MRSA in Ghana.
- **Aims:**
  - To phenotypically and genotypically characterize some selected resistance mechanisms and virulence factors in a selected sample of *S. aureus* isolates from a hospital in Ghana.
- Study investigated the antibiotic susceptibility and molecular characterization of MRSA isolates from clinical samples in a Ghanaian hospital

METHODS
Collection and Identification of Isolates

- A total of 91 *S. aureus* isolates from different patients were collected from Tertiary Teaching Hospital in Ghana between May and September 2015.
- Isolates obtained from blood (60.3%), urethral swabs (8.6%), urine (6.9).
- Initial identification (colony morphology and slide coagulase test) was conducted at the Microbiology Unit of the hospital.
- Isolates were subjected to further tests including mannitol fermentation and the tube coagulase test.
- Identity of isolates confirmed by the automated VITEK 2 system

METHODS
Detection of MRSA and Antibiotic Susceptibility Profiles

- The cefoxitin test was used to screen for MRSA according to CLSI guidelines (CLSI 2014).
- MICs for MRSA isolates were determined using the automated VITEK 2 system against 14 antibiotics.
- *S. aureus* ATCC 25923 was used as the reference strain.
- Inducible clindamycin resistance was tested by the 'D-zone'.

METHODS:
DNA extraction, PCR and sequencing of virulence and resistance genes

- Genomic DNA was extracted (Roche High Pure PCR Template Preparation Kit).
- The resistance genes *mecA*, *blaZ*, *aac-aph*, *ermC*, *tetK* and the virulence genes *lukS/F-PV*, *hla*, *hld* and *eta* were amplified by PCR using already described primers.
- The PCR products were sequenced (Inqaba Biotech, Pretoria, South Africa) to confirm the identity of the genes.
- Analysis of the sequences was done using ChromasPro 1.9.9. (Technelysium, Queensland Australia), BioEdit and BLAST 2.0.

METHODS:
Pulsed Field Gel Electrophoresis

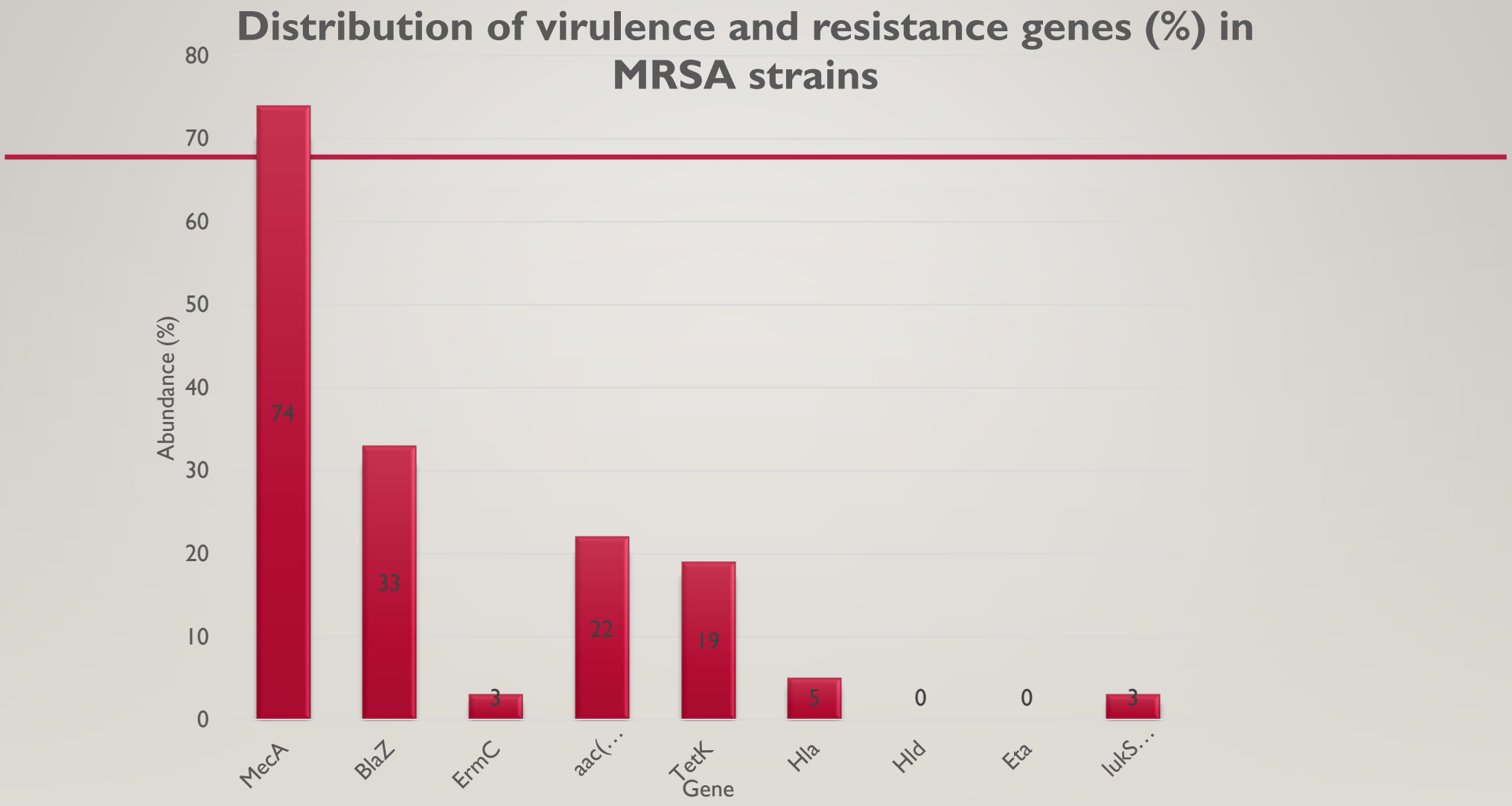
- Strain typing was conducted using pulsed field gel electrophoresis (PFGE) using contour-clamped homogeneous electric field apparatus (CHEF DR-III; BioRad, Hercules, CA).
- Restriction was done with the SmaI restriction enzyme for *S. aureus*.
- Analysis of results was done using BioNumerics software version 6.6 (Applied Maths NV, Belgium).
- Two putatively novel nucleotide sequences for *hla* and *blaZ* genes were submitted to GenBank and the following accession numbers were assigned; KY056259 (*hla*) and KY056260 (*blaZ*)

RESULTS AND DISCUSSION:
Antibiotic susceptibility profile of MRSA isolates (n=58)

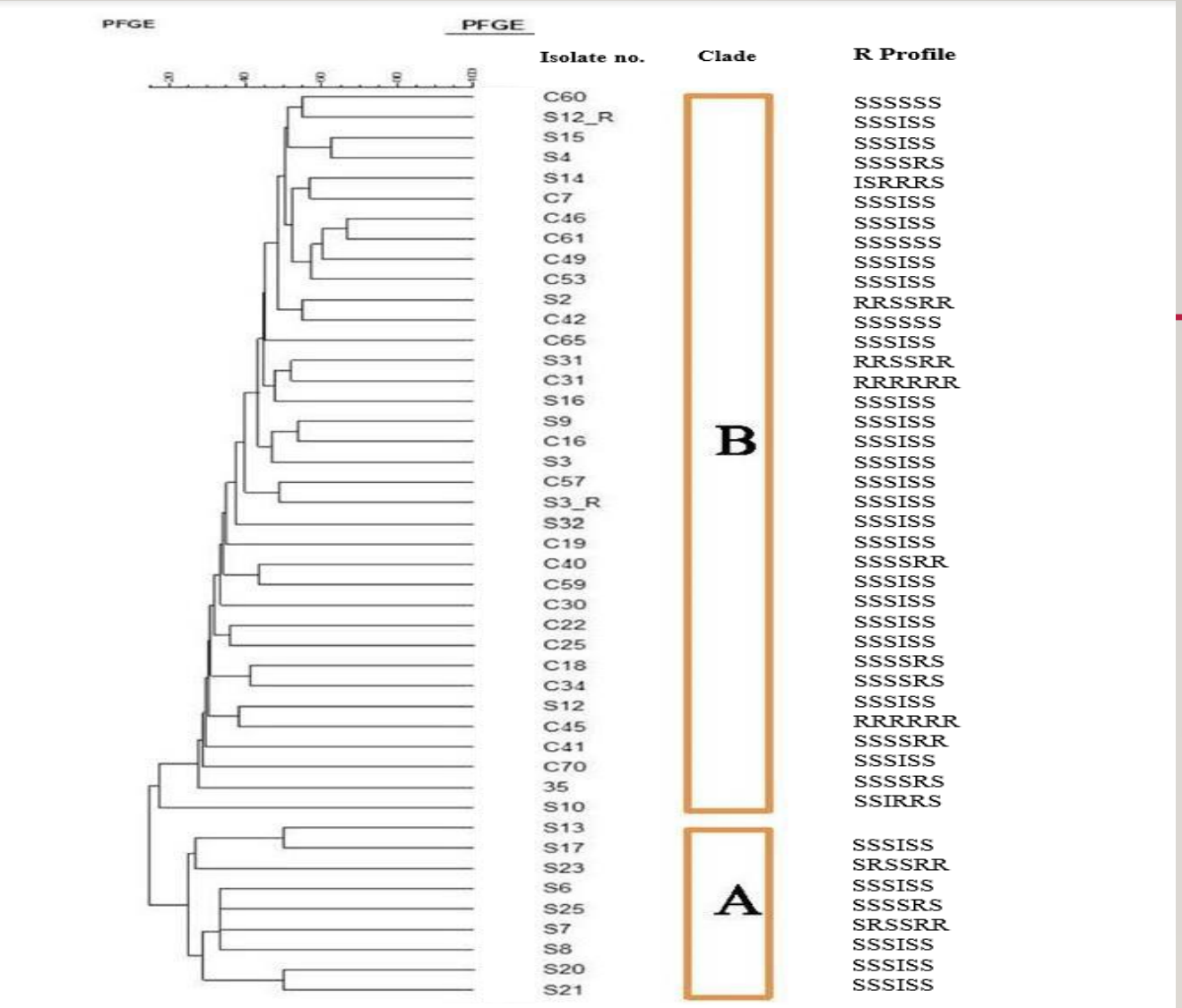
Antibiotic	No. (%) Sensitive	No. (%) Intermediate	No. (%) Resistant
Oxacillin	0 (0.0)	0 (0.0)	58 (100.0)
Gentamicin	49 (84.5)	2 (3.4)	7 (12.1)
Ciprofloxacin	49 (84.5)	1 (1.7)	8 (13.8)
Moxifloxacin	54 (93.1)	0 (0.0)	4 (6.9)
Erythromycin	49 (84.5)	1 (1.7)	8 (13.8)
Clindamycin	18 (31.0)	31 (53.4)	9 (15.5)
Linezolid	58 (100.0)	0 (0.0)	0 (0.0)
Teicoplanin	58 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	58 (100.0)	0 (0.0)	0 (0.0)
Tetracycline	36 (62.1)	0 (0.0)	22 (37.9)
Tigecycline	58 (100.0)	0 (0.0)	0 (0.0)
Fusidic acid*	15 (26.3)	42 (73.7)	0 (0.0)
Rifampicin*	37 (65.0)	16 (28.0)	4 (7.0)
Trimethoprim/sulphamethoxazole	46 (79.3)	1 (1.7)	11 (19.0)

*n=57 as MICs could not be determined for 1 isolate each.

RESULTS: Distribution of virulence and resistance genes



RESULTS: PFGE



The S, I and R (susceptible, intermediate and resistant) indicate resistance or susceptibility to gentamicin, ciprofloxacin, erythromycin, clindamycin, tetracycline and trimethoprim/sulfamethoxazole respectively

DISCUSSION

- There was an MRSA prevalence of 63%, 43 (74%) of which were genotypically confirmed as MRSA (alternate mechanisms of methicillin resistance *mecB*, *mecC*, new methicillinase, over-production of β-lactamases etc).
- Previous Ghana study reported MRSA prevalence of 5.7% (Egyir et al., 2013).
- Our study was comparable however to the 55.0%, 52.0% and 45.0% reported in Ethiopia, Egypt and Algeria respectively (Falagas et al., 2013).
- High tetracycline resistance (*tet*K) was evident.
- None of the isolates resistant to all antibiotics tested and all isolates were fully susceptible to linezolid, teicoplanin, tigecycline, fusidic acid and vancomycin (options for treatment).

DISCUSSION

- Relatively high susceptibility levels against the ciprofloxacin (84.5%) and moxifloxacin (93.1%), erythromycin (84.5%), gentamicin (84.5%) and trimethoprim-sulphamethoxazole (79.3%).
- Inducible clindamycin resistance not detected.
- The observation that some isolates were tetracycline-resistant and yet showed no *tetK* genes upon genotypic screening could be due to fact that their resistance to tetracycline may be mediated by other tetracycline resistant genes like *tetM*.
- Low prevalence of PVL not surprising considering they are clinical isolates.
- None of the isolates harbored the gene for the exfoliative toxin, *eta*. This is consistent with data from another study in Ghana (Egyir et al, 2015).

DISCUSSION

- The LukS/F-PV gene commonly associated with community-acquired MRSA. There was an insertion of G (8) and an SNP (G→C [429]) in the *lukS/F-PV* gene.
- Three SNPs were observed for isolate S15, viz, in the *hla* gene (C→T [12], T→A [15] and T→C [204]).
- A missense mutation of N41S was observed in the *hla* protein (compared with the hla protein of S. aureus gb|AIG51324.1)
- The effects of SNPs at different positions of the codon may affect the expression and functioning of this gene.
- A study conducted identified SNPs in the *hla* gene at positions 2376, 2483 and 2484 from the start codon, associated with alpha toxin hyper-production (Liang et al, 2011)

CONCLUSIONS AND LIMITATIONS

- Results indicated a fairly high occurrence of MRSA.
- The high occurrence of MRSA found in this study is worrying, considering that there are limited treatment options for antibiotic-resistant *S. aureus* in Ghana.
- There is an urgent need to institute effective surveillance mechanisms to monitor MRSA and implement stringent infection prevention and control programmes to forestall its spread.
- Detailed patient demographic data was unavailable, making the discussion of clonal relatedness as well as hospital-associated and community-acquired comparisons challenging