



# PREVALENCE AND ANTIBIOGRAM OF MULTI-DRUG RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG PREGNANT WOMEN ATTENDING ANTE-NATAL CLINICS IN OWERRI, IMO STATE, NIGERIA

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## ABSTRACT

**Background:** Multi-drug resistant (MDR) *Staphylococcus Aureus* (SA) is on the rise. The prevalence and antibiogram of MDR *Staphylococcus Aureus* among pregnant women attending Ante-Natal Clinics in Owerri, Imo State is being studied.

**Aim and Objective:** The prevalence of *Staphylococcus aureus* (*S.aureus*) among pregnant women was studied using urine and High Vaginal Swab(HVS) from pregnant women attending antenatal clinics.

**Materials and Methods:** The pregnant women attending at three hospitals; Federal Medical Centre (FMC) Owerri, Imo State University Teaching Hospital (IMSUTH) Orlu and General Hospital Okigwe. A total of 1, 197 (619 Urine and 578 HVS) samples were used. Samples were analysed in the Microbiology Laboratories of the different hospitals, within thirty minutes of collection.

**Results:** The results revealed that 121 (19.5%) of the Urine samples harboured *S.aureus* while 87 (15.1%) of the HVS were positive (mean: 17.4%). Antimicrobial susceptibility tests revealed that 126 (60.5%) of the *S.aureus* isolated from both the Urine and HVS samples across the state were multi-drug resistant. The predominant MAR index was 0.27 as 33.2% of isolates were resistant to 3 / 11 of antibiotics tested while the least 0.64 (7/11) was shown by 0.01% of the isolates. Generally, the highest resistance was to Ceftriazone (63.8%), followed by Gentamicin (63.0%), while Ciprofloxacin gave the highest susceptibility (95.2%) followed by Ofloxacin (92.3%). Questionnaires revealed that 75.7% (87), 72.9%(43) and 76.5% (26) (mean: 75%) that tested positive for the organism at FMC Owerri, IMSUTH Orlu and General Hospital Okigwe respectively were asymptomatic. Result analysis revealed a significant prevalence of resistant *S.aureus* among these out-patients who were mostly asymptomatic and unsuspecting carriers.

**Conclusion:** The need for routine surveillance of pregnant women for *S.aureus* to reduce maternal and child morbidity caused by the organism has therefore been revealed by this study.

**Key words:** prevalence, Vaginal Swab, *Staphylococcus aureus*, pregnant, asymptomatic, resistance

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*“To reduce maternal and child morbidity/mortality there is a need for routine surveillance of pregnant women for microbial pathogens especially multi-drug resistant strains as a part of their ante-natal care”*

## INTRODUCTION

Pregnant women attend ante-natal clinics to ascertain their good health and those of their foetuses from time to time hence they are prone to contact with microorganisms that are of clinical importance. Nosocomial infections therefore can be contracted by these unassuming ordinarily "healthy patients" i.e. not necessarily presenting any symptom of disease.

*Staphylococcus aureus* has been implicated as the most notorious organism associated with nosocomial infection. It is a Gram positive versatile aerobe which is a normal flora of the skin but when opportuned cause many infections<sup>1, 2, 3</sup> of which nosocomial infection have posed a scourge to pregnant women over the years.<sup>1,2</sup>

*S. aureus* is a Gram positive coccus that occurs in grape-like clusters. It is a eubacterium found on the surface of the human skin and mucous membranes<sup>4</sup>. It is also found in other areas of human contact such as air, dust and food products<sup>4,5</sup>. Cheesborough<sup>2</sup> describes the *Staphylococcus* species as a group of non motile, non capsulate, Gram positive cocci of uniform size (about 1µm in diameter) that occur characteristically in clusters/groups but also singly and in pairs. *S. aureus* is an opportunistic pathogen in man and animals<sup>4</sup>. Most strains become infectious usually when the skin or mucous membrane is punctured by variety of objects such as needles, blades, surgical devices etc.<sup>4,2</sup> Little wonder then, that it is a serious threat within the hospital.

Generally, the organism presents three broad disease types; a variety of superficial infections such as pimples, boils and toxic epidermal necrolysis (characterized by outer layer of the skin separating from the deeper layers); systemic infections such as *endocarditis* (inflammation of heart valves), *osteomyelitis* (inflammation of bone or bone marrow) etc<sup>5</sup>.

The "special" conditions of pregnancy which includes a distended stomach, short urethra,

release of specific moisture laden hormones etc may predispose them to microbial infections<sup>6,7,8</sup>.

Several physiologic changes occur during pregnancy that cause otherwise healthy women to be susceptible to serious sequelae from urinary tract infections<sup>9</sup>. Asymptomatic bacteriuria increases the risk for symptomatic bacteriuria. Treatment reduces the risk of a symptomatic infection<sup>10</sup>.

The widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multidrug resistant bacteria, increased number of nosocomial (hospital) and community-acquired infections; and increased health care costs<sup>11</sup>. Multi drug resistant *Staphylococcus aureus* raises greater concern because it is a pathogen with high virulence<sup>12</sup>, high ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions<sup>13</sup>.

Antibiotic resistance which is the character of bacteria to withstand the inhibitory or lethal effects of antibiotics, purely demonstrates the versatility of microbes in their struggle for existence<sup>13</sup>. It has been observed that antibiotics susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment<sup>14</sup>. This therefore demands the need for periodic screening of common bacterial pathogens for their antibiogram in different communities<sup>15</sup>.

This study is therefore set to determine the prevalence of the Multidrug resistant forms of *S.aureus* among the unassuming pregnant women in Owerri with a view to alerting the women and the entire populace on the presence of such organism and its public health implications as well as suggest preventive and control measures.

## MATERIALS AND METHODS

### Study location

The research was carried out using antenatal patients of Federal Medical Centre, Owerri, Imo State University teaching Hospital Orlu and General Hospital, Okigwe which are the three major government owned hospitals representing the three geopolitical zones (Owerri, Orlu and Okigwe) of Imo State, Eastern Nigeria.

### Sample and Study population

A total of six hundred and twenty five (625) which include 322, 200 and 100 pregnant women aged 15 years and above attending antenatal clinics at FMC Owerri, IMSUTH Orlu and General hospital Okigwe respectively were recruited for the study. Five hundred and seventy eight, 578 (317, 161 and 100 from Owerri, Orlu and Okigwe respectively) high Vaginal Swab (HVS) samples and 619 (322, 197 and 100 from Owerri, Orlu and Okigwe respectively) mid-stream Urine samples were used for the study. This implies a total of 1,197 samples. The subjects' informed consent was obtained for the research.

### Materials

Standards materials for isolation, identification and characterization of bacteria were used in the research including consumables and non consumables.

Interviewee and interviewer administered questionnaires were also used.

### Sample collection

The HVS samples were collected by obstetricians with the aid of cuscus speculum. For the urine samples each subject was given a sterile wide mouth-screw capped leak-proof container to collect at least 2mls of their mid-stream urine after being tutored on vulval cleaning and aseptic collection. The name, date and collection time for each patient was labeled accordingly on each sample container.

### Isolation of bacteria

The streaking method of culturing as described by <sup>2</sup> was used for the isolation. This was done in duplicates. The plates were incubated at 37°C for

For 18-24 hours. After incubation, the colonies formed were observed and the morphology recorded.

### Identification/Characterization of Isolates

Isolates obtained were stained using Gram Staining procedure as described by Cheesborough<sup>2</sup> for preliminary identification of the isolates. To further characterize the isolates, all isolates that were Gram positive cocci were plated onto Mannitol salt agar selective medium and further subjected to the following biochemical tests as described by <sup>2</sup>. The results were recorded.

### Antimicrobial susceptibility/sensitivity test

Mueller Hinton Agar (MHA) medium and commercial multidisc sensitivity discs were used for the susceptibility test following the Kirby Bauer method described by <sup>2</sup>. The disc contains the following antibiotics; cotrimoxazole 25µg, nitrofurantoin 200µg, gentamicin 10µg, ciprofloxacin 10µg, ofloxacin 5µg, augumentin 30µg, tetracycline 30µg and amoxicillin 25 µg and pefloxacin 5µg. Single discs containing nalidixic acid, were also added alongside the multidisc.

Zones of inhibition were used to extrapolate the level of sensitivity of the isolates to the test antibiotics.

### Multiple Antibiotic Resistant (MAR) Index

Multiple Antibiotic Resistance (MAR) index value of all the strains were calculated. The MAR index applied to a single isolate is defined as a / b, where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was subjected.

Results are presented using tables and analyzed using chi square ( $\chi^2$ ) statistical test, percentages and averages.

Ethical approval and informed consent of the hospital management, laboratory scientists and antenatal patients respectively were sought and obtained.

## RESULTS

### Isolation and identification results

All the isolates that grew on Mannitol salt agar medium were suspected to be *S.aureus* and were confirmed as such using biochemical methods. They were coagulase, catalase, and methyl red positive, and non lactose fermenting.

Two hundred and eight (17.4%) (121 (19.5%) and 87 (15.1%) Urine and HVS samples) of the 1197 (619 Urine and 578 HVS) samples used were positive for *S.aureus*. The distribution of samples and prevalence rate according to the zones are shown in table 1 below.

All the questionnaires administered were completed and returned. The analysis of the questionnaires returned revealed that 75.7% (87), 72.9%(43) and 76.5% (26) that tested positive for the organism at Federal Medical Centre (FMC) Owerri, IMSUTH Orlu and General Hospital Okigwe respectively were not aware of any infection during the time of sample collection.

Table1: Distribution of samples and prevalence rate of *S.aureus* across the zones of Imo state

Zones	URINE		HVS		TOTAL	
	NS	NI(%)	NS	NI(%)	NS	NI(%)
Owerri	322	65 (20.2)	317	50 (15.8)	639	115 (18)
Orlu	197	39 (19.8)	161	20 (12.4)	358	59 (16.5)
Okigwe	100	17 (17.0)	100	17 (17.0)	200	34 (17.0)
IMO	619	121 (19.5)	578	87 (15.1)	1197	208 (17.4)

Key: NS = number screened.

NI (%) = number infected (percentage infected)

Imo = total

### Antimicrobial susceptibility test results

The antimicrobial susceptibility test results are presented in table 2 below summarized as the percentage effectiveness of the antimicrobials on the test organisms.

Table2: Showing Percentage Effectiveness of Antibiotic on *Staphylococcus aureus*

Antibiotic	Disc Potency(µg)	No.Sensitive	No. Resistant Isolates	% sensitive Isolates	% Resistant Isolates
Augumentin	30	96	112	46.2	53.8
Ceftriazone	30	76	132	36.2	63.8
Nitrofurantoin	200	116	92	55.8	44.2
Gentamicin	10	77	131	37.0	63.0
Cotrimoxazole	25	155	53	74.5	25.5
Ofloxacin	5	192	16	92.3	7.7
Amoxicillin	25	91	117	43.8	56.2
Ciprofloxacin	10	198	10	95.2	4.8
Tetracycline	30	90	118	43.3	56.7
Pefloxacin	5	188	20	90.4	9.6
Nalidixic acid	30	190	18	91.3	8.7

Key: R = Resistant + = Susceptible

### MAR Index Result

About 93% of the isolates were resistant to at least two of the antibiotics used. The MAR index value ranged between 0.18 and 0.82. The predominant MAR index was 0.27 as 33.2% of isolates were resistant to 3 / 11 of antibiotics tested while 16.3% and 12.0% were resistant to 4 / 11 (MAR index – 0.36) and  $\geq 5 / 11$  (MAR index –  $\geq 0.45$ ). The least number 0.01% showing MAR index of 0.64 (7/11) to antibiotics tested respectively.

## DISCUSSION AND CONCLUSION

There has been an increasing concern about the prevalence of multi drug resistant bacterial strains<sup>13</sup>. Several workers have reported the multiple antibiotic resistance patterns of *S. aureus* from clinical samples<sup>14, 15, 16</sup>. When low doses of antibiotics are used against bacteria, they inhibit

Table3: Prevalence of MDR Staphylococcus aureus isolates from HVS and Urine of pregnant women from Imo State

Samples Used	No(%) Isolate d	No Resistant To:			Total MDR	% MDR
		3 Drugs	4 Drugs	≥5 Drugs		
HVS	87 (41.8)	30	13	10	53	60.9
Urine	121 (58.2)	39	21	13	73	60.3
<b>Total</b>	208 (100)	<b>69 (33.2)</b>	<b>34 (16.3)</b>	<b>23 (11.1)</b>	<b>126</b>	<b>60.5</b>

the growth of susceptible bacteria, leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells then eventually affecting other cells<sup>17</sup>. From the result obtained, the prevalence of the organism in pregnant women in Imo State is 17.4. The prevalence depicts a resistance profile of >50% to almost half (i.e. 5 out of 11 = 0.46) of the antibiotics tested, some of which form the first line of treatment for most patients in cases of self medication. It is worthy to note that multidrug resistance has serious implication for the empiric therapy of infections caused by microbes.

This study has established that ciprofloxacin, nalidixic acid, ofloxacin and pefloxacin (to which < 20%age resistance of the organism was below 10% respectively) are effective drugs that can be used for treatment of *S.aureus* infections at their in-use concentrations corroborating<sup>15</sup> while cotrimoxazole and nitrofurantoin which showed up to 50% susceptibility of organisms may be sparingly effective, augumentin, amoxicillin, tetracycline, gentamicin and ceftriazone will hardly be efficacious since >50% of the isolates showed resistance to these drugs. The high resistance 63.0% to gentamicin is worrisome as it mostly forms the first line treatment for most people with symptoms of staphylococcal infection and has been thought to be a broad spectrum antibiotic for Gram positive organisms. It is also thought to be safe in pregnancy.

Amoxycillin, tetracycline and gentamicin are the most common over the counter antibiotics in this part of the world which can explain the high resistance achieved by isolates as being in tandem with drug abuse and self medication. Recently the quest for augumentin therapy especially among the middle class residents of Imo state for varying ailments has been on the rise<sup>17</sup> and may also explain the high resistance obtained. The study corroborates earlier studies in which most of *S.aureus* isolates were highly susceptible to ofloxacin, ciprofloxacin, and pefloxacin<sup>18, 19</sup> but disagrees with their reports of high susceptibility of the organism to gentamicin. This may have geographical / habit implications. The fluoroquinolones are newer drugs with mode of action central on inhibition of the DNA replication which stops the multiplication of the bacteria cells and are relatively expensive therefore they are more likely less available for abuse. The level of multi-drug resistance shown by the *S.aureus* isolates from these mostly asymptomatic pregnant women is of great concern. About 62% of the isolates were resistant to at least three antibiotics, 96% were resistant to at least one antibiotic and only 4% were susceptible to all the antibiotics tested. These observations confirm the postulation that healthy members of the community are the highest reservoir of antimicrobial resistant bacteria<sup>18, 19</sup>.

This also shows that multidrug resistance is truly spreading rapidly among the microbial population and could be traced to indiscriminate use of antibiotics along with poor hygiene and infection control (risk factor for antibiotic resistance in bacteria) which are highly prevalent in Nigeria and other developing countries<sup>20, 21, 22</sup>. Generally the resistance pattern observed in this study is in conformity with previous observations that most isolates of *S. aureus* are resistant to a large number of commonly prescribed antibiotics<sup>22</sup>.

Though a high percentage of the women were asymptomatic carriers, it is worthy to note that asymptomatic infections predispose to symptomatic infections. Treatment of asymptomatic bacteriuria reduces the risk of a symptomatic infection<sup>10</sup>. Hence the positive women need to be treated. Furthermore, these infections if not identified early and properly treated may lead to infant mortality / morbidity as they may be associated with low birth weight, premature labour, and/or pre-eclampsia<sup>6,10</sup>.

Conclusively, it has been revealed from the findings of this research that though the prevalence of *S.aureus* among pregnant women in Imo state is <20% as against 30 to 50 % obtained in other areas<sup>18</sup>, multidrug resistant strains dominate the population observed among these women most of who are asymptomatic carriers. This is of serious public health import as the organism is an opportunistic pathogen and can perpetrate harm at the slightest conducive opportunity to both the unassuming pregnant woman and/or her fetus. The high multidrug resistance maybe closely linked to the fact that the society is presently characterized with inappropriate prescription, unethical dispensing and indiscriminate use of antibiotics. The rate at which most antibiotics are losing the battle against resistant organisms should be of immense concern to the health professionals and calls for effective measures (including trainings) to promote rational use of antibiotics and thereby prolong their 'life expectancy'<sup>18</sup>, There is necessity for constant antimicrobial sensitivity surveillance. There is also need for routine surveillance of pregnant women to ensure that the organism does not cause maternal and child morbidity. The antibiotic susceptibility pattern of the organism should also be ascertained before administering drugs in cases of infection.

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