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Research Article

Antibiotic- Resistant Pattern of *Staphylococcus aureus* amongst Pregnant Women Attending Antenatal Clinic at PAAUTH, Anyigba, Kogi State, Nigeria

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Abstract

Antimicrobials are drugs or medicines, including antibiotics, antivirals, antifungals, and antiparasitics, used to prevent and treat infectious diseases in humans, animals, and plants. This study is aimed at determining the Antibiotic - Resistant Pattern of *Staphylococcus aureus* among Pregnant Women Attending Prince Abubakar Audu University Teaching Hospital, Anyigba, Kogi State, Nigeria. One hundred and eighty-two (182) 'catch clean' midstream urine samples were aseptically collected and analyzed using standard bacteriological and biochemical Techniques. Of the 182 urine samples analyzed, 65 showed significant mix microbial growth indicating 35.7%[65/182]. 44 samples were confirmed to be *Staphylococcus aureus* (24.2%[44/182]). 13 antibiotics commonly prescribed for patients in the hospital were tested on the forty-four (44) *S. aureus* isolates. Ampicillin (29.5%) 13/44, Cotrimoxazole (38.6%) 17/44, Erythromycin (47.7%)21/44, Tetracycline (43.2%) 19/44, Cefotaxime (29.5%) 13/44, Ciprofloxacin (31.8%) 14/44, Ofloxacin (40.9%) 18/44, Streptomycin (43.2%) 19/44, Roxithromycin (31.8) 14/44, Cloxacillin (36.4%) 16/44, Gentamycin (31.8%) 14/44, Levofloxacin (27.3%) 12/44 and Cefoxitin (56.8%) 25/44. $MARI = 215 / (13 \times 44) = 215 / 572 = 0.37$.

Keywords: Antibiotic Resistant Pattern, *Staphylococcus aureus*, Pregnant Women

INTRODUCTION

Antimicrobials are drugs or medicines which includes antibiotics, antivirals, antifungals, and antiparasitics that are used to prevent and treat infectious diseases in humans, animals and plants. Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become difficult or impossible to treat, thereby, increasing the risk of disease spread, severe illness, disability and death ¹. AMR is a natural process that happens over time through genetic changes in pathogens. Its emergence and spread is accelerated by human activity, mainly the misuse and overuse of antimicrobials to treat, prevent or control infections in humans, animals and plants ¹.

Staphylococcus aureus, a gram-positive coccoid bacterium, is one of the most common human opportunistic pathogens ². *S. aureus* persistently colonizes 20–40% of the general population ². The spectrum of *S. aureus* is broad, ranging from asymptomatic or mild skin and soft tissue infections to severe life-threatening systemic infections ³. Infections due to *S. aureus* can be susceptible or resistant to antibiotics ⁴. Globally, antibiotic resistance has become a growing concern in the public health sector. This is because resistance often results in treatment failure, which can have serious consequences especially in critically ill patients ⁵.

Resistant- bacteria may spread and create broader infection control problems, both within healthcare institutions and in the community. Multiple antibiotic

resistant *Staphylococcus aureus* are major threats to patients care, owing to their stubborn intransigence to chemotherapy and disinfection⁶. Prolonged therapy with methicillin may lead to the development of low-level resistance that compromise therapy, but may not be detected by routine susceptibility testing methods used in hospital laboratories⁷. *S. aureus* has been implicated in a wide range of infections ranging from acute to chronic infections such as boils, bacteriuria, osteomyelitis, pneumonia, endocarditis, meningitis, septicemia and arthritis. This organism is a leading cause of human bacterial infection worldwide and is endemic in hospitals and communities⁸. The bacterium is frequently found on the skin and anterior nares of healthy individuals. However, it predominates in surgical wound infections with prevalence ranging from 4.6% to 54.4%⁹. Antimicrobial resistance (AMR) occurs when microorganisms like bacteria, viruses, fungi, and parasites evolve to resist the drugs designed to kill them, such as antibiotics and antivirals. This makes infections harder to treat, increasing the risk of severe illness, death, and the spread of disease¹⁰. AMR is a major global health threat driven by natural selection and accelerated by the overuse and misuse of antimicrobials in human medicine, animal farming, and agriculture. Antimicrobial resistance (AMR or AR) occurs when microbes evolve mechanisms that protect them from antimicrobials, which are drugs used to treat infections¹⁰. This resistance affects all classes of microbes, including bacteria (antibiotic resistance), viruses (antiviral resistance), parasites (antiparasitic resistance), and fungi (antifungal resistance). Together, these adaptations fall under the AMR umbrella, posing significant challenges to healthcare worldwide¹¹. Misuse and improper management of antimicrobials are primary drivers of this resistance, though it can also occur naturally through genetic mutations and the spread of resistant genes¹². Antibiotic resistance, a significant AMR subset, enables bacteria to survive antibiotic treatment, complicating infection management and treatment options¹¹. Resistance arises through spontaneous mutation, horizontal gene transfer, and increased selective pressure from antibiotic overuse, both in medicine and agriculture, which accelerates resistance development¹³. The burden of AMR is immense, with nearly 5 million annual deaths associated with resistant infections¹⁴. Infections from AMR microbes are more challenging to treat and often require costly alternative therapies that may have more severe side effects¹⁵. Preventive measures, such as using narrow-spectrum antibiotics and improving hygiene practices, aim to reduce the spread of resistance¹⁶. Microbes resistant to multiple drugs are termed multidrug-resistant (MDR) and are sometimes called superbugs¹⁷.

The World Health Organization (WHO) claims that AMR is one of the top global public health and development threats, estimating that bacterial AMR was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths¹⁸. Moreover, the WHO and other international bodies warn that AMR could lead to up to 10 million deaths annually by 2050 unless actions are taken¹⁹. Global initiatives, such as calls for

international AMR treaties, emphasize coordinated efforts to limit misuse, fund research, and provide access to necessary antimicrobial in developing nations. However, the COVID-19 pandemic redirected resources and scientific attention away from AMR, intensifying the challenge²⁰.

MATERIAL AND METHODS

Study Design

A cross sectional study design was used to obtain data from the pregnant women attending antenatal at the Prince Abubakar Audu University Teaching Hospital, Anyigba, Kogi State, Nigeria.

Study Area

This study was carried out at Prince Abubakar Audu University Teaching Hospital (PAAUTH), Anyigba, in the Eastern Senatorial Zone of Kogi State. The Eastern Senatorial Zone consist of Nine (9) Local Government Areas out of the Twenty – one (21) Local Government Areas of the state. PAAUTH is the only tertiary hospital in the senatorial zone thereby serving as the main Referral Centre to all other health facilities in the zone²¹. Kogi State is a state in the North Central region of Nigeria, bordered to the west by the states of Ekiti and Kwara, to the north by the Federal Capital Territory, to the northeast by Nasarawa State, to the northwest by Niger State, to the southwest by the states of Edo and Ondo, to the southeast by the states of Anambra and Enugu, and to the east by Benue State. It is the only state in Nigeria to border ten other states. Named for the Hausa word for river, the state was formed from parts of Benue State, Niger State, and Kwara State on 27 August 1991. The state is nicknamed the "Confluence State" as the confluence of the River Niger and the River Benue occurs next to its capital, Lokoja²². Of the 36 states of Nigeria, Kogi is the thirteenth (13th) largest in area and twentieth (20th) most populous with an estimated population of about 4.5 million as of 2016. The climate of the state has an annual rainfall total of between 1,100mm and 1,300mm. The rainy season lasts from April to October each year while the dry season last from November to March. Kogi is a multi-ethnic state with over multiple indigenous languages spoken in the state. The main language is Igala then Epira and Okun. Other common languages include Nupe, Kakanda, Bassa, Kupa, Bassa Nge, Hausa, Osayen Ogori in Kogi central speak Oko language²².

Study Population

The study populations were the pregnant women attending the antenatal clinic in the department of Obstetrics and Gynecology at the Prince Abubakar Audu University Teaching Hospital, Anyigba, Kogi State, Nigeria.

Inclusion and Exclusion Criteria

1. Inclusion Criteria

All Pregnant women coming for the first time ANC bucking were enrolled into the study.

2. Exclusion Criteria

All the pregnant women currently on Methicillin treatment for *Staphylococcal* infection or any other infections were excluded.

Ethical Approval

An ethical approval was obtained from the Research and Ethic Committee of Prince Abubakar Audu University Teaching Hospital (PAAUTH). The approval No: **KSUTH/ETHICS/005/ VOL.1/34**

Sample Size.

The sample size was determined using the sample size calculation formula by Andrew Fisher as shown below.

$$n = \frac{z^2 p(q)}{d^2}$$

where

n = sample size of the study population,

z = a confidence level of 95% = 1.96,

p = previous prevalence rate of 12.5% = 0.125²³

q = 1 - p = 1 - 0.125 = 0.875

d = confidence interval of ±5%

Therefore,

$$n = \frac{(1.96 \times 1.96) \times 0.125(1 - 0.125)}{0.05 \times 0.05}$$

$$= \frac{3.8416 \times (0.125 \times 0.875)}{0.0025 \times 0.0025}$$

$$= \frac{3.8416 \times 0.1094}{0.00000625}$$

$$= 63216.1$$

$$n = 168.1 + 16.8 \text{ (10\% attrition rate)} = 184.9 \approx 185^{24}$$

Sample Collection

Simple random and systematic sampling methods were adopted in recruiting participants for this study. To obtain a 'clean- catch' midstream urine specimen, all the research subjects were properly orientated during the antenatal morning 'Health Talk' on how to wash their hands and clean the area around the urethral opening, allow the area to dry and collect the urine, with the labia held apart. Under the supervision of the female nurses attached to the ANC, a 5ml amount of the midstream urine samples each were collected into the sterile urine container gently tight - locked and labeled appropriately with the participant's identification number. The urine samples were transported to the Laboratory in leak - proofed zip lock bags for laboratory analysis. A total of 182 urine samples were collected within this study period.

Laboratory Analysis

Urine Macroscopy

Macroscopic examination of the urine samples were done visually, noting their physical appearance such as color,

clarity, and the presence of any visible abnormalities like blood or sediment. This physical examination was necessary in order to get initial clues about the participant's health conditions.

Urine Microscopy

Microscopic examination of the urine samples were done to provide more detailed information about the presence of microorganisms in the urine. This involved the use of a microscope to identify and quantify various elements. This techniques, permits the visualization of bacterial cells, red and white blood cells, epithelial cells, casts, crystals, and other components in the urine which were hitherto not visible to the naked eyes. It helps in diagnosing urinary tract infections (UTIs), kidney disorders, and other conditions.

Urine Culture

The urine samples were aseptically inoculated on Mannitol Salt broth and incubated at 37°C for 18 - 24 hours. A loopful of the inoculated mannitol salt broth were later streaked on mannitol salt agar and incubated at 37°C for 24 hours. The plates were observed for creamy golden colonies typical of *S. aureus*. These suspected *S. aureus* isolates were further characterized using conventional/standard bacteriological and biochemical techniques such as colony morphology, Gram-staining, catalase test, Dnase test and coagulase test, which screened *S. aureus* from other *Staphylococcus* spp.

Biochemical Characteristics of the *S. aureus* Isolates

Primarily, biochemical tests used to identify *Staphylococcus aureus* are the catalase test and the coagulase test. The catalase test differentiates *Staphylococcus* from *Streptococcus*, while the coagulase test distinguishes *S. aureus* from other *Staphylococcus* species. Additionally, tests for hemolysins and thermostable deoxyribonuclease are also routinely used.

Catalase Test

Principle- This test detects the presence of the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen.

Procedure- A small amount of bacterial culture is mixed with hydrogen peroxide. If bubbles of oxygen are produced, the test is positive (catalase-positive).

Result- *Staphylococcus* species are typically catalase-positive, while most *Streptococcus* species are catalase-negative.

Coagulase Test

Principle -This test detects the presence of coagulase, an enzyme that causes blood plasma to clot.

Procedure -The two types of coagulase tests involves mixing bacterial culture with plasma on either a clean grease - free glass slide or in a clean sterile glass tube. A positive result is indicated by clotting.

Result - *S. aureus* is typically coagulase-positive, while most other *Staphylococcus* species are coagulase-negative.

Hemolysin Production

S. aureus can produce hemolysins, which are proteins that can lyse red blood cells. This can be detected by growing the bacteria on blood agar plates.

Thermostable DNase

S. aureus can produce thermostable deoxyribonuclease, which degrades DNA. This was detected by the use of a DNase test.

Mannitol Fermentation

S. aureus typically ferments mannitol's phenol red indicator to a golden yellow colony as observed on mannitol salt agar.

Gram Stain

S. aureus is a Gram-positive coccus, and therefore, stained purple with Gram stain. From the results of the biochemical tests, the Gram staining and colony morphology, *Staphylococcus aureus* isolates were reliably identified.

Antibiotic Resistant Pattern of *S. aureus*

The Antibiotic Resistant patterns of *S. aureus* isolates were determined by the Kirby Bauer disc diffusion

method as recommended by CLSI ²⁵. Each of the isolate were standardized to 0.5 McFarland equivalent and aseptically inoculated on prepared Muller-Hinton agar plates using sterile standard wire loop. The inoculated plates were allowed to stand for 10–15 minutes. Antibiotic impregnated discs namely Ampicillin (20µg), Co- Trimoxazole (25µg) Tetracycline (30µg), Cefotaxime (30µg), Ciprofloxacin (30µg), Ofloxacin (5µg), Streptomycin (10µg), Roxithromycin (30µg), Cloxacillin (5µg), Gentamicin (15µg), Levofloxacin (5µg), Erythromycin (15µg), and Cefoxitin (30µg) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 h after which the zones of inhibition around each disc were measured to the nearest mm with a meter rule, recorded manually and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines ²⁵.

Data Statistical Analysis

The data obtained from this study were analyzed using IBM@SPSS, Frequency and percentages for demographic variables and were presented using tables, and charts for easy explanations.

RESULTS

Table 1: Prevalence Distribution of Microbial Isolates Among Pregnant Women Attending Antenatal Clinic, Based on Pregnancy Duration.

Pregnancy duration	No. of Samples Examined	Significant microbial growth (%)	<i>S. aureus</i> isolates (%)	no growth (%)
1 ST Trimester	9	4 (44.4)	3 (33.3)	5 (55.6)
2 ND Trimester	33	20 (60.6)	10 (30.3)	13 (39.4)
3 RD Trimester	140	41 (29.3)	31 (22.1)	99 (70.7)
Total	182	65 (35.7)	44 (24.2)	117 (64.3)

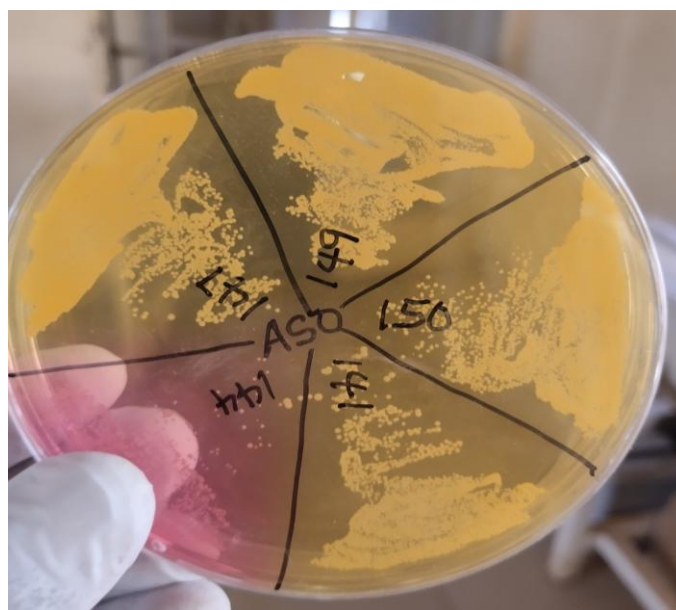


Plate 1. Mixed Microbial growth on MSA.

Table 2: Antibiotic Resistant Pattern of *S. aureus* to the Commonly Prescribed Antimicrobial Drugs at Prince Abubakar Audu University Teaching Hospital, Anyigba.

Anti-microbial drugs	Disc content	No. of Isolates Examined	No. of I (%)	No of R (%)	No of S (%)
Ampicillin	20mcg	44	0 (0.0)	13 (29.5)	31 (70.5)
Cotrimoxazole	25mcg	44	1 (2.3)	17 (38.6)	26 (59.1)
Erythromycin	15mcg	44	1 (2.3)	21 (47.7)	22 (50.0)
Tetracycline	30mcg	44	1 (2.3)	19 (43.2)	24 (54.5)
Cefotaxime	30mcg	44	0 (0.0)	13 (29.6)	31 (70.5)
Ciprofloxacin	30mcg	44	2 (4.5)	14 (31.8)	28 (63.6)
Ofloxacin	5mcg	44	1 (2.3)	18 (40.9)	25 (56.8)
Streptomycin	10mcg	44	0 (0.0)	19 (43.2)	25 (56.8)
Roxithromycin	30mcg	44	1 (2.3)	14 (31.8)	29 (65.9)
Cloxacillin	5mcg	44	1 (2.3)	16 (36.4)	28 (63.6)
Gentamycin	15mcg	44	0 (0.0)	14 (31.8)	30 (68.2)
Levofloxacin	5mcg	44	0 (0.0)	12 (27.3)	32 (72.7)
Cefixitine	30mcg	44	0 (0.0)	25 (56.8)	19 (43.2)

Zones are interpreted according to CLSI Standard (Bakthavatchalam, *et al.*, 2024) Ed34.

I=Intermediate, R= Resistant and S= Susceptible



Plate 2: Antibiotic Resistant Pattern of *S. aureus* MHA

DISCUSSION

The Multiple Antibiotic-Resistant pattern of *Staphylococcus aureus* of the 13 commonly prescribed Antimicrobial Drugs at Prince Abubakar Audu University Teaching Hospital, Anyigba against the forty-four (44) *S. aureus* isolates was carried out and the results were as follows:

Ampicillin (29.5%) 13/44, Cotrimoxazole (38.6%) 17/44, Erythromycin (47.7%) 21/44, Tetracycline (43.2%) 19/44, Cefotaxime (29.5%) 13/44, Ciprofloxacin (31.8%) 14/44, Ofloxacin (40.9%) 18/44, Streptomycin (43.2%) 19/44, Roxithromycin (31.8%) 14/44, Cloxacillin (36.4%) 16/44, Gentamycin (31.8%) 14/44, Levofloxacin (27.3%) 12/44 and Cefixitine (56.8%) 25/44. The Multiple Antibiotic Resistant (MAR) Index, for the 44 *Staphylococcus aureus* isolates to which

13 antibiotic drugs were tested was 0.375, given the prevalence of 37.5%. A MAR index of 1 means an organism is resistant to all the antibiotic tested. Generally, an MAR index ≥ 0.2 suggests a high - risk source of contamination, implying significant antibiotic use or abuse in that facility/ environment. This study revealed possible abuse of these drugs, poor hospital or personal hygiene and the need for better enlightenment campaign against 'self medication', having and complying with functional IPC (Infection Prevention and Control) and a safe and standard antibiotic use policy in the hospital, the State and the country to comb the looming danger anticipated from Antibiotic Resistance. This result is lower than the result of the study conducted at Obafemi Awolowo University Teaching Hospital, Ile Ife ²⁶ where the susceptibility pattern of *Staphylococcus aureus* to 10 conventional antibiotics being frequently prescribed in Ile-Ife, South Western Nigeria showed an overall prevalence of 50.0% (28/56) to be resistant to methicillin, while 18 (32.1%) were sensitive. The Multiple Antibiotic Resistant (MAR) Index, for the 44 *Staphylococcus aureus* isolates to which 13 antibiotic drugs were tested, the formula below was used;

$$a / (b \times c)$$

Where; a = Aggregate Antibiotic Resistant Score = 215 (see Table 2),

b = Number of Antibiotic Drugs used = 13,

c = Total number of samples (*S. aureus*) tested = 44

Therefore; MARI = $215 / (13 \times 44) = 215 / 572 = 0.37$.

CONCLUSION

The Multiple Antibiotic Resistant (MAR) Index, for the 44 *Staphylococcus aureus* isolates to which 13 antibiotic drugs were tested was 0.375, given the prevalence of 37.5%. A MAR index of 1 means an organism is resistant to all the antibiotic tested. Generally, an MAR index ≥ 0.2 suggests a high - risk source of contamination, implying significant antibiotic use or abuse in that facility/ environment. This study revealed possible abuse of these drugs, poor hospital or personal hygiene and the need for better enlightenment campaign against 'self medication', and complying with functional IPC (Infection Prevention and Control) and a safe and standard antibiotic use policy in the hospital, the State and the country to comb the looming danger anticipated from Antibiotic Resistance.

Recommendation

1. Periodic determination of the Antimicrobial Susceptibility Pattern or Multiple Antibiotic Resistant Index should be done, documented and properly communicated to the clinics and any other patient caregiver.
2. Clinical health talks should be by professional health educators and should focused on the danger of antibiotic abuse / 'self medication', poor personal and / or environmental hygiene, and IPC compliance by staff, patients and their visitors.

Limitation

1. Non-response and deliberate concealing of information by the respondents. To overcome this limitation, proper orientation was given to the research subjects on the aim and the objectives of the research in addition to assuring them of the confidentiality of the information and the importance of accurate data to the reliability of the variables being investigated.
2. The non- compliance of the study subjects to the precautionary measures in ensuring the quality of samples collected. To overcome this limitation, the female nurses who were recruited as research assistants helped to supervised and ensured that the sample collection were in compliance with the quality guidelines.

Conflicts of Interest: The researchers honestly declared that there is no conflicts of interest.

Ethical Approval: An ethical approval was obtained from the Research and Ethic Committee of Prince Abubakar Audu University Teaching Hospital (PAAUTH). The approval No: KSUTH/ETHICS/005/ VOL.1/34

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