# Antimicrobial Susceptibility and Beta-lactamase detection of MRSA in Osogbo. SW Nigeria

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**ABSTRACT：** A total of 156 *Staphylococci aureus* isolates from different human clinical specimens comprising urine, stool, skin, wound swabs, ear and nose / nasal swab and blood obtained from patients at two large referral hospitals in Osogbo, Nigeria were screened for their antibiograms and plasmid profiles. A total of six antibiotic resistance profiles were obtained with ten of the isolates showing multi-drug resistance. Plasmids of three size ranges were detected in the isolates. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 6.5 – 23.2kb. Very high resistance levels (87.5%) were detected against penicillin and tetracycline while vancomycin and gentamicin recorded the least resistance levels of (62.5%) and (6.3%) respectively among the isolates. The starch paper analysis confirmed the presence of beta-lactamase production in all the isolates tested. [Nature and Science. 2007;5(3):44-48]. (ISSN: 1545-0740).

**Keywords:** Antibiogram; plasmid profile; clinical specimen.

## Introduction

Humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter, Payne, et al., (1966). Infections caused by *Staphylococcus aureus* resistant to methicillin (MRSA) are increasing in prevalence in adults and children. Nosocomial infections account for morbidity and mortality of millions of patients annually, worldwide. *Staphylococcus aureus* especially Methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections ( Mansouri et. al., 1997). MRSA is of concern not only because of its resistance to Methicillin but also because it is generally resistant to many other chemotherapeutic agents. Its now a major nosocomial pathogen in hospitals today due to prolonged hospital stay, intravenous drug abuse, and carriers of MRSA, especially in the nasal cavity, even along medical personnel (Vidhani et. al.,2001). Community-acquired MRSA infections in the absence of identified risk factors have been reported infrequently (Mansouri. et al., 1997). This study was undertaken to document the prevalence of methicillin resistance among *S.aureus* isolates at tertiary care teaching hospital, and even among healthy individual in this region. The pattern of susceptibility of both methicillin sensitive and methicillin resistance isolates to the commonly used antimicrobials were also analysed. The betalactamase production of this bacteria was also investigated using starch paper method. This work deals with prevalence of MRSA in a tertiary institution and its beta-lactamase production.

## Materials and Method

This study was carried out in Osogbo, Ladoke Akintola University of Technology College of Health Sciences, South Western Nigeria, from August-2004 to October 2004, we processed the samples of Pus, Urine, Blood, high vaginal swabs, Sputum, throat swabs, and ear swabs received from patients and

healthy individuals. A total of 106 Staphylococci were isolated out of which 28 were from healthy individuals. The isolates were confirmed by standard microbiological procedures (Cowan and Steel, 1954)

**Laboratory Methods**

Specimens were screened by preliminary Gram's stain and were inoculated on 10% sheep blood agar and MacConkey's agar. Staphylococcus aureus was identified by conventional techniques Layton et al., (1995). Antimicrobial sensitivity was performed on all the *Staphylococcus aureus* isolates by Kirby-Baur's disc diffusion method (Duguide et al., 1996). Oxacillin sensitivity was performed on Muller-Hinton agar with 4% sodium chloride. The strains were reported as sensitive, or resistant, to Oxacillin with inhibition zone diameter equal or more than 13mm and less than or equal to 10mm respectively. Disk diffusion testing was performed as recommended by the National Committee for Clinical Laboratory Standards; briefly, a broth culture suspension of the isolate to be tested was prepared in trypticase soy broth and turbidity adjusted to a 0.5 McFarland standard. The zone sizes were read after 24 hours of incubation in ambient air at 35°C. Isolates were classified as either susceptible or not susceptible Bauer et al. (1966). All the resistant strains were subjected to testing for beta lactamase production by starch paper method. NCCCL (1993). The MRSA isolates were tested for susceptibility to the following additional antibiotics: clindamycin, erythromycin, gentamicin, penicillin, tetracycline and vancomycin.

**Results**

Out of 156 isolates of Staphylococci collected, 28 (17.9%) were from healthy individuals. The results showed 67 were coagulase positive and 89 (57.1%) were coagulase negative. Out of 67(42.9%) coagulase positive Staphylococci 32 (47.8%) isolates were resistant to Methicillin. Table 1 shows the prevalence distribution of various clinical specimens of samples with methicillin resistance staphylococcus aureus and coagulates positive *staphylococcus aureus*. Highes prevalence of samples was isolated from wound from both MRSA and COSA, while stool sample had the least distribution for both. Table 2 shows the antibiotic resistance pattern of Methicillin resistant and sensitive strains of *Staphylococcus aureus.* As many as ten (14.9%) Staphylococcus strains were resistant to all antibiotics tested and three (4.4%) were resistant to all other antibiotics except Methicillin. Multidrug resistance was found to be less common amongst the methicillin sensitive Staphylococcci aureus (MSSA) strains. Maximum resistance was observed against tetracycline in this study and penicillin, (87.5% %) respectively. Least resistance was observed against gentamicin (62.5%) and vancomycin (6.3%) among MRSA. MIC values to Oxacillin > or equal to 250ugm/ml were found only in one strain, while majority of the strains MIC ranged between 2ugm/ml to 10 ugm/ml. Although the mean value of majority of the strains (21 out of 32) showed MIC values of 6ug/ml.

Table 1. Prevalence Rate Of Mrsa And Cosa From Clinical Specimen

| SOURCE | Coagulase positive Staphylococcus aureus | MRSA ( %) |
|---|---|---|
| SKIN | 12 | 6 (9.0) |
| BLOOD | I4 | 6 (9.0) |
| STOOL | 3 | 2 (3.0) |
| URINE | 6 | 1 (1.5) |
| WOUND SWAB | 18 | 13(19.4) |
| EAR SWAB/ NASAL SWAB | 14 | 4 (5.9) |
| TOTAL | 67 | 32 (47.8%) |

Table 1 b. Prevalence rate of MRSA

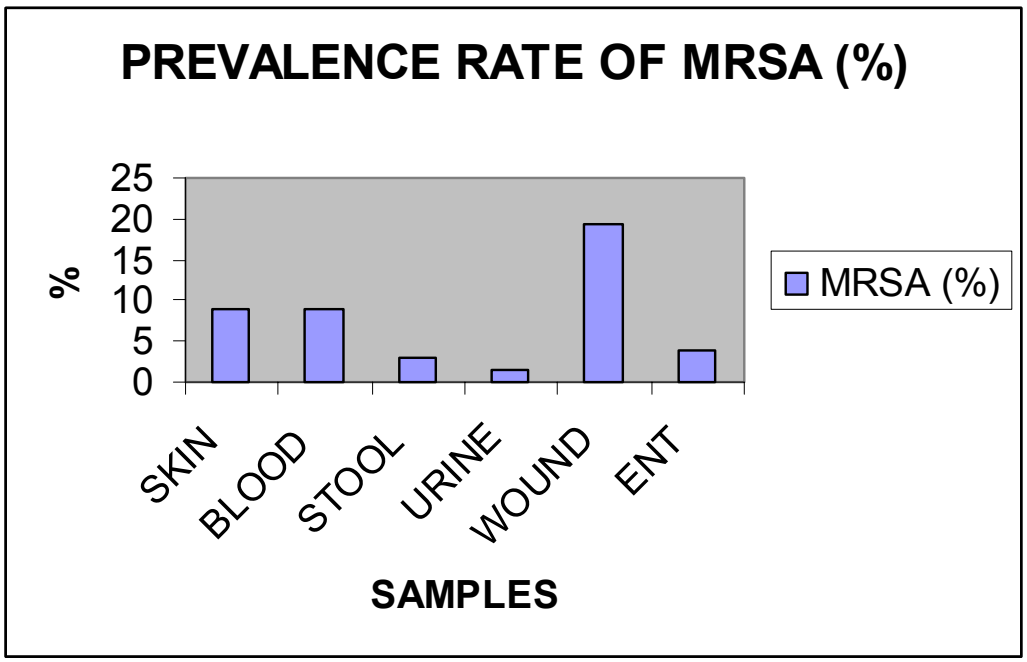


Table 2. Antibiotics

| ANTIBIOTICS | MRSA (N=32) | MSSA (N=35 | P.VALUE | |
|---|---|---|---|---|
| PENICILLIN | 28 (87.5%) | 24 (68.6%) | >0.05 | NS |
| ERYTHROMYCIN | 26 (81.1%) | 20 (57.1%) | <0.05 | NS |
| GENTAMICIN | 20 (62.5%) | 16 (45.7%) | <0.05 | NS |
| CHLORAMPHENICOL | 24 (75%) | 19 (54.35) | >0.05 | S |
| TERACYCLIN | 28 (87.5) | 12 (34.3%) | 0.05 | S |
| VANCOMYCIN | 2 (6.3%) | --1 (2.9%) | 0.05 | Significant |

P.Value = Indicates statistically significance difference between methicillin resistance and sensitive strains.
NS= NOT SIGNIFICANT
S= SIGNIFICANT

**Discussion**

Because of the ability of staphylococci to change over time, the MRSA will continue to be a problem in the future, as it has been in the past and still is, at present. Despite, intensive efforts to control resistant organisms by aggressive infections control methods antibiotic-resistant Staphylococci, especially MRSA has become the most common cause of hospital acquired infections worldwide. Chaudhary et al., (1999); Anupurba et al., (2003); (Taiwo et al., 2005). In this study, the frequency of MRSA was determined, (47.8%), which is almost similar but higher to other cases reported in pervious findings by (Taiwo et al., 2005) yet it is very high in comparison to the records of Zaman et al., (1994), Majumder et al., (2001) and Anupurba., (2003)**.** The injudicious use of antibiotics in Osogbo public hospitals and because of the easy availability of antibiotics without prescription, the chances of the emergence of resistant strains is enhanced. Lack of public awareness has contributed to the degeneration of the situation. Many investigators have reported an increase in the incidence of MRSA during recent years, most of which originated from wounds (pus). Vidhani et a.l (2001). We also found a high rate of MRSA isolates i.e. thirteen (40.6%) from the clinical specimens also showed multiple drug resistance. In our study one (2.9%) MSSA (Methicillin sensitive *Staphylococcus*

*aureus*) isolates were resistant to all other antibiotics tested. We have observed in our study that resistance to different antibiotics among MRSA strains was significantly higher than those, which were sensitive to methicillin. The values of MIC observed in Oxacillin resistance is almost similat to previous work of (Tahnkiwale et al., 2002). Vancomycin which was proposed as the drug of choice in several chronic cases of MRSA seems to be a better drug when compared to other antibiotics used in this study though with a low resistance of (6.3%) was observed for MRSA. The rapid detection of colouration by the starch paper method by all the isolates tested, confirmed the presence of beta-lactamase in all the isolates tested, and further confirmed the indiscriminate use of antibiotics in this area. This is perhaps due to the different clonally expansion and drug pressure in the community.

Since complete eradication of MRSA may not be possible, control of transmission seems to be the appropriate goal. The efficacy of some controlling methods are widely recognized and recommended by most authors. The first and the most effective way among these are to avoid transmission through hand contamination from personnel even to patients. The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore chemotherapy should be guided by sensitivity of the probable causative organism. Accurate detection of MRSA by clinical laboratories is of great importance, also awareness should be created about the route of its transmission in the community and the risk factors for infection such as antimicrobial and parental drug use.

**Conclusion**

Abused and injudicious use of antibiotics will lead to development of drug resistance. Also there is resistance to other antibiotics in Methicillin resistant strains. Timely detection of methicillin resistant strain will help in prevention of hospital-acquired infections. Control of MRSA infections is essential, and it can be achieved by proper implementation of hospital control measures and regular surveillance activity for proper documentation and control measures aimed at combating spread and control.

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