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Antibiotic susceptibility and phenotypic profile of *Staphylococcus* species isolated from different clinical samples from health facilities: A cross-sectional study

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Abstract

Background: *Staphylococcus* species are widely distributed in nature and found in various human body sites.

Objectives: To determine the antibiotic susceptibility pattern of *Staphylococcus* species isolated from different clinical samples.

Methods: This cross-sectional study was conducted on 400 clinical specimens from conveniently sampled patients seeking healthcare at two health facilities in sulaimani / Iraq. Bacterial isolation and identification were done using conventional techniques, after which the antibiotic susceptibility profile of *Staphylococcus* species commonly prescribed antibiotics used in treating infections at the facilities was done using the disc diffusion method. Finally, *MecA*, methicillin-resistant *Staphylococcus aureus* and macrolides-lincosamide and streptogramin genes with mupirocin-resistant, beta-lactamase and vancomycin-resistance phenotypes were identified.

Results: *Staphylococcus aureus* was the prevalent isolated species ($n = 197$, 49.3%), followed by *Staphylococcus hemolyticus* ($n = 115$, 28.8%), *Staphylococcus epidermidis* ($n = 49$, 12.3%), *Staphylococcus hominis* ($n = 9.0$, 2.3%), *Staphylococcus sciuri* ($n = 8.0$, 2.0%) and *Staphylococcus lentus* ($n = 4.0$, 1.0%). All isolated species resisted Penicillin G, Ampicillin, Cefotaxime and Cefoxitin. Most of the isolates, 89.5% ($n = 358$) had the beta-lactamase phenotype, 18.0% ($n = 72$) had the MecA gene, 2.8% ($n = 11$) the Mupirocin-resistant phenotype, and 2.0% ($n = 8.0$) the vancomycin-resistance phenotype. Additionally, 12 isolates had both methicillin-resistant *Staphylococcus aureus* (66.7%) and macrolides-lincosamide and streptogramin (65.2%) genes. The majority of the patients, 43% ($n = 172$) were >50 years old and 52.25% ($n = 209$) males. Also, most samples were from patients with urinary tract infection ($n = 73$), wound ($n = 71$), blood ($n = 35$), sputum ($n = 29$), pus ($n = 28$), seminal fluid ($n = 27$), cerebrospinal fluid ($n = 1.0$) and stool ($n = 1.0$). Most isolates that had the MSLb gene were highly significantly resistant to both Clindamycin (94.6%) and Erythromycin (84.7%) ($p < 0.001$).

Conclusions: *Staphylococcus aureus* was the predominant *Staphylococcus* species isolated from the clinical samples, most of which were resistant to most commonly prescribed antibiotics and had developed resistant genes and phenotypes.

Keywords

Gram-negative bacteria, antibiotic susceptibility test, incidence, various patient sample

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Introduction

Staphylococcus species are non-sporforming, heat-resistant, spherically shaped Gram-positive bacteria that can grow in aerobic and anaerobic conditions.[1] The *Staphylococcus* genus encompasses more than 47 species and 23 subspecies broadly classified into coagulase-positive and negative groups. Among the coagulase-positive species, *Staphylococcus aureus*

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is notable for causing food-borne infections in humans, soft tissue infection, pneumonia and urinary tract infection (UTI), with >1 million deaths in 2019.[2,3] At the same time, *Staphylococcus epidermidis* and *Staphylococcus hemolyticus* are among the coagulase-negative species known to cause hospital-associated infections.[4] The diversity of the *Staphylococcus* genus reflects its varying roles, from being harmless commensals to pathogenic organisms that cause serious infections.[5]

In clinical settings, various samples, such as pus from abscesses, ear discharge, blood, nasal swabs, throat swabs and urine, are commonly analysed to study these bacteria, each offering unique insights into their prevalence and resistance patterns.[6] In sputum samples, the presence of *S. aureus* can indicate oropharyngeal or pulmonary infections, especially among immunocompromised individuals.[7]

For analysis of suspected tissue samples, a polymerase chain reaction assay can definitely help determine the bacterial profile. For blood samples, the culturing technique is a suitable method for bacterial isolation, specifically methicillin-resistant *S. aureus* (MRSA), which complicates clinical management and signifies the adaptability of bacteria to therapeutic pressures. MRSA is most commonly known to carry *MecA*.[8]

Resistance to macrolides-lincosamide and streptogramin (MLSb) in *Staphylococcus* species is associated with three main mechanisms, including methylation of rRNA (target modification), active efflux and enzymatic inactivation of drugs.[9] The resistance is further complicated by beta-lactamase (BLACT) enzyme secretion that effectively neutralizes broad-spectrum antibiotics, highlighting a dynamic arms race between bacterial survival strategies and the development of antibiotic agents.[10] Bacterial chromosomal genes produce this enzyme and can be transferred between bacteria, binding extracellularly to prevent antibiotics from reaching their intracellular targets.[9]

The emergence of strains resistant to vancomycin is a growing crisis in managing severe infections by staphylococci that necessitates the need for novel antimicrobial discoveries and the prudent use of existing antibiotics.[11] The management of MRSA encompasses various strategies, evolving with new diagnostic and treatment approaches that start with initial management, assessing the MRSA infection type and severity to determine the appropriate treatment. Antibiotic therapy is tailored based on infection type and local antibiotic resistance patterns.[12] Thus, this study was conducted to determine the *Staphylococcus* species in various clinical samples and their susceptibility to multiple antibiotics.

Patients and methods

Study design and setting

Using a convenient sampling technique, this cross-sectional study was conducted on 400 samples from 400 patients (112 samples were out-patient and 288 were from in-patient) with various diseases between October 2023 and April 2024. Those patients were admitted to the Anwar Shexa Medical City and the Smart Health Tower, Sulaimaniyah, Iraq. These two health facilities are general private hospitals with more than 15 departments (such as radiology, surgery, microbiology, immunology, serology, internal medicine, paediatrics, obstetrics, orthopaedics, pathology and so on) and 100 beds in the centre of Sulaimaniyah city with more than 300 healthcare staff.

Inclusion criteria

Samples from in-patients and out-patients aged ⩾18 with various clinical diseases.

Exclusion criteria

Samples from unknown sources and improperly labelled/stored/collected samples were excluded.

Study protocol

Different clinical samples (wound, vaginal, nasal, ear, throat swabs, blood, urine, pus, sputum, bronchial wash, catheter tip, stool, endotracheal aspiration, seminal fluid, cerebrospinal fluid, bile fluid, pleural fluid, peritoneal fluid, synovial fluid, nipple discharge and central vein line) were collected directly from patients with various infections. Then, samples were processed immediately after reaching the Bacteriology Laboratory using standard bacteriological techniques, such as culturing on various culture agars under aerobic conditions at 37°C for 48 h. Later, bacterial identification was done by colonial morphology (round, convex, and 1–4 mm in diameter with a sharp border), Gram staining (Gram-positive purple colour), catalase test (catalase positive), and coagulase test (*S. aureus* and *Staphylococcus intermedius* are coagulase-positive, while other staphylococci are coagulase-negative). Additionally, automated detection of bacterial isolates was done using the BD Phoenix™ M50 System (BD, USA). Through a specific panel for Gram-positive bacteria and based on criteria provided by the manufacturer, antibiotic susceptibilities were determined using minimum inhibitory concentrations. Simultaneously, the detection of *MecA*, MRSA and MLSb genes with mupirocin-resistant (MR), BLACT and Vancomycin-resistance (VR) phenotypes was performed. The antibiotics used were selected according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[13]

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS; IBM, Chicago, IL, USA, version 26). Analysed data presented as numbers and percentages. The Chi-square test was used to determine the relationship between the categorical variables. A p-value ⩽0.05 was considered as significant.

Table 1. Sociodemographic characteristics of the patients with collected sample types.

Patient characteristics	Frequency (%)
Age (years)	
<30	80 (20)
30–50	148 (37)
>50	172 (43)
Gender	
Female	191 (47.75)
Male	209 (52.25)
Type of sample	
Urine	73 (18.3)
Wound swab	71 (17.8)
Blood	35 (8.8)
Sputum	29 (7.3)
Pus	28 (7.0)
Seminal fluid	27 (6.8)
Tissue	26 (6.5)
ETA	18 (14.5)
Vaginal swab	14 (3.5)
Catheter tip	11 (2.8)
BAL	9.0 (2.3)
Central vein line	8.0 (2.0)
PE	8.0 (2.0)
Ear swab	7.0 (1.8)
Nasal swab	7.0 (1.8)
PF	8.0 (2.1)
Bile fluid	6.0 (1.5)
Throat swab	6.0 (1.5)
Synovial fluid	4.0 (1.0)
Nipple discharge	3.0 (0.8)
CSF	1.0 (0.3)
Stool	1.0 (0.3)

BAL: bronchial wash; CSF: cerebrospinal fluid; ETA: endotracheal aspiration; PF: peritoneal fluid; PL: pleural fluid.

Results

Sociodemographic characteristics of the patients with collected sample types

The mean age of the patients from which samples were collected was 46.65 ± 19.90 years. Most patients were aged >50 years old (43%) and males (52.25%). In addition, most samples were from patients with UTI ($n=73$), followed by wound ($n=71$), blood ($n=35$), sputum ($n=29$), pus ($n=28$) and seminal fluid ($n=27$), while the least were from patients' cerebrospinal fluid (CSF) and stool ($n=1.0$ each; Table 1).

Prevalence of Staphylococcus *species isolated from the clinical samples*

In all, 17 different *Staphylococcus* were isolated from the 400 clinical samples. Among them, *S. aureus* was prevalent ($n=197$, 49.3%), followed by *S. hemolyticus* ($n=115$, 28.8%), then *S. epidermidis* ($n=49$, 12.3%), *Staphylococcus hominis* ($n=9.0$, 2.3%), *Staphylococcus sciuri* ($n=8.0$, 2.0%), *Staphylococcus lentus* ($n=4.0$, 1.0%), *Staphylococcus capitis* ($n=3.0$, 0.8%), *Staphylococcus saprophyticus* ($n=3.0$, 0.8%), *Staphylococcus intermedius* ($n=2.0$, 0.5%), *Staphylococcus schleiferi* ($n=2.0$, 0.5%), and *Staphylococcus warneri* ($n=2.0$, 0.5%). Other species, such as *Staphylococcus caparae*, *Staphylococcus cohnii*, *Staphylococcus lugdunensis*, *Staphylococcus pasteuri*, *Staphylococcus simulans*, and *Staphylococcus xylosus* were also isolated (0.1% each). *S. aureus* was prominently isolated from wound swabs ($n=38$, 19.3%), *S. hemolyticus* in urine samples ($n=37$, 32.2%), *S. epidermidis* in urine and blood ($n=9.0$, 18.4%), *S. hominis* in blood ($n=5.0$, 55.6%), *S. sciuri* in urine, wound and sputum ($n=2.0$, 25%) and *S. lentus* ($n=1.0$, 0.25%) from urine, wound swab, blood and vaginal swab (Table 2).

Antibiotic profile of isolated Staphylococcus *species*

About 96.4% ($n=190$) of *S. aureus* isolates were resistant to Ampicillin (AM), 92.4% ($n=182$) to Penicillin G (PG), 68.5% ($n=135$) to Clindamycin (CLI), 71.1% ($n=140$) to Erythromycin (ERT)/Tetracycline (TET), 52.8% ($n=104$) to Cefotaxime (CTX) and 49.2% ($n=97$) to Cefoxitin (CFX). Also, 95.7% ($n=110$) of the *S. hemolyticus* isolates were resistant to PG, 91.3% ($n=105$) to AM, 83.5% ($n=96$) to CFX, and 82.6% ($n=95$) to CTX. All the *S. epidermidis* isolates were resistant to PG, 95.9% ($n=47$) to AM, and 75.5% ($n=37$) to OX/CTX. All the *S. hominis* isolates were resistant to AM/PG and 66.7% ($n=6.0$) to CFX/CTX/OX/ERT. All the *S. sciuri* isolates were resistant to CFX/CTX/AM/PG and 87.5% ($n=7.0$) to Cefotaxime/Oxacillin (CFT/OX). All the *S. lentus* isolates were resistant to CFX/CTX/AM/PG/OX/TET and 75% ($n=3.0$) to ERT. Regarding the sensitivity incidence, *S. lentus* had the highest rate of sensitivity (100%) to seven antibiotics (DA, TEI, VA, LINZ, NIT, TER and TIG), followed by *S. hominis* to six antibiotics (DA, TEI, VA, LINZ, MUR and NIT), then *S. sciuri* to four antibiotics (DA, VA, LINZ and MUR; Table 3).

Correlation of isolated Staphylococcus *species to phenotypic characteristics*

The majority, 89.5% ($n=358$) of the isolates had BLACT phenotype, 18.0% ($n=72$) had the MecA gene, 2.8% ($n=11$) had the MR phenotype and 2.0% ($n=8.0$) had the VR phenotype. Among 17 isolated species, 14 had BLACT phenotype, 4 had MR phenotype and 3 had *MecA* gene and VR phenotype. *S. aureus* and *S. hemolyticus* had BLACT, VR and MR phenotypes with the *MecA* gene. On the other hand, *S. warneri*, *S. lugdunensis* and *S. pasteuri* had neither phenotypic expression (BLACT, VR and MR) nor gene holding (Table 4). Moreover, 12 species had both MRSA ($n=267$, 66.7%) and MLSb ($n=261$, 65.2%) genes together, of which

Table 2. Common Staphylococcal isolates in relation to type of samples.

Type of sample	*Staphylococcus* species, Frequency (%)					
	S. aureus: 197 (49.3%)	*S. hemolyticus*: 115 (28.8%)	*S. epidermidis*: 49 (12.3%)	*S. hominis*: 9.0 (2.3%)	*S. sciuri*: 8.0 (2.0 %)	*S. lentus*: 4.0 (1.0%)
Urine ($n=73$)	19 (9.6)	37 (32.2)	9.0 (18.4)	0.0 (0.0)	2.0 (25)	1.0 (0.25)
Wound swab ($n=71$)	38 (19.3)	21 (18.3)	3.0 (6.1)	2.0 (22.2)	2.0 (25)	1.0 (0.25)
Blood ($n=35$)	16 (8.1)	3.0 (2.6)	9.0 (18.4)	5.0 (55.6)	0.0 (0.0)	1.0 (0.25)
Sputum ($n=29$)	23 (11.7)	3.0 (2.6)	1.0 (2.0)	0.0 (0.0)	2.0 (25.0)	0.0 (0.0)
Pus ($n=28$)	18 (9.1)	5.0 (4.2)	3.0 (6.1)	1.0 (11.1)	0.0 (0.0)	0.0 (0.0)
Seminal fluid ($n=27$)	5.0 (2.5)	18 (15.7)	3.0 (6.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Tissue ($n=26$)	13 (6.6)	5.0 (4.3)	5.0 (10.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
ETA ($n=18$)	14 (7.1)	4.0 (3.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Vaginal swab ($n=14$)	7.0 (3.6)	6.0 (5.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.0 (0.25)
Catheter tip ($n=11$)	3.0 (1.5)	2.0 (1.7)	5.0 (10.2)	0.0 (0.0)	1.0 (12.5)	0.0 (0.0)
BAL ($n=9$)	6.0 (3.0)	2.0 (1.7)	0.0 (0.0)	0.0 (0.0)	1.0 (12.5)	0.0 (0.0)
CV line ($n=8$)	3.0 (1.5)	2.0 (1.7)	3.0 (6.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PL ($n=8$)	3.0 (1.5)	2.0 (1.7)	2.0 (4.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Ear swab ($n=7$)	7.0 (3.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Nasal swab ($n=7$)	6.0 (3.0)	1.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PF ($n=7$)	3.0 (1.5)	0.0 (0.0)	4.0 (8.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Bile fluid ($n=6$)	1.0 (0.5)	3.0 (2.6)	0.0 (0.0)	1.0 (11.1)	0.0 (0.0)	0.0 (0.0)
Throat swab ($n=6$)	6.0 (3.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Synovial fluid ($n=4$)	3.0 (1.5)	0.0 (0.0)	1.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Nipple discharge ($n=3$)	2.0 (1.0)	0.0 (0.0)	1.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
CSF ($n=1$)	0.0 (0.0)	1.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Stool ($n=1$)	1.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

BAL: bronchial wash; CSF: cerebrospinal fluid; ETA: endotracheal aspiration; PL: pleural fluid; PF: peritoneal fluid.

S. aureus reported the highest, followed by *S. hemolyticus*, then *S. epidermidis* (Table 5). Furthermore, most of the isolates that had the MRSA gene had no *MecA* gene ($n=197$, 73.78%; $p<0.001$; Table 6). Most of the isolates that had the MSLb gene were highly significantly resistant to both Clindamycin (CLN; $n=243$, 94.6%) and ERT ($n=244$, 84.7%; $p<0.001$; Table 7).

Discussion

The present study demonstrates that *Staphylococcus* infections remain a significant public health concern. *Staphylococcus* species with varying antibiotic susceptibility profiles were isolated from the clinical samples of patients presenting with various diseases. However, the distribution of *Staphylococcus* species varied significantly across different age groups. For instance, *S. epidermidis* was commonly found in preterm neonates,[14] while older adults had higher rates of MRSA. These observations may have important implications for age-specific prevention and treatment strategies targeting Staphylococcal infections.[15]

In this study, the mean age of patients was 46.65 ± 19.90 years, and the majority (43%) were aged >50 years and were males (52.25%). These results agreed with Thorlacius-Ussing et al. who found that age was strongly related to *Staphylococcus* infection incidence and males had a twofold higher risk of acquiring infection than female patients.[16] These might be related to the fact that the immune systems of the elderly may not be able to fight infection, and people with chronic diseases may spend time in hospitals where they are exposed to infectious agents. In addition, the male gender is considered a risk factor for *Staphylococcus* infection as a high rate of bacterial colonization is related to free testosterone.[16]

Sample types are related to the prevalence rate of specific *Staphylococcus* species. Frequent detection of *S. aureus* in the blood reflects the bacteria's ability to cause severe systemic infections. Meanwhile, urine samples were more likely to contain methicillin-resistant coagulase-negative staphylococci, suggesting their role in UTIs. Thus, in this study, most *Staphylococcus* isolates were detected in UTI samples, followed by wound swabs, and the least were detected in CSF and stool samples. In this regard, Sigudu et al. reported the highest incidence of *Staphylococcus* species in the skin (39.7%), followed by urinary specimens (29.8%) and blood (20.5%).[17] These disparities between studies might be related to the sample size, sample type, patients' age, incidence of infection and type of infection.

Moreover, in this study, *S. aureus* was prevalent, followed by *S. hemolyticus*, then *S. epidermidis*, while *S. caparae*, *S. cohnii*, *S. lugdunensis*, *S. pasteuri*, *S. simulans* and *S. xylosus* were the least. These results agreed with that of Sigudu et al., who found the predominance of *S. aureus* (74.4%), followed

Table 3. Antibiotic profile of isolated *Staphylococcus* species.

Antibiotics	*Staphylococcus* species, Frequency (%)						
	S. aureus: 197 (49.3%)		*S. hemolyticus*: 115 (28.8%)	*S. epidermidis*: 49 (12.3%)	*S. hominis*: 9.0 (2.3%)	*S. sciuri*: 8.0 (2.0 %)	*S. lentus*: 4.0 (1.0%)
GN	I	1.0 (0.5)	1.0 (0.9)	2.0 (4.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	33 (16.8)	59 (51.3)	14 (28.6)	1.0 (11.1)	3.0 (37.5)	2.0 (50)
	S	163 (82.7)	55 (47.8)	33 (67.3)	8.0 (88.1)	5.0 (62.5)	2.0 (50)
CFX	I	5.0 (2.5)	2.0 (1.7)	2.0 (4.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	97 (49.2)	96 (83.5)	33 (67.3)	6.0 (66.7)	8.0 (100)	4.0 (100)
	S	95 (48.2)	17 (14.8)	14 (28.6)	3.0 (33.3)	0.0 (0.0)	0.0 (0.0)
CTX	R	104 (52.8)	95 (82.6)	37 (75.5)	6.0 (66.7)	8.0 (100)	4.0(100)
	S	93 (47.2)	20 (17.4)	12 (24.5)	3.0 (33.3)	0.0 (0.0)	0.0 (0.0)
CFT	I	5.0 (2.5)	21 (18.3)	4.0 (8.2)	1.0 (11.1)	0.0 (0.0)	2.0 (50)
	R	75 (38.1)	70 (60.9)	27 (55.1)	5.0 (55.6)	7.0 (87.5)	2.0 (50)
	S	117 (59.4)	24 (20.9)	18 (36.7)	3.0 (33.3)	1.0 (12.5)	0.0 (0.0)
AM	R	190 (96.4)	105 (91.3)	47 (95.9)	9.0 (100)	8.0 (100)	4.0 (100)
	S	7.0 (3.6)	10 (8.7)	2.0 (4.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
PG	R	182 (92.4)	110 (95.7)	49 (100)	9.0 (100)	8.0 (100)	4.0 (100)
	S	15 (7.6)	5.0 (4.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
OX	R	92 (46.7)	93 (80.9)	37 (75.5)	6.0 (66.7)	7.0 (87.5)	4.0 (100)
	S	105 (53.3)	22 (19.1)	12 (24.5)	3.0 (33.3)	1.0 (12.5)	0.0 (0.0)
DA	I	1.0 (0.5)	0.0 (0.0)	1.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	12 (6.1)	7.0 (6.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	S	184 (93.4)	108 (93.9)	48 (98)	9.0 (100)	8.0 (100)	4.0 (100)
SXT	I	1.0 (0.5)	1.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	40 (20.3)	43 (37.4)	20 (40.8)	5.0 (55.6)	3.0 (37.5)	2.0 (50)
	S	156 (79.2)	71 (61.7)	29 (59.2)	4.0 (44.4)	5.0 (62.5)	2.0 (50)
TEI	I	0.0 (0.0)	4.0 (3.5)	1.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	13 (6.6)	8.0 (7.0)	4.0 (8.2)	0.0 (0.0)	1.0 (12.5)	0.0 (0.0)
	S	184 (93.4)	103 (89.6)	44 (89.8)	9.0 (100)	7.0 (87.5)	4.0 (100)
VA	I	2.0 (1.0)	1.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	9.0 (4.6)	2.0 (1.7)	3.0 (6.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	S	186 (94.4)	112 (97.4)	46 (93.9)	9.0 (100)	8.0 (100)	4.0 (100)
CLI	I	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	3.0 (37.5)	0.0 (0.0)
	R	135 (68.5)	80 (69.5)	27 (55.1)	1.0 (11.1)	4.0 (50)	2.0 (50)
	S	62 (31.5)	35 (30.4)	22 (44.9)	8.0 (88.1)	1.0 (12.5)	2.0 (50)
ERT	I	3.0 (1.5)	5.0 (4.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	140 (71.1)	92 (80)	33 (67.3)	6.0 (66.7)	5.0 (62.5)	3.0 (75)
	S	54 (27.5)	18 (15.7)	16 (32.7)	3.0 (33.3)	3.0 (37.5)	1.0 (25)
LINZ	R	9.0 (4.6)	6.0 (5.2)	3.0 (6.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	S	188 (95.4)	109 (94.8)	46 (93.9)	9.0 (100)	8.0 (100)	4.0 (100)

(Continued)

Table 3. (Continued)

Antibiotics	*Staphylococcus* species, Frequency (%)						
	S. aureus: 197 (49.3%)		*S. hemolyticus*: 115 (28.8%)	*S. epidermidis*: 49 (12.3%)	*S. hominis*: 9.0 (2.3%)	*S. sciuri*: 8.0 (2.0 %)	*S. lentus*: 4.0 (1.0%)
MUR	R	14 (7.1)	10 (8.7)	3.0 (6.1)	0.0 (0.0)	0.0 (0.0)	2.0 (50)
	S	183 (92.9)	105 (91.3)	46 (93.9)	9.0 (100)	8.0 (100)	2.0 (50)
NIT	I	3.0 (1.5)	1.0 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	14 (7.1)	8.0 (7.0)	1.0 (2.0)	0.0 (0.0)	1.0 (12.5)	0.0 (0.0)
	S	180 (91.4)	106 (92.2)	48 (98)	9.0 (100)	7.0 (87.5)	4.0 (100)
CIP	I	1.0 (0.5)	1.0 (0.9)	2.0 (4.1)	1.0 (11.1)	0.0 (0.0)	0.0 (0.0)
	R	105 (53.3)	68 (59.1)	24 (49)	4.0 (44.4)	3.0 (37.5)	1.0 (25)
	S	91 (46.2)	46 (40)	23 (46.9)	4.0 (44.4)	5.0 (62.5)	3.0 (75)
LEV	I	28 (14.2)	19 (16.5)	6.0 (12.2)	0.0 (0.0)	1.0 (12.5)	0.0 (0.0)
	R	70 (35.5)	44 (38.3)	14 (28.6)	4.0 (44.4)	2.0 (25)	1.0 (25)
	S	99 (50.3)	52 (45.2)	29 (59.2)	5.0 (55.6)	5.0 (62.5)	3.0 (75)
MOX	I	5.0 (2.5)	2.0 (1.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	25 (12.7)	22 (19.1)	5.0 (10.2)	1.0 (11.1)	1.0 (12.5)	1.0 (25)
	S	167 (84.8)	91 (79.1)	44 (89.8)	8.0 (88.9)	7.0 (87.5)	3.0 (75)
RIF	I	1.0 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	26 (13.2)	18 (15.7)	7.0 (14.3)	1.0 (11.1)	1.0 (12.5)	1.0 (25)
	S	170 (86.3)	97 (84.3)	42 (85.7)	8.0 (88.9)	7.0 (87.5)	3.0 (75)
TET	I	3.0 (1.5)	3.0 (2.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	R	140 (71.1)	76 (66.1)	33 (67.3)	4.0 (44.4)	5.0 (62.5)	4.0 (100)
	S	54 (27.4)	36 (31.3)	16 (32.7)	5.0 (55.6)	3.0 (37.5)	0.0 (0.0)
TIG	I	2.0 (1.0)	12 (10.4)	3.0 (6.1)	1.0 (11.1)	0.0 (0.0)	0.0 (0.0)
	R	7.0 (3.6)	24 (20.9)	5.0 (10.2)	1.0 (11.1)	1.0 (12.5)	0.0 (0.0)
	S	188 (95.4)	79 (68.7)	41 (83.7)	7.0 (77.8)	7.0 (87.5)	4.0 (100)

GN: Gentamycin; CFX: Cefoxitin; CTX: Cefotaxime; CFT: Ceftazidime; AM: Ampicillin; PG: Penicillin G; OX: Oxacillin; DA: Daptomycin; SXT: Trimethoprim + Sulfamethoxazole; TEI: Teicoplanin; VA: Vancomycin; CLI: Clindamycin; ERT: Erythromycin: LIN: Linezolid; MUR: Mupirocin; NIT: Nitrofurantoin; CIP: Ciprofloxacin; LEV: Levofloxacin; MOX: Moxifloxacin; RF: Rifampicin; TET: Tetracycline; TG: Tigecycline.

Table 4. Relation of the phenotypic features with isolated *Staphylococcus* species.

Staphylococcus species	*MecA* gene		Mupirocin-resistant phenotype		Beta-lactamase phenotype		Vancomycin resistant phenotype	
	Negative: 328 (82.0)	Positive: 72 (18.0)	Negative: 389 (97.3)	Positive: 11 (2.8)	Negative: 42 (10.5)	Positive: 358 (89.5)	Negative: 392 (98.0)	Positive: 8.0 (2.0)
	Frequency (%)							
S. aureus	131 (66.5)	66 (33.5)	193 (98)	4.0 (2.0)	16 (8.1)	181 (91.9)	191 (97.0)	6.0 (3.0)
S. hemolyticus	110 (95.75)	5.0 (4.3)	110 (95.7)	5.0 (4.3)	14 (12.2)	101 (87.9)	114 (99.1)	1.0 (0.9)
S. epidermidis	49 (100)	0.0 (0.0)	48 (98.0)	1.0 (2.0)	3.0 (6.1)	46 (93.9)	49 (100)	0.0 (0.0)
S. hominis	9.0 (100)	0.0 (0.0)	9.0 (100)	0.0 (0.0)	1.0 (11.1)	8.0 (88.9)	9.0 (100)	0.0 (0.0)
S. sciuri	8.0 (100)	0.0 (0.0)	8.0 (100)	0.0 (0.0)	1.0 (12.5)	7.0 (87.5)	8.0 (100)	0.0 (0.0)
S. lentus	4.0 (100)	0.0 (0.0)	4.0 (100)	0.0 (0.0)	0.0 (0.0)	4.0 (100)	4.0 (100)	0.0 (0.0)
S. capitis	3.0 (100)	0.0 (0.0)	2.0 (66.7)	1.0 (33.3)	1.0 (33.3)	2.0 (66.75)	3.0 (100)	0.0 (0.0)
S. saprophyticus	3.0 (100)	0.0 (0.0)	3.0 (100)	0.0 (0.0)	2.0 (66.7)	1.0 (33.3)	3.0 (100)	0.0 (0.0)
S. intermedius	2.0 (100)	0.0 (0.0)	2.0 (100)	0.0 (0.0)	0.0 (0.0)	2.0 (100)	2.0 (100)	0.0 (0.0)
S. schleiferi	1.0 (50)	1.0 (50.0)	2.0 (100)	0.0 (0.0)	0.0 (0.0)	2.0 (100)	2.0 (100)	0.0 (0.0)
S. warneri	2.0 (100)	0.0 (0.0)	2.0 (100)	0.0 (0.0)	2.0 (100)	0.0 (0.0)	2.0 (100)	0.0 (0.0)
S. caparae	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	0.0 (0.0)	1.0 (100)	1.0 (100)	0.0 (0.0)
S. cohnii	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	0.0 (0.0)	1.0 (100)	1.0 (100)	0.0 (0.0)
S. lugdunensis	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)
S. pasteuri	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)
S. simulans	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)
S. xylosus	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)	0.0 (0.0)	1.0 (100)	1.0 (100)	0.0 (0.0)

Table 5. Relation of MRSA and MLSb phenotype with isolated *Staphylococcus* species.

Staphylococcus species	MRSA gene		MLSb gene	
	Negative: 133 (33.3)	Positive: 267 (66.7)	Negative: 139 (34.8)	Positive: 261 (65.2)
	Frequency (%)			
S. aureus	97 (49.2)	100 (50.8)	58 (29.4)	139 (70.6)
S. hemolyticus	13 (11.3)	102 (88.7)	33 (28.7)	82 (71.3)
S. epidermidis	12 (24.5)	37 (75.5)	24 (49)	25 (51.0)
S. hominis	3.0 (33.3)	6.0 (66.7)	8.0 (8.9)	1.0 (11.1)
S. sciuri	1.0 (12.5)	7.0 (87.5)	4.0 (50.0)	4.0 (50.0)
S. lentus	0.0 (0.0)	4.0 (100)	2.0 (50)	2.0 (50.0)
S. capitis	1.0 (33.3)	2.0 (66.7)	1.0 (33.3)	2.0 (66.7)
S. saprophyticus	1.0 (33.3)	2.0 (66.7)	2.0 (66.7)	1.0 (33.3)
S. intermedius	0.0 (0.0)	2.0 (100)	1.0 (50)	1.0 (50)
S. schleiferi	0.0 (0.0)	2.0 (100)	0.0 (0.0)	2.0 (100)
S. warneri	2.0 (100)	0.0 (0.0)	2.0 (100)	0.0 (0.0)
S. caparae	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)
S. cohnii	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)
S. lugdunensis	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)
S. pasteuri	0.0 (0.0)	1.0 (100)	1.0 (100)	0.0 (0.0)
S. simulans	1.0 (100)	0.0 (0.0)	1.0 (100)	0.0 (0.0)
S. xylosus	0.0 (0.0)	1.0 (100)	0.0 (0.0)	1.0 (100)

MRSA: methicillin resistance *S. aureus*; MLSb: macrolides-lincosamide and streptogramin.

by *S. epidermidis* (11%), and then *S. hemolyticus* (3.3%).[17] These findings might be related to the virulence factors available in these species that cause several clinical conditions in humans. *S. aureus* can spread from person to person by direct contact, through contaminated objects or, less often, by inhalation of infected droplets dispersed by sneezing or coughing.[17]

Furthermore, in this study, all isolates were resistant to PG, AM, CTX and CFX antibiotics but sensitive to DA, VA

Table 6. Correlation between MRSA with *MecA* genes.

MRSA gene: Frequency (%)	*MecA* gene: Frequency (%)		
	Negative: 328 (82)	Positive: 72 (18.0)	*p*-Value
Negative 133 (33.3)	131 (98.4)	2.0 (1.6)	<0.001[a]
Positive 267 (66.7)	197 (73.78)	70 (26.22)	

MRSA: methicillin resistance *Staphylococcus aureus.*
[a]Highly significant difference using Chi-square test.

Table 7. Correlation between MLSb phenotype with Clindamycin and Erythromycin.

Antibiotic	MLSb: Frequency (%)			*p*-Value
	Negative: 139 (34.8)	Positive: 264 (65.2)	Total	
CLI				
I	3.0 (75.0)	1.0 (25.0)	4.0 (1.0)	<0.001[a]
R	14 (5.4)	243 (94.6)	257 (64.3)	
S	122 (87.8)	17 (12.2)	139 (34.8)	
ERT				
I	4.0 (44.4)	5.0 (55.6)	9.0 (2.3)	<0.001[a]
R	44 (15.3)	244 (84.7)	288 (72.0)	
S	91 (88.3)	12 (11.7)	103 (25.8)	

MLSb: macrolides-lincosamide and streptogramin; CLI: Clindamycin; ERT: Erythromycin.
[a]Highly significant difference using Chi-square test.

and LINZ. In this regard, the highest resistance was observed against Cloxacillin (70.3%) and the lowest resistance against Colistin (0.1%).[18] In addition, *Staphylococcus* species showed high resistance to ERT (83%), CFT (68%) and AM (62%). These variations might be related to the type of isolated species, its virulence and its pathogenicity.

This investigation involved extensive phenotypic characterization of various clinical isolates belonging to different *Staphylococcus* species, focusing on MRSA prevalence rates, *MecA*-mediated resistance expression levels, BLACT production rates, and MLSb resistance frequencies. Most *Staphylococcus* isolates (89.5%) had BLACT phenotype, followed by *MecA* gene (18%), then MR phenotype (2.8%), while the least had VR phenotype (2.0%). Moreover, 12 species had both MRSA (66.7%) and MLSb (65.2%) genes. Most isolates that had the MRSA gene had no *MecA* gene (73.78%), and most isolates that had the MSLb gene were highly significantly resistant to both CLN (94.6%) and ERT (84.7%). A significant prevalence of the *MecA* gene was found in *S. aureus*, highlighting a high incidence of MRSA, corresponding to an enhanced risk for severe infections resistant to standard therapies. The fact that many different species of *Staphylococcus* produce BLACT suggests that there is still a long way to go in resisting beta-lactams, which complicates the application of wide-spectrum antibiotics and requires more specific therapeutic methods. Since *Staphylococcus* species frequently exhibit MRSA and MLSb phenotypes, treatment should develop plans reflecting these patterns depending on where they practice medicine; otherwise, their prescriptions will fail to work against infections caused by drug-resistant organisms, which might lead to many people being infected.

The study's limitations included that the data were collected over a specified period and may not capture temporal changes or long-term trends in *Staphylococcus* species prevalence and resistance patterns. In addition, the study was conducted in two institutions, which may limit its external validity to other healthcare facilities. In addition, the calculation and justification of the sample size selected for this study were not done. Consequently, conducting a broader study for a longer duration in multiple health centres is recommended, using more advanced techniques to determine the precise prevalence, species and resistance of isolated bacteria, especially *Staphylococcus* species.

Conclusions

Various *Staphylococcus* species were common among sample patients with variable diseases, especially aged males. *Staphylococcus* species exhibit a significant range of antibiotic resistance, with notable prevalence among *S. aureus* and *S. hemolyticus*. The high incidence of *MecA* and MLSb resistance across the species underscores the ongoing challenge of antibiotic resistance in clinical settings. The variation in resistance profiles calls for enhanced surveillance and personalized antibiotic therapy approaches to manage infections effectively. In addition, the study highlights the importance of continuous monitoring and developing novel strategies to

combat the evolving resistance patterns of *Staphylococcus* species to ensure adequate healthcare interventions.

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Author contributions

KAA: Conceptualization, supervision, study registration, editing of the final draft; SMS: Study design, data collection, writing of the original manuscript; SMA: Analysis and interpretation of results, writing of the original manuscript; AJMA: Methodology, investigation, study administration; MKQ: Writing of the original manuscript, resources.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability statement

Due to privacy and ethical restrictions, the data supporting this study's findings are available upon request from the corresponding author.

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Ethics approval

The Ethical Committee of the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq (No. 130 on 21 January 2024) approved the study protocol. The Institutional Board Review (IBR) of Anwar Shexa Medical City also approved it (No. 02 on 20 January 2024). Patients were given written informed consent before sample collection began.

Informed consent

Written informed consent was obtained from all subjects before the study.

Trial registration

Not applicable.

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