

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF STAPHYLOCOCCUS AUREUS TO DIFFERENT ANTIMICROBIAL AGENTS ISOLATED AS CLINICAL SAMPLES AT CERTAIN GENERAL HOSPITALS IN SANA'A CITY, YEMEN

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ABSTRACT

Background: *Staphylococcus aureus* has become an important cause of persistent nosocomial and community acquired infections by worldwide spread of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the modern day. **Objective:** The study aims to assess the antibiotic susceptibility pattern of *S. aureus* from different hospitals clinical specimens and determining prevalence of MRSA. **Methods:** A retrospective cross-sectional study of 500 samples *S. aureus* isolates from clinical samples of patients admitted to Al-Gumhury Hospital, 48 Model Hospital, Al-Thawra General Hospital, and National Center of Public Health Laboratories (NCPHL) in Sana'a city. Kirby-Bauer disk diffusion, antimicrobial susceptibility testing (AST), is used. **Results:** The results revealed that the proportion of *S. aureus* infection, 334 (66.8%) were males and 166 (33.2%) were females. Moreover, Al-Thawra General Hospital had 198 (39.6%) *S. aureus* isolates, then (NCPHL) 112 (22.4%), Al-Gumhouri Teaching Hospital 109 (21.8%) and 48 Model Hospital 81 (16.2%). A statistically significant association between hospitals and MRSA was found. MRSA strains were predominantly isolated from pus and ear swab 32.4% and 24%

respectively. Moreover, the prevalence of MRSA among all *S. aureus* isolates was (20.8 %). In addition, a certain antimicrobial such as Linezolid, Vancomycin, Clindamycin and Amikacin had the highest sensitivity rate. Conversely, Penicillin, Methicillin, Oxacillin, Cefoxitin, and others showed the highest resistance rates. **Conclusions:** The study reveals a moderate prevalence of MRSA representing nearly quarter of *S. aureus* cases at certain general hospitals in Sana'a city, Yemen, indicates ongoing screening and follow-up programs and suggestions for the development of antimicrobial stewardship programs in Sana'a, Yemen.

KEYWORDS: Antimicrobial susceptibility patterns, *S. aureus*, MRSA, Yemen.

INTRODUCTION

Global resistance to antimicrobials is increasing for many reasons, the most important of which is the increase in prescriptions, dispensation in developing countries, and indiscriminate use.^[1] According to estimates by the World Health Organization and United Nations report, deaths due to antimicrobial resistance could increase with the time.^[2-3]

Recently, antimicrobial resistance is a major public health threat and antimicrobial resistance bacteria in different hospital departments are increasing dramatically all over the world and in Yemen this problem is more extensive and complex.^[4-5]

In Yemen, most studies focused on studying the sensitivity to antibiotics for each bacterium separately, while few studies studied the resistance to all bacterial isolates.^[4] It has been predicted that if appropriate control and prevention measures are not taken, antimicrobial resistance will become one of the leading causes of death among hospitalized or non-hospitalized patients in developing and developed countries.^[6]

According to the WHO Global Action Plan on Antimicrobial Resistance, it is essential to increase awareness of antimicrobial resistance throughout research and monitoring programs in all parts of the world.^[7]

Staphylococcus aureus is one of the common pathogens that cause nosocomial infection and community infection. It was found that *S. aureus* was the second leading pathogen of antimicrobial resistance-related deaths in 2019.^[8] The prevalence of MRSA varies greatly between nations, as well as from one hospital to another within a single nation. The most

recent data from the WHO on MRSA incidence showed rates surpassing 20% in all WHO regions and even as high as 80% in some countries.^[9]

Currently, there have been many studies showing that *S. aureus* is increasingly resistant to antibiotics and spread of multidrug resistant (MDR). Notably, methicillin resistant *S. aureus* (MRSA) is a global health problem ,causing many difficulties in treatment and prognosis.^{[10-}

^{11]} Therefore, determining the antibiogram of *S. aureus* will help clinicians to treat cases with the most appropriate drug(s). The main objective of this study is to assess the antimicrobial susceptibility patterns of *S. aureus* isolated from clinical samples at certain general hospitals in Sana'a city, Yemen.

SUBJECTS AND METHODS

This was a retrospective study of clinical samples of patients who were laboratory diagnosed to had *S. aureus* infection. from January 2023 to December 2023 at four referral major hospitals in Sana'a: Al-Thawra General Hospital, Al-Gumhouri Teaching Hospital, 48 Model Hospital and National Center for Public Health Laboratories (NCPHL).

The sample size of this study was 500 based on electronic medical records (EMR) of *S. aureus* isolated from different clinical specimens at four major referral hospitals in Sana'a city as shown in Table 1.

Table 1: Distribution of clinical samples according to hospitals.

Clinical samples	Pus	V. swab	Sputum	C.S.F	Urine	Semen	Wound swab	Ear swab	Blood	Milk aspiration	Total
Hospital											
48 Model Hospital	68	0	6	0	3	1	0	3	0	0	81
National Center of Public Health Laboratories	93	3	2	0	0	5	2	6	0	1	112
AlGumhouri Teaching Hospital	53	5	0	0	17	18	0	16	0	0	109
Al-Thawra General Hospital	48	3	14	3	112	6	3	0	9	0	198
Total	262 52.4%	11 2.2%	22 4.4%	3 0.6 %	132 26.4%	30 6%	5 1%	25 5%	9 1.8%	1 0.2%	500 100%

***S. aureus* identification**

Phenotypic approaches had used to identify *S. aureus* isolates and assess their antibiotic susceptibility (AST). To accomplish this, each specimen was examined using a variety of identification techniques, such as Gram-stained smear light microscopy, observation of colony morphology and growth patterns on different media ,such as deoxyribonuclease agar and mannitol salt agar, and manual biochemical reactions, such as catalase and coagulase tests.^[7]

Antibiotic sensitivity

The modified Kirby-Bauer disc diffusion method was used to assess the antibiotic sensitivity of bacterial isolates on Mueller-Hinton agar. The inhibitory zone diameter was interpreted in accordance with the 2017 Clinical Laboratory Standards Institute guidance, CLSI.^[12-13]

Detection of MRSA

Cefoxitin disc diffusion was used to detect MRSA strains as recommended by CLSI to detect methicillin resistance.^[14] Cefoxitin can be utilized to screen diverse MRSA populations since it is a more effective inducer of *mec-A gene* expression than oxacillin or methicillin.^[12]

Statistical analysis

Data analysis was done using SPSS statistical program, Version 26. The association of MRSA with baseline characteristics of clinical samples received for *S. aureus* were determine by calculating X^2 and *P* value. Different antibiotic resistance patterns and their frequency were calculated and difference rate and significance of resistant to different antibiotics were calculated. The significance of the observed difference between groups was assessed using the X^2 test with a threshold *P*=.05.

Ethical issues: Following a commitment to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Sana'a University. No patients were directly involved in this retrospective study, and the secondary data were analyzed based on routine microbiological analysis.

RESULTS AND DISCUSSION

Demographic characteristics of the study population are presented in Table 2. Among these 500 *S. aureus* isolates, 334 (66.8%) were males and 166 (33.2%) were females. Moreover, Al-Thawra General Hospital had 198 (39.6%) *S. aureus* isolates, then National Center of

Public Health Laboratories (NCPHL) 112 (22.4%), Al-Gumhour Teaching Hospital 109 (21.8%) and 48 Model Hospital 81 (16.2%).

The rate of isolation for *S. aureus* was highest from pus samples 262 (52.4%) followed by urine 132 (26.4%), and the lowest was milk aspiration 1(0.2%).

Table 2: Demographic characteristics of the study population.

Characteristic	Frequency (%)
Gender	
Male	334 (66.8%)
Female	166 (33.2%)
Hospital	
48 Model Hospital	81 (16.2%)
National Center of Public Health Laboratories	112 (22.4%)
Al-Gumhour Teaching Hospital	109 (21.8%)
Thawra General Hospital	198 (39.6%)
Total	500 (100.0%)
Clinical Samples	
Pus	262 52.4%
Vaginal swab	11 2.2%
Sputum	22 4.4%
C.S.F	3 0.6 %
Urine	132 26.4%
Semen	30 6%
Wound swab	5 1%
Ear swab	25 5%
Blood	9 1.8%
Milk aspiration	1 0.2%
Total	500 100%

The overall prevalence of MRSA among all *S. aureus* isolates was 104/500 (20.8 %), as shown in Table 3.

MRSA were present at the National Center of Public Health Laboratories (NCPHL) followed by 48 Model Hospital, but not in other hospitals. This result was statistically significant association between hospitals and MRSA ($\chi^2 = 382.3$ & $P = 0.00$). Moreover, MRSA strains were predominantly isolated from pus cultures and ear swab that is 32.4% and 24% respectively and this result was not statistically significant association between clinical samples and MRSA ($\chi^2 = 66.9$ & $P = 6.2$) as shown in Table 3.

Table 3: Association of *Staphylococcus Aureus* Strains in Hospitals According to Clinical Samples.

Variable	MRSA <i>n</i> (%)	MSSA <i>n</i> (%)	Total <i>n</i> (%)	χ^2	<i>P</i>
Hospital					
48 Model Hospital	7 8.6% 1.4% *	74 91.4%	81	382.3	0.0
National Center of Public Health Laboratories	97 86.60% 19.4% *	15 13.40%	112		
Al-Gumhuri Teaching Hospital	0 0%	109 100%	109		
Al-Thawra General Hospital	0 0%	198 100%	198		
Total	104 20.8%	396 79.2%	500 100%		
Clinical samples					
Pus	85 (32.4%)	177 (67.6%)	262	66.9	6.2
Vaginal swab	3 (32.4%)	8(32.4%)	11		
Sputum	2 (9%)	20 (91%)	22		
C.S.F	0 (0%)	3 (100%)	3		
Urine	0 (0%)	132 (100%)	132		
Semen	5 (16.7%)	25 (83.3%)	30		
Wound swab	2 (40%)	3 (60%)	5		
Ear swab	6 (24%)	19 (86%)	25		
Blood	0 (0%)	9 (100%)	9		
Milk aspiration	1 (100%)	0 (0%)	1		
Total	104 20.8%	396 79.2%	500 100%		

* % whole total

S. aureus isolated in this study were highly resistant to Penicillin, methicillin, oxacillin (100%), followed by Cefoxitin (98.1%) Ampicillin (89.0%), Cefuroxime (80.5%), Fusidic

acid (80%) and Ceftazidime (78.9%). Moxifloxacin and Fosfomycin was the sole drug that exhibited 100% sensitivity to this pathogen, as shown in Table 4 & Figure 1.

Table 4: Antimicrobial Susceptibility Patterns of *S. Aureus* to Different Antimicrobial Agents.

Antimicrobials	Resistance		Sensitive		Intermediate		Total	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Penicillin	180	100.0%	0	0.0%	0	0.0%	180	100.0%
Methicillin	12	100.0%	0	0.0%	0	0.0%	12	100.0%
Ampicillin	138	89.0%	14	9.0%	3	1.9%	155	100.0%
Amoxicillin	33	68.8%	14	29.2%	1	2.1%	48	100.0%
Augmentin	202	55.3%	144	39.5%	19	5.2%	365	100.0%
Cephalexin	36	34.3%	57	54.3%	12	11.4%	105	100.0%
Cefuroxime	107	80.5%	21	15.8%	5	3.8%	133	100.0%
Cefotaxime	153	61.9%	58	23.5%	36	14.6%	247	100.0%
Ceftazidime	97	78.9%	20	16.3%	6	4.9%	123	100.0%
Meropenem	22	37.9%	31	53.4%	5	8.6%	58	100.0%
Imipenem	53	34.4%	97	63.0%	4	2.6%	154	100.0%
Vancomycin	12	3.5%	326	95.9%	2	0.6%	340	100.0%
Doxycycline	69	34.3%	116	57.7%	16	8.0%	201	100.0%
Amikacin	16	22.2%	56	77.8%	0	0.0%	72	100.0%
Gentamicin	78	29.9%	163	62.5%	20	7.7%	261	100.0%
Erythromycin	111	46.3%	111	46.3%	18	7.5%	240	100.0%
Azithromycin	126	51.4%	98	40.0%	21	8.6%	245	100.0%
Ciprofloxacin	122	43.4%	147	52.3%	12	4.3%	281	100.0%
Linezolid	1	0.8%	119	98.3%	1	0.8%	121	100.0%
Moxifloxacin	0	0.0%	16	100.0%	0	0.0%	16	100.0%
Levofloxacin	36	31.0%	80	69.0%	0	0.0%	116	100.0%
Cefepime	32	40.0%	43	53.8%	5	6.3%	80	100.0%
Fusidic acid	4	80.0%	1	20.0%	0	0.0%	5	100.0%
Co-trimoxazole	17	32.7%	35	67.3%	0	0.0%	52	100.0%
Tetracycline	19	33.9%	36	64.3%	1	1.8%	56	100.0%
Clindamycin	9	15.0%	51	85.0%	0	0.0%	60	100.0%
Ceftriaxone	41	59.4%	28	40.6%	0	0.0%	69	100.0%
Cefoxitin	104	98.1%	2	1.9%	0	0.0%	106	100.0%
Oxacillin	5	100.0%	0	0.0%	0	0.0%	5	100.0%
Fosfomycin	0	0.0%	1	100.0%	0	0.0%	1	100.0%
Ofloxacin	12	60.0%	8	40.0%	0	0.0%	20	100.0%

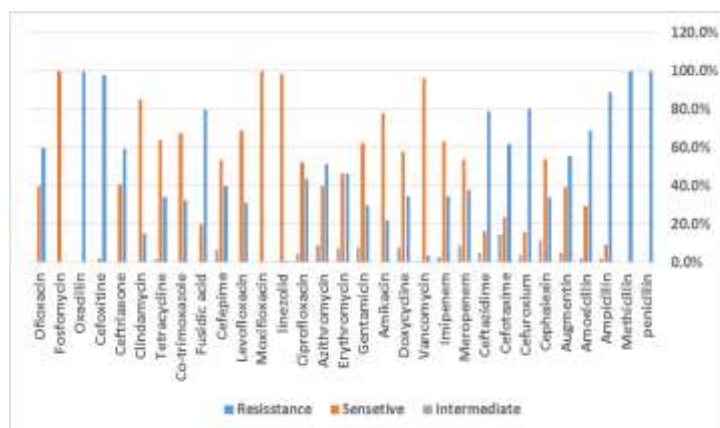


Fig. 1: Antimicrobial Susceptibility Patterns of *S. Aureus* to Different Antimicrobial Agents.

S. aureus is a common bacterial pathogen that affects everyone, both male and female. In this study, the distribution of *S. aureus* among gender was found to be higher in male than female patients (66.8% vs 33.2%).

The finding resembled with the research conducted in China^[15] and in Egypt.^[16] In addition, the result obtained from the present study was contrast to previous studies, which showed the incidence of *S. aureus* was much higher in females than in males.^[17-11]

The reason could be partly due to the infection rate between genders depending on geography and year of study. Therefore, gender and age are not risk factors for the acquisition or colonization of MRSA.

The present investigation depicted that the prevalence of MRSA and MSSA isolated from pus was the highest in comparison to other clinical samples, despite the fact that there was no statistical association between the isolation rates of MRSA and MSSA with any of the analyzed clinical samples.

This result is in line with the findings from various study settings in Nepal^[18] and Afghanistan.^[19]

The present study found that most of *S. aureus* isolates particularly MRSA from National Center of Public Health Laboratories (NCPHL). The reason is (NCPHL) regarded as a major referral central laboratory in Yemen that received most clinical samples at Sana'a city.

The present study revealed a prevalence of 20.8% MRSA, which is comparable to the studies, conducted in other regions of Yemen, including 37.4% at the 48 Military hospital in Sana'a city & 17.6% at the private hospitals in Sana'a city, Yemen.^[10-20] This shows that there is a difference in the MRSA prevalence pattern in various areas of the country. A reason for the difference in MRSA spectrum may be due to injudicious use of antibiotics. However, several investigations carried out in region and elsewhere in the world reported a greater prevalence of MRSA.^[21-22] Although it is quite challenging to reconcile these contradictory data with respect to both time and location, these variations could be due to variances in the circulating clones, infection control procedures and trends for antibiotic prescription in various hospital settings. Additionally, the decline in prevalence in this study might be brought on by a reduction in hospital visits and less exposure to *S. aureus* infections because of COVID- 19-induced travel restrictions.

AST of all 500 *S. aureus* isolates against 31 commonly used antibiotics showed that the overall resistance to antibiotics was alarmingly higher in Penicillin, methicillin, oxacillin (100%), followed by Cefoxitin (98.1%) Ampicillin (89.0%), Cefuroxime (80.5%), Fusidic acid (80%) and Ceftazidime (78.9%). Moxifloxacin & Fosfomycin was the sole drug that showed 100% sensitivity to this pathogen.

The higher rate of resistance observed in penicillin (100%) is in line with the results in the studies conducted in a tertiary care hospital in eastern Nepal that the drug was 100% resistant against this pathogen is corroborated by a study elsewhere in the world.^[11-22-23]

The prevalence of MRSA strains, which have been shown to be resistant to vancomycin in earlier investigations, varies substantially depending on the study location, from none (0.0%) in different parts of Yemen to (3.50%) in this study and in private hospitals at Sana'a city (7.80%).^[10-6] Alarmingly higher (29.4%) in Ethiopia and 62.5% in south- west Nigeria.^[24-25]

A Kenyan hospital in 2018 reported sensitivity against vancomycin and linezolid of 95% and 97.3% respectively which similar to our findings in this study.^[26]

The control of MRSA transmission seems to be the only hope to complete eradication of MRSA. The most effective way to control MRSA is good hand hygiene along with environmental cleaning of hospital rooms to reduce nosocomial rates of infection. The results of current study showed that there were highly susceptible to newer drugs such as

Fosfomycin and Linezolid. This finding differs from a study done in Iran which showed 5.5% of MRSA isolates were resistant to Linezolid.^[25-27]

CONCLUSION

The present study reveals a moderate prevalence of MRSA representing nearly quarter of *S. aureus* cases at general hospitals in Sana'a city, Yemen. The accurate diagnosis of MRSA strains in hospitals, patients and health care workers is an important need. In addition, the dissemination of MRSA and MSSA strains with high resistance to different antibiotics in Sana'a hospitals is a warning for public health. Accurate and continuous surveillance of antibiotic resistance patterns among *S. aureus* strains should be considered in health programs.

Recommendations

Further research through multicenter studies and integrating clinical data is warranted to guide evidence-based strategies against this critical healthcare challenge.

Limitations

The study duration encompasses only a year. A longer study duration could have been helpful to determine the trend of MRSA over the years. Due to the retrospective nature of the investigation, we discovered some missing data and only 104 of the 500 samples were analyzed for a thorough examination of MRSA prevalence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

Following a commitment to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Sana'a University. No patients were directly involved in this retrospective study, and the secondary data were analyzed based on routine microbiological analysis.

Author's contribution

The second and fourth author, who collect all clinical data. While the third and first author had analyzed the data, wrote, reviewed and edited the paper.

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