Prevalence and antimicrobial resistance pattern of *Staphylococcus aureus* and Methicillin Resistance *Staphylococcus aureus* isolated from waste bin handles in the Tamale Metropolis and Tolon District

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ABSTRACT

There has been an increased incidence of *Staphylococcus aureus* colonization on several fomites with implicated transmission and infection. *S. aureus* was predisposed to developing antibiotic resistance due to the rise in antimicrobial overuse and genetic mutation, which contributes to formation of methicillin-resistant *S. aureus* (MRSA). MRSA is known to cause several illnesses in hospitals, and infection in the community. This study aims to determine the prevalence and antimicrobial susceptibility of *Staphylococcus aureus* and MRSA on waste bin handles in the Tamale metropolis and Tolon district, Ghana. Hundred (100) swabs samples were taken from the handles of waste bins at Homes and streets in the Tamale metropolis and Tolon. Swab samples were inoculated and isolated on Baird Parker agar at 37°C for 24-48 hours, and further confirmed using Staph aureus plus latex agglutination assay. Disk diffusion method was employed for the antibiotic susceptibility testing using EUCAST guidelines. Isolates resistant to oxacillin were classified as Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA). Fifty-six (56%) of samples were contaminated with *S. aureus*, of which 41% were MRSA isolates. Fifty (50%) of isolates were classified as Methicillin Susceptible *Staphylococcus aureus* (MSSA). The percentage of *S. aureus* resistant to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin were 29, 38, 57, 68, 62, and 80% respectively. Waste bin handles serve as habitat for multidrug resistant *S. aureus*. This may lead to a high rate of CA-MRSA infection. Further study should be done to determine the prevalence of other pathogenic bacteria on waste bin handles.

Keywords: Antibiotics; Ghana; Methicillin-Resistant *Staphylococcus aureus* (MRSA); Methicillin Susceptible *Staphylococcus aureus* (MSSA); Tamale
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Introduction

Staphylococcus aureus survive on living and inanimate surfaces and present high risk to the human populace due to their high survival rate (Creech *et al*., 2015). These bacteria can survive on the skin of mammals, nasal cavities of animals, humans and non-living inhabitants due to their facultative nature (Brown *et al*., 2013). A study by (Fritz *et al*., 2014) showed the risk associated with *S. aureus* transmission from carriers of these bacteria to various household environments. Methicillin Resistant

Staphylococcus aureus (MRSA) was identified on bed side rails, curtains and hands of patients with MRSA infection (Kurashige *et al*., 2016). Another study conducted to investigate MRSA in nasal carriage of diabetic and non-diabetic patients in Ghana revealed high percentage of *S. aureus* (31.0%) isolates with MRSA being 3.3% (Anafo *et al*., 2021). Similar to MRSA is Methicillin Susceptible *Staphylococcus aureus* (MSSA) whose mortality has increased with a recent study showing 72% death rate within 30 days after diagnosis (Quiñonez-Flores *et al*., 2024). A research at the Dental Pedagogical clinics of Fernando Pessoa University revealed that MSSA on surfaces of equipment used by staff increased from 40% to 53.3% after patient care (Goncalves *et al*., 2020). Inadequate awareness creation about MRSA and MSSA infection is a contributing factor to this bacteria's high prevalence in the community (Andersen *et al*., 2015).

One of the means of managing waste properly is the use of waste bins for storing waste. Waste bins are often colonized by diverse bacteria (Hossain *et al*., 2013; Mello *et al*., 2019). *S. aureus* could spread to humans through contact that may lead to fatal infections (Rosenberg Goldstein *et al*., 2012). The high resistance of these bacteria to several antibiotics worsens the control of *S. aureus* (Peacock & Paterson, 2015). Also, mutation from natural occurrence within genes such as *murA, uhpT and glpT* is another contributing factor of MRSA resistance (Lee *et al*., 2020). However, there is very little awareness of the mode of spread of MRSA and its control through good hand washing practices. Research by (Dayie *et al*., 2022), showed how new strains of MRSA are resistant to antibiotics such as Penicillin. Therefore, a lot is required, particularly, in the Northern Ghana where the risk of *S. aureus* infection is high (Addaney & Oppong, 2015). The aim of this study is to determine the prevalence and antimicrobial resistance pattern of *S. aureus* and MRSA isolated from waste bin handles in the Tamale Metropolis and Tolon district.

Materials and Methods

Study area and sampling

The study was carried out in five different zones in the Tamale metropolis which lies between latitude 9º 25'N and longitude 0º 58'W (Ochire-Boadu *et al*., 2020). A total of 100 samples were randomly collected from homes and streets of four areas in Tamale (North, East, South and Central), and Tolon district within four months from 17th January to 10th April 2018. Sterile cotton swabs moistened in saline solution were used to rub gently on each waste bin handle, kept back into its case, labeled, and finally transported on ice to the laboratory for analysis. Laboratory analysis was carried out at the One Health Lab in the Spanish Laboratory Complex, located in the Nyankpala campus of the University for Development Studies.

Inoculation, isolation, and confirmation

Samples collected were spread on Baird Parker medium supplemented with Rabbit Plasma Fibrinogen (RPF) (Oxoid, UK) and incubated at 37°C for 48 hours. Colonies confirmed to be *S. aureus*, were further subjected to Staphaurex plus latex agglutination (Thermo Scientific™, R30950102). Positive *S. aureus* isolates were then streaked on nutrient agar and incubated at 37°C for 24 hours to obtain pure cultures for further analysis.

Antibiotic susceptibility testing

Following the disk diffusion method and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines,

antibiotic susceptibility test was performed for the isolated *S. aureus*. A 24-hour culture of the isolate was scooped in bits and dissolved in a test tube containing 2 mL of normal saline. Suspension turbidity was compared and adjusted to 0.5 McFarland using DEN 1B densitometer (UK). Sterile cotton swab was aseptically dipped into the uniform suspension created, pressed against test tube wall to remove excess water, and spread uniformly on labeled prepared Mueller Hinton agar (Oxoid, UK) in plate. With the aid of sterile forceps, antibiotic discs were aseptically placed on surface inoculated agar in plates, following incubation at 35°C for 24 hours, and diameter of zone of inhibition measured. Result was interpreted as susceptible, intermediate and resistance according to EUCAST breakpoint guidelines (Matuschek *et al*., 2014). Antibiotics used in the study were ciprofloxacin (5µg), gentamicin (10µg), erythromycin (15µg), tetracycline (10µg), chloramphenicol (10µg) and streptomycin (30µg). Oxacillin (1µg) was used to determine MRSA isolates. Multidrug resistance was classified as isolate resistance to three or more antibiotics. All isolates were stored in eppendorf tubes 1.5 mL each of 20% glycerol with Brain-Heart Infusion (Oxoid, UK) at -20 °C. Isolates resistant to one or more antibiotics were classified as MRSA whiles *S. aureus* strains susceptible to any of the antibiotics used were classified as MSSA.

Data Analysis

The statistical tool for data analysis and graphical representation of data was Microsoft excel 2013 and Statistical Products and Services Solutions (SPSS), version 24. Descriptive statistics was used in comparing results represented on bar chart such as antimicrobial results and *S. aureus* prevalent at 95% confidential interval. Bivariate analysis was used in comparing MRSA and MSSA distribution.

Results and Discussion

In total, 100 samples from waste bin handles in five towns at Tamale and Tolon District were randomly collected. Moistened cotton swabs were gently rubbed ones on waste bin handles. More samples were collected from Tolon district (40) due to availability based on convenience. Fifty-six samples (56%) were found to be contaminated with *S. aureus* from a total of 100 samples collected from waste bin handles. Out of the fifty-six isolates, 33.93% were recorded from Tamale central and 5.36% were recorded from Tamale East **(**Table 1**).**

TABLE 1

Positive isolates of S. aureus

Location	Total samples	Positive isolates	Percentage (%)
Tamale south	10	8	14.29
Tamale Central	20	19	33.93
Tamale East	10	3	5.3
Tamale North	20	7	12.50
Tolon	40	19	33.93
Total	100	56	100

(Standard Deviation (SD), Mean) = 10.95, 20 samples, p = 0.045 (Significant at 0.05 alpha level)

Antibiotic resistance pattern of S. aureus

Out of the fifty-six isolates confirmed to be *S. aureus*, resistance to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin were 29%, 38%, 57%, 68%, 63% and 80% respectively. In Tamale south, a total of eight (14.29%) isolates were obtained of which resistance to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin were 0%, 25%, 63%, 50%, 63%, 63%. Nineteen (33.93%) isolates were confirmed in Tamale central with resistance to the above antibiotics being 37%, 26%, 42%, 47%, 53% and 74% respectively. Seven (12.50%) isolates were isolated from Tamale North with the resistance pattern to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin being 43%, 43%, 71%, 100%, 86% and 100% respectively. Resistant isolates in Tolon district were in the following order 29%, 35%, 43%, 89%, 67% and 100% for ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin respectively, while in Tamale East, the total resistant isolates were 33%, 33%, 100%, 67%, 67% and 100% for ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin.

Prevalence of MRSA from S. aureus

The number of *S. aureus* isolates was 56 (56%) with 23 (41%) being MRSA. P-test to compare the significant difference between number of *S. aureus* isolates and MRSA prevalence from the areas where samples were collected showed $p < 0.05$ ($p = 0.037$), demonstrating a significant difference between isolates of *S. aureus* and MRSA.

TABLE 2

MRSA isolates confirmed from S. aureus strains from each town

Town	Positive isolate	%	MRSA	%
Tamale South	8	14.23	1	4.35
Tamale Central	19	33.93	8	34.78
Tamale North	7	12.5	3	13.04
Tamale East	3	5.36	2	8.70
Tolon	19	33.93	9	39.13

Positive isolate (Standard Deviation, Mean=6.58, 11.20. MRSA isolates (Standard Deviation, Mean) =3.26, 4.60. $p < 0.05$ ($p = 0.037$)

Multidrug resistance pattern of S. aureus

Isolates that were resistant to three or more antibiotics categories were defined to be multi drug resistant. Of the total fifty-six isolates, 35 comprising 63% were multidrug resistant strains. Isolates that were resistance to only two antibiotics were twelve (21%). There were 12 (21%) isolates that were resistant to only two antibiotics. Six (17%) isolates were resistant to four antibiotics while twelve (34%) isolates were resistant to five antibiotics. Moreover, 15 (43%) isolates out of the 35 (63%) multidrug resistant strains were obtained in Tolon district, while the remaining three (9%), three (9 %), 11 (30%) and three (9%) were found in Tamale East (9%), Tamale North (9%), Tamale Central (30%) and Tamale South (9%) respectively.

Antibiotic resistance pattern of MRSA isolates

Out of the fifty-six confirmed *S. aureus* isolates, twenty-three (41%) were MRSA. From this, the resistance pattern of the isolates to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and

streptomycin was 44%, 44%, 61%, 74%, 57%, 87% respectively. Seventeen (74%), out of the twenty-three MRSA isolates were multidrug resistant. MRSA isolates that were resistant to only three antibiotics were five (29%), whiles resistant to four, five and six antibiotics were five (29%), five (29%) and two (13%) respectively.

Antibiotic resistance pattern of MSSA isolates

Methicillin Susceptible *Staphylococcus aureus* (MSSA) was 28 (50%) out of the total *S. aureus* isolates. Isolates of MSSA resistant to ciprofloxacin, gentamicin, erythromycin, tetracycline, chloramphenicol, and streptomycin were 18%, 39%, 54%, 61%, 64% and 64% respectively. Multidrug resistant isolates from the MSSA were 19 (68%). MSSA resistant to three, four, five and six antibiotics were 4 (21%), 8 (42 %), 7 (37%) and 0% respectively, while MSSA resistant to only two and one antibiotics were three (11 %) and 6 (21 %) respectively.

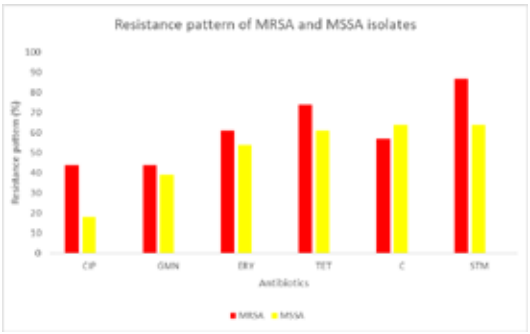

Fig. 1: Resistance pattern of MRSA and MSSA isolates. GMN; Gentamicin, CIP; Ciprofloxacin, STM; Streptomycin, C; Chloramphenicol, ERY; Erythromycin, TET; Tetracycline

This study was conducted to determine the presence of *S. aureus* and MRSA from waste bin handles at different sub areas in the Tamale metropolis and Tolon district as well as to determine the antibiotic resistance pattern of these isolates. It appears to be the first of its kind in the country. Contamination of waste bin handles with *S. aureus* was isolated more from samples taken in the street (82%) as compared to those from households (18%). This could be explained by the high level of contact by waste bins at open places in the street, capable of transferring *S. aureus* (Grace & Fetsch, 2018). Our study supports similar outcomes of high prevalence rate of MRSA. There were high isolates of *S. aureus,* (56%) comparatively higher than a study by (Chatziprodromidou *et al*., 2017), who took 99 swab samples from litter handles across different areas in South-western Greece, with *S. aureus* prevalence being 7.8%. Moreover, the results of MRSA (41%) was high when compared to that of Pesewu *et al*. (2014) who collected 65 samples from surfaces and workers in a hospital setting and recorded 9.1% isolates of MRSA. The MSSA isolates recorded in the study (50%) was also comparably lower than the results obtained by (Saba *et al*., 2017), who isolated (83%) of MSSA from 120 swab samples of contact surfaces at three major hospitals in the Tamale metropolis.

The result of our study is an indication of the possible high reoccurrence of MRSA infection due to contamination on object of high contact (Miller *et al*., 2015). Moreover, it is an indication that when hand hygiene practices are not enforced in such areas, the possibility of MRSA transmission and infection will be high (Chun *et al*., 2015; Kim *et al*., 2013). The increasing rate of antibiotic resistance has been a major global challenge particularly with the evolving trends of MRSA. From our study, the susceptible isolates (MSSA-50%) were more than the resistant isolates (MRSA, 41%),

however, most MRSA isolates demonstrated resistance to streptomycin (87%) which was comparatively higher than the result by (Ben Slama *et al*., 2011), who recorded 11% resistance by MRSA against streptomycin in a study conducted in Tunisia. The study also showed a higher multidrug resistance 35 (63%) which is higher than the outcome by (Umar Puma *et al*., 2018) who obtained 30.7% multidrug resistance by MRSA from a total 140 samples collected from waste sources. More of the MRSA demonstrated multidrug resistant (74%), as compared to that of MSSA (68%) which is higher than the result by Saba *et al*. (2017) who recorded five (63%) and four (10%) of multidrug MRSA and MSSA respectively on various fomites at three major hospitals in the Tamale metropolis.

Conclusion and Recommendation

To my knowledge, this study is the first research conducted on the prevalence of *S. aureus* on waste bin handles in Ghana. There was high prevalence of MRSA from samples collected in the Tamale metropolis and Tolon District. *S. aureus* isolates were resistant to most of the antibiotics used whiles two MRSA isolates were multidrug resistant to all the six antibiotics used. To reduce the spread of these bacteria, it is suggested that waste bins should be kept clean to reduce the presence of MRSA. Effective washing of hands with soap and water and reducing antibiotics overuse can reduce *S. aureus* infection. The study provides more information on the survival of MRSA on waste bin handles and how their prevalence can lead to infection. It is recommended that further study should be done to determine other bacteria prevalence on waste bin handles.

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