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Efflux and *mecA/mecC*-mediated-resistance in multi-drug-resistant *Staphylococcus aureus* clinical isolates from selected Ghanaian primary and tertiary hospitals

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Abstract:

Background: Globally, methicillin-resistant *Staphylococcus aureus* (MRSA) infections are associated with increased morbidity and mortality amongst patients; however, reports on MRSA infections in Ghana are mostly from tertiary healthcare facilities. This study determined the prevalence of methicillin and multiple efflux resistance mechanisms in *S. aureus* isolates from five selected primary and tertiary healthcare facilities in Ghana.
Methodology: Different clinical samples (nasal, urine and wound) were collected from patients in four primary and one tertiary hospitals in Ghana. *Staphylococcus aureus* was isolated on mannitol salt agar and preliminarily identified using conventional biochemical tests and confirmed by PCR amplification of *Spa1113* gene and Matrix Assisted Laser Desorption Ionisation–Time of Flight Mass Spectrometry (MALDI-TOF). Antibiotic susceptibility test for each isolate to selected antibiotics was determined by the Kirby-Bauer disk diffusion method, and multi-drug resistance (MDR) was defined as resistance to three or more antibiotic classes. The *mecA and mecC* genes were detected by polymerase chain reaction (PCR). Multiple efflux-pump activity of multidrug resistant isolates was assessed against ampicillin and ciprofloxacin by microbroth dilution assay with reserpine, an efflux pump inhibitor.
Results: From a total of 626 clinical samples, *S. aureus* was confirmed in 68 (10.9%). MDR was detected in 46 (67.6%) of the 68 isolates, and 28 (41.2%) were phenotypic MRSA, with *mecA* gene confirmed in 9 (32.1%) of the MRSA isolates while *mecC* gene was absent in all the isolates. MRSA isolates were more susceptible to trimethoprim-sulfamethoxazole (50.0%) compared to other selected antibiotics. Multiple efflux-pump activity was detected as contributing to resistance in 6 of 25 (24.0%) *S. aureus* isolates.
Conclusion: The high prevalence of MRSA in clinical samples mediated by *mecA* gene and possible efflux pump activity call for re-evaluation of treatment protocols of *S. aureus* infections at both primary and tertiary healthcare facilities in Ghana.

Key words: MRSA, *mecA* gene, *mecC* gene, *Staphylococcus aureus*, efflux-pump

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Efflux et résistance médiée par *mecA/mecC* chez des isolats cliniques de *Staphylococcus aureus* multirésistants provenant d'hôpitaux Ghanéens de soins primaires et tertiaires sélectionnés

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Résumé:

Contexte: À l'échelle mondiale, les infections à *Staphylococcus aureus* résistant à la méthicilline (SARM) sont associées à une augmentation de la morbidité et de la mortalité chez les patients. Cependant, au Ghana, les données relatives aux infections à SARM proviennent principalement d'établissements de soins tertiaires. Cette étude a déterminé la prévalence de la résistance à la méthicilline et des mécanismes de résistance à de multiples antibiotiques chez des isolats de S. aureus provenant de cinq établissements de soins primaires et tertiaires sélectionnés au Ghana.
Méthodologie: Différents prélèvements cliniques (nasaux, urinaires et de plaies) ont été collectés auprès de patients dans quatre hôpitaux primaires et un hôpital tertiaire au Ghana. *Staphylococcus aureus* a été isolé sur gélose au mannitol et au sel et identifié préliminairement par des tests biochimiques conventionnels, puis confirmé par amplification PCR du gène *Spa1113* et par spectrométrie de masse MALDI-TOF. La sensibilité aux antibiotiques de chaque isolat a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer. La multirésistance (MDR) a été définie comme une résistance à trois classes d'antibiotiques ou plus. Les gènes *mecA* et *mecC* ont été détectés par réaction en chaîne par polymérase (PCR). L'activité des pompes d'efflux des isolats multirésistants a été évaluée vis-à-vis de l'ampicilline et de la ciprofloxacine par un test de microdilution en bouillon avec la réserpine, un inhibiteur des pompes d'efflux.
Résultats: Sur un total de 626 échantillons cliniques, Staphylococcus aureus a été confirmé dans 68 cas (10,9%). Une MDR a été détectée dans 46 de ces 68 isolats (67,6%), et 28 d'entre eux (41,2%) présentaient un phénotype de SARM. Le gène mecA a été confirmé dans 9 de ces isolats de SARM (32,1%), tandis que le gène mecC était absent dans tous les isolats. Les souches de SARM étaient plus sensibles au triméthoprime-sulfaméthoxazole (50,0%) qu'aux autres antibiotiques testés. Une activité de pompes d'efflux multiples a été détectée, contribuant à la résistance chez 6 des 25 souches de S. aureus (24,0%).
Conclusion: La forte prévalence du SARM dans les échantillons cliniques, liée au gène mecA et à une possible activité de pompes d'efflux, justifie une réévaluation des protocoles de traitement des infections à S. aureus dans les établissements de soins primaires et spécialisés du Ghana.

Mots-clés: SARM, gène *mecA*, gène *mecC*, *Staphylococcus aureus*, pompe d'efflux

Introduction:

Bacterial infections are a major cause of morbidity and mortality worldwide. For several decades, antibiotics have increased the survival rate of patients. However, emergence of resistant bacteria has become a significant problem within the health sector (1). *Staphylococcus aureus* infection is reported as a menace in hospitals in most parts of the world (2). In Ghana, it has been reported as one of the most occurring MDR pathogen amongst patients reporting to regional and teaching hospitals for clinical care (3). The anterior nares and external skin surfaces of humans are frequently colonized by staphylococci causing skin, soft tissue and invasive infections (4).

The occurrence and spread of methicillin resistant *Staphylococcus aureus* (MRSA), a strain resistant to methicillin and/or oxacillin (5) in communities and health facilities is particularly worrisome (1). Synthesis of altered penicillin-binding protein (PBP) in MRSA lowers binding affinity to β-lactam antibiotics (6). Until the discovery of *mecC* gene, detection of *mecA* was the 'gold standard' in identification of MRSA (7). Although the sequence of *mec*C gene is about 70% identical to *mecA*, the strain could be missed during phenotypic testing because it contains a type XI staphylococcal-cassette-chromosome with genetic makeup similar to oxacillin-susceptible MRSA (8), hence its detection requires genetic/molecular testings.

Studies have also reported MRSA genotypes that possess neither *mecA* nor *mecC* genes suggesting involvement of other mechanisms, including mutation in the PBP genes, hyper production of β-lactamases and multidrug efflux pump activity, in resistance to oxacillin and MRSA expression (9). In Ghana, very few studies have reported prevalence of MRSA and possible mechanisms of their resistance. Continuous monitoring of prevalence and antibiograms of MRSA in healthcare facilities is essential for effective management and infections control. This study aimed to determine the prevalence and susceptibility patterns of *S. aureus* isolates and identify possible mechanisms responsible for the resistance among the isolates in selected healthcare facilities in Ghana.

Materials and method:

Study sites:

The study sites were four primary hospitals and a tertiary hospital in Ghana; Kwame Nkrumah University of Science and Technology (KNUST) Hospital, Manhyia Government Hospital, Suntreso Government Hospital, Agogo Presbyterian Hospital, and Komfo Anokye Teaching Hospital.

Ethical consideration:

The study was approved by the Committee on Human Research, Publication, and Ethics (CHRPE) of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, with approval number CHRPE/AP/354/17.

Permissions for the study were granted by the authorities of the selected hospitals, and informed consent of selected participants and their caregivers were obtained. The study was performed in accordance with the Declaration of Helsinki (10).

Sample collection and identification:

Clinical samples (333 urine, 119 wound and 174 nasal swabs) were collected from a total of 626 patients in the five primary and tertiary hospitals in Ghana, who were selected by systematic random sampling from patients with suspected cases of infection for which microbiological assessment had been requested. The samples were inoculated into 10 mL nutrient broth and incubated at 37°C for 24 hours. A loopful was streaked on mannitol salt agar (MSA) and incubated at 37°C.

Golden-yellow colonies from MSA plates were further sub-cultured on nutrient agar and incubated at 37°C, followed by Gram staining and conventional biochemical tests on the colonies, including haemolysis on blood agar, catalase and coagulase tests, for presumptive identification of *S. aureus*, with *S. aureus* ATCC 25923 used as positive control (11). Preliminary confirmation of the *S. aureus* isolates was performed using Matrix Assisted Laser Desorption Ionisation–Time of Flight (MALDI-TOF) mass spectrometry.

Molecular confirmation of *S. aureus* and detection of *mecA* and *mecC* genes:

Isolates were further confirmed by PCR amplification of *Spa1113* gene (F: 5'-TAA AGA CGA TCC TTC GGT GAGC-3', R: 5'-CAG CAG TAG TGC CGT TTG CTT-3). A simplex PCR reaction was carried out in thermal cycler (Gene Amp, ThermoFisher Scientific, Waltham, MA, USA USA), with PCR reaction in a final volume of 25µL containing 2µL of DNA template, 12.5µL of OneTaq master mix, 2µL (0.8µM) each of forward and reverse primers and 6.5µL of nuclease-free water. The PCR consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 10 min. Amplicon size ranged from 180 to 600 bp.

PCR amplifications of *mecA* and *mecC* genes were performed following the procedure described by Boamah et al., (13), using the primers *mec*A (F: 5`-ACG GTA ACA TTG ATC GCA ACG-3`, R: 5`-GGC CAA TTC CAC ATT GTT TCG-3') and *mec*C (F: 5`-GAA AAA AAG GCT TAG AAC GCC TC-3', R: 5`-GAA GAT CTT TTC CGT TTT CAG C-3'). The PCR reaction was carried out in a final volume of 25µL containing 2µL DNA template, 12.5µL OneTaq master mix, 2µL (0.8 µM) of each primer and 6.5µL nuclease-free water. The PCR reaction consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59°C and 55°C for 1 min for *mecA* and *mecC* genes, respectively and extension at 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products for were separated using a 1.5% $^{w}/_{v}$ agarose gel at 100 V and visualized under UV light. Amplicon sizes were detected at 176 and 138 bp, respectively (12).

Antibiotic sensitivity testing:

Antibiotic susceptibility of the *S. aureus* isolates was determined following the guidelines of Clinical and Laboratory Standards Institute (CLSI). The inoculum of *S. aureus* isolate was prepared by suspending 5 to 7 well-isolated colonies in sterile normal saline and standardized to the density of 0.5 McFarland standard. Eight antibiotic disks (Oxoid Ltd, Basingstoke, UK) in eight antibiotic classes including erythromycin (ERY-15µg), oxacillin (OXA-1µg), ciprofloxacin (CIP-5µg), chloramphenicol (CHL-30µg), trimethoprim-sulfamethoxazole (SXT-25µg), tetracycline (TET-30µg), gentamicin (CN-10µg), and cefoxitin (FOX-100µg), were placed on the surface of inoculated Mueller-Hinton agar plates and incubated at 37°C for 24 hours. Zones of growth inhibition were measured, and breakpoints for sensitive or resistant estimated according to CLSI guideline (13).

Detection of multiple efflux pump activity by microdilution assay:

The efflux pump activity of 25 MDR *S. aureus* isolates was assessed using the efflux pump inhibition assay utilising the microdilution method. The MICs of ampicillin and ciprofloxacin were determined in the presence and absence of reserpine (an efflux pump inhibitor) using 96-well microtiter plates. The inoculum was prepared by suspending 5 to 7 well-isolated colonies in sterile normal saline to the density of 0.5 McFarland standard. Using a dilution factor of 2, different concentrations of the antibiotics were prepared from 0.8 to 12.8µg/mL. One hundred microliters (100µL) of double strength nutrient broth were dispensed into 5 wells. This was followed by the addition of appropriate volumes of ciprofloxacin for a given concentration (500 µg/mL) which was serially diluted by 2-fold into each well, followed by the introduction of 20 µL of the inoculum. Sterile water was then added to make a final volume of 200 µL.

The plates were incubated for 24 hours at 37°C after which 20µL of 1.25mg/mL 3-(4,5

dimethylthiazol-2-yl)-2, 5 -diphenyltetrazolium bromide (MTT) was added to the wells. Wells that showed purple colour after 30 min incubation at 37°C indicated bacteria growth and those that remained yellowish indicated inhibitory activity. The MICs of ampicillin and ciprofloxacin were re-determined in the presence of 50µg/mL concentration of the plant alkaloid reserpine (reserpine stock solution was prepared to 500µg/mL) (14,15). A two-fold reduction in the MICs of ampicillin and ciprofloxacin for the MDR isolates indicates presence of multiple efflux pump activity.

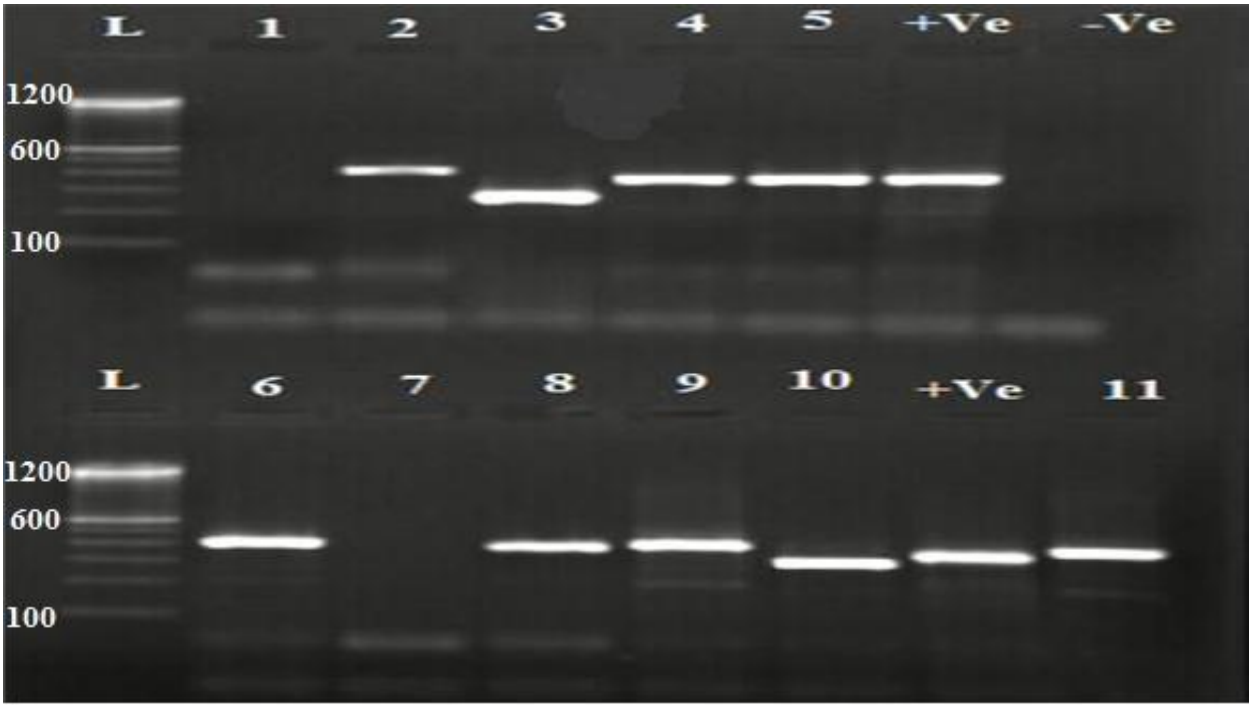
Statistical analysis:

The data generated was extracted into an MS-office Excel software Version 1908 (Microsoft Corporation Copyright 2016). Data was later analyzed using Statistical Package for the Social Sciences Version 22 (SPSS, SPSS Inc., Chicago, IL, USA) Susceptibility data were compared by using Chi-square analysis with GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). A level of significance (*p* value) less than 0.05 was considered statistically significant.

Results:

Distribution of *S. aureus* and MDR isolates in the hospitals:

Based on phenotypic growth characteristics on culture media, biochemical properties, *Spa* gene detection (Fig 1) and MALDI-TOF analysis, a total of 68 (10.9%) *S. aureus* isolates were confirmed. *S. aureus* was more frequently isolated from urine (14.7%, n=49/333) than from nasal (10.3%, n=18/174) and wound swabs (0.8%, n=1/119) (x^2=17.496, p=0.0002) (Table 1). *S. aureus* was also more frequently isolated from Agogo Presbyterian Hospital (15.3%, n=25/163) than KNUST (14.7%, n=16/109), KATH (12.0%, n=12/184), Suntreso (5.7%, n=3/53) and Manhyia (1.7%, n=2/117) (x^2= 16.843, p=0.0021) (Table 1).



L: DNA ladder; +ve: *S. aureus* 25923, -ve: RNase free water, number 1 to 11: representative *S. aureus* isolates

Fig 1: Gel image showing a 180-600 bp PCR amplicons of *Spa* gene in *Staphylococcus aureus* isolates

Table 1: Distribution of multi-drug and methicillin resistant *Staphylococcus aureus* isolates in clinical samples from primary and tertiary care hospitals in Ghana

Sources/Samples	**Total (n=626)**		**KATH (n=184)**		**Agogo (n=163)**		**KNUST (n=109)**		**Suntreso (n=53)**		**Manhyia (n=117)**	
Total *S. aureus* isolates	68 (10.9)	%	22 (12.0)	%	25 (15.3)	%	16 (14.7)	%	3 (5.7)	%	2 (1.7)	
MDR *S. aureus*	46	67.6	14	63.6	17	68.0	13	84.0	1	33.3	1	50.0
MRSA	28	41.2	7	31.8	12	48.0	9	56.3	0	0	0	0
Urine samples	**333**		**63**		**125**		**0**		**47**		**98**	
S. aureus isolates	49 (14.7)		19 (30.2)		25 (20.0)		0		3 (6.4)		2 (2.0)	
MDR *S. aureus*	31	63.2	12	63.1	17	68.0	0	0	1	33.3	1	50.0
MRSA	18	36.7	6	31.5	12	48.0	0	0	0		0	0
Nasal swab samples	**174**		**66**		**22**		**86**		**0**		**0**	
S. aureus isolates	18 (10.3)		3 (4.5)		0		15 (17.4)		0		0	
MDR *S. aureus*	15	83.3	2	66.6	0		13	86.6	0	0	0	0
MRSA	10	55.6	1	33.3	0		9	60.0	0	0	0	0
Wound swab samples	**119**		**55**		**16**		**23**		**6**		**19**	
S. aureus isolates	1 (0.8)		0		0		1 (4.3)		0		0	0

KATH: Komfo Anokye Teaching Hospital; Agogo: Agogo Presbyterian Hospital; KNUST: Kwame Nkrumah University of Science and Technology Hospital; Suntreso Government Hospital; Manhyia Government Hospital. n; number of samples.

Table 2: Susceptibility and resistance profiles of *Staphylococcus aureus* isolates from primary and tertiary healthcare facilities in Ghana

Isolate ID	**Source**	**ERY-15**	**OXA -1**	**CIP-15**	**CHL -30**	**SXT -25**	**TE-30**	**CN-10**	**FOX-30**	**MDR**
N106	Nasal	R	R	S	S	S	S	S	R	MDR
N112	Nasal	S	S	S	S	S	R	S	S	-
N114	Nasal	R	R	S	R	R	R	R	R	MDR
N116	Nasal	R	R	R	S	S	S	S	R	MDR
N119	Nasal	R	R	S	R	R	R	S	R	MDR
N126	Nasal	R	S	R	R	S	I	S	S	MDR
N134	Nasal	S	S	S	S	S	S	S	S	-
N138	Nasal	R	R	R	S	S	R	R	R	MDR
N139	Nasal	R	R	S	S	S	I	R	S	MDR
N141	Nasal	R	R	R	R	S	R	R	R	MDR
N145	Nasal	R	R	R	R	R	R	R	R	MDR
N149	Nasal	S	R	R	S	S	R	S	S	MDR
N153	Nasal	R	R	R	R	S	R	R	R	MDR
N169	Nasal	S	S	S	S	S	S	S	S	-
N175	Nasal	R	R	R	S	R	R	R	R	MDR
N34	Nasal	R	S	S	S	S	S	S	S	
N42	Nasal	S	S	R	S	S	R	S	S	
N47	Nasal	S	R	S	R	S	R	S	R	MDR
S02	Urine	I	R	S	S	R	S	S	S	-
S03	Urine	S	S	R	S	S	R	S	S	-
S28	Urine	S	R	S	R	S	R	S	R	MDR
S29	Urine	R	S	S	R	S	R	R	S	MDR
S30	Urine	R	S	R	R	S	R	S	S	MDR
S32	Urine	R	R	R	S	R	R	R	R	MDR
S40	Urine	S	S	S	R	S	R	S	S	
S42	Urine	R	R	R	R	R	R	R	R	MDR
S44	Urine	R	S	S	R	S	S	S	S	MDR
S48	Urine	S	R	S	R	S	R	S	R	MDR

S49	Urine	S	S	S	S	S	S	S	S	-
S50	Urine	S	R	S	R	R	R	R	R	MDR
S54	Urine	R	S	S	R	S	R	S	S	MDR
S55	Urine	R	S	S	S	S	R	S	S	MDR
S56	Urine	S	R	S	R	R	R	R	R	MDR
S59	Urine	S	S	S	R	S	I	S	S	-
S60	Urine	S	S	S	S	S	S	S	S	-
S79	Urine	S	S	S	S	S	S	S	S	-
Sa01	Urine	S	R	S	R	S	S	S	S	MDR
Sa03	Urine	S	S	S	S	S	S	S	S	-
Sa25	Urine	S	S	S	S	S	S	S	S	-
Sa29	Urine	S	S	S	R	S	R	S	S	-
Sa30	Urine	S	S	S	R	S	R	S	S	MDR
Sa35	Urine	S	S	S	R	S	R	S	S	MDR
Sa36	Urine	S	R	R	R	R	R	R	R	MDR
Sa40	Urine	S	R	S	R	S	R	S	S	MDR
Sa41	Urine	S	R	S	R	S	R	S	R	MDR
Sa43	Urine	R	S	S	R	S	S	S	S	MDR
Sa44	Urine	R	S	S	R	S	S	S	S	MDR
Sa48	Urine	R	R	R	R	R	R	R	R	MDR
Sa49	Urine	S	R	R	R	S	R	R	R	MDR
Sa54	Urine	R	R	S	R	R	R	S	S	MDR
Sa55	Urine	S	S	S	S	S	R	S	S	-
Sa77	Urine	S	S	S	R	S	S	S	S	-
Sa79	Urine	S	S	S	S	S	S	S	S	-
U01	Urine	R	R	R	R	R	R	R	R	MDR
U02	Urine	R	R	R	R	R	R	R	R	MDR
U09	Urine	S	R	S	S	S	R	S	S	-
U21	Urine	R	R	S	R	R	R	R	R	MDR
U24	Urine	S	S	S	S	S	R	S	S	-
U26	Urine	S	S	S	R	S	R	S	S	-
U29	Urine	R	R	R	R	S	R	R	R	MDR
U38	Urine	S	R	S	S	S	S	S	S	-
U42	Urine	R	R	R	S	S	I	R	R	MDR
U45	Urine	R	R	S	S	S	I	R	R	MDR
U49	Urine	R	R	S	S	S	R	R	R	MDR
U50	Urine	R	R	R	S	S	R	R	S	MDR
U52	Urine	S	R	S	R	S	S	S	R	MDR
U59	Urine	S	R	S	S	S	R	S	S	-
W119	Wound	S	S	S	R	S	S	S	S	-
Number of resistant isolates (%)		31 (45.5)	38 (55.8)	21 (30.9)	38 (55.8)	15 (22.1)	43 (63.2)	23 (33.8)	28 (41.2)	46 (67.6)

ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; CHL-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamicin 10ug; FOX-30: Cefoxitin-30

Antibiogram profile of MSSA and MRSA isolates from the healthcare facilities:

Of the 68 *S. aureus* isolates confirmed, 28 (41.2%) were MRSA while 40 (58.8%) were MSSA. The MRSA isolates were more prevalent in the samples from KNUST Teaching hospital (56.3%, n=9/16) compared to the others (x^2= 6.277, p=0.1794) and they were more prevalent in nasal samples (55.6%, n=10/18) compared to urine samples (36.7%, n=18/49) (OR= 0.4645, 95% CI = 0.1552 - 1.391, p = 0.2635) (Table 1). However, the MRSA prevalence differences between the hospitals and the samples were not statistically significant.

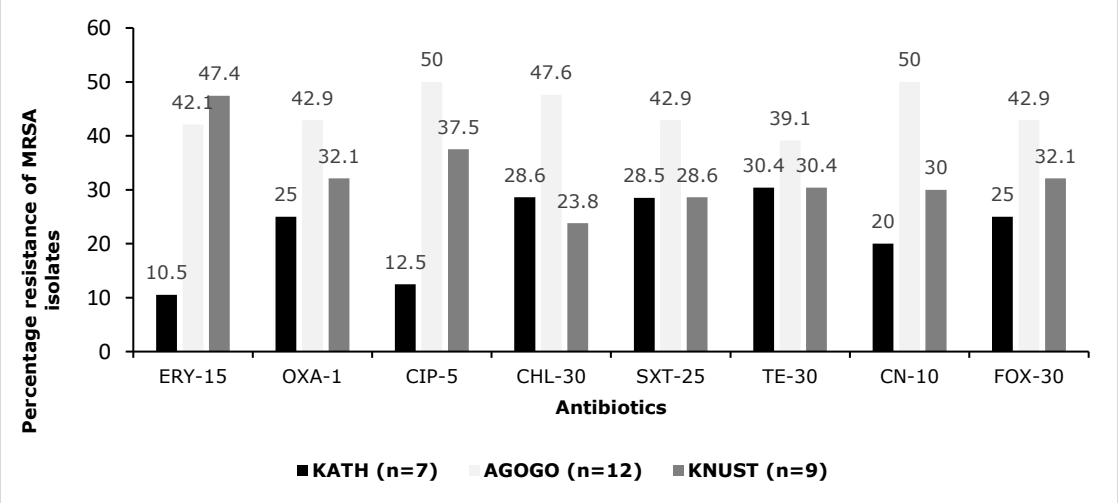
The susceptibility rates of the *S. aureus* to antibiotics tested were in the range of 28 to 84% while resistance ranged from 16 to 72% (Table 2). Most of the *S. aureus* isolates were resistant to tetracycline (63.0%, n=43/68) while the isolates showed least resistant to trimethoprim-sulfamethoxazole (22.0%, n= 15/68). Forty-six (67.6%) of the *S. aureus* isolates were multi-drug resistant (Table 2).

The MRSA isolates were resistant to all selected antibiotics, ranging between 60 to 100.0%, but were susceptible to trimethoprim-sulfamethoxazole (50.0%, n=14/28) (Fig 2a). MRSA isolates from KATH showed relatively low resistance to the different antibiotics compared to those from Agogo and KNUST hospitals (Fig 2a). Six (21.4%) of the MRSA isolates were resistant to all the eight antibiotics tested (Table 3). Erythromycin and ciprofloxacin showed relatively higher activity against the MRSA isolates from KATH while trimethoprim-sulfamethoxazole was comparatively more active against isolates from KNUST and Agogo (Fig 2a). Compared to the methicillin sensitive *S. aureus*, MRSA isolates exhibited higher resistance rates to all the antibiotics tested (Fig 2b).

Table 3: Antibiotic resistance profiles of MRSA isolates from the five selected primary and tertiary care hospitals in Ghana

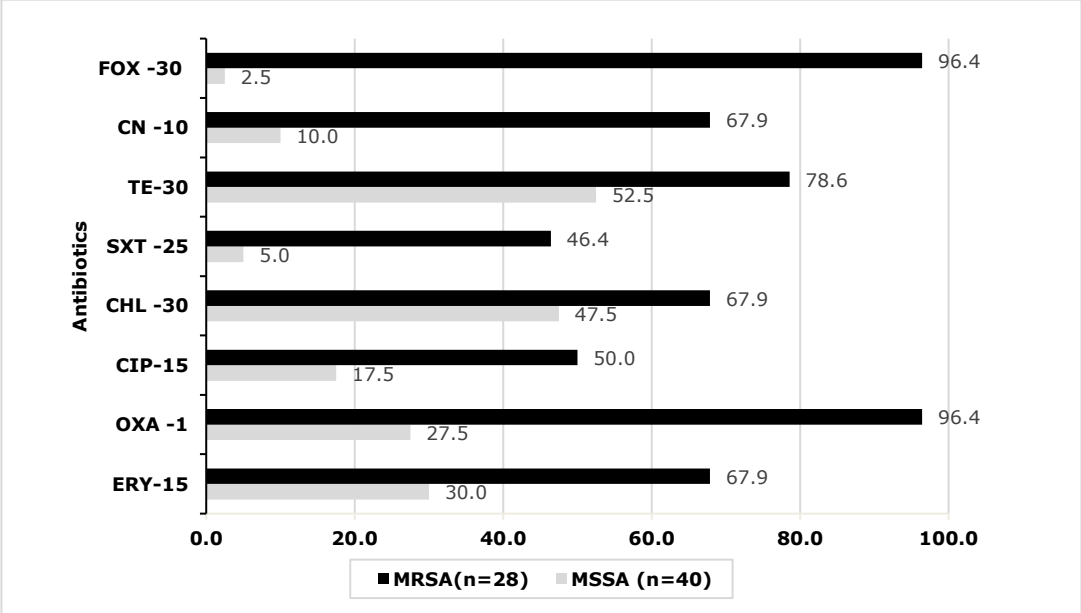
Resistance pattern	Clinical MRSA isolates (ID)+	Number of MRSA (%)
ERY-OXA-FOX	N106	1 (3.6)
OXA-CHL-FOX	U52+	1 (3.6)
ERY-OXA-CIP-FOX	N116	1 (3.6)
ERY-OXA-CN-FOX	U45	1 (3.6)
OXA-CHL-TET-FOX	S28+, N47, Sa41, S48	4 (14.3)
ERY-OXA-CIP-CN-FOX	U42	1 (3.6)
ERY-OXA-TET-CN-FOX	U49	1 (3.6)
ERY-OXA-CHL-SXT-TET-FOX	N119+	1 (3.6)
OXA-CIP-CHL-TET-CN-FOX	S49	1 (3.6)
OXA-CHL-SXT-TET-CN-FOX	S50+, S56	2 (7.1)
OXA-CIP-CHL-SXT-TET-CN-FOX	Sa36	1 (3.6)
ERY-OXA-CHL-SXT-TET-CN-FOX	U21, N114	2 (7.1)
ERY-OXA-CIP-CHL-TET-CN-FOX	U29, N141+	2 (7.1)
ERY-OXA-CIP-SXT-TET-CN-FOX	S32, N138, N175	3 (10.7)
ERY-OXA-CIP-CHL-SXT-TET-CN-FOX	Sa48+, N153+, N145, U02, S42+, U01	6 (21.4)
Total		**28 (100.0)**

ERY: Erythromycin 15 µg; OXA: Oxacillin 1 µg; CIP: Ciprofloxacin 5 µg; CHL: Chloramphenicol 30 µg; SXT: Trimethoprim-sulphamethoxazole 23.75ug; TET: Tetracycline 30ug; CN: Gentamycin 10ug; FOX: Cefoxitin-30ug; + presence of *mecA* gene.



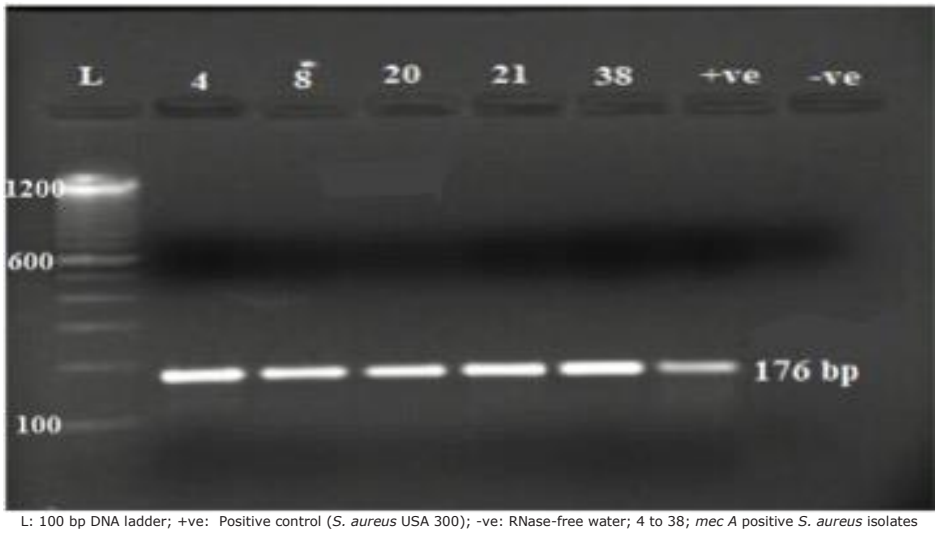
ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; *CHL*-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamycin 10ug; FOX-30: Cefoxitin-30ug; Agogo: Agogo Presbyterian Hospital; KNUST: Kwame Nkrumah University of Science and Technology Hospital; KATH: Komfo Anokye Teaching Hospital.

Fig 2a: Antibiotic susceptibility of methicillin resistant *Staphylococcus aureus* isolates from three healthcare facilities in Ghana



ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; C*HL*-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamycin 10ug; FOX-30: Cefoxitin-30ug; MRSA: Methicillin resistant *S. aureus*. MSSA: Methicillin sensitive *S. aureus*.

Fig 2b: Comparative antibiotic resistance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from healthcare facilities in Ghana



L: 100 bp DNA ladder; +ve: Positive control (*S. aureus* USA 300); -ve: RNase-free water; 4 to 38; *mec A* positive *S. aureus* isolates

Fig 3: Gel image confirming the 176 bp PCR amplicon of the *mecA* gene in MRSA isolates

Prevalence of resistant genes in multi-drug-resistant *S. aureus* strains:

Of the 68 *S. aureus* isolates, 9 (13.0%) contained *mec A* gene but none possessed *mec* C gene (Fig 3). All the 9 isolates were among the 28 (32.1%) phenotypic MRSA isolates.

Efflux pump activity amongst MDR *S. aureus* isolates:

The MICs of ciprofloxacin and ampicillin for the 25 MDR *S. aureus* tested ranged from 0.8 to 12.8 µg/mL. In the presence of reserpine (an efflux pump inhibitor), a two-fold reduction in the MIC was recorded in 10 of the MDR *S. aureus* isolates and this occurred in 28.6% (n= 8/28) of the MRSA isolates. This reduction in MIC was observed in 10 of 25 (40.0%) isolates tested against ciprofloxacin, 6 of 25 (24.0%) isolates tested against ampicillin, and 6 of 25 (24.0%) isolates tested against both ciprofloxacin and ampicillin, indicating multiple efflux pump activity (Table 4).

Table 4: Effect of efflux pump inhibition (reserpine) on the minimum inhibitory concentration of *Staphylococcus aureus* isolates

MDR *S. aureus* isolate	Minimum inhibitory concentration (µg/mL)					
	Cip alone	Cip+ reserpine	Fold change in MIC	Amp alone	Amp + reserpine	Fold change in MIC
S1	6.4	6.4	1	12.8	12.8	1
S2	6.4	6.4	1	12.8	12.8	1
S3	0.8	0.8	1	12.8	12.8	1
S4	1.6	0.8	2↓	3.2	1.6	2↓
S5	3.2	1.6	2↓	3.2	1.6	2↓
S6	6.4	6.4	1	12.8	12.8	1
S7	3.2	1.6	2↓	6.4	3.2	2↓
S8	1.6	0.8	2↓	6.4	3.2	2↓
S9	0.8	0.8	1	6.4	6.4	1
S10	0.8	0.8	1	12.8	12.8	1
S11	1.6	0.8	2↓	6.4	3.2	2↓
S12	0.8	0.8	1	3.2	3.2	1
S13	0.8	0.8	1	6.4	6.4	1
S14	1.6	0.8	2↓	0.8	0.8	1
S15	6.4	6.4	1	3.2	3.2	1
S16	3.2	3.2	1	3.2	3.2	1
S17	6.4	3.2	2↓	3.2	3.2	1
S18	1.6	1.6	1	6.4	6.4	1
S19	1.6	0.8	2↓	3.2	3.2	1
S20	0.8	0.8	1	6.4	6.4	1
S21	1.6	1.6	1	6.4	6.4	1
S22	3.2	1.6	2↓	12.8	6.4	2↓
S23	0.8	0.8	1	12.8	12.8	1
S24	12.8	12.8	1	12.8	12.8	1
S25	1.6	0.8	2↓	0.8	0.8	1

↓: Reduction in MIC; MIC: minimum inhibitory concentration

Discussion:

Increasing antibiotic resistance among pathogenic bacteria calls for more studies on the prevalence and resistance mechanisms among these bacteria. Continuous monitoring of prevalence, susceptibility patterns and resistance mechanisms of pathogenic bacteria, including MRSA in health facilities is also necessary for effective management and control of bacterial infections (1). Most of the reported surveillance on antimicrobial resistance of pathogens worldwide and in Ghana often involve samples obtained from tertiary or large regional healthcare facilities and teaching hospitals (3,16,17-22) with very few from secondary and primary healthcare facilities. In this study, samples were collected from one regional/teaching hospital and four secondary healthcare facilities including one university hospital and three district hospitals in order to report on antimicrobial resistance at the district levels as well.

In this study, 41.2% of *S. aureus* were identified as MRSA by the cefoxitin disk diffusion test. This value is higher than 28-34.8% previously reported in Ghana (3,16,18). Most studies define MRSA as *S. aureus* isolates resistant to oxacillin, possession of either *mecA* or *mecC* gene or a latex agglutination test for detection of PBP2a (19,20). These could account for the differences seen between these studies. However, in agreement , a review (17) has reported

MRSA prevalence of 23-73% from clinical samples across Europe.

A high number of the MDR-MRSA isolates were identified in the nasal samples (n=10, 35.7%). This relatively high prevalence in nasal samples (nasal carriage of MRSA) could lead to transmission to other individuals visiting the healthcare facilities and within communities (1,22,23). The antibiograms of the *S. aureus* isolates revealed an appreciable level of resistance to the selected antibiotics mostly to oxacillin and cefoxitin (100.0%), tetracycline (82.1%) and chloramphenicol (75.0%). Similarly, high resistance of *S. aureus* to β-lactams (oxacillin) have been reported in Ghana (16,18,20). Half of the isolates tested were however susceptible, to a large extent, to trimethoprim-sulfamethoxazole (50.0%), which is comparable to previously reported studies (21,24).

In this study, *mecA* gene was detected in 9 (32.1%) of the 28 MRSA isolates, representing 13.2% of the total *S. aureus* isolates. A previous study in Ghana reported 28.0% prevalence of *mecA* genes in *S. aureus* isolated from samples from patients at the burns unit of a teaching hospital (22), higher than that observed in our study. The difference could be due to the fact that patients with burns and those on admission in intensive care units tend to be more susceptible to MRSA infections (25). *mecC* genes were not detected in any of the *S. aureus* strains in our study. Globally, the prevalence of *mecC* genes in *S. aureus* is quite low; between 0.4% and 0.7% (24,26,27). *mecC* genes have not yet been reported among clinical *S. aureus* isolates in Ghana.

Aside *mecA* and *mecC* genes, factors such as the presence of multiple efflux pumps have also been identified to contribute to resistance development in *S. aureus* (26,28,29,30), hence the MICs of ciprofloxacin and ampicillin in the presence and absence of an efflux pump inhibitor (reserpine) was used to assess the presence of efflux pump activities among selected isolates not possessing *mecA*. The MICs of ampicillin and ciprofloxacin in the presence of an efflux pump inhibitor (reserpine), reduced by a factor of two in 24.0% and 40.0% of isolates respectively. Similar results of a two-fold reduction in MIC values for norfloxacin in the presence of reserpine has been reported by Dang et al., (27). The reduction in MIC values obtained may signify the presence of multidrug efflux pumps in the MRSA isolates. Efflux pump genes have been found in MRSA isolates from clinical samples in Iran (28,29).

Among the 28 MRSA isolates, 19 (67.9%) had neither *mecA*, *mecC* nor exhibited active efflux pump activity, indicating other mechanisms are responsible for resistance evolution in those MRSA isolates. Studies suggest mutations in the PBP genes and polymorphisms in the *mec* genes could account for such resistance expression. Also, hyper-production/overexpression of β-lactamase antibiotic degrading enzymes could enhance resistance in the MRSA strains (9,27,28). Identification of other possible mechanisms responsible for the multidrug resistance in the *S. aureus* isolates especially in MRSA strains could improve infection management (1).

Conclusion:

The prevalence of *S. aureus* isolates from clinical samples in our study was 10.9%, with 41.2% being phenotypic MRSA isolates. The isolates were least resistant to trimethoprim-sulfamethoxazole among the selected antibiotics. *mecA* gene-mediated-resistance was identified as one of the mechanisms responsible for multidrug resistance in 32.1% of MRSA isolates while *mecC* genes were not detected in the isolates. Active efflux was also identified to be partly responsible for resistance in the isolates.

Contribution of authors:

CNZ, CA, and VEB were in the study conceptualization; CNZ and YDB were involved in data curation; CNZ, HO and VEB were involved in formal analysis: CNZ, YDB and VEB were involved in the study investigation; CNZ, YDB, VEB and CA were involved in the study methodology; CNZ, CA, VEB, and YDB were involved in project administration; CNZ, CA, VEB, and YDB provided resources; CA, YDB and VEB were involved in study supervision; CNZ and HO wrote the original manuscript draft; HO, CNZ, VEB and YDB reviewed and edited the manuscript. All authors reviewed and approved the manuscript submitted for publication.

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Authors declare no conflict of interest

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