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# Evaluation of Community Acquired Methicillin Resistant *Staphylococcus Aureus* (CAMRSA) from different Sources at Selected Events and viewing Center in Kano Metropolitan, Nigeria

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## ABSTRACT

**Introduction**: Methicillin resistant *Staphylococcus aurous* (MRSA) is a threat to both the hospital and community. Community-acquired methicillin resistant *Staphylococcus aureus* (CAMRSA) is increasing rapidly leading to increase in morbidities and mortalities rate in the world.

**Aim:** The aim of this work was to survey for CAMRSA from Event and Viewing Centers in Kano Metropolis, Nigeria.

**Methodology**: A total of 780 samples (192 and 588 from indoor air and human and fomite-surfaces respectively) were collected "between" November, 2019 to March, 2020. Cultural isolation of *Staphylococcus aureus* was done on Mannitol salt Agar (MSA) and Blood Agar. Gram stain, Catalase and Coagulase test were done to determine the suspected colonies of *Staphylococcus aureus*. 30ug of cefoxitin disc diffusion on a Prepared Nutrient Agar (NA) were also used to assessed the present of resistant strains of Methicillin. All isolates were further assessed to determine the multi-drugs resistant strains from various antibiotics which included; Gentamicin, Amoxicillin, Ceftriaxone, Ceftazidine, Erythromycin, Chloramphenicol, Oflaxacin, Cefuroxime and Ciprofloxacin. Chi-square was used to analyzed weather there is significant differences or not, set calculated p-value at <0.05.

**Result**: Of 505 isolates of *Staphylococcus aureus* recovered from 780 non-clinical samples of air, surface, nose and hands, 58 out of 505 isolates of S. aureus was detected to be CAMRSA in the present Study. 130 (26.15%), 199 (40.04%), 85 (17.10%) and 91 (18.31%) of *S. aureus* were found to be in air samples, Surfaces, Nose and Hand respectively. Surface was observed to have the highest CAMRSA resistant strains with 30/58(51.72%) out of samples recorded. Of 505 isolates, viewing center has to be recorded with highest *Staphylococcus aureus* and MRSA occurrence with 262 and 37 respectively. For the susceptibility of an antibiotics, Ciprofloxacin and gentamicin had highest antibacterial profile with 253/262 (96.6%) and 233/243 (95.9%) in viewing and event center respectively. Greatest level of resistance was observed with ESBL antibiotics (cefuroxime) showed from Event and Viewing Centers with 194/243 (80.2%) and 210/262 (79.8%) respectively.

**Keywords:** Community-acquired, Methicillin resistant *Staphylococcus aureus* (MRSA), Event and viewing center

## INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) was described for the first time in England in 1961 [1]. *S. aureus* is frequently associated with skin infections, pneumonia, surgery wounds, bacteremia, osteomyelitis and endocarditis, being considered one of the most important pathogens of the human being, both at the community level and at nosocomial infections [2]. According to the European Centre for Disease Prevention and Control (ECDC) in its annual report of the priority program of infections and resistances to antimicrobials, the percentage of MRSA is decreasing in Portugal, with 47.4, 46.8, 43.6 e 39.2% in the years 2014, 2015, 2016 and 2017 respectively, Portugal is considered one of the countries with the highest MRSA percentage in Europe (ECDC, 2017) [3]. *S. aureus* strains are still responsible for a lot of pathologies at oral and perioral surfaces such as: angular cheilitis; oral ulcers; failure of treatment with implants; prosthetic stomatitis; facial

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abscesses; xerostomia; osteomyelitis of the jaws among others, in addition to generalized infections discussed before, making it a public health problem [4,5]. Aerosols contribute as a route of direct or surface contamination leading to the increase of these strains during patient attendance in dentistry and consequently to a higher probability of cross infection [6].

## RESEARCH PROBLEM

The fact that there are always various events for people to show-off, from weddings, graduation parties, reunions, anniversaries, business meetings, dinner party and so on. These events are usually conducted in halls that provide convenient facilities for guests than would have been achieved in a home or office. In addition, an average football fan in Nigeria may not even be able to afford a satellite TV to watch matches of their favourite clubs, while even if they can afford, they prefer viewing centers. This is because it gives them the opportunity to team up with other club fans. Crowded environments have been reported to significantly increase the risk of MRSA colonization [7]. According to Al-Ruaily [8] Colonization has been reported as an important step in the chain of events that lead to *S. aureus* infections. CAMRSA is emerging as a serious threat to public health worldwide and has significantly increased morbidity and mortality rate of patients along with the increased length of hospital stay.

## AIM AND OBJECTIVES

### Aim of the Study

The aim of study was to survey for the presence community-acquired methicillin resistant *Staphylococcus aureus* from Event and Viewing Centers in Kano Metropolis, Nigeria.

### Objectives of the Study

1. To isolate and identify Methicillin resistant *Staphylococcus aureus* from human hands, noses, fomites, objects and indoor air of some Event and Viewing Centers.
2. To determine the susceptibilities of the isolates to various antibiotics.
3. To determine the distribution of *Staphylococcus aureus* and MRSA from both Event and Viewing Centers.

## METHODOLOGY

### Study Area

This study was carried out at some event and viewing centers of Kano Metropolis, Kano State, Nigeria. It's located between latitudes 11°52'N and 12°07'N and longitudes 8°24'E and 8°38'E. It's a conurbation of eight Local Government Areas (LGAs) around main city, which metamorphose to form the present Kano metropolis. The LGAs are Dala, Fagge, Gwale, Kano Municipal, Kumbotso, Nasarawa, Tarauni and Ungoggo. These eight LGAs formed the study area for this study.

### Study Design

A cross Sectional study

### Ethical approval

Ethical approval for the study was obtained from the ethics committee of Kano State Censorship Board, in 15 November, 2019; an ethical approval to conduct the study was issued.

## SAMPLES COLLECTION

### Swab stick Sampling

A total of 588 Samples were collected from apparently healthy humans' hand and nasal mucosa, using sterile swab sticks [9]. Samples were also collected from the surfaces of door handles, Handset keypads, locker/drawer handles, surfaces like Tables, Air conditioners, TV Screen and TV set remote control. Surfaces was swabbed using a sterile swab sticks, and the sampled swab sticks were put in to polyethylene bags inside ice box. All the samples were immediately taken to Microbiology Laboratory, Department of Microbiology, Bayero University Kano, for microbiological analysis as described by CLSI, (2018).

### Indoor Air Sampling

Sampling were conducted twice a day early before starting the Event and Viewing and during the Event and Viewing, in accordance with the direction of prevailing winds, on the height of 1.5m above the ground level to maintain uniform collection of air samples. A total of 192 samples were collected in all Event and Viewing Centers. To collect the samples, Mannitol Salt Agar (MSA) plates were exposed for 30 min at the height of 1.5m above the ground level. After the exposure, the plates were taken to the Microbiology Laboratory, Department of Microbiology, Bayero University Kano, and incubated at 37°C for 24 h [10].

### Bacterial Isolation

All Swab stick samples were also streaked on a prepared Mannitol Salt Agar (MSA) plate in a Zig zag motion and incubated at 37°C for 24 h. After overnight incubation on Mannitol Salt Agar, yellow colonies were picked and sub cultured into sterile Blood Agar (BA) plate and further incubated at 37ºC for 24 h, the organisms formed milky and some yellowish colonies and changing the red bloody color of the medium to yellow with hemolysis on MSA and BA respectively are characterizing as *S. aureus* and used for further analysis (Hilliard and Reddy, 2018) **(Table 1).**

**Table 1.** Gram Stain and Biochemicals Test.

| Test | Methods | Procedure |
|---|---|---|
| Gram staining | Slide | Cheesebrough [10] |
| Catalase | Slide | Cheesebrough [10] |
| Coagulase | Test tube | Cheesebrough [10] |

**Antibiotics Susceptibility Testing**

Antibiotics susceptibility testing was carried out using the disc diffusion method (CLSI, 2018). The culture inoculum was first standardized by emulsifying a loopful of discrete colonies of *S. aureus* in 3 ml of physiological saline and form a standard culture suspension. The culture suspension was adjusted and a turbidity equivalent to 0.5 Mcfarland Standard (equivalent to 1.5 x $10^8$ cfu/ml). Mueller Hinton agar (MHA) plates were then inoculated with the test bacteria (0.5 Mcfarland turbidity standard) using a sterile swab stick. The plates were allowed to dry for 10 min and then commercial antibiotics discs, gentamicin (30µg), cefoxitin (30µg), ceftriaxone (30µg), cefuroxime (30µg), ceftazidime (30µg), ofloxacin (5µg), amoxicillin (20µg), chloramphenicol (30µg) and erythromycin (5µg) were applied aseptically to the surface of the agar. After 30 min of applying the discs, the plates were inverted, and incubated at 37°C for 24 h. The zone of inhibition was measurement and interpreted as resistant and sensitive based on the guidelines of CLSI, (2018).

**Detection of Community Acquired Methicillin Resistant *Staphylococcus aureus* (CAMRSA)**

A 0.5 Mc Farland standard suspension of the isolate was made and a lawn culture was done on MHA plate. Cefoxitin 30µg discs were placed and plates were incubated at 37°C for 24 h and zone diameter was measured. An inhibition zone diameter ≤21 mm was reported as methicillin resistant and ≥22 mm was considered as methicillin susceptible (CLSI, 2018).

**Statistical analysis**

Chi-square was used to see if there was a significant difference in the occurrence of *S. aureus* and MRSA isolated from event and viewing centers. Calculated p-value of >0.05 were considered to be statistically not significant.

**RESULT AND DISCUSSION**

Refer to the **Table 2** showing the overall occurrences of *S. aures* and MRSA. Of total 780 bacterial samples survey from different event and viewing center within Kano metropolitan, Surface has highest number of 199(40.04) of *Staphylococcus aureus* with 30(51.72) Methicillin resistant strains in which viewing center showed the highest number of MRSA. Furthermore, the highest number of resistant strains in viewing and event center were all obtained during program, this was not surprising because aerosols contribute as a route of indirect transmission or surface contamination in congested places and consequently to a higher probability of cross infection [11] Of 58 Methicillin resistant *Staphylococcus aureus* (MRSA) isolates, 45(77%) were all detected during program, this was much higher than 13.2% recorded by [12] in a study conducted in Federal University Dutse, Jigawa State, Nigeria. Factors such as overcrowding and poor personal and environmental hygiene can all influence the viability of MRSA in an indoor environment [13].

**Table 2.** Percentage Occurrences of *S. aureus* and MRSA from Sample sites.

| Sample site | No. of sample | No. of *S. aureus* n (%) | No. of MRSA n (%) |
|---|---|---|---|
| Air samples | 192 | 130 (26.15) | $7^{DP}$ (12.06) |
| Surface | 323 | 199 (40.04) | $13^{BP}$+$17^{DP}$ (51.72) |
| Nose | 108 | 85 (17.10) | $13^{DP}$ (22.40) |
| Hand | 157 | 91 (18.31) | $8^{DP}$ (13.79) |
| Total | 780 | 505 | $13^{BP}$+$45^{DP}$=58 |

*Key: DP: During program; BP: Before program*

In **Table 3** when comparing the number and percentages of *S. aureus* in the Event and Viewing Centers, the rate of *S. aureus* was higher in viewing centers 262(51.8%) compared with event centers 243(48.1%). However, no significant difference (P value = 0.764617). Likewise, if we look at the distribution of MRSA isolates between different event and viewing centers, we found them to be 37(63.8%) in viewing centers and 21(36.2%) in event centers out of a total 58.

However, there is significant difference (P value = 0.032077) in the distribution of MRSA. This higher occurrence of *S. aureus* and MRSA in viewing centers could be due to various associated risk factors such as overcrowding, poor hygienic condition, antibiotic treatment, underlying immune-compromised conditions which predispose an individual to MRSA acquisition. These studies observed a decrease of contamination level in Event Centers than Viewing Centers_this may be attribute to the proper structural design, good hygienic condition, restriction of movement and the people entering event centers are need, it may be responsible for the lower rate of *S. aureus* and MRSA in event centers. Since, the P value = 0.032077 is lower than 0.05, hence there is statistically difference between the MRSA isolated in Event and Viewing Centers. In this present study, most of the samples collected were from fomite-surfaces and has to be with highest isolates for both event and viewing centers in Kano metropolitan, the results are similar to those reported by [8]. Here, the result also shows that fomite-surfaces (door handle) are more prone to *S. aureus*. This might be due to high chances of contact with door handles and also *S. aureus* is a normal flora of the human skin and due to the fact that human being always interact with their environment, is possible that the organism can leave human body to live on his environment. It is was reported that microbes can persist on an-animate object for a long period of time ranging from less than a minute to about a month or more as reported by Kramer [13] in their work, they reported that some organism can persist for a long period on surfaces and air. Crowded environments have been reported to significantly increase the risk of MRSA colonization. Colonization has been reported as an important step in the chain of events that lead to *S. aureus* infections [7,8]. Methicillin resistance *Staphylococcus aureus* isolation rate from nasal, hand and fomite found in this study is similar to findings of Ghidey [14] which have suggested that nasal colonization is not the only route for pathogenesis of CA-MRSA infection and that skin-skin and skin-fomite contact could represent important transmission routes. Nasal carriage of MRSA is an important risk factor for subsequent MRSA infection and transmission as the bacterium is transmitted to the nares by contaminated hands and from surfaces where it can survive for months [15].

**Table 3.** Distribution of *S. aureus* and MRSA isolates from air, human and formites-surface at different event and viewing center in Kano metropolitan.

| Sample site | No. of sample | No. of *S. aureus* n (%) | No. of MRSA n (%) |
|---|---|---|---|
| Event center | | | |
| Air samples | 96 | 60 (24.69) | 3 (14.28) |
| Surface | 179 | 94 (38.68) | 10 (47.68) |
| Nose | 50 | 39 (16.04) | 4 (19.05) |
| Hand | 86 | 50 (20.57) | 4 (19.05) |
| Total | 411 | 243 | 21 |
| Viewing center | | | |
| Air samples | 96 | 70 (27.77) | 4 (10.81) |
| Surface | 144 | 105 (41.60) | 20 (54.05) |
| Nose | 58 | 46 (18.15) | 9 (24.32) |
| Hand | 71 | 41 (16.27) | 4 (10.81) |
| Total | 369 | 262 | 37 |

For the susceptibility test of *S. aureus* showed that ciprofloxacin and gentamicin had the greatest activity with 253/262 (96.2%) and 233/243 (95.9%) in viewing and event center respectively. In other hand cefuroxime from β-lactams antibiotics was the highest resistant antibiotic with 194/243(80.2) and 210/262(79.8%) in viewing and event center respectively (**Table 4**). Ciprofloxacin and gentamicin seem to be the only antimicrobial agent that showed the highest level of susceptibility against *S. aureus* in the study which is consistent with previous report by Ibrahim [16] and However, it was higher than those reported by Kadora [17] and Kumurya and Ado [18] in their study in Kano. Hence, these antibiotics may be used as the drug of choice for treating MDR-MRSA infections. However, frequent monitoring of their sensitivity and routine testing should be carried out. Use of ciprofloxacin and gentamicin should be

limited to preserve their values. The high rate of resistance to cefuroxime, ceftriaxone, ceftazidime and erythromycin shows that these antibacterial agents would be unreliable and also most of the isolates showed resistance towards commonly β-lactams antibiotics. This agrees with previous reports that most isolates of *S. aureus* are resistant to a large number of commonly prescribed antibiotics [19]. Self-medication and indiscriminate use of antibiotics have severally been reported as promoters of antibiotic resistance and frequent administration of systemic antibiotics modified nasal *S. aureus* from MSSA to MRSA. Moreover, the differences in sensitivity profile of the bacteria isolates among the sources may be attributed to practices of self-medication, drug abuse and indiscriminate use of antibiotics. Similarly, Onanuga and Temedie [20] also confirmed the occurrence of high multiple antibiotic index in their study.

**Table 4.** Susceptibility profile of *S. aureus* from Events and Viewing center to various antibiotics used.

| Drugs | Event; n=243 | Center | Viewing; n=262 | Center |
|---|---|---|---|---|
| | R (%) | S (%) | R (%) | S (%) |
| AMX | 21 (8.6) | 222 (91.4) | 40 (15.3) | 222 (84.7) |
| GNC | 10 (4.1) | 233 (95.9) | 26 (10.0) | 236 (90.0) |
| CAX | 192 (79.0) | 51 (20.9) | 51 (20.9) | 191 (72.9) |
| CRX | 29 (1.9) | 214 (88.1) | 166 (63.4) | 96 (36.6) |
| ERT | 78 (32.1) | 165 (67.9) | 108 (41.2) | 154 (58.8) |
| CHC | 57 (23.5) | 186 (76.5) | 81 (30.9) | 181 (69.1) |
| OFL | 63 (29.9) | 180 (70.1) | 88 (33.6) | 174 (66.4) |
| CXM | 194 (79.8) | 49 (20.2) | 49 (20.2) | 52 (19.8) |
| AZM | 24 (9.9) | 219 (90.1) | 219 (90.1) | 205 (78.2) |
| CPX | 17 (7.0) | 226 (93.0) | 9 (3.4) | 253 (96.6) |

*Key: AMX: Amoxicillin; GNC: Gentamicin; CAX: Ceftriaxone; CRX: Ceftazidine; ERT: Erythromycin; CHC: Chloramphenicol; OFL: Oflaxacin; CXM: Cefuroxime; CPX: Ciproflaxacin; AZM: Azithromycin*

## CONCLUSION

The study recorded the overall 505 *S. aureus* with 58 methicillin resistant, about half and quarter of resistant strains was recovered during programmed, this study provides a vital information of the presence of Community acquired methicillin resistant *Staphylococcus aureus* (CAMRSA). In addition to that it was found that Ciprofloxacin and Gentamicin was active against almost mediated CAMRSA isolates. Hence, the use of those antibiotics may be used as drugs of choice for treating MDR-MRSA infections.

## RECOMMENDATION

Education /awareness on strict adherence to aseptic procedure and regular screening of event and viewing centers for the presence of MRSA to control colonization and infection. Also, proper building design of event and viewing centers are needed to aid ventilation and reduce overcrowding.

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