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Incidence of Multidrug Resistant (MDR) *Staphylococcus aureus* Isolated from Urban Population and Private Health Clinics in the Federal Capital Territory; Abuja, Nigeria

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ABSTRACT

This study was carried out to ascertain the distribution of *Staphylococcus aureus* around satellite towns of Abuja, which is the Nigerian capital territory. A survey of their susceptibility profile to commercially available antibiotics was also carried out. Six hundred and ninety-seven (697) individuals were examined, 78(14.63%) were positive for *Staphylococcus aureus* based on growth morphology. With respect to sex, females (17.03%) were more predisposed to contracting the infection than males (11.31%). Both the males and the females' aged ≤10-30yrs had the highest infection rate of 12.87-33.33% and 20.0-33.33% respectively. The least infected persons were aged ≥ 50 yrs (10.34%) in males and 41-50yrs (7.55%) in females. Statistically, age was a determining factor in the distribution of infection (Cal. χ^2 df.4 =24.6952 >Tab. χ^2 = 0.711 (Significant, p< 0.05). The socio-demographic data of individuals showed that persons with no formal education 17(36.17%; 32.53 – 39 – 81) and artisans (22.22%), had higher infection rate. The highest percentage occurrence obtained of *S. aureus* was from ear, HVS and wound swabs 28%, 27%, and 17%, respectively. *S aureus* was more common during the rainy season 31(18.02%; Confidence Interval: 15.11 – 20.93). *Staphylococcus aureus* prevalence was high in symptomatic and healthy participants 12.50%: 33.97% respectively. Though all study sites had *S. aureus*, Nyanya had the highest prevalence, 32.5% and Wuse had the lowest 7%. Antibiogram of the 18 *Staphylococcus aureus* isolates was determined. The resistance profile of the isolates was Cotrimoxazole (100%) followed by Augmentin (77.78%), then Streptomycin (66.67%) and Chloramphenicol (66.67%) respectively. In addition, the antibiotic resistance profile of isolates was examined against 11 frequently used antibiotics, from which the list if 11 (representative of many antibiotic classes), were chosen and analyzed to determine the multiple antibiotic resistance (MAR) indices of isolates. The MAR values obtained ranged from 0.91(Highest) to 0.09(lowest).

INTRODUCTION

Staphylococcus aureus is recognized as one of the most important bacterial pathogens (Gupta *et al.,* 2013) seriously contributing to the problems of a hospital and community-acquired infections all over the world (Vysakh and Jeya, 2013). The name *Staphylococcus* comes from the Greek word 'Staphyle', meaning a bunch of grapes, and ‘kokkos', meaning berry. They are Gram-positive, facultative anaerobic, usually unencapsulated cocci). *S. aureus* is a common skin and nasopharynx commensal, a frequent causative agent of wound sepsis. It produces pustules, carbuncles, and otitis and is also a common causative agent of infection in hospitals most liable to infect newborn babies, surgical patients, old and malnourished persons, diabetic and chronic disease patients (Chan *et al.,* 2011; Boyanova and Mitov, 2013). *Staphylococcus aureus* is a common cause of infection in people. In recent years, experts have become very concerned about the increased incidence of strains of the bacteria that fail to succumb to all but a few antibiotics. This is known as antibiotic resistance, and most experts think that it is due to the worldwide overuse of antibiotics (Alumran*et al.,* 2013). Until a few years ago, only nosocomial acquired isolates were found to show such resistance but later on, even community-acquired strains have shown such antibiotic resistance (Chan *et al.,* 2011). In addition, several investigations have been conducted to study the carriage rate and the antimicrobial resistance pattern of *S. aureus* (Junaid*et al.,* 2006; WHO, 2012) have reported that multidrug-resistant *S. aureus* exists in many locations in Nigeria. Chigbu and Ezeronye (2003) have reported as high as 80% *S. aureus* prevalence in Abia state, Nigeria. Iroegbu*et al.* (1997) reported the antibiotic sensitivity pattern of nasal isolates during the *S. aureus* surveillance in Nsukka, Nigeria. The results showed a sensitivity of 30.9% - 58.6% to Penicillin, Ampicillin, Tetracycline, and Chloramphenicol.

Due to the indiscriminate usage of antibiotics by individuals and resistance by *S. aureus* to these antibiotics, the over-crowding in many emerging slums around most developing cities in Nigeria is ensuring the spread *S. aureus*(Okeke*et al.*, 1999; Onwuliri*et al.,* 2006). The challenges of hygiene are becoming a concern in Abuja Municipal Area, where incessant demolition of illegal structures and the exorbitant accommodation cost in main cities and Satellites have forced many residents to the suburbs. Many of these suburbs are becoming crowded with poor environmental waste disposal and management systems. This is unhygienic and creates breeding sites for bacteria.

This study was aimed at ascertaining the prevalence and multidrug resistance indices of *Staphylococcus aureus* isolated from Abuja using commercially available antibiotics.

MATERIALS AND METHODS

Sample collection

Clinical samples were collected from In/Outpatients who were attending hospitals within the FCT (Abuja) to access medical care. Specimens were obtained from wound, urine, skin, ear and high vaginal swabs, as these are potential sources of *S. aureus*. All samples were collected in sterile containers and processed aseptically in Biosafety Class II level cabinet.

Bacteriological analysis

Laboratory analyses were carried out in Departments of Microbiology and Biotechnology of the National Institute for Pharmaceutical research and development (NIPRD) Abuja.

Using streak plate method, specimens were cultured on to Mannitol Salt agar plate medium. Incubation was carried out at 37°C for 24 hours. The cultures were observed after 24 hours. (Cheesbrough, 2006).

Morphological identification and biochemical analysis

Standard Bacteriological methods including Gram staining were employed for specific identification of *Staphylococcus aureus*. Biochemical tests employed include *Coagulase* test and *Catalase* test.

Antibiogram and multidrug resistance indices

Sensitivity tests were carried out to determine the antibiotic susceptibility of isolated *S. aureus* strains, using conventional antibiotics; which were mainly from the Penicillin's, aminoglycosides and glycopeptide groups using the disc diffusion test (Kirby-Bauer sensitivity test).

The isolates were enriched for 8hrs in Peptone water broth. The enriched cultures were aseptically streaked on Mueller Hinton agar plates and the antibiotic discs placed on the agar surface. After 24 hours incubation, the inhibition Zones were recorded and resistance data

tabulated. A zone size interpretation chat was used to determine the resistance pattern. (Iroha et al., 2012)

The determination of sensitive, immediate or resistant isolates depends on the zone of growth inhibition diameter of CSLI breakpoint. *Staphylococcus aureus* (ATCC25932) standard strain was included in each batch analysis as the control strain. Methicilin resistance expression was determined by disk diffusion method, using both oxacillin and cefoxin discs. Antibiotic discs (Becton Dickson and Company, Sparks USA), were placed at least 15 mm apart and from the edge of the plates to prevent the overlapping of the inhibition zones. The antibiotics used were Gentamicin (GEN), Sparfloxacin (SPA), Ciprofloxacin (CIP), Ofloxacin (OFX). Perflacine (PEF), Augmentin (AU), Streptomycin (ST), Amoxicillin (AMX), Cotrimoxazole (SXT) and Chloramphenicol (CHL). The susceptibility of the various isolates, including the control to the antibiotics, was determined. The plates were incubated at 37^{o}C for 24hrs, and the diameters of zones of inhibition were determined and compared with recorded diameters of the control organism *Staphylococcus aureus* ATCC25922 that was obtained from the stock culture collection of the National Veterinary Research Institute, NVRI, Vom Plateau State Nigeria.

DETERMINATION OF MULTIDRUG RESISTANCE INDICES

Multiple antibiotic resistance indices of all strains were ascertained. The MAR index was applied to a single isolate as defined by the relationship between numbers of antibiotics to which an isolate is resistant compared to all the antibiotics used according to (Cheesbrough, 2006). MAR was calculated based on 10 most common antibiotics used in the treatment of *S. aureus* around the study communities.

Multidrug resistance (MDR) index

$$MAR = a/b$$

Where a = number of antibiotics to which the isolate was resistant to

b = number of antibiotics to which the isolate was subjected

RESULTS

Table 1: Distribution of *S. aureus* in The Study Population-Based On Age Group of Study Subjects

Age Group	Male	Female	Total	
	No infected/ (%) positive	No infected /(%) positive	No infected/(%) positive	Confidence Interval (95% CI)
≤10 – 20 (n=11)	2(33.33)	1(20.00)	3(27.27)	23.41 – 31.13
21 – 30 (n=184)	13(12.87)	25(33.33)	38(20.65)	17.14 – 24.16
31 – 40 (n=193)	8(11.59)	15(12.10)	23(11.92)	9.11 - 14.73
41 – 50 (n=95)	5(11.90)	4(7.55)	9(9.47)	6.93 – 12.01
≥ 51 (n=50)	3(10.34)	2(9.52)	5(10.00)	7.40 – 12.60
Total (n=533)	31(11.31)	47(17.03)	78(14.63)	11.57 – 17.69

Cal. χ^2 df.$_4$ =24.6952 >Tab. χ^2 = 0.711 (Significant, p< 0.05)

Table 2: Educational Status of Study Subjects

Type of Education	No Screened	No Positive (%)	95% C.I
No formal education	47	17(36.17)	32.53 – 39. 81
Primary	268	21(7.84)	5.80 – 9.88
Secondary	293	20(9.89)	7.63 – 12.15
Tertiary	89	11(12.40)	9.90 – 14.90

Table 3: Distribution of *S. aureus* in the Study Population Based On Occupational Distribution inStudy Participants

Occupation	No Screened	No Positive (%)	95% C.I
Civil servant	121	7(5.79)	3.95 – 9.67
Student	79	9(11.39)	8.32 – 16.26
House wife	38	12(31.58)	19.91 – 35.83
Unemployed	72	11(15.28)	13.75 – 19.07
Force men	34	5(14.71)	11.30 – 17.94
Trading	114	11(9.65)	7.17 – 13.41
Farming	23	6(26.09)	17.52 – 28.26
Driving	22	6(27.27)	18.26 – 29.10
Artisan	9	4(44.44)	39.07 – 47.37
Unknown	21	7(33.33)	29.30 – 37.98
Total	**533**	**78(14.63)**	**11.57 – 17.69**

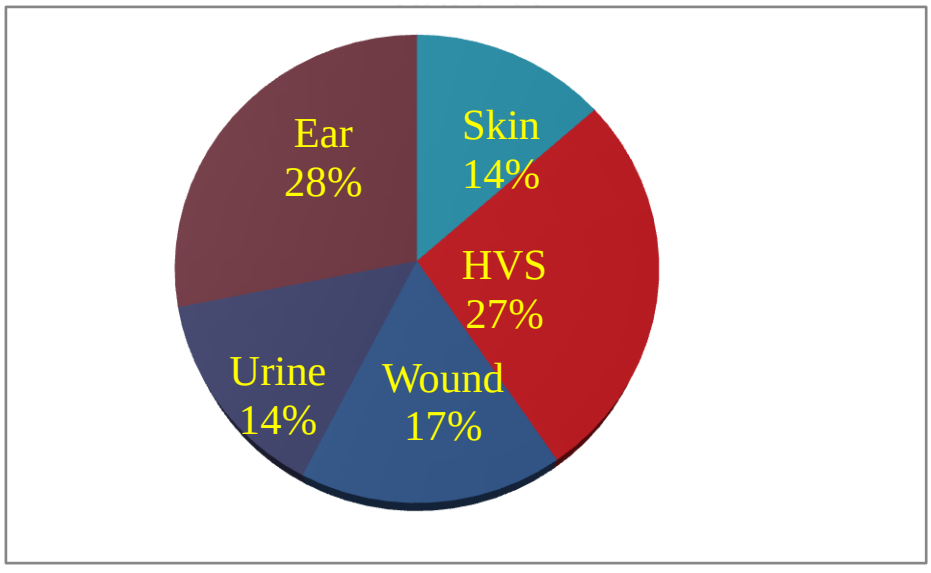

Figure 1: Occurrence of *Staphylococcus aureus*in different clinical specimens in study subjects

Key: HVS: High vaginal swab

Table 4: Seasonal Prevalence of *Staphylococcus aureus in* Residents of Federal Capital Territory, FCT Abuja

Season	Number samples	Number infected	Percentage (%)	Confidence interval (95% CI)
Early Rainy season	174	10	5.75	3.99 – 7.51
Rainy Season	172	31	18.02	15.11 – 20.93
Early dry season	188	19	10.11	7.83 – 12.39
Dry Season	163	18	11.04	8.67 – 13.41

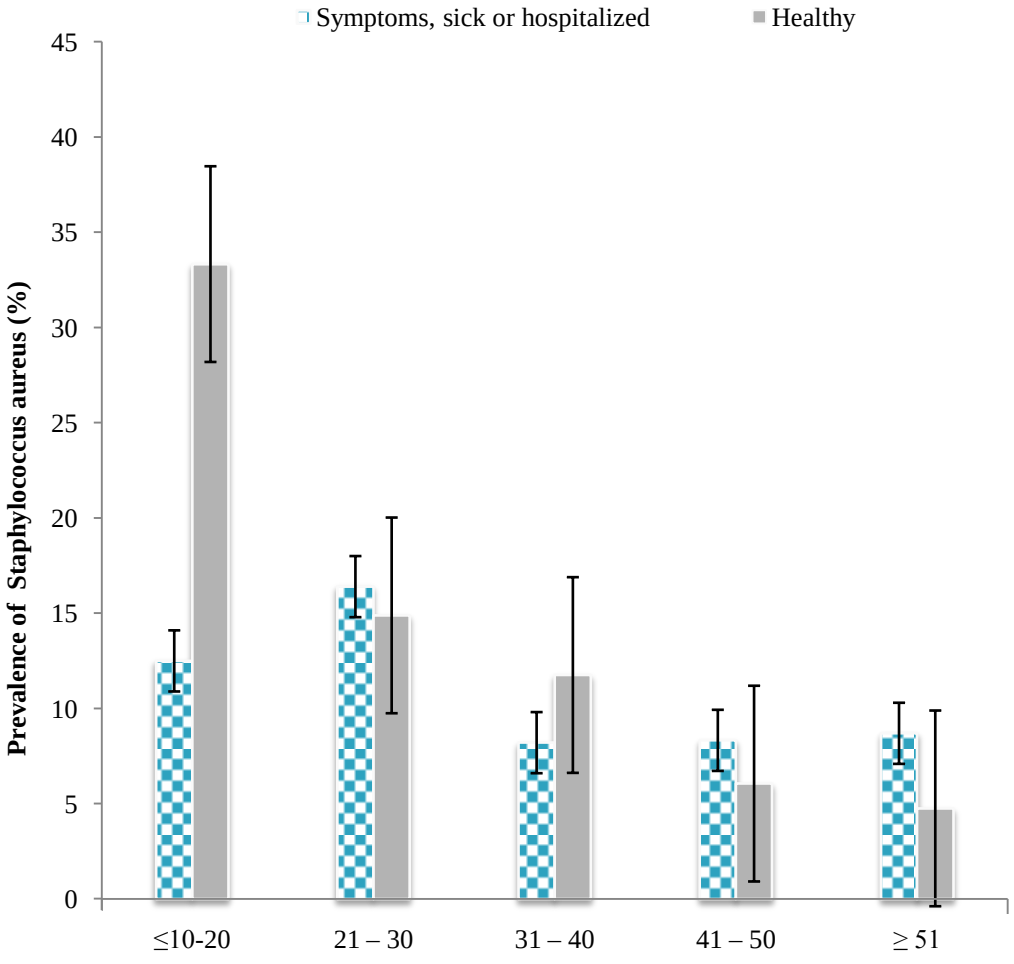

Fig. 2: Prevalence of *Staphylococcus aureus* in relation to the health status of study participants

Table 5: Distribution of *S. aureus*with respect to location

Location	Incidence of *Staphylococcus aureus*
Nyanya	30
Kubwa	28
Bwari	14
Gwagwalada	10
Kuje	10
Wuse	8

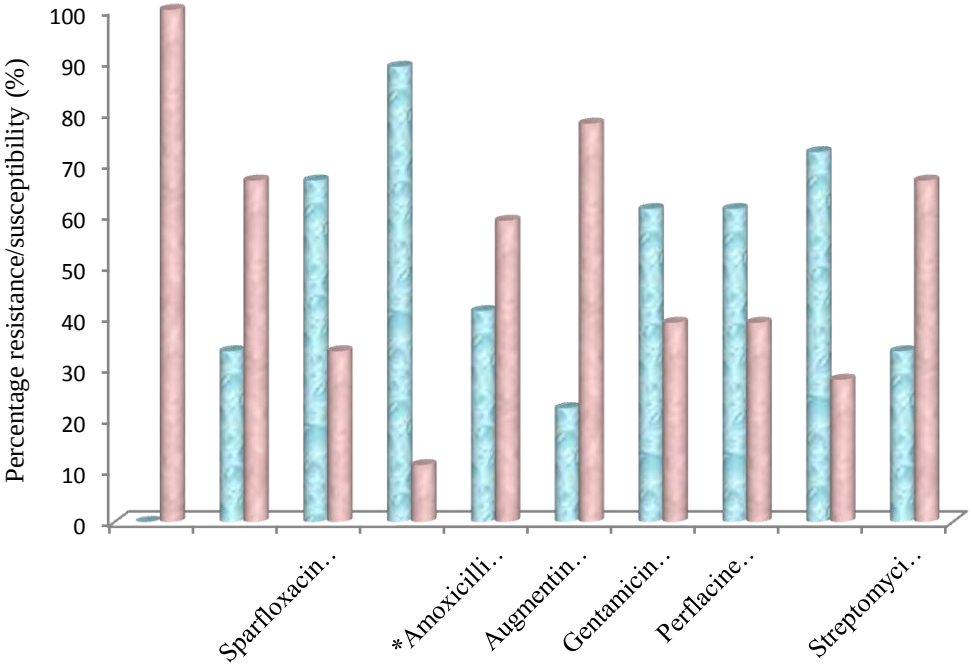

Fig. 3: Antibiogram of circulating *S. aureus* isolates from Abuja

Table 6: Antibiotics Resistance and Multiple Antibiotic Resistance (MAR) Index of Circulating *S. aureus* Isolates in the Study Population

***S. aureus* isolate (ID)**	**Degree of resistance (No of antibiotics)**	**Antibiotics isolate is resistant to**	**MAR Index**
Isolate – 11	1	CIP	0.09
Isolate – 17	2	CIP OFX	0.18
Isolate - 07, 12, 15, 26	3	SPA, CIP, OFX	0.27
Isolate - 03, 04, 14, 18, 24	4	SPA, CIP, GEN, OFX	0.36
Isolate - 01, 08, 21	5	SPA, CIP, GEN, OFX, AMX	0.45
Isolate - 09, 10	8	SXT, OFX, CIP AMX, CHL, SPA, GEN, ST	0.73
Isolate - 06, 22	9	OFX, SPA GEN AMX, CHL,AU, PEF, ST SXT	0.82
Isolate – 05	10	SXT, CHL, SPA, CIP , AMX, AU, GEN, PEF, OFX, ST	0.91

Key: SXT= Cotrimoxazole, CHL= Chloramphenicol, SPA= Sparfloxacin, CIP = Ciprofloxacin, AMX=Amoxicillin, AU= Augumentin, GEN= Gentamicin, PEF = Perflacine, OFX = Ofloxacin, ST = Streptomycin

MAR = a/b

Where: a = number of antibiotics to which the isolate was resistant

b = number of antibiotics to which the isolate was subjected to.

DISCUSSION

In this study, a total of 697 persons, revealed an occurrence of 78(14.63%) *Staphylococcus aureus* based on growth morphology. This is consistent with National Nosocomial Infections Surveillance System (Saana*et al.,* 2013)**.** Diekema*et al.,* 2001, noted that *Staphylococcus aureus* causes a diverse spectrum of infections in humans, ranging from superficial skin infections, bone and joint infections, septic shock, bovine and bovine mastitis. It has also been reported as the leading cause of bloodstream, lower respiratory tract, skin/soft-tissue infections and serious infections including pneumonia, bacteremia, and endocarditis (Lowry, 1998; Feil*et al.*, 2003; Saana*et al.*, 2013). Komolafe and Adegoke (2008) also reported that *S. aureus* is responsible for worsening of some already existing superficial infections; which include boils (skin abscess), impetigo (pus-filled blisters on the skin), styes, pneumonia, osteomyelitis, acute bilateral endocarditis and scalded skin syndrome in very young children that causes skin to strip off (denude). It is agreed that *S. aureus* is one the most important human pathogens largely due to its ubiquitous occurrence as a colonizer in humans, domestic animals, and livestock (Morgan, 2008; Gupta *et al.*, 2013). Between 25% and 35% of healthy human carry *S. aureus* on the skin or mucous membranes and are the primary source of infection in hospitals (Wertheim*et al.,* 2005).

In relation to socio-demographic factors, the participants' sexually active population age group, especially females, had higher *S. aureus* 47(17.03%) than the males 31(11.31%). This may be due to more work and risk exposures or sexual activities since the reproductive age are more prone to many reproductive tract infections, including *Staphylococcus aureus*. This finding agrees with Patel *et al.,* (2003) and Stanley *et al.,* (2013), who observed sexual activity in young female adults as the major risk to contracting *Staphylococcus aureus* infection and other reproductive tract infections. Specimens linked to individuals with no formal education (36.17%) and artisans (22.22%), revealed higher infection. Poor socio-economic status and low literacy level have been reported in the higher prevalence of *Staphylococcus aureus* infection. This is often under poor hygienic conditions in homes and hospitals. Low income, population density, populated environment, overcrowding, lack of knowledge of the organism aid transmission among in-patients and healthy individuals. This is, however, consistent with Thinkhamrop*et al.,* (2002), who reported poor economic status as a risk factor for contracting urinary tract infections (UTI) and reproductive tract infections caused by *S. aureus*.

The highest numbers of S. aureus isolates were from the ear and HVS; followed by wound with percentage occurrences of 28% and 27% and 17%, respectively; compared to 14% in urine or skin respectively. *S aureus* was more common during the rainy season 31(18.02%). This is low compared to Tula *et al., (*2011) who obtained higher prevalence of *S. aureus* isolation from wounds (23.8%); skin (37.8%) and beds (35.1%). Other studies also reported higher prevalence's of 28.6% and 34.7% in Kano and Ilorin, respectively (Taiwo*et al.,* 2004; Nwankwo*et al.,* 2010). These reports confirm FCT with low prevalence observed in this present study. Tula *et al.,* (2011) who recorded higher prevalence in isolation of *S. aureus* in his study blamed his results to the wrongful identification of other species of *Staphylococcusas S. aureus.*

As high as 62.5% *S. aureus* has previously been recorded in seminal fluids (Okon*et al.,* 2008). Even in developed economies such as the United States (USA), studies have shown that 10%-20% of the general population is persistent carriers of *S. aureus*, while up to 50% are intermittent carriers (Lowy, 1998). Furthermore, carrier rates of 25% were reported among the hospital staff (Haddadin*et al.,* 2002). *S. aureus* is transmitted directly or indirectly through fomites and by inhaling the air-borne droplets (Lowy, 1998).

Prevalence of Antibiotic Resistance in Circulating *S. aureus*

In relation to antibiotic resistance, a high multi-antibiotic resistance of circulating *S. aureus*was observed from their antibiogram. This was highest in Cotrimoxazole, where none (100%) of the isolates was susceptible. 66.67% - 77.78% isolates exhibited high multi-antibiotic resistance to Augmentin, Streptomycin, and Chloramphenicol. This reflects a true Nigerian statistic where multidrug-resistant *S. aureus* have been reported in the hospital and non-hospital population (Chigbu and Ezeronye, 2003). About 80% *S. aureus* are resistant to more than one clinically used antimicrobial agent as reported in Abia State, Nigeria (Chigbu and Ezeronye, 2003). Some other studies in Nigeria have also reported antibiotic sensitivity pattern of 43.3%, 58.6%, 41.4% and 30.9% for Penicillin, Ampicillin, Tetracycline, and Chloramphenicol, respectively (Iroegbu*et al.,* 1997). In the present studies, the least resistance was recorded for Ciprofloxacin (11.11%); where only 2 isolates showed significant resistance to the antibiotics ($p < 0.05$). This means that most *Staphylococcus aureus* is susceptible to Ciprofloxacin antibiotic with 88.89% inhibited by its treatment (16 of 18) isolates challenged. A 65% *S. aureus* sensitivity Perfloxacin (Ciprofloxacin) was reported by Amadi*et al.,* (2007); who used the high sensitivity Perfloxacin suggested better choice in the

treatment of *S. aureus* in the study area. This disagrees 1 in 10 with the 100% sensitivity of *S. aureus* isolates to Perfloxacin (Obi *et al.,* 1996; Chalita*et al.,* 2004). In the same vain Uwaezuoke and Aririatu (2006) reported 85.4% sensitivity of Ciprofloxacin to *S. aureus* strains isolated from Owerri, Nigeria. Similarly, Farzana*et al.* (2004). On the other hand, prior investigations had reported the high level of resistance of *S. aureus* to Ciprofloxacin and this is in line with the result of this study (Chigbu and Ezeronye 2003). The 70.37% resistance of *S. aureus* isolates to Septrin reported in this study is also in conformity with the findings of Astal*et al.*, (2002). A 2-3 in every 5-resistance sensitivities of *S. aureus* isolates to Amoxicillin (58.82%). Uwaezuoke and Aririatu (2006) reported 74% % sensitivity of *S. aureus* isolates to Ampicillin in Abuja, Nigeria. Similarly, Farzana*et al.,* (2004), observed that these variations could most likely be attributable to strain differences. Nevertheless, 73.6% - 100% amoxicillin resistant strains of *S. aureus* were reported (Adewoye and Lateef, 2005). Gentamicin, Perflacine, Ofloxacin, and Sparfloxacin are likely more potent antibiotics, as only 3 of 10 isolates were resistant to circulating antibiotics used.

Multiple Antibiotics Resistance (MAR) Indices of Circulating S.*aureus*

The high and multiple antibiotics resistance index was observed as most isolates of *S. aureus* tested against circulating antibiotics; were resistant to more than half (multiple) as many antibiotics they were exposed. This calls for urgent need for antibiotics surveillance and intensified control measure of antibiotics use. The monitoring of both antibiotic consumption and MAR is necessary for effective containment of this important public health associated infections (Brown, *et al.,* 1991). This is because emerging and rising resistance to newer and otherwise potent antibiotics may compound the whole community problem (Kamat*et al.,* 2008). The 90.91% and 45.46 - 81.81% prevalence obtained for these *S. aureus* isolates is consistent with those workers who reported low resistance profiles for non-quinolones and aminoglycosides (Lilenbaum*et al.,* 1998). This may have implications on the effectiveness of the local antibiotic resistance control and reveals an obvious compromise of antibiotics routinely used in the clinical treatment of many infections in this environment. The clearly visible high rate resistance of isolates to penicillin corroborates documented increasing *penicillinase*-producing *B-lactamases* (Wenzel and Edmond, 2000; Chambers, 2001; Kamat*et al.,* 2008).

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