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Research Paper

Antibiotics Susceptibility Profile of *Staphylococcus Aureus* Isolated from Clinical Patients in Abuja, Nigeria

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This study evaluated the antibiotics susceptibility profile of *Staphylococcus aureus* isolated from human clinical samples in Abuja, Nigeria. Sample collection and identification of isolates were performed using standard microbiological techniques. Agar disc diffusion method was employed to determine the antibiotics susceptibility profile of the isolates. The result showed that out of 192 samples collected from 6 area councils in Abuja, the prevalence rate of S. *aureus* was 38% (73/192). The highest isolation rate of S. *aureus* was observed in wound sample (53.1%), followed by Ear (47.4%) and urethral swab (47.1%) while the least was observed in high vaginal swab (30.8%). The age group mainly affected were ages 20-29 (24), 30-39 years (21) and ≤ 10 (16). The least isolation rate was observed in age groups 0-19 (8) and ≥ 40 (5). Samples from female patients had the highest occurrence of S. *aureus* 64.38% (47) compared to the male 35.62% (26). Statistically, there was no significant difference in the occurrence of S. *aureus* from the various area councils sampled at P value ≤ 0.05. The antibiotics susceptibility study showed that Vancomycin was the most effective drug of choice (100%) for the treatment of infections associated with S. *aureus*. High resistance was observed against Cefoxitin (86.3%), Amoxicillin (84.9%), Oxacillin (71.2%), Tetracycline (67.1%), Erythromycin (61.6%), Ceftazidime (56.2%), Clindamycin (38.4%), Ciprofloxacin (34.2%) and Linezolid (26%). Significant numbers of the isolates (24.7%) were multidrug resistant based on class of antibiotics while 65.8% of the isolates had multiple antibiotics resistance index (MARI) ≥ 0.3.

Keywords: Staphylococcus *aureus*, antibiotics resistance, clinical samples, Abuja.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most important bacterial pathogens in medicine today, accounting for a high proportion of cases of severe infection in both hospital and outpatient medical care. In hospital setting, *Staphylococcus* was first identified in 1880 in Aberdeen, United Kingdom by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint (Ogston, 1984).In hospital setting, Staphylococci produce diseases through their ability to multiply and spread widely in tissues and also through the production of extracellular substances. Staphylococci produce diseases through their ability to multiply and spread widely in tissues and also through the production of extracellular substances. *Staphylococcus aureus* is a common pathogen associated with serious acquirable infections such as boils, styes, pustules, impetigo, infections of wounds, ulcer and burns, osteomyelitis, mastitis, septicaemia, meningitis, furuncles and carbuncles, pneumonia and pleural emphysema (Cheesbrough, 2002; Normanno *et al.,* 2005).

The global sentry antimicrobial surveillance program, a network of approximately 100 sentinel hospitals in Canada, the United States (US), Latin America, Europe and the West Pacific, demonstrated that *S. aureus* was the most prevalent cause of nosocomial and community-acquired bloodstream infections, skin and soft tissue infections (SSTIs) and pneumonia in almost all geographic areas, (Diekema *et al.,* 2001). Onanuga *and* Onalapo, (2008) reported 36% and 40% prevalence in women's urine in two centers in Nigeria.

Treatment of *S. aureus* infections are majorly by the administration of antibiotics especially methicillin and other betalactames, erythromycin, vancomycin, clindamycin, linezolide, daptomycin-qumopristine etc. In the use of the betalactames, the alteration of the penicillin-binding protein in *S. aureus* does not allow the drug to bind well to the bacteria cell causing resistance to β-lactamase antimicrobial agent (Pantosti *et al.,* 2007). Methicillin resistant *Staphylococcus aureus* (MRSA) strains cause hospital infections particularly in wound infections and septicaemia. Reports have shown that infections associated with MRSA impose high economic and time burden on health care resources, and most frightening are emergence of MRSA infections in patients with no apparent risk factors in the community and hospital setting (Udo *et al.,* 1993). Numerous studies have indicated that *S. aureus* is among the most frequently encountered microorganisms in microbiology laboratories in Nigeria (Shittu *et al.,* 2006). However, data on the antibiotic susceptibility patterns of this pathogen in North Central Nigeria are inadequate, and in most cases, isolates are screened against commonly available antibiotics. The African data on *S. aureus*, particularly on antibiotic susceptibility, are extremely limited (Ako-Nai, *et al.,* 1991). Hence, this study evaluates the antibiotic susceptibility profile of *S. aureus* isolated from Hospital samples in Abuja, Nigeria.

MATERIALS AND METHODS

Study area

The study area was carried out in the six Area Councils in Abuja, Federal Capital Territory (FCT). These six area councils are Abuja Municipal, Gwagwalada, Abaji, Kuje, Kwali, and Bwari. Abuja is the capital city of Nigeria It is located in the center of Nigeria. According to 2006 census, Abuja had a total population of 776,298 but from 2000 and 2010, this population grew by almost 140% with more recent estimate showing the population now exceed 2.4 million (World Urbanization Prospect, 2017).

Ethical approval

Ethical clearance with assertion number FHREC/2014/01/65/05-11-14 was obtained from Health Research Ethics Committee of Federal Capital Territory, Health and Human Services Secretariat, Area 11, Garki Abuja. Individual consent was obtained using the information leaflet and consent form during sampling survey.

Sample collection

A total of 192 skin and soft tissue samples *were* collected from February 2015 to November 2015 from hospital patients in the six area councils of Federal Capital Territory, Abuja Nigeria. Samples were collected from pus, wound sites, urethra, cervix, vagina and ear by qualified medical laboratory scientists and Clinicians.

Media

The media used for Morphological characterization were Mannitol salt agar (Oxoid Ltd., England), Nutrient agar (Oxoid Ltd., England), Oxacillin resistant screening agar base (Oxoid Ltd., England), nutrient broth (Fluka Spain), Muller Hilton agar (Oxoid Ltd., England), Dnase agar (Oxoid Ltd, England), and peptone water water (Fluka Spain). All biological media were prepared according to manufacturer's instructions.

Isolation, identification and biochemical characterization of *S. aureus*

The characteristics golden yellow distinct colonies Mannitol salt agar plates were aseptically isolated and further identified using established microbiological methods that include colonial morphology. ***S. aureus*** was identified and differentiated from related organisms based on the colony morphology, Gram stain reaction, and their biochemical reactions using established microbiological standard by (Cheesbrough, 2004). Gram Stain reaction: An evenly spread smear of the specimen was made on a slide. The smear was air dried and heat fixed by passing through the flame of a Bunsen burner three times. The heat-fixed smears were then covered with crystal violet for one minute and rinsed rapidly with clean water. The smears were covered with Lugol's iodine for one minute and also washed off with water. They were decolorized rapidly with alcohol for a few seconds and rinsed with water. They were then flooded with Safranin red for 30 seconds and then rinsed out. Finally, the smears were placed in a draining rack for air-drying. The smears were then examined microscopically, first with ×40 objective to obtain the level of distribution of material and then with oil immersion × 100 objectives to observe the microscopic appearance of the bacterial cells. Suspected isolates showed purple-coloured cocci in clusters.

Catalase test

Catalase test was used to differentiate those bacteria that produced the enzyme catalase e.g. Staphylococci from non-catalase producing bacteria e.g. Streptococci. Using sterile wooden sticks, golden yellow colonies of the isolates were emulsified in test tubes containing 2 ml of hydrogen peroxide solution. The test tubes were then observed for immediate bubbling which is an indication of positive result.

Coagulase test

This test was used to identify *S. aureus* which produces the enzyme coagulase. A drop of distilled water was placed on two separate slides. The isolates were then emulsified in each of the drops, forming thick suspensions. A loop full of plasma was then added to the suspensions and mixed gently, then observed for clumping. Coagulase tube test was used to detect some MRSA. Presence of clumping within ten seconds (10 seconds) was recorded as positive for *S. aureus.*

Dnase test

This was used for identification of *S. aureus* which produces deoxyribonuclease (Dnase) enzymes. DNase agar plate was divided into four by marking the underside of the plate. Isolates and control organism (ATCC 25923) were cultured on DNase agar and incubated for 24 h at 37ºC. Excess of 1N HCL was then poured over the agar surface (approximately 15 ml), and excess acid removed with vacuum pipette. Clear zone around bacterial colonies within 5 min of adding the acid was an indication of production of deoxyribonuclease.

Antibiotic susceptibility testing

Susceptibility profiles of the isolates were performed using ten commonly available antibiotics according to Chesbrough, (2002) agar disc diffusion method. Colonies of an overnight culture of *S. aureus* isolates were inoculated into a normal saline suspension of 5ml to match 0.5 McFarland standards. *S aureus* ATCC 25923 was used as the control strain. Antibiotics used their concentrations in µg and their interpretation chats according to CLSI, (2016) shown in (Table 1).

Statistical analysis

Distribution and percentages were calculated for categorical variables. The demographic analyses were compared with the use of chi square and one way anova statistical package. A p-value of less than or equal to 0.05 is considered to be statistical significant.

RESULTS

High isolation rate was observed in age group 20-29 years (32.9%) followed by 30-39 years (28.8%) and ≤ 10 years (%).Samples from female patients had the highest occurrence of *S. aureus* 64.38% (47) compared to the male 35.62% (26) (Table 2). Statistically, there was no significant difference in the occurrence of *S. aureus* from the various area councils sampled at P value = 0.286.

Antibiotics Resistant of *S. aureus* Isolated from Clinical Samples in Abuja, Nigeria.

The antibiotics susceptibility study showed that Vancomycin was the highest effective drug of choice (100%) for the treatment of infections associated with *S. aureus* in Abuja, Nigeria (Figure 1) as High resistance was observed against Cefoxitin (86.3%), Amoxicillin (84.9%), Oxacillin (71.2%), Tetracycline (67.1%), Erythromycin (61.6%),Ceftazidime (56.2%), Clindamycin (38.4%), Ciprofloxacin (34.2%) and Linezolid (26%) (Figure 2).

Multidrug resistance *staphylococcus aureus* isolated from clinical samples in Abuja, Nigeria

Twenty eight point eight percent (28.8%) of the isolates were not multidrug resistant while seventy one point two percent (71.2%) were resistant to more than 3 classes of the antibiotics tested.

Antibiotics resistant pattern of *S. aureus* isolated from clinical samples

On evaluation of resistant pattern of 73 *S. aureus* isolated from hospital patients, the isolated samples had irregular resistant pattern. The most occurring resistant pattern among the isolated was FOX,OX,AML,E of 4.76% followed by FOX,OX,AML,CAZ,TE,E and FOX,OX,AML,CAZ,TE of 3.81% respectively .The isolates were resistant to more than three antibiotics tested.

DISCUSSION

The distribution of the suspected MRSA by gender among *S. aureus* isolated showed that 72.91% (35) were from female study group while 27.08% (13) were from male- Table 3. This study concurs with the report of Omoregies *et al*., 2008 in Benin City, Nigeria, who reported that close proximity of the female urethra meatues to the anus, shorter urethra, sexual intercourse, use of water and hands during washing after toilet are among factors that influences the prevalence of *S. aureus* in women.

On evaluating the isolation rate of *S. aureus* from various clinical specimens obtained, 30.16% (19/63) were

Table 1. Antibiotics used and their interpretative criteria.

Antibiotics	Abbreviation	Sensitivity (S)	Intermediate (I)	Resistant
Cefoxitin (30 µg)	FOX	≥22	–	≤ 21
Oxacillin (1µg)	OX	≥22	–	≤21
Amoxycillin (30µg)	AML	≥17	14-16	≤13
Ceftazidime (30 µg)	CAZ	≥21	18-20	≤17
Erythromycin (15µg)	E	≥23	14-22	≤13
Ciprofloxacin (5 µg)	CIP	≥21	16-20	≤15
Clindamycin (2 µg)	DA	≥	15-20	≤14
Tetracycline (30 µg)	TE	≥	15-18	≤14
Vancomycin (30 µg)	VA	–	–	–

Table 2. Distribution of *S. aureus* among age group studied.

AGES	CLINIC (FOOD HANDLERS)						TOTAL
	AMAC	BWARI	KUJE	KWALI	ABAJI	GWAS	
< 10	4	3	0	1	3	5	16
10 - 19	0	0	1	3	2	1	7
20 – 29	2	2	10	6	2	2	24
30 – 39	4	8	0	2	2	5	21
40 – 49	1	1	0	0	0	0	2
50 – 59	1	0	0	0	0	0	1
60 – 69	0	0	0	0	0	2	2
TOTAL	12	14	11	12	9	15	73

Table 3. Gender distribution of *S. aureus* among clinical patients.

AGE	CLINIC						TOTAL
	AMAC	BWARI	KUJE	KWALI	ABAJI	GWAS	
Male	3	4	5	3	7	7	29
Female	9	10	4	9	4	8	44
TOTAL	12	14	9	12	11	15	73

from urine, 53.12% (17/32) from wound, 47.36% (9/19) from Ear, 47.05 % (8/17) from Urethral Swab, 36.36% (8/22) from ECS, and 30.77% (12/39) from HVS (Table 4). In a similar study carried out by Shittu and Lin, (2006) more than 80% of the total number of isolates recovered were from infected wounds. Adetayo *et al*. (2014) in Ibadan reported that 52.2% of *S. aureus* from clinical specimens were recovered from urine specimens, 30.4% from wounds swab, 13.0% ear swab and 4.3% from nasal swab. These findings showed that *S. aureus* is ubiquitous in its distribution, and although it is a normal flora of the anterior nares, skin, and genital tract of humans, it can cause infections in virtually any organ or system of the body; infections of wounds, skin, soft tissue, blood, and the lower urinary tract are particularly common.

The result of the antibiotic resistant profile of *S. aureus* isolated to the ten antibiotics used in this study indicated high resistance to cefoxitin (86.3 %), Amoxicillin (84.9 %), Oxacillin (71.2%), Tetracycline (67.7 %), Erythromycin (61.64 %), and Ceftadizime (56.2 %), mild resistant to Clindamycin (38.35%) and Ciprofloxacin(34.24%) but highly susceptible to Linezolide (73.97%) and Vancomycin (100 %) (Figure1). The findings observed in this study correlate with other findings that percentage resistance of *S. aureus* varies by different geographical location (Fridkin, 2001; Hiramatsu *et al.,* 2001; Ikeh, 2003; Onanuga *et al.,* 2005; Olayinka *et al.,* 2005). The high resistance observed against CAZ, TE, AML and E are supported by the findings of Gross-Schulman *et al.,* (1998); Weems, (2001); Adetayo *et al.,* (2014); Ogbimu and Omu, (1986) and Uwaezuoke and Aririatu (2004),who reported that MRSA strains shows significant resistant to all beta-lactam, tetracycline and erythromycin antibiotics.

The findings on vancomycin and linezolid substantiated other reports that Vancomycin remains the reference standard for the treatment of systemic infection caused by MRSA, as a result of its relatively clean safety profile, its durability against the development of resistance, and, for many years, the lack of other approved alternatives (Scott, 2007). In an event of failure, new treatment

Table 4. Distribution of *S. aureus* by samples sources in clinical specimens.

Source	No sampled	No of isolate	% of isolate
Urine	63	19	30.15
Wound	32	17	53.12
HVS	39	12	30.76
Ear	19	9	47.36
ECS	22	8	36.36
US	17	8	47.05
Total	192	73	38.02

Table 5. Multiple Antibiotic Resistant Index of *Staphylococcus aureus* isolated from Clinical samples in Abuja, Nigeria.

MARI	Number of Occurrence	Percentage (%)
0.1	1	1.4
0.2	19	26.0
0.3	23	31.5
0.4	18	24.7
0.5	11	15.1
0.6	1	1.4

Key: Multiple antibiotic resistance index (MAR I) was calculated according to Krumperman (1983),MAR index=x/y, where x is the number of antibiotics resistant to and y is the number of antibiotics tested. The figure 72.6% was calculated from 23+18+11+1=53/73 and 27.4%=1+19=20/73 as explained from the formula

options for invasive methicillin resistant *Staphylococcus aureus* infections have been reported to include linezolid, daptomycin, tigecycline, and quinupristin/dalfopristin (Scott, 2007). The reasons for clinical failure of vancomycin are many and have been hypothesized to include poor penetration of the drug to certain tissues (Albanèse *et al.,* 2000), loss of accessory gene, regulator function in methicillin resistant *Staphylococcus aureus* (Sakoulas *et al.,* 2006), and potential escalation of vancomycin minimum inhibitory concentrations (MICs), (Robert *et al.,* 2006). The work of Kulkarni *et al.*(2014) supported the claim that linezolid could be used as alternative drug to Vancomycin as it has been found to showcase promising therapeutic option in an era of rapidly growing antibiotic resistance (Arora *et al.,* 2003). Linezolid is a synthetic oxazolidinone that inhibits the initiation of protein synthesis at the 50S ribosome (Weigelt *et al.,* 2005). It is currently approved by the Food and Drug Administration (FDA) for the treatment of complicated skin and skin-structure infections (SSSIs) and nosocomial pneumonia caused by susceptible pathogens, including MRSA.

Further evaluation of the susceptibility profile of the *Staphylococcus aureus* isolates in this study showed that 65.8% (48) were multidrug resistant (Resistance to four or more of the antibiotics tested) and none was susceptible to all the tested antibiotics. Twenty eight point eight percent (28.8%) of the isolates were not multidrug resistant while seventy one point two percent (71.2%) were resistant to more than 3 classes of the antibiotics tested (Figure 2).The high percentage of multidrug resistant might be an indication that a large proportion of the bacteria isolates have been pre-exposed to several antibiotics. High percentage (72.6%) of the isolates had MARI ≥ 0.3 while 27.4% had MARI ≤ 0.2 (Table 5). The high percentage of multidrug resistance 71.2% (Figure 2) might be an indication that a large proportion of the bacteria isolates have been pre-exposed to several antibiotics. The MARI result showed that 100% of the methicillin resistant *S.aureus* isolates had MARI ≥ 0.3 with irregular pattern of resistant. The most common pattern was FOX,OX,AML,CAZ,TE,E,DA (5.6%) and FOX,OX,AML,E (5.6%), indicating that some of the isolates have been exposed to a combination of microbial characteristics such as selective pressure on antimicrobial usage, societal and technological changes that enhance the transmission of drug resistant organisms (Orozova *et al.,*2008). Other reasons could be due to increase in irrational consumption rate of antibiotics and transmission of resistant isolates between people.

The highest percentage of MRSA was in urine and wound samples (31.25% and 20.83% respectively). This finding is in agreement with the report of Kulkarni *et al.* (2014) who observed highest percentage (82.38) of methicillin resistant *S.aureus* strains from urine sample

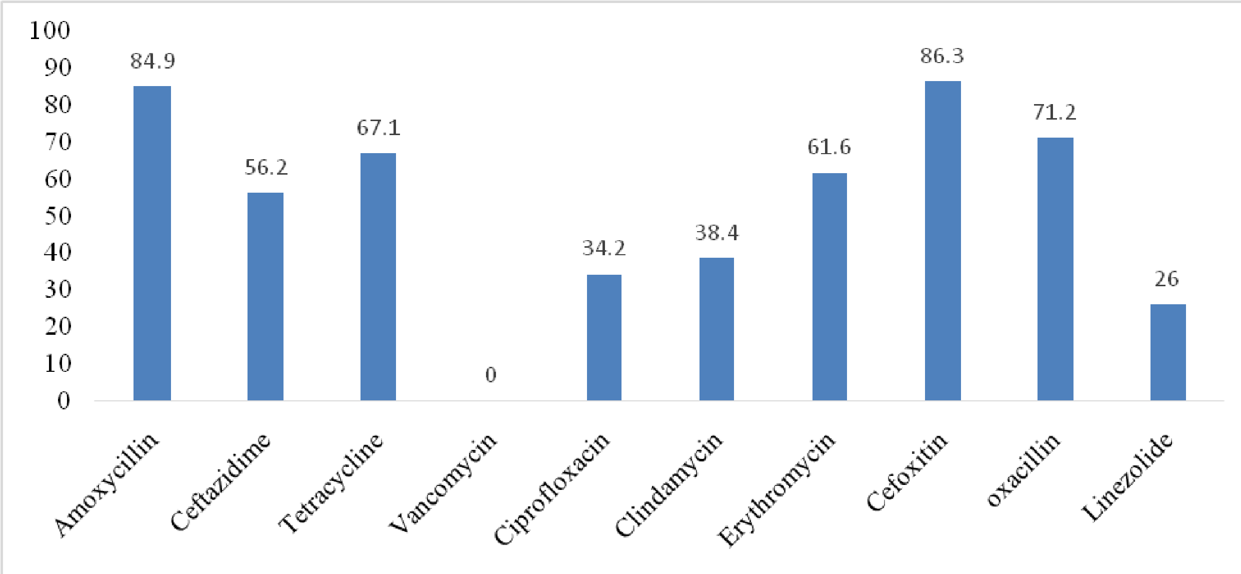

Figure 1. Percentage Antibiotics Resistant of *S. aureus* Isolated from Clinics Samples in Abuja, Nigeria.

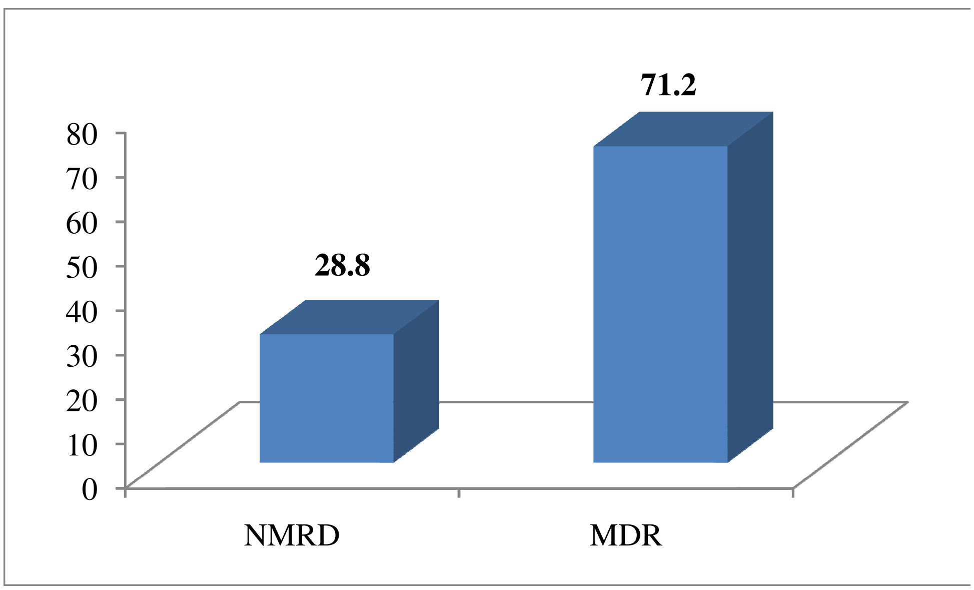

Figure 2. Level of Multidrug Resistance *Staphylococcus aureus* Isolated from Clinical Samples in Abuja, Nigeria.
Keys: NMDR = not multidrug resistant, MDR = multidrug resistant. Note: MDR is resistance to 3 or more classes of antibiotics tested.

while pus had 64.67% and blood sample had 62.69% . Also, the study conducted by Anupurba *et al.* (2003) supported this finding that MRSA is predominate in urine sample compared to other sample sources. The report of Onanuga *et al.* (2006), who observed 71.7% prevalence of MRSA among healthy women volunteers in Abuja, supported the possibility of isolating methicillin resistant *S. aureus* in the studied area. The study conducted by Adetayo *et al.* (2014) in Ibadan indicated the ineffectiveness of commonly prescribed antibiotics among MRSA isolates and calls the need to reassess the policies on antibiotic usage in hospital and the environment. The presence of isolates with this resistant characteristic negate significant impact as methicillin resistant *S. aureus* has been reported to be endemic in many hospitals causing excess mortality and economic burden compared to methicillin-susceptible isolates (Cosqrove *et al.,* 2005).

Conclusion

The finds of this study showed a high prevalence of methicillin-resistance *S. aureus* from clinical samples. Urban regions with populations of lower socioeconomic status and evidence of overcrowding appear to be at high risk for the emergence of MRSA in Abuja. Drugs such as beta lactames, tetracyclines, erythromycin, gentamicin and quinolones commonly prescribed in various hospitals and pharmaceutical outlets were observed to have reduced therapeutic effects against methicillin resistant *S. aureus* isolates but drugs such as linezolid had high therapeutic activity and could be used as surrogate/substitute to vancomycin during MRSA outbreak in Abuja. These findings indicate the need for intensive training/retraining of healthcare workers. The finds of this study also call for the development of models that could assist in limiting the spread of drug resistant bacteria.

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