



Prevalence and Antibigram of Methicillin Resistant *Staphylococcus aureus* Nasal Carriage among Apparently Healthy University Staff and Students in Kaduna, Nigeria

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ABSTRACT: Methicillin Resistant *Staphylococcus aureus* (MRSA) has been frequently implicated in healthcare-associated infections. Hence, the objective of this paper was to investigate the prevalence and antibiogram of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage among apparently healthy University staff and students in Kaduna, Nigeria. A total of 250 nasal swab samples were collected alongside demographic data. Gram staining and biochemical tests were carried out to identify the *Staphylococcus aureus*. Methicillin-resistance was determined phenotypically using cefoxitin disc. Antibiotics susceptibility testing of the MRSA isolates was carried out using agar diffusion method and the multidrug resistance determined. Out of the 250 nasal samples collected, 41 (16.4%) were confirmed to be *S. aureus*, out of which 25(61.0%) were methicillin resistant. Students from Faculties of Science and Medicine harboured higher percentages of MRSA (69.2% and 60.0%, respectively) in their nasal cavity. Gentamicin (84%) and ciprofloxacin (72%) were the most active antibacterial agents against the MRSA isolates. High level of resistance was recorded amongst the MRSA isolates to amoxicillin (80.0%), cotrimoxazole (52.0%), erythromycin (32.0%) and streptomycin (32.0%). Multidrug resistance was recorded in 48% of the MRSA isolates. In conclusion, there was a high prevalence of MRSA (61.0%) among Staff and students of Kaduna State University. Irrational use of antibiotics especially in the community and without prescription might be responsible for this.

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Staphylococcus aureus has remained a major human pathogen; it is estimated that 20% of the human population are long-term carriers of *S. aureus*. It colonizes healthy individuals, and can colonize any part of the mucous membranes, human nose, especially the anterior nares (Wertheim et al, 2005). It is responsible for causing a variety of human infections, which may range from minor skin diseases to life-threatening infections. *S. aureus* isolates worldwide are increasingly resistant to a greater

number of antimicrobial agents; inevitably this has left fewer effective bactericidal antibiotics to treat these often life-threatening infections. As rapidly as new antibiotics are introduced, staphylococci have developed efficient mechanisms to neutralize them (Klytmans *et al.*, 1997). Methicillin-resistant *Staphylococcus aureus* (MRSA), first reported in the early 1960s in the United Kingdom, are strains of *S. aureus* that through the process of natural selection developed resistance to all available

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penicillins and other β -lactam antimicrobial drugs (David *et al.*, 2010). Although the evolution of such resistance does not cause the organism to be more intrinsically virulent, resistance does make MRSA infections more difficult to treat and thus more dangerous, particularly in hospitalized patients and those with weakened immune systems (Kundu *et al.*, 2012). MRSA can be spread from one person to another through casual contact or through contaminated objects, it is common in hospitals, schools, prisons, and nursing homes, where people with open wounds, invasive devices such as catheters, and weakened immune systems are at greater risk of healthcare associated infection (CDC, 2002). A strain acquired in a hospital or health care setting is called health care-associated methicillin-resistant *S. aureus* (HA-MRSA) (Kundu *et al.*, 2012).

MRSA is thought to be restricted to the hospital setting, not until the late 1990s when MRSA infection was discovered among healthy individuals in the community with no history of hospitalization, intravenous drug use, prior antimicrobial use, and underlying illnesses such as cardiovascular and pulmonary disease, diabetes, malignancy, and chronic skin diseases (Charlebois *et al.*, 2004). This strain called community acquired or associated MRSA (CA-MRSA) was found to be non-susceptible to beta lactams antibiotics, harbor different *SCCmec* class IV and V and a phage-borne pantone valentine leucocidin (PVL) toxin incriminated in skin and soft tissue infection in healthy children and adults (Montanaro *et al.*, 2016). The main mode of transmission of MRSA is via hands which may become contaminated by contact with colonized or infected patients, colonized or infected body sites of the personnel themselves, or devices, items, or environmental surfaces contaminated with body fluids containing MRSA (CDC, 2002). In school setting, risk factors for CA-MRSA infection include exposure to an individual with MRSA, usually skin-to-skin contact, and exposure to environments favorable to crowding (Herman, 2008) or a lack of cleanliness. (Deleo, 2010). Community-acquired MRSA is more common among the athletes (Deresunski, 2005) military personnels (Roberts, 2009) and prison inmates. In the community, MRSA tends to affect younger, healthier people such as college students compared to other groups of people (Rohde, 2009; Morita, 2007). MRSA strain is of concern not only because of its resistance to methicillin but also because of its general resistance to many other chemotherapeutic agents (Vidhani *et al.*, 2001). Resistance in MRSA is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin binding protein called PBP2a or PBP21. Penicillin-binding proteins are

targets for beta-lactam antibiotics. PBP2a has a decreased affinity for binding beta-lactam antibiotics resulting in resistance not only to methicillin but also to all beta-lactams including penicillins and cephalosporins (Weems, 2001; Onanuga *et al.*, 2005). MRSA continues to be a major cause of serious infection to man, both in hospitals and in the community. Living in crowded household and having household members with MRSA-colonized persons have been found to increase the risk of becoming infected by MRSA (David *et al.*, 2010; Stevens, 2010; Boubaker, 2004). Students can be infected by MRSA by a household member who is infected or a carrier through person to person contact, contact of surfaces, or contaminated household items, and become a carrier. Infection with MRSA among students can lead to a variety of sequelae, including ventilator-associated pneumonia, chronic wound infection, bloodstream infection (bacteremia), and septic conditions, which in some circumstances can lead to death and to some extent is transferable among students and university staffs. MRSA infections has also been known for its morbidity, long hospital stay, difficult to treat infections, and increased cost of treatment.

The emergence of MRSA has posed a serious therapeutic challenge in the treatment of *S. aureus* infections by β -lactam and related group of antibiotics. Moreover, the overall incidence of MRSA isolation is gradually increasing. There are reports on the prevalence of HA-MRSA in some parts of Nigeria (Adeiza *et al.*, 2020; Obajuluwa *et al.*, 2015; Udobi *et al.*, 2013), but only few reports exist on nasal carriage of MRSA among students, among which are: 61.8% from undergraduate students of a private university in Ogun State (Alli *et al.*, 2022); 32.7% from healthy students of University of Jos (Olorunfemi *et al.*, 2021); 4% from medical students in a private university in Ogun State (Ajani *et al.*, 2020); 22.6% among staff and students of Faculty of Pharmaceutical Sciences, Nnamdi Azikwe University Awka (Ike *et al.*, 2016). No case of MRSA among students in Kaduna State has been reported. Therefore investigation, documentation and understanding of the prevalence of MRSA infections in different parts of Nigeria are of great importance. These will control indiscriminate and irrational use of antibiotics and will be useful for implementation of measures to control the spread of MRSA in hospitals and community. Therefore the objective of this study was to investigate the prevalence and antibiogram of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage among apparently healthy university staff and students in Kaduna, Nigeria.

MATERIALS AND METHODS

Ethical Consideration: Ethical approval was given by the Health Research Ethics Committee (HREC) of Kaduna State Ministry of Health after reviewing the research protocol (Approval number: NHREC/17/03/2018).

Informed consent: Informed consent was obtained from each of the participants after adequately informing them about the background and the objective of the study. The confidentiality and privacy of the patients was preserved throughout the study period. Questionnaires were also administered.

Inclusion/Exclusion criteria: Staffs and students who were on antibiotics therapy or had used antibiotics

within the last four weeks of the sampling time were excluded from the study.

Study Area: The study area is Kaduna State University (KASU), it was officially established on the 24th of May, 2004. It is located at Tafawa Balewa way Kaduna, Kaduna State. Kaduna is about 162 km away from the Federal Capital Territory (FCT) Abuja and 234 km away from Kano, the capital of Kano state. Tafawa Balewa is located on latitude 10.44565N and longitude 7.4565N.

Sample collection: A total number of 250 nasal swab samples were collected from staffs and students of KASU. The detailed sampling according to the category/gender and the distribution according to Faculty is presented in Table 1.

Table 1: Distribution of respondent according to gender and Faculties

Faculty	Number of students tested			Number of staff tested		
	Male	Female	Total	Male	Female	Total
Pharmaceutical science	15	25	40	4	6	10
Science	24	16	40	5	5	10
Social science	18	24	42	3	5	8
Art	26	18	44	4	2	6
Medicine	30	18	48	2	0	2
Total	113	101	214	18	18	36

Collection of Sample and Pretreatment: A total of 250 nasal swab samples were collected from students and staff of Kaduna State University using sterile swab stick moistened with sterile normal saline. The samples were transferred to Pharmaceutical Microbiology Laboratory of the Faculty of Pharmaceutical Sciences, Kaduna State University in an ice pack. Each sample was transferred to sterile nutrient broth and incubated for 24 hours at 37°C. Growth from the overnight cultures was streaked on Mannitol salt agar and incubated at 37°C for 24 hours.

Isolation and Identification of *Staphylococcus aureus*: Colonies that grew on mannitol salt agar plates with golden yellow and smooth edge or surface was sub-cultured in nutrient agar. Gram staining was done in accordance with standard Gram staining procedure (Cheesbrough, 2006), and microscopic examination carried out. Catalase test, coagulase test, indole, oxidase test and sugar fermentation (lactose, fructose and glucose) were carried out according to Cheesbrough (2006).

Determination of MRSA: Cefoxitin disc (30µg) which had been described as surrogate marker for detection of *mecA* gene was used in this study. Sterile Mueller Hinton petri dishes were inoculated with a standardized overnight culture *S. aureus* equivalent to McFarland standard. Cefoxitin discs were aseptically

placed on each inoculated petri dishes, diffusion period was allowed and the plates were incubated at 37°C for 24 hours. Inhibition zones diameter were measured and interpreted as in the guideline recommended by CLSI (2018). Inhibition zone diameter < 21 mm was reported as MRSA and >21 mm as methicillin sensitive *S. aureus* (MSSA. (CLSI, 2018).

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was carried out on each purified *S. aureus* isolates using CLSI modified disc-diffusion method as described by Cheesbrough (2006). The sterile Mueller Hinton agar plates were inoculated aseptically by the standardized (0.5 Mc Farland) inoculums of each isolate (approx. 10⁸ CFU/ml) using sterile cotton swabs. Sterile forceps was thereafter used to place the antibiotic discs in a circular pattern on the media. The process was carried out for all the identified isolates, the plates were kept on the bench for about 30 minutes to allow the antibiotics to diffuse into the agar, the plates were thereafter incubated at 37°C for 24 hours. After incubation, the zone of inhibition in diameter for each antibiotic was measured and interpreted as either sensitive, intermediate or resistant according to CLSI guidelines (CLSI, 2018). The following antibiotics were tested: Pefloxacin 10µg, Gentamicin 10µg, Amoxicillin 30µg, Ceftriazone 30µg, Ciprofloxacin 10µg,

Streptomycin 30µg, Cotrimaxazole 30µg, and Erythromycin 10µg.

RESULTS AND DISCUSSION

Health history of the sampled staffs and students showed that 148 of the respondents had used antibiotics in the past 3 months (Figure 1). They either sourced their drugs from open market, pharmacy stores or patent medicine stores (Figure 2). Out of the 250 samples collected, 41 (16.4%) were identified as *S. aureus* with Faculty of Science having the highest occurrence 13 (26%) as presented in Table 2.

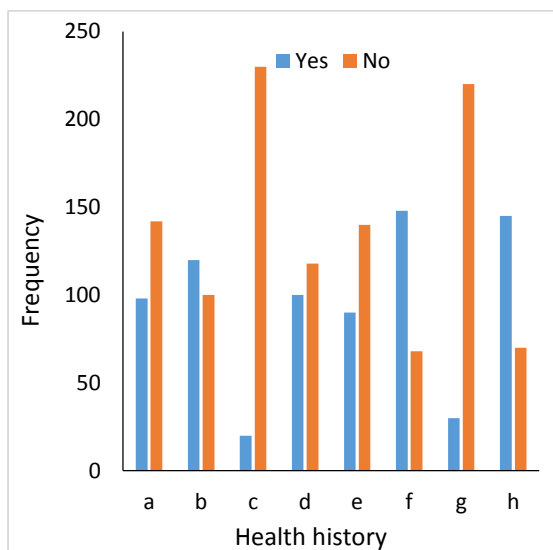


Figure 1: History of health of Respondents
 a = History of chronic disease; b = Hospital attendance in the past 12 months; c = Had surgery in the past 12 months; d = History of boil or skin infection in the past 12 months; e = History of contact with health worker in the past 12 months; f = Used antibiotics in the past 3 months; g = Asthmatic; h = History of respiratory disorder or infection

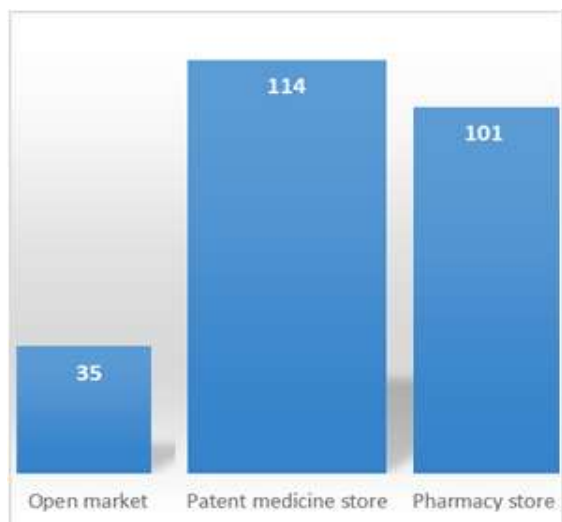


Fig 2: Respondents' source of drugs

Out of the 41 *S. aureus* isolates obtained, 25(61.0%) were methicillin resistant *Staphylococcus aureus* and 16(39.0%) were methicillin sensitive *Staphylococcus aureus*. The distribution of MRSA among staffs and students of KASU according to gender and Faculties are shown in Tables 3 and 4. The results of the antibiotics susceptibility of the MRSA isolates showed that gentamicin was the most active (84%), followed by ciprofloxacin (72%). The MRSA isolates were highly resistant to amoxicillin (80%), (Table 5). The MRSA isolates that showed resistance to at least one antibiotic from 3 or more antimicrobial classes were classified as multidrug resistant, that is 12/25 (48%). The details are presented in Table 6.

Table 2: Distribution of *Staphylococcus aureus* according to different Faculties

Faculty	Number of students sampled	Number of staff sampled	<i>Staphylococcus aureus</i>
Pharmaceutical sciences	40	10	6(12%)
Science	40	10	13(26%)
Social sciences	42	8	7(14%)
Art	44	6	5(10%)
Medicine	48	2	10(20%)
Total	214	36	41(16.4%)

Table 3: Distribution of Methicillin resistant *Staphylococcus aureus* among staff and students of Kaduna State University according to gender

Category	Number of <i>S. aureus</i> isolates	Methicillin resistant <i>Staphylococcus aureus</i>
Male students	13	9(69.2%)
Female student	23	12(52.2%)
Male staff	3	2(66.6%)
Female staff	2	2(100%)
Total	41	25(61.0%)

Table 4: Distribution of Methicillin resistant *Staphylococcus aureus* (MRSA) among staff and students from different faculties

Faculty	No of <i>S. aureus</i> isolates from students	No of <i>S. aureus</i> isolates from staff	MRSA
Pharmaceutical sciences	4	2	4
Science	12	1	9
Social sciences	7	0	4
Art	4	1	2
Medicine	9	1	6
Total	36	5	25

Staphylococcus aureus has long been recognized as a major human pathogen responsible for a wide range of infections, ranging from mild skin infections to nose and ear infections. Out of the 41 isolates of *S. aureus* investigated for methicillin resistance in this study, only 25 (61.0 %) isolates were phenotypically confirmed to be MRSA. This is very high compared to

previous similar studies conducted both within and outside Nigeria. The prevalence rates of MRSA as reported by some researchers in different parts of Nigeria include, a study by Iroha *et al.*, (2015) on prevalence of methicillin resistant *Staphylococcus aureus* among apparently healthy students in Afikpo, Ebonyi state, 43.4% prevalence was reported; In a study by Azeez *et al.*, (2008) on distribution and antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates in University of Calabar, 37.5% prevalence was reported. In a study by Ogefere and Ogunleye, (2019) on prevalence of MRSA among apparently healthy students attending tertiary institution, 37.2 % prevalence was reported. In Imo state, southeast Nigeria, Amadi *et al.* (2013) reported a prevalence rate of 27 % isolates of *S. aureus* from nasal specimen that were methicillin resistant.

Table 5: Percentage antibiotic susceptibility of Methicillin resistant *S. aureus* isolates from staff and students of Kaduna State University (KASU)

S/N	Antibiotics	Resistant (%) n= 25	Intermediate (%)	Sensitive (%)
1.	Pefloxacin 10µg	6(24.0)	6(24.0)	13(52.0)
2.	Gentamicin 10µg	2(8.0)	2(8.0)	21(84.0)
3.	Amoxicillin 30µg	20(80.0)	-	5(20.0)
4.	Ceftriazone 25µg	5(20.0)	4(16.0)	16(64.0)
5.	Ciprofloxacin 10µg	4(16.0)	3(12.0)	18(72.0)
6.	Streptomycin 30µg	8(32.0)	3(12.0)	14(56.0)
7.	Cotrimaxazole 30µg	13(52.0)	7(28.0)	5(20.0)
8.	Erythromycin 10µg	8(32.2)	1(4.0)	16(64.0)

Our study however observed a comparatively higher MRSA among students of Faculties of Science and Medicine in comparison with other Faculties. Though reasons for this may not be very clear, medical students usually have compulsory postings in the hospital, thereby increasing their risk of exposure to these resistant bacterial strains among patients, hospital items, and specimens. A report study by Alli *et al.*, (2022) also had a higher prevalence of MRSA (50%) from medical students of a private university in Ogun State, Nigeria compared with other faculty students. A previous study in Thailand which evaluated carriage rate of *S. aureus* among students in preclinical classes by collecting nasal swabs prior to working in the hospital (the first), following the first rotation (the second) and at the end of the rotation schedule in the hospital (the last) observed an increasing carriage rate of 29.7%, 30.5% and 39.4%, respectively (Treesirichod *et al.*, 2014). This may

explain the higher prevalence observed among medical students in this study.

Table 6: Antibiotics resistance pattern of Methicillin resistant *S. aureus* isolated from sample collected from staffs and students of Kaduna State University (KASU)

S/N	Isolates code	Antibiotic Resistance Pattern	NART	NCART	CR
1.	M4A	AM, S, E	3	3	MDR
2.	M14A	AM, SXT, PEF	3	3	MDR
3.	M16B	AM, PEF, SXT, S	4	4	MDR
4.	M19B	AM, CPX, S, SXT	4	4	MDR
5.	M1C	SXT, AM	2	2	No MDR
6.	M9C	AM, SXT, E	3	3	MDR
7.	M18D	AM, SXT, S	3	3	MDR
8.	M9E	AM, S	2	2	No MDR
9.	F29E	AM	1	1	No MDR
10.	F1A	PEF, AM, R, SXT, E	5	4	MDR
11.	F10A	R	1	1	No MDR
12.	F17A	AM, PEF	2	2	No MDR
13.	F24A	E, AM, CPX	3	3	MDR
14.	F33A	AM, CPX, E, SXT	4	4	MDR
15.	F3B	AM, E	2	2	No MDR
16.	F3C	AM, R, SXT, S	4	3	MDR
17.	F5C	SXT	1	1	No MDR
18.	F24C	AM, R	2	1	No MDR
19.	F24D	AM, CPX, SXT	3	3	MDR
20.	F13E	CN	1	1	No MDR
21.	F21E	S, E	2	2	No MDR
22.	S4B	AM, SXT	2	2	No MDR
23.	S6B	PEF, AM	2	2	No MDR
24.	S17D	CN	1	1	No MDR
25.	S3E	SXT, AM, R, E, PEF, S	6	5	MDR

Key: NART= Number of Antibiotics Resistance; NCART= Number of Class of Antibiotics Resistance; CR= Classification of resistance; MDR= Multidrug resistance; Pefloxacin 10µg – PEF, Gentamicin 10µg –CN, Amoxicillin 30µg –AM, Ceftriazone 25µg –R, Ciprofloxacin 10µg –CPX, Streptomycin 30µg –S, Cotrimaxazole 30µg –SXT, Erythromycin 10µg –E

Also, the alarming high MRSA prevalence observed in Faculty of Science students in this study might be due to the fact that several of these students are living in overcrowded environments, also the lecture room is most times congested with no adequate ventilation. Thus, having members colonized with MRSA will increase the risk of others becoming colonized. Colonization has also been reported as an important step in the chain of events that lead to *S. aureus* infections (Ugwu *et al.*, 2015; Chih-Jung *et al.*, 2011). 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 (Alaklobi *et al.*, 2015). The anterior nares are the main ecological niche for *Staphylococcus aureus* and high prevalence of methicillin resistant *Staphylococcus aureus* was observed may also be due

to activities like nose picking. So, transmission occurs mainly through person-to-person contact as the nose, ear and open skin areas are considered the most important sites for colonization.

The high prevalence of MRSA observed in this study might be related to the abuse of antimicrobial agents by the students and staff of Kaduna State University where the study was conducted as well as possible poor personal hygiene amongst the students under study. Another possible reason for the high prevalence might be due to the fact that people often practice self-medication as a way of meeting some of their primary health care needs, 14% of the respondents agreed that they practice self-medication while 22% seek medical advice from friends and relation. In some cases; physicians adopt to blind treatment prior to getting the susceptibility test result of their sick patients; and this phenomenon together with the acquisition of antibiotics over-the-counter (OTC) even without a doctor's prescription might lead to pathogenic bacteria (including *S. aureus*) developing resistance to these drugs over time. From the antibiotic sensitivity test carried out, gentamicin (84.0%) was the most active against the MRSA isolates followed by ciprofloxacin (72.0%), erythromycin (64.0%), ceftriaxone (64.0%) and streptomycin (56.0%). The susceptibility profile of MRSA to gentamicin in this study is similar to a previous study in Benin City on prevalence of MRSA among apparently healthy students attending tertiary institution where 89.1% susceptibility to gentamicin was reported and also for Erythromycin (52.7%) and ceftriaxone (5.5%) (Ogefere *et al*, 2019). Similarly, in a study by Iroha *et al*, (2015) on prevalence of methicillin resistant *Staphylococcus aureus* among apparently healthy students in Afikpo, Ebonyi state, 82.0% susceptibility to gentamicin, 61.0% to ciprofloxacin and 58.0% to pefloxacin was observed. The highest rate of resistance in the MRSA isolates was observed in amoxicillin (80.0%) followed by cotrimaxazole (52.0%). However, streptomycin and erythromycin showed less resistance (32.0%) each compared with amoxicillin. The high resistance level of the MRSA isolates to amoxicillin observed in this study might be connected with the fact that amoxicillin was mostly used by the respondents in the last 3 months before this study and the increasing rate of availability of cheap different brands of generic amoxicillin in the market which might have probably led to misuse of it (Christopher *et al*. 2013). Methicillin-resistance in staphylococci is strongly associated with resistance to beta-lactam antibiotics (Abadi *et al.*, 2015; Ibadin *et al.*, 2017). The increasing frequency of drug resistance has been attributed to combination of microbial characteristics, selective pressure of antimicrobial use and societal and

technological changes that enhance the transmission of drug resistant organisms (Orozova *et al.*, 2008). The reason for the high resistance to antibiotics may also be due to increase in an irrational consumption rate, transmission of resistant isolates between people, self-medication and non-compliance with medication and sales of substandard drugs.

The increasing prevalence of MRSA bacteria and their associated wide spectrum of resistance to some commonly used antibiotics (as reported in this study) are of public health concern; and this calls for effective measures including public enlightenment to reduce irrational use of antibiotics and detection of MRSA bacteria from clinical and environmental samples in order to contain the emergence and spread of such pathogens. *Conclusion:* The presence of methicillin-resistant *S. aureus* among University staff and students in Kaduna is a potential serious health hazards to the University community. The high prevalence of MRSA means an extensive distribution of the organism in the nostrils of these staff and students. These MRSA isolates were highly resistant to beta lactam class of antibiotics especially amoxicillin but greatly susceptible to gentamicin. The spread of high resistant strains maybe attributed to the wide use of antibiotics among the staff and students.

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