# Prevalence of methicillin resistant *Staphylococcus aureus* among primary, secondary school students and traders in Ekpoma, Esan West Local Government Area, Edo State.

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## ABSTRACT

The incidence of methicillin resistant *Staphylococcus aureus* (MRSA) among primary and secondary school children and traders in Ekpoma, Edo State was evaluated in this study. One hundred and sixty nasal samples from apparently healthy subjects were screened for MRSA using standard microbiological methods. Results revealed that 60 samples (37.5%) were identified as *Staphylococcus aureus,* 68 (42.5%) as coagulase negative Staphylococci and 32 samples (20.0%) showed no growth. The distribution of MRSA between males and females were 41.7% and 58.3% respectively. The nasal carriage rate of MRSA and methicillin sensitive *Staphylococcus aureus* (MSSA) was high (41.7%) in the age range of 11 – 15 years and low (4.2%) in the age group greater than 40 years. Antibiotics susceptibility pattern of MRSA isolates revealed that gentamicin was 100% sensitive. The strain was also sensitive to erythromycin, streptomycin and pefloacin at 83.3%, 75.0% and 91.7% respectively. The result of this study further stresses the need for good personal hygiene if the infection by MRSA is to be reduced in the community.


**Key words**: Methicillin, Resistance, *Staphylococcus, aureus,* antibiotics, specimen, Susceptibility.

## INTRODUCTION

*Staphylococcus aureus* is a bacterium that colonizes the human skin and nasal passage. *Staphylococcus aureus* has two mechanisms to cause damage to human; active tissue invasion through the building of abscesses, and the release of toxins that can kill cells[1].

Methicillin – resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* which is resistant to methicillin and related penicillins and is particularly difficult to treat because it is also resistant to most other common antibiotics[1]. Although *Staphylococcus aureus* infections were historically treatable with common antibiotics, but the emergence of drug–resistant strains are now a major concern. MRSA was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s[2, 3]. Staphylococci are Gram positive cocci occurring characteristically in groups of clusters but also singly and in pairs. They are non–motile and non–capsulated.[1] *Staphylococcus aureus* is the most medically important member in terms of pathogenicity of the group[4]. *Staphylococcus* is present in the nose of 30% of healthy people and could be found on the skin. It causes infection most commonly at sites of lowered host resistance, through damaged skin or mucous membrane[5]. Although 50–60% of patients with MRSA are merely colonized (i.e. they carry the bacteria but do not have symptoms or an illness), serious infections such as those involving the blood stream, respiratory tract and bones or joints do occur[5]. *Staphylococcus aureus* causes boils, pustules, burns, osteomyelitis, mastitis,


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septicaemia, meningitis and pneumonia. It also causes toxic food poisoning presenting with vomiting, diarrhea but no fever, toxic shock syndrome and toxic skin exfoliation.[1] MRSA are potential source for the spread of nosocomial infections and healthcare workers with a huge cost burden in terms of longer hospital admission, expensive drug treatment as well as effect on the community [6]. This study was carried out to determine the prevalence, distribution and antibiotic susceptibility pattern of MRSA amongst apparently healthy market traders, primary and secondary school students in Ekpoma, Edo State.

## MATERIAL AND METHODS

### STUDY AREA

This study was carried out among primary, secondary school children and traders in Ekpoma markets, Ekpoma, the administrative headquarter of Esan West LGA of Edo State, Nigeria. The area lies between latitudes 6°43' and 6°45' North of the Equator and longitude 6°6' and 6°8' East of the Greenwich Meridian.[7] Ekpoma has a population of 89,680 and 127,718 at the 1991 and 2006 population census respectively (NPCN, 2012), majority of which are civil servants, traders, business men/women, transporters, farmers, teachers/lecturers and students by occupation.

### SAMPLE SIZE

Sample size was determined using the formula reported by Hu and Rosenberg[8]. One hundred and sixty (160) nasal swabs from apparently healthy traders in Ekpoma was used as subjects in this study. Informed consent was obtained from all participants involved in the study after a proper orientation of the basics involved. Infants and toddler (0-4) and adults above the age of 60 years were excluded from the research.

### SAMPLE COLLECTION

One hundred and Sixty (160) nasal swab specimens used were collected randomly from apparently healthy traders in Ekpoma. Nasal swabs were collected in good light vision from subjects bending their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick (moistened with sterile saline). Both right and left nostrils were swabbed bearing labels as swab code number and date of collection. The swab sticks were carefully returned to their sterile containers, sealed with adhesive tape and labeled accordingly. Collected specimen were taken to the laboratory were bacteriological analysis was carried out immediately.

### SAMPLE ANALYSIS

The collected specimens were cultured in mannitol salt agar (selective and differential for cultivation and recovery of pathogenic staphylococci) and nutrient agar. The plates were then incubated at 37°C for 24hours. Growth were observed after incubation. This was followed by Gram staining. Standard biochemical tests (Catalase and Coagulase test) were also carried out to identify *Staphylococcus aureus.*

### ANTIBIOTICS SENSITIVE TEST

The staphylococcal isolates that were positively identified using the culture based methods were subjected to antibiogram characterization. All the bacterial isolates were tested for resistance or sensitivity to different antibiotics using the standard disc diffusion method (Kirby Bauer test). For the disc diffusion assay, bacteria were grown between 18 and 24 h on Mueller-Hinton agar, harvested and then suspended in 0.85% sterile physiological saline solution adjusted to a 0.5 McFarland turbidity standard, corresponding to $10^8$cfu $mL^{-1}$. The inoculum was streaked onto plates of Mueller-Hinton agar using a sterile cotton swab and impregnated with appropriate antibiotics (table 7). The results were recorded after 24 h of incubation at 37 $^{o}$C. commercially available antibiotics discs, obtained from Abtek Biological Ltd were used to determine the resistance patterns of the isolates. The diameter of the zone of inhibition around each disc was measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standard Institute[17].

**RESULTS**

A total of 160 nasal swabs which comprised of 80 males and females each were collected and analysed. 60 (37.5%) were identified as Coagulase positive *Staphylococcus aureus,* 68 (42.5%) as coagulase negative *Staphylococcus* and 32 (20.0%) showed no growth (Table 1). Nasal carriage of coagulase positive *Staphylococcus aureus* was slightly higher in females 32 (20.0%); while the male harboured more of the coagulase negative *Staphylococcus* 44 (27.5%) than the females. Also, more females (15.0%) had no growth as shown in Table 1. Among the various age groups screened, the prevalence of *Staphylococcus aureus* was higher in the age range of 11 – 15 years with (41.7%) prevalence and age ranged 21 – 25 and 36 – 40 years had the lowest (Table 2).

Table 1: Distribution of *Staphylococcus* Species among studied subjects by Sex

| Sex | N | *S. aureus* (%) | CNS (%) | No Growth (%) |
|---|---|---|---|---|
| **Male** | 80 | 28 (35.0) | 44 (55.0) | 8 (10.0) |
| **Female** | 80 | 32 (40.0) | 24 (30.0) | 24 (30.0) |
| **Total** | 160 | 60 (37.5) | 68 (42.5) | 32 (20.0) |
| **P value** | | | <0.05 | |

$X^2$ = 14.15, Degree of freedom = 2, P value = 0.00085, $P<0.05$
N = Total number of isolate
CNS = coagulase negative *Staphylococcus*
% = percentage.

Table 2: Distribution of *Staphylococcus* species among studied subjects by age groups

| Age (Years) | N | *S. aureus* (%) | CNS (%) | No Growth (%) |
|---|---|---|---|---|
| **5 – 10** | 26 | 10 (38.5) | 12 (46.1) | 4 (15.4) |
| **11 – 15** | 48 | 20 (41.7) | 18 (37.5) | 10 (20.8) |
| **15 – 20** | 16 | 4 (25.0) | 9 (56.7) | 3 (12.5) |
| **21 – 25** | 12 | 3 (25.0) | 5 (41.7) | 4 (33.3) |
| **26 – 30** | 26 | 12 (46.2) | 10 (38.5) | 4 (15.3) |
| **31 – 35** | 16 | 4 (25.0) | 8 (50.0) | 4 (25.0) |
| **36 – 40** | 6 | 3 (50.0) | 3 (50.0) | 0 (0.0) |
| **>40** | 10 | 4 (40.0) | 3 (30.0) | 3 (20.0) |
| **Total** | 160 | 60 (37.5) | 68 (42.5) | 32 (20.0) |

$X^2$ = 8.34, Degree of freedom = 14, P value = 0.88, $P>0.05$
N = Total number of isolate

CNS = coagulase negative *Staphylococcus*
% = percentage.

The distribution of *Staphylococcus aureus* amongst study subjects by occupation revealed nasal carriage to be highest among traders for both coagulase observed to be highest among traders 28 (46.7%) as can be seen in table 3. The distribution of MRSA between male and females were 41.7% and 58.3% respectively. This can be seen in Table 4.

Table 3: Distribution of *Staphylococcus* species among studied subjects by occupation

| Occupation | N | *S. aureus* (%) | CNS (%) | No Growth (%) |
|---|---|---|---|---|
| Primary School | 50 | 18 (36.0) | 22 (44.0) | 10 (20.0) |
| Secondary School | 50 | 14 (28.0) | 18 (36.0) | 18 (36.0) |
| Trader | 60 | 28 (46.7) | 28 (46.7) | 4 (6.7) |
| Total | 160 | 60 (37.5) | 68 (42.5) | 32 (20.0) |

P Value <0.05
$X^2 = 15.92$, Degree of freedom = 4, P value = 0.003, $P<0.05$
N = Total number of isolate
CNS = coagulase negative *Staphylococcus*
% = percentage.

Table 4: Distribution of Methicillin-resistant and sensitive *Staphylococcus* species among studied subject by sex

| Sex | MRSA (%), N = 24 | MSSA (%), N = 36 | P value |
|---|---|---|---|
| Male | 10 (41.7) | 18 (50.0) | |
| Female | 14 (58.3) | 18 (50.0) | >0.5 |
| Total | 24 (40.0) | 36 (60.0) | |

$X^2 = 0.402$, Degree of freedom = 1, P value = 0.526, $P>0.05$
N = Total number of isolate
MRSA = Methicillin – Resistant *Staphylococcus aureus*
MSSA = Methicillin – Sensitive *Staphylococcus aureus*
% = percentage.

This distribution of Methicilin – resistant and sensitive *Staphylococcus aureus* among studied subjects by age groups is shown in Table 5. MRSA nasal carrier rate was higher (25.0%) in the age range of 11 – 15 years and lower (4.2%) in above 40 years age groups (Table 5). As can be seen in table 5 also, the peak of nasal carrier rates of both MRSA and MSSA was found in the age groups of 11 – 15 years after which carrier rates decreased progressively with increasing age.

On occupation wise analysis, the nasal carriage rate MRSA and MSSA showed that primary school students have the highest occurrences (Table 6).

Table 5: Distribution of Methicillin-resistant and sensitive *Staphylococcus* species among studied subject by age group

| Age | MRSA (%), N = 24 | MSSA (%), N = 36 | P value |
|---|---|---|---|
| 5 – 10 | 3 (12.5) | 7 (19.4) | |
| 11 – 15 | 6 (25.0) | 14 (38.9) | |
| 16 – 20 | 2 (8.3) | 2 (5.6) | |
| 21 – 25 | 3 (12.5) | 0 (0.0) | |
| 26 – 30 | 3 (12.5) | 9 (25.0) | |
| 31 – 35 | 4 (16.7) | 0 (0.0) | >0.5 |
| 36 – 40 | 2 (8.3) | 1 (2.8) | |
| >40 | 1 (4.2) | 3 (8.3) | |
| Total | 24 (40.0) | 36 (60.0) | |

$X^2 = 14.31$, Degree of freedom = 7, P value = 0.045, $P<0.05$
N = Total number of isolate
MRSA = Methicillin – Resistant *Staphylococcus aureus*
MSSA = Methicillin – Sensitive *Staphylococcus aureus*
% = percentage.

Table 6: Distribution of *Staphylococcus* species among studied subjects by occupation

| Occupation | MRSA (%), N = 24 | MSSA (%), N = 36 | P value |
|---|---|---|---|
| Primary school | 10 (41.7) | 8 (22.2) | |
| Secondary school | 6 (25.0) | 8 (22.2) | |
| Trader | 8 (33.3) | 20 (55.6) | >0.05 |
| Total | 24 (40.0) | 36 (60.0) | |

$X^2 = 33.86$, Degree of freedom = 2, P value = 0.184, $P>0.05$
N = Total number of isolate
MRSA = Methicillin – Resistant *Staphylococcus aureus*
MSSA = Methicillin – Sensitive *Staphylococcus aureus*
% = percentage.

Antibiotic susceptibility patterns of MRSA isolate indicated that Gentamycin had 100% sensitivity. This strain was also sensitive to Erythromycin, Streptomycin and Pefloxacin at 83.3%, 75.0%, and 91.7% respectively. Nonetheless, resistance was observed against Ciprofloxacin (100%), Septrin (100%) and Amoxacillin (70.8%) as shown in Table 7. Similarly, MSSA strain showed a high level of resistant to Ciprofloxacin, Septrin, Rocephin and Amoxicillin. The level of resistance shown by MRSA to other antibiotics when compared with that of MSSA was by far higher as seen in Table 7.

Table 7: Antibiotic susceptibility patterns of *Staphylococcus aureus* isolate

| Antibiotic | MSSA (%), N = 36 | | MRSA (%), N = 24 | |
|---|---|---|---|---|
| | S | R | S | R |
| Erythromycin | 32 (88.9) | 4 (11.1) | 20 (83.3) | 4 (16.7) |
| Gentamycin | 36 (100) | 0 (0.0) | 24 (100) | 0(0.0) |
| Streptomycin | 34 (94.4) | 2 (5.6) | 18 (75.0) | 6 (25.0) |
| Ampiclox | 33 (91.7) | 3 (8.3) | 8 (33.3) (66.7) | 16 |
| Pefloxacin | 17 (47.2) | 19 (52.8) | 22 (91.7) | 2 (8.3) |
| Zinnact | 12 (33.3) | 24 (66.7) | 3 (12.5) (87.5) | 21 |
| Ciprofloxacin | 3 (8.3) | 33 (91.7) | 0 (0.0) | 24 (100) |
| Septrin | 6 (16.7) | 30 (83.3) | 0 (0.0) | 24 (100) |
| Rocephin | 2 (5.6) | 34(49.4) | 2 (8.3) | 22 (91.7) |
| Amoxacilin | 14 (38.9) | 22 (61.1) | 7 (29.2) | 17(70.8) |
| P value | <0.05 | | <0.05 | |

With MSSA: $X^2$ = 186.12, Degree of freedom = 9, P value = 0.000000, $P<0.05$
With MRSA: $X^2$ = 140.57, Degree of freedom = 9, P value = 0.000000, $P<0.05$
N = Total number of isolate
MRSA = Methicillin – Resistant *Staphylococcus aureus*
MSSA = Methicillin – Sensitive *Staphylococcus aureus*
R = Resistant
S = Sensitive
% = percentage.

## DISCUSSION

In this study, a total of 60 (37.5%) among 160 apparently healthy individuals from primary, secondary schools and traders screened for MRSA were nasal carriers. There was however a statistical significant difference ($P<0.05$) in the nasal carriage rate of methicillin resistant *Staphylococcus aureus*, coagulase negative staphylococci (CoNS) and individuals who do not harbour staphylococci in the nasal cavity (no growth) based on gender or sex. The high percentage of no growth in both males and females may probably indicate colonization of other organisms in the anterior areas other than *Staphylococcus aureus* according to sex wise showed that female with 40.0% was highest as against their male counterpart with 35.0%, and this is in total agreement with report of Onyemelukwe *et al*.,[9] and Arch *et al*.,[10].

*Staphylococcus aureus* occurrences among the age group was highest in age group 11 - 15 with 33.3% and this agreed with the work of Smith *et al*.,[11] and Johnson[12] who reported same in their various independent researches in different areas, but this findings was in contrast to the report of Okodua *et al*.,[13] who reported high prevalence of *Staphylococcus aureus* was among 40 – 45 age group.

According to the distribution of *Staphylococcus aureus* among occupation, traders with 46.7% had the highest occurrence as against other subjects from primary school and secondary or high school and this could be attributed to their exposure to several environmental elements as suggested by Okodua *et al*.,[13] as they opined that individuals that are exposed to several environment like civil servants and others are more prone to carry *Staphylococcus aureus* in their nasal passages. An occupation wise analysis of the prevalence of staphylococcus species was not statistically significant (*P*>0.05) in their difference in nasal carriage rate.

The prevalence of MRSA in female were more than male with 58.7%, though not significant, the high nasal carriage of *S. aureus* and MRSA among female as compared to their counterparts was in agreement with the previous findings of Onyemelukwe *et al*.,[9] Arch *et al*.,[10] respectively. The relatively lower colonization rate among old subject in the study was in agreement with Arch *et al*.,[10] A sex wise analysis of methicillin – resistant versus sensitive strains of *S. aureus* showed that statistically, there was no significant difference in the nasal carriage rate (*P*>0.05) among the various age groups. MRSA nasal carrier rate was high (25.5%) in age group of 11 – 15 years and less (4.2%) in age group greater than 40years. Similar findings were reported by Majumdar *et al*.,[14] in their study in East Sikkin and according to age wise analysis, it was found to be statistically significant (*P*<0.05). It has been reported that the older adults are less likely to be colonized with *S. aureus* than the younger ones but, when colonized, are more likely to have MRSA strains. Therefore, older adults with suspected staphylococcal infection may need antibiotic coverage against resistant strains. This might be attributed to relatively less number of elderly participants in the study.

The nasal carriage rate between MRSA and MSSA was not statistically significant (*P*>0.05) and was highest among the primary school students with 41.7%. This result was in consonance with the report of Tejero *et al*., [15] who claimed that the older adults are less likely to be colonized with *S. aureus* than their younger ones. However, when they are colonized, they are more likely to harbour MRSA strains.

Antibiotics susceptibility pattern of MRSA and MSSA isolates revealed that the best drug of choice for treatment of their infection is Gentamicin (100% sensitive). Other antibiotics which can also be used against MRSA and MSSA strains are Erythromycin and Streptomycin. MSSA strains are resistant to Pefloxacin but MRSA strains are susceptible. MRSA and MSSA strains were resistant to antibiotics such as Ampiclox, Zinnact, Ciprofloxacin, Septrin, Rocephin and Amoxicillin and this findings were in agreement with the report of Okodua *et al*.,[13] but were contrary to that reported by Akpaka *et al*.,[16] who revealed that all MRSA isolate were resistant to Gentamycin and Erythromycin. Antibiotic analysis of sensitive and resistant MRSA and MSSA isolates showed that there was a statistical significant difference (*P*<0.05) in the susceptibility patterns.

It is evident from the results obtained in this study that the rate of nasal carriage of methicillin – resistant *Staphylococcus aureus* (MRSA) among apparently healthy primary, secondary schools and traders subjects in Ekpoma is 40.0%. There is also a high level of resistance shown by MRSA isolates to other antibiotics compared with that of methicillin – sensitive *Staphylococcus aureus* (MSSA). However, there seems to be a steady rise of MRSA isolates to commonly used antibiotics but the present rate is still high in comparison to values in some other studies. The result of this study further stresses the need for good personal hygiene if the infection by MRSA is to be reduced in the community.

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