

# Antimicrobial Resistance Patterns and Categorization of *Staphylococcus aureus* in Sudan

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**Abstract** Treating staphylococcal infection has become a challenge to the physicians, as this bacterium formed one of the multidrug resistant superbugs. The rapid and wide spread of resistant strains demand in depth investigation of resistance patterns of this organism. This study aimed to investigate the resistance patterns of *Staphylococcus aureus* and determine the incidence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains in Sudan. The study was designed as cross-sectional study, conducted over a time span of 15 months collected from five different states in Sudan. A total of 335 *Staphylococcus aureus* isolates were collected from different sources. The isolates were identified by microbiological tests and PCR. Antimicrobial susceptibility testing was performed using the Standard Disc Diffusion method (Kirby-Bauer's) and the isolates categorized into MDR, XDR, or PDR according to their resistance patterns following the recommendation and definitions obtained by Clinical and Laboratory Standards Institute (CLSI), European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) USA. The highest rates of resistance among the fifteen antimicrobial categories tested were for the  $\beta$ -lactam and  $\beta$ -lactam/ $\beta$ -lactamase inhibitors category, in which 307 (91.6%) isolates were resistant to penicillin, 299(89.3%) to oxacillin, 235 (70%) to ceftazidime, 193 (57.6%) and 169 (50.4%) to Amoxicillin/Clavulanic and Amoxicillin/Sulbactam respectively. Followed by the Macrolides category, in which 154(46%) were Erythromycin resistant. Resistance to Fluoroquinolones category was the third highest rate with 89(26%). Resistance to Tetracyclines, Aminoglycosides, Carbapenems, Trimethoprim/Sulphamethoxazole, Clindamycin, Rifampicin, Chloramphenicol and Phosphonic acid ranged from 26% to 13.1%. The lowest resistance was for Oxazolidinones (Linezolid), Anti-MRSA Cephalosporins (Ceftaroline) and Glycopeptide (Teicoplanin) with percentages of 2.4%, 0.9% and 0.6% respectively. Based on the results of antimicrobial susceptibility, 20(6%) of the isolates categorized as non- Multidrug resistant (non-MDR), 310 (93.7%) as multidrug resistant (MDR), 2(0.6%) was extensively drug resistant (XDR) and no pan-drug resistant (PDR) was found. The MDR strains are expanded in all Sudan and with resistance patterns near to XDR, which make the evolution into XDR and even PDR possible in the near future. This is one of the challenging matters demanding immediate action.

**Keywords:** antimicrobial categorization, multidrug resistance, extensively drug resistance, pan-drug resistance

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## 1. Introduction

Several centuries ago, *Staphylococcus aureus* was first recognized as an etiological agent of suppurative abscesses in humans. Infections range from mild skin and soft-tissue infections to life-threatening conditions such as endocarditis, chronic osteomyelitis, pneumonia, and

bacteremia [1]. In addition to causing almost all types of infections in humans, *S. aureus* has acquired a considerable medical significance due to its ability to resist multiple types of antimicrobial agents through various pathways. Therefore, the early identification of resistance patterns in *S. aureus* is an important task to efficiently track the multidrug resistant (MDR) strains associated with high levels of morbidity and mortality [2]. After penicillin was discovered, *S. aureus* infections

seemed controllable; however, resistance soon arose. In 1959, methicillin was introduced as the first designer anti-penicillinase antibiotic of its kind. However, within just three years, methicillin-resistant *S. aureus* (MRSA) started appearing, which led to several other multiantibiotic-resistant strains to emerge, and currently, the acronym stands for multidrug-resistant *S. aureus* [3]. The Centers for Disease Control and Prevention (CDC) and European Center for Disease Prevention and control (ECDC) had worked together to develop a standardized international terminology for describing acquired resistance profiles in a group of bacteria, including *S. aureus*, through a joint initiative between the two organizations. They categorized bacteria into three groups according to their resistance patterns: (1) **Multidrug resistant (MDR)**: Resistance to at least one agent in three or more antimicrobial categories, (2) **Extensively drug resistant (XDR)**: Resistance to at least one agent in all but two antimicrobial categories, and (3) **Pan-drug resistant (PDR)**: Resistance to all agents in all antimicrobial categories [4].

Despite limited laboratories capacity to monitor AMR, available data suggest that Sudan shares the worldwide trend of increasing drug resistance. The lack of adherence to the antimicrobial treatment protocol authorized by the World Health Organization (WHO) and the Ministry of Health put Sudan in high risk of developing extreme patterns of antimicrobial resistance (AMR). This fact should be investigated to understand the current situation of AMR in Sudan. This work aimed at investigating the resistance patterns of *S. aureus* isolates to determine the frequencies of MDR, XDR and PDR and if there is significant correlation to some patients' criteria [2].

## 2. Methodology

### 2.1. Study Design and Data Collection

The present work was conducted prospectively as cross-sectional study. *S. aureus* isolates collected from seventeen microbiology laboratories in five Sudanese States (Khartoum, Gedaref, North Kordofan, Kassala, and El Gazira), through fifteen months (from December 2019 to March 2021). Information about the patients was extracted from medical records in the Laboratories Information Systems included the following patients' characteristics: age, gender, specimen types, and hospitalization status (ICU admission/inpatient/ outpatient).

The estimated sample size of 335 ( $\pm 25$  error) was determined based on the statistical formula of Fisher for sample size calculation [5]. A total population of 862 *S. aureus* isolates were collected, from which 335 study samples were selected based on being viable upon delivery to National University-Sudan Microbiology Laboratory, the confirmation of identification results and the completion of sample's information.

### 2.2. Microbiological and Molecular Identification

The isolates were identified using Chromogenic agar (HiCrome™ Universal Agar, Mumbai, India)

and conventional biochemical tests. To confirm the identification to the species level, we used PCR assay that targets the *S. aureus* genus-specific 16S rRNA gene (which serves as an internal control) with primers Staph756F (5'-AACTCTGTTATTAGGGAAGAACA-3') and Staph750R (5'-CCACCTTCCTCCGGTTTGTACC-3'), both produce 756 bp amplicon [6]. The DNA was extracted from 24 hr bacterial growth on Luria-Bertani (LB) media. A single colony was emulsified in a 100  $\mu$ L of distilled water and heated at 95°C for 10 min. The suspensions were then centrifuged at 1500 rpm for 5 min [7]. PCR was performed in a reaction mixture of 2  $\mu$ L of the extracted DNA, 0.2  $\mu$ L of each 0.07  $\mu$ M 16S rRNA primers (Staph756F and Staph750R), 10  $\mu$ L of distilled water added to 4  $\mu$ L of the PCR Master Mix (5x HOT FIREPol® Blend-Solis BioDyne, Estonia). Amplification was carried out with the following thermocycling conditions (using Aeris Machine Pelter technology, Thermo Assist): initial denaturation step at 94°C for 10 min then 10 cycles of amplification consisting of denaturation at 94°C for 45 sec, after that annealing at 55°C for 45 sec and 72°C for 75 sec, finally 25 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 75 sec [6]. PCR products were separated on 2% agarose gel along with a 100bp DNA ladder (APSLABS, India) and stained using ethidium bromide. The amplified products were then visualized with a transilluminator (Biometer an analytical Jena company) [7].

### 2.3. Antimicrobial Susceptibility Testing

Phenotypic antimicrobial susceptibility of the isolates was studied using the Standard Disc Diffusion method (Kirby-Bauer's) and the inhibition zone diameters were measured in (mm) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines M100 27<sup>th</sup> [8]. In order to detect MRSA, 10  $\mu$ g Oxacillin disc applied on 6% NaCl Mueller-Hinton agar inoculated with the tested isolates. To categorize the isolates into MDR, XDR and PDR, the recommendations and definitions obtained by CLSI, European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) USA were applied [4]. The criteria for defining MDR, XDR and PDR in *S. aureus* (one or more of these must apply) were:

MDR: (i) an MRSA is always considered MDR by virtue of being an MRSA, and/or (ii) non-susceptible to  $\geq 1$  agent in  $\geq 3$  antimicrobial categories.

XDR: non-susceptible to  $\geq 1$  agent in all but 2 categories.

PDR: non-susceptible to all antimicrobial agents listed. Resistance to oxacillin predicts non-susceptibility to all other  $\beta$ -lactam antimicrobials, except for cephalosporins anti-MRSA (i.e., all categories of penicillins, cephalosporins,  $\beta$ -lactamase inhibitors and carbapenems) [4]. Antimicrobial categories and agents used to define MDR, XDR and PDR *S. aureus* were listed in (Table 3). The sizes of the zones of inhibition were interpreted by Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints of the NCCLS M100-S12, by Hi media interpretative chart as susceptible, intermediate, and

resistant. The quality control strain *S. aureus* 25923 was tested along with the samples [8].

## 2.4. Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics v.28.0.0.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics used to calculate frequencies and percentages for qualitative variables and means with standard deviations for quantitative variables. Chi-square test was used to compare categorical variables, and differences considered statistically significant when *p*-value was less than 0.05. Odd ratio and 95% CI were estimated by multinomial logistic regression for risk of developing MDR strains. Hierarchical analysis was used to identify the relatedness between isolates according to their resistance patterns using the dendrogram diagram.

## 2.5. Ethics Statement

The study was approved by the Research Ethics Committee (REC) of the National University Biomedical Research Institute, National University-Sudan (Approval No: NU-REC/01-021/04), Khartoum, Sudan. The samples that were studied were residual, and the data about the patients were collected anonymously, and did not include any identifying information about the patients.

## 3. Results

### 3.1. Demographic Characteristics of the Patients with *S. aureus* Infections

From a total of 862 *S. aureus* isolates collected, 335/862 (38.8%) were included in the study. 527/862 (61.2%) were excluded; of which 289/527(54.8%) were found to be mis-identified as *S. aureus* by the laboratories after re-identification and confirmation of identification by PCR, 140/527(26.5%) had incomplete information about the patients and 98/527(18.5%) yielded no growth upon subculture.

Most of the isolates, 286/335(85.4%) collected from Khartoum State. The males predominated at 224/335 (66.9%) compared to the females at 111/335 (33.1%). The most frequent age group was 19 to 64 years, with minimum age of 1 week old and maximum of 89 years old, giving a mean age of 51 years, with SD  $\pm$  22. Of the participants, 174/335 (52%) were outpatients, 123/335 (36.7%) were inpatients and 38/335 (11.5%) were in the intensive care unit (ICU). *S. aureus* strains were isolated from fourteen different sources. The most common source type was wound swabs 127/335(37.9%), plus 12/335 (3.6%) samples were post-surgical wound swab, followed by urine and body fluid with frequency of 46/335 (13.8%) for each. Other sources were sputum 31/335 (9.3%), blood 15/335(4.5%), eye 2/335 (0.6%), ear 12/335 (3.6%) and nasal swabs 1/335 (0.3%), Cerebrospinal fluid 4/335 (1.2%), tissues 6/335 (1.8%), catheter's tip 5/335(1.5%), and high vaginal swab 5/335(1.5%), (Table 1).

**Table 1. Demographic characteristics of patients regarding distribution of *S. aureus* strains; 2019-2021**

Characteristics	<i>S. aureus</i> strains (n=335)	
	No.	%
<b>Gender</b>		
Male	224	66.9%
Female	111	33.1%
<b>Age group: minimum= 1 week, maximum=89yr, mean= 51yr, SD <math>\pm</math>22</b>		
Under 1 year	10	3%
1-18 years	21	6.2%
19-65 years	207	61.8%
66 years and older	97	29%
<b>Patient's status</b>		
Inpatients	123	36.7%
ICU patients	38	11.3%
Outpatients	174	52%
<b>Source of the clinical isolates</b>		
Wound swab	127	37.9%
Post-surgical wound swab	12	3.6%
Urine	46	13.8%
Sputum	31	9.3%
Blood	15	4.5%
Body Fluid aspirate	46	13.8%
Eye swab	2	0.6%
Ear swab	12	3.6%
High vaginal swab (HVS)	5	1.5%
Pus	22	6.6%
Cerebrospinal fluid (CSF)	4	1.2%
Tissue	6	1.8%
Catheter's tip	5	1.5%
Nasal swab	1	0.3%
<b>States</b>		
El Gazira	22	6.6%
Gedaref	7	2.1%
Kassala	10	3.0%
Khartoum	286	85.4%
North Kordofan	10	3.0%

### 3.2. Antimicrobial Susceptibility Results

To investigate the resistance patterns of the isolates and determine the incidence of the MDR, XDR and PDR in Sudan, 22 antimicrobial agents from 15 antimicrobial categories were tested for their activities on the isolates. The highest rates of resistance were against the  $\beta$ -lactam and  $\beta$ -lactam/ $\beta$ -lactamase inhibitors category, 307(91.6%) were resistant to penicillin, 299(89.3%) for oxacillin, 235 (70%) for ceftazidime, 193(57.6%) and 169(50.4%) for Amoxicillin/Clavulanic and Amoxicillin/Sulbactam respectively. Followed by the Macrolides category, in which 154(46%) isolates were Erythromycin resistant. Fluoroquinolones category showed the third highest resistance rate of 89(26%). Tetracyclines, Aminoglycosides, Carbapenems, Trimethoprim/ Sulphamethoxazole, Clindamycin, Rifampicin, Chloramphenicol and Phosphonic acid showed resistance rate ranging from 26% to 13.1%. The lowest resistance rates were for Oxazolidinones (Linezolid), Anti-MRSA Cephalosporins (Ceftaroline) and Glycopeptide (Teicoplanin) with percentages of 2.4%, 0.9% and 0.6% respectively. Vancomycin in Glycopeptides category yielded the highest intermediate susceptibility patterns among the antimicrobial agents, 31(9.3%), (Table 2).

**Table 2. Antimicrobial susceptibility patterns of *S. aureus* (N=335) isolates**

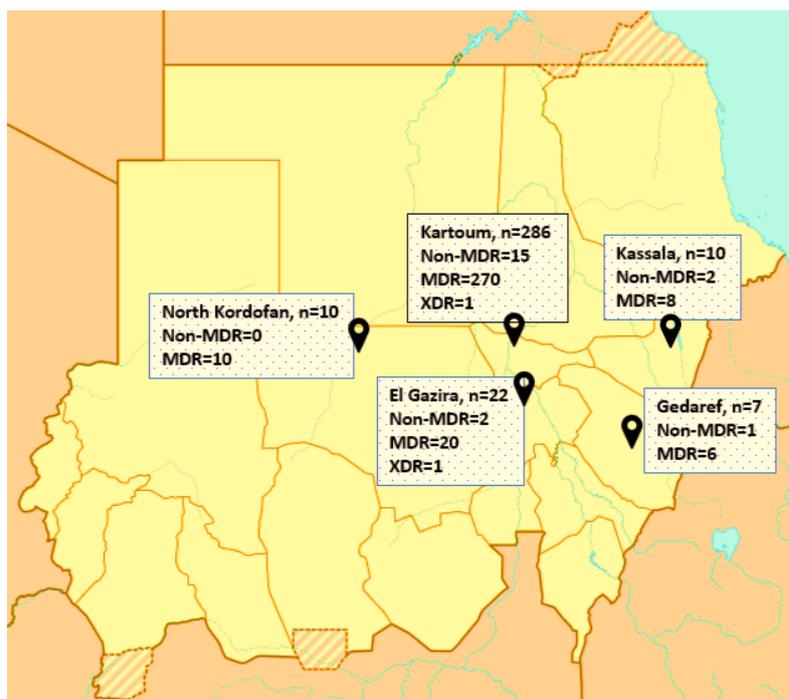
Antimicrobial category	Antimicrobial agents	<i>S. aureus</i> n (%)		
		S	I	R
β-lactam + β-lactam/β-lactamase inhibitors	Penicillin (10U)	28(8.4%)	-	307(91.6%)
	Oxacillin (10μg)	36 (10.7%)	-	299(89.3%)
	Ceftazidime (30μg)	85(25.5%)	15(4.5%)	235(70%)
	Amoxicillin/Clavulanic acid ([20/10] 30μg)	142(42.4%)	-	193(57.6%)
	Amoxicillin/Sulbactam ([10/10] 20μg)	166(49.6%)	-	169(50.4%)
Anti-MRSA Cephalosporins	Ceftaroline (30μg)	332(99.1%)	-	3(0.9%)
Glycopeptides	Vancomycin (30μg)	295(88.1%)	31(9.3%)	9(2.9%)
	Teicoplanin (30μg)	330(98.5%)	3(0.9%)	2(0.6%)
Macrolides	Erythromycin (15μg)	180(53.7%)	1(0.3%)	154(46%)
Lincosamides	Clindamycin (2μg)	299(89.3%)	4(1.2%)	31(9.3%)
Tetracyclines	Tetracycline (30μg)	246(73.4%)	6(1.8%)	83(24.8%)
	Doxycycline (30μg)	241(71.9%)	15(4.5%)	79(23.6%)
Carbapenems	IMP (10μg)	282(84.2%)	1(0.3%)	52(15.5%)
Phenicol	Chloramphenicol (30μg)	316(94.3%)	-	19(5.7%)
Folate pathway inhibitors	Trimethoprim/Sulphamethoxazole (25μg)	274(81.8%)	25(7.5%)	36(10.7%)
Fucidanes	Phosphonic acids (200μg)	321(95.8%)	-	14(4.2%)
Oxazolidinones	Linezolid (30μg)	327(73.4%)	-	8(2.4%)
Fluoroquinolones	Ciprofloxacin (5μg)	246(73.4%)	-	89(26%)
Aminoglycosides	Gentamicin (10μg)	256(76.4%)	-	78(23.3%)
Glycylcyclines	Tobramycin (10μg)	291(86.9%)	-	44(13.1%)
	Tigecycline (15μg)	323(96.4%)	-	12(3.6%)
Ansamycins	Rifampicin (5μg)	296(88.4%)	11(3.3%)	28(8.4%)

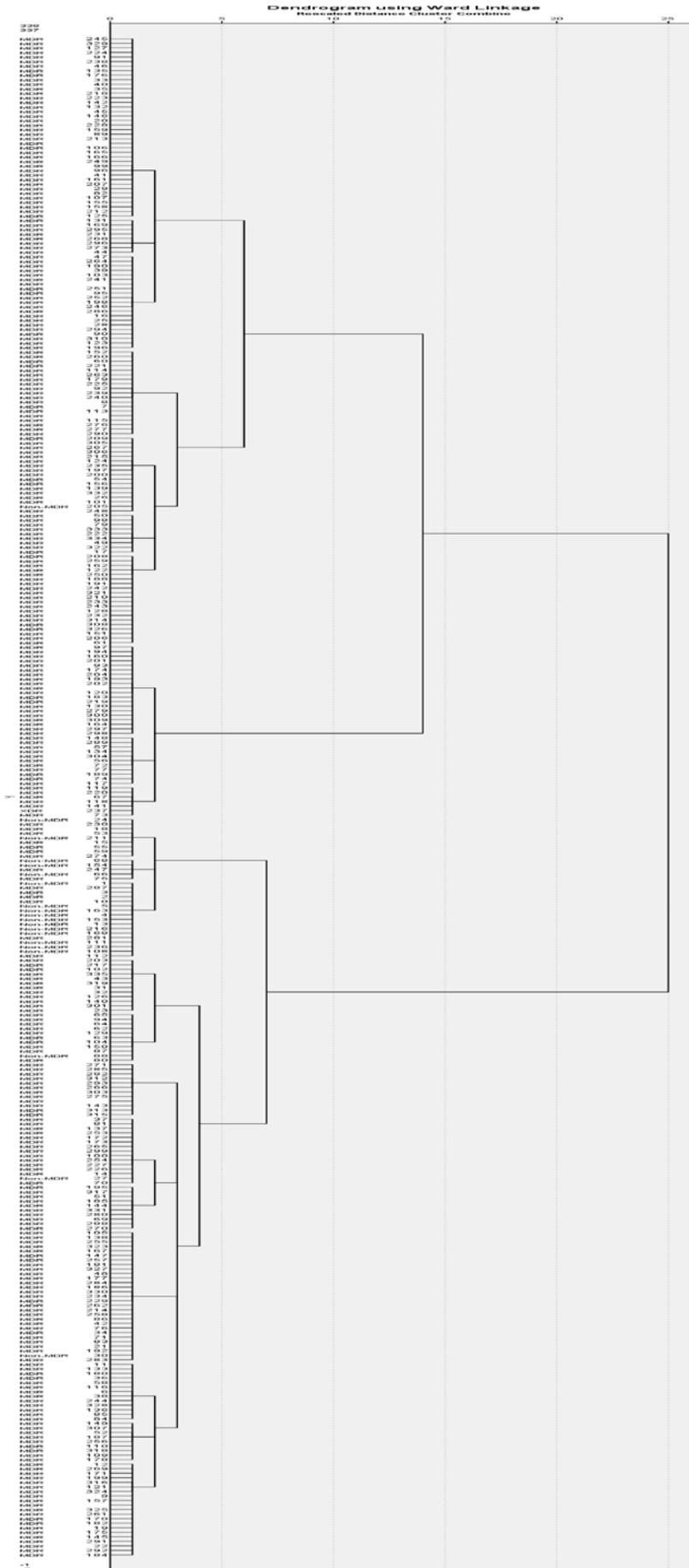
Notes: n: number of isolated *S. aureus* strains; %: percentage of susceptibility patterns; S: sensitive; R: resistant; I: intermediate; Inhibition zone diameter by mm.

**Table 3. Frequencies of MDR, XDR and PDR among *S. aureus* strains:**

Resistance profile	Number of antimicrobial resistance categories	n (%) N=335
<b>Non-MDR</b>	0-2 categories but not including Oxacillin	20(6%)
<b>MDR</b>	3-12 categories= 232(69.3%) or 1-2 including Oxacillin= 81 (24.1%)	313(93.4%)
<b>XDR</b>	13 categories or more	2(0.6%)
<b>PDR</b>	All agents in the 15 categories	0

MDR: Multi-drug resistant, XDR: Extensive-drug resistant, PDR: Pan-drug resistant.

**Figure 1. Geographical distribution of antimicrobial resistance categories in Sudan**



**Figure 2.** Dendrogram, constructed using IBM SPSS Statistics v.28.0.0.0, showing the relatedness of *S. aureus* isolates (n=335) according to their antimicrobial resistance categorization across the five states. MDR=multidrug-resistance; XDR=extended drug-resistance

**Table 4. Statistical correlation and odd ratios of patients' demographic characteristics and antimicrobial resistance categorization of the isolates**

Patient characters	MDR-SA (n=315)	Non MDR-SA (n=20)	P-value <sup>1</sup>	OR <sup>2</sup> (95% CI <sup>3</sup> )
<b>Gender</b>				
Male (n=224)	210	14	0.485	1.16(0.46-2.93)
Female (n=111)	105	6		
<b>Patients' status</b>				
Inpatients (n=161)	150	11	0.341	1.32(0.56-3.10)
Outpatients (n=174)	165	9		
<b>Province resides</b>				
Khartoum (n=291)	275	16	0.260	1.04(0.94-1.15)
outside Khartoum (n=44)	40	4		
<b>Source of clinical isolates</b>				
Wound swab (n=139)	131	8	0.041*	2.66(1.04-6.79)
Body Fluid aspirate (n=47)	44	3	0.898	0.92(0.26-3.27)
Urine (n=46)	41	5	0.140	0.45(0.15-1.30)
Blood culture (n=31)	28	3	0.744	0.81(0.22-2.88)
Pus (n=21)	21	0	0.478	2.79(0.16-47.78)
Sputum (n=15)	14	1	0.911	0.89(0.11-7.10)
Ear swab (n=12)	12	0	0.739	1.62(0.09-28.42)
Tissue (n=7)	7	0	0.994	0.99(0.54-17.95)
Vaginal swab (n=5)	5	0	0.822	0.71(0.04-13.38)
Cerebrospinal fluid (n=4)	4	0	0.772	0.58(0.03-11.23)
Eye swab (n=2)	2	0	0.472	0.32(0.02-6.99)
Nasal swab (n=1)	1	0	0.321	0.19(0.01-4.93)
<b>MRSA</b>	299	0	<0.001*	2.25((1.29-7.33)

**Notes:** MDR-SA: Multidrug Resistant-*S. aureus*; Non-MDR-SA: Non-Multidrug Resistant-*S. aureus*; 1. P\* value <0.05 => statistical significance; 2. Odd Ratio, proportion of contribution to explain the outcome variable; 3. 95% confidence interval for the odd ratio.

Based on the results of antimicrobial susceptibility, 20(6%) of the isolates categorized as non-multidrug resistant (non-MDR), 310(93.7%) as multidrug resistant (MDR), 2(0.6%) was extensively drug resistant (XDR) and no pan-drug resistant (PDR) was found (Table 3). Figure 1 illustrates the geographic distribution of antimicrobial resistance categories in Sudan.

Regarding the correlation of the demographic characteristics of the patients and the resistance patterns along with the antimicrobial resistance categorization of the isolates, it was insignificant correlation, except for the source of the sample ( $p$ -value=0.041, OR=2.66, 95% CI=(1.04-6.79), (Table 4). Dendrogram diagram used to show the hierarchical clustering of the isolates according to their resistance patterns. The dendrogram presented two main clusters of the isolates and 22 related sub-clusters, (Figure 2 Dendrogram.docx).

## 4. Discussion

The response of bacteria to antimicrobial agents continues to change over time and from place to place rapidly. Therefore, the emergence of bacterial resistance imposes the need for continuous surveillance to guide therapy. Sudan is characterized by diverse nature, the weather, terrain, lifestyle of the people and even the types of domestic livestock and wildlife in each region vary widely. All these factors substantially affect the types of diseases exist in each area. This appeared clearly in this study, as the clinical source of the samples showed significant correlation with regions ( $p$ -value=0.005).

Previous studies inside and outside Sudan reported *S. aureus* as a major causative agent of skin, wound, urinary track and body fluids infections [9,10,11,12,13,14]. Our results supported these findings since wound swabs were the most frequent sample type, additionally, other samples related to skin, like pus, catheter tips and tissues, had considerable frequencies along with body fluids and urine. *S. aureus* is also known to cause meningitis in neonates aged less than 1 month [15] and that was reported in this study as well, four strains were isolated from the cerebrospinal fluid of neonates less than 3 weeks old. It is interesting to note that *S. aureus* invasive cases are more prevalent in neonates than in older children in territories with limited resources [16,17].

Like many studies conducted in Sudan, we also found that males with *S. aureus* infections were more than females [14,15,18,19]. Zhang in China, 2017, emphasized that the rate and type of infections differ between males and females due to the differences between them in anatomy and physiology, health behaviors, smoking, exposure to risk, environmental experiences, and stress [18].

Infections with *S. aureus* are more prevalent amongst people over fifties, and this is similar to the findings of Sudanese, Abidjani, and Chinese researchers [12,13,14,18]. Elderly patients are particularly susceptible to staphylococcal infections, especially MRSA, due to their frequent use of antibiotics and weakened immune systems.

Different rates of MRSA prevalence were determined in many studies in Sudan, ranging from 35%, and reached up to 77% over the 10 last year [9,11,13,14], which is still quite higher than worldwide records. For example, a

surveillance in Europe reported that the mean MRSA percentage was 18%, and some countries in this surveillance had reported MRSA percentages above 25% [19]. In South Africa, MRSA prevalence was 30.9%, and in India was 31.3% between 2017 and 2020 [20,21]. We reported 89.3% resistance to methicillin which exceeded both local and international prevalence records. MRSA now considered a MDR, fortunately, the anti-MRSA-Ceftaroline, Linezolid, and Teicoplanin, still have good action on MRSA, and provide excellent treatment choices, but the most prescribed drug for MDR-Gram-positive cocci in Sudan is Vancomycin. Despite the good results of this drug in treating MRSA, it was frustrating to find nearly 10% of isolates exhibited intermediate sensitivity to Vancomycin, a warning sign of further resistance.

The isolates showed varying resistance rates to the antimicrobial categories tested, from susceptible to all 15 categories to resistant to 14 of them. However, it was a shocking to find only 6% of isolates were non-MDR and 93.4% were MDR. The prevalence of MDR has been greatly influenced by the prevalence of MRSA ( $p$ -value less than 0.001, Odd ratio 2.25), as 24.1% of MDR isolates were categorized as MDR for just being MRSA, according to the guidelines of CDC and ECDC [13], and this justified the high rate of MDR in Sudan compared to other countries, as mentioned before MRSA incidence in Sudan was higher than worldwide reports. Although the XDR rate was low in our study, emerging from MDR to XDR is always a concern. Worldwide studies reported quite differences in MDR, XDR, and PDR incidences. In India, out of total 252 *S. aureus* strains studied, 35.3% were non-MDR, 49.6% were MDR, 15.1% strains were XDR, and no PDR reported [21]. In 2018, Indonesian researchers reported 66.6% of studied samples were non-MDR, 28.7% of samples collected were MDR, and 4.7% were XDR [22]. It was difficult to compare our findings of antimicrobial resistance categorization with studies in Sudan, since no studies that determined the frequencies of drug resistance categories (MDR, XDR and PDR) were published in our country. Additionally, many studies in Sudan and worldwide, followed different approaches for investigating the antimicrobial resistance patterns and categorization. This may justify the huge differences in findings between this study and the others.

The distribution of resistance across Sudan seems to be homogenized. Giving the fact that many patients from other states seek for medical care in Khartoum State. They travel to Khartoum to access secondary and tertiary medical services which they lack in their home states. It is common for those patients to receive antibiotics before attending the hospital from general practitioners or from over-the-counter sellers who often sell antibiotics at improper doses, increasing the risk for developing antimicrobial resistance.

Despite the accounted variation in the study area and the demographics criteria of the patients, still these factors have insignificant impacts on the resistance patterns of isolates, except for sample's source ( $p=0.04$ ). However, there was significant correlation between some study variables and sample's source (reflects the types of *S. aureus* infections) which is correlated significantly with the admission to ICU ( $p=0.001$ ), gender ( $p=0.04$ ), and sample collection area ( $p=0.005$ ). It means a certain *S.*

*aureus* strain in a particular region in Sudan is more likely to cause a certain type of infection that can lead to ICU admission.

Hierarchical cluster analysis, and the dendrogram constructed, illustrated how staphylococcal strains are clustered based on their antimicrobial resistance patterns. Surprisingly, the dendrogram showed MDR and Non-MDR bacteria clustering together in the same subgroups of comparable resistance patterns, this can be explained by the fact that some non-MDR bacteria can change their resistance pattern and become MDR. This bacterium is notorious for becoming progressively resistant to antibiotics; hence, they often tend to cause outbreaks in epidemic waves following the emergence of a few resistant clones which may explains the resistance clustering [23].

## 5. Conclusion

Although the prevalence of XDR and PDR *S. aureus* in Sudan is not as high as the worldwide reports, MDR strains are outspread in all Sudan and with resistance patterns near to XDR, which make the evolution into XDR and even PDR possible in near future. This is one of the challenging matters demanding immediate action.

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## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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