How to Cite:
Obaid, A. J., Mohammed, M. K., & Jabbar, A. D. (2022). Characterization of clinical and community acquired staphylococcus aureus, local isolates. *International Journal of Health Sciences*, *6*(S7), 6895-6902. https://doi.org/10.53730/ijhs.v6nS7.13687

Characterization of clinical and community acquired staphylococcus aureus, local isolates.

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Abstract*---**It was found that 46.6% of *S. aureus* were collected from nose swaps followed by ear (41.6%) and catheter tubes (11.8%). Results showed the highest resistance 100% for both classes of Aminopenicillins (Amoxicillin), cephalosporins 3Gr (Cefotaxime, Ceftriaxone, and Cefexime, and Carboxypencillin, as well as one group as 93.5% for class the β-lactam combination agents (Amoxicillin/ clavulanic acid). Many virulence factors and antibiotics resistance genes also being considered in this study, including erm, *luk*, *hlg*, *fbl*, *Sea*, *MecA*, *seb*, vat and *tet*. The highest frequency was in *MecA* genes 53 **(**88%) followed by ***sea genes 20 (33%), ***hlg*** and ***seb*** genes 18 (30%) for each one, ***erm*** genes 14 (23.3%) and ***luk*** genes 2 (3.3%), genes ***fbl***, ***vat*** and ***tet*** were not detected in any isolate. Results shows no correlation concerning age, gender or the site of infection with either antimicrobial resistance or virulence factors genes.

Keywords*---Staphylococcus aureus*, antibiotic resistance, virulence genes, pathogenicity.

Introduction

One of the most aggressive antibiotic resistant bacteria strains is multidrug resistant *Staphylococcus aureus* as well as methicillin resistant ones (Gordon *et al.*, 2021). Methicillin-resistant *Staphylococcus aureus* is a strain of Staphylococcus aureus that is resistant to beta-lactam antibiotics (Lowy , 2003).Over the last decade, the prevalence of *S. aureus* infections has apparently

increased due to higher rates of colonization, immunosuppression, a greater use of surgical implants, and dramatic increases in antibiotic resistance (Belthur *et al.*, 2012 and Montanaro *et al* ., 2011). The source of *S. aureus* as pathogens is still not fully diagnosed whether it is endogenous or exogenous particularly with regard to the extent of its resistance to antibiotics. Survey of literature showed little information about studies focused on the epidemiology of Staphylococcus infection of otitis media, and catheters contamination (Roemer *et al* ., 2013). Hence, this project aimed to both evaluate the bacterial etiology of otitis media, catheters and the antibiotic resistance patterns of *S. aureus* and investigate the molecular features and genetic background of *S. aureus* from Wasit/Iraq to understanding the role of different virulence factors in the development of staphylococcal infections. These findings will certainly help to understand the pathogenicity and proper management, thus decreasing the improper use of antibiotics. This paper was intended to determinate the prevalence of *S. aureus* and its resistance to antimicrobials and the presence of virulence genes.

Methods

Clinical samples were collected from 160 males and 30 females as controls. The emergence of private clinics carriers and four health care hospitals Workers like staff, nurses and in these hospitals from both Different genders and ages. Sterile scarves were worn during sample collection to prevent bacterial skin contamination. Nose samples (both frontal carvings) and Tonsils, ears and catheters were collected using sterility swabs are located in the mode of transported to the lab Immediately.

Samples and Processing

In this study, (190) samples were obtained. Samples were collected from 160 males and 30 females (tonsils, ear, nasal swabs and catheter tubes swabs were taken from operations Hall, Inpatient Hall, Recovery room). Each swab was carefully taken from the infection sites and placed in tubes containing ready-made medium to keep the swab moist until it was brought to the lab then inoculated on blood agar medium and Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. to study profile, consistency of growing colonies, capability of blood hemolysis and MSA turns to yellow color. (Makinde *et al.*, 2019).

Bacterial isolation

Samples collected were cultivated on MacConkey and blood agar, then incubated at 37°C for 24 h. When a growth appears on the blood and MacConkey agar, the samples are diagnosed by the shape, size, color, odor, beta hemolytic on blood agar, pigment production and also by oxidase and catalase test (positive). And then take single colony from growth to culture on cetrimide agar Base contain Nalidixic acid (is bactericidal and inhibits gram negative bacteria but pseudomonas aeruginosa is usually resistant) aerobically incubated overnight at 37°C. pseudomonas aeruginosa diagnosis on this media by growth with or without the production of the bluish green dye. Then all selected colonies were subjected to Catalase, Oxidase, Haemolysis, Clumping factor tests.

Detection of resistances and virulence genes:

All primers (listed in table 1) used in this study were synthesized by (AlphDNA/ Canada) they were diluted to give (10 pmol/ ul) as final concentration.

Primers	**Sequence**		**r**eference
mecA gene	F	ACGAGTAGATGCTCAATATAA	Al-Talib *et al* 2009
	R	CTTAGTTCTTTAGCGATTGC	
erm	F	AAG CGG TAA ACC CCT CTG A	Roberts *et al* 2011
	R	TTC GCA AAT CCC TTC TCA AC	
tet K	F	GTA GCG ACA ATA GGT AAT AGT	Strommenger *et al* 2003
	R	GTA GTG ACA ATA AAC CTC CTA	
vat A	F	TGG TCC CGG AAC AAC ATT TAT	
	R	TCC ACC GAC AAT AGA ATA GGG	
hlg	F	GCC AATCCGTTATTAGAAAATGC	
	R	CCATAGACGTAGCAACGGAT	
luk-pv	F	ATCATTAGGTAAAATGTCTGGACATGATCCA	
	R	GCATCAASTGTATTGGATAGCAAAAGC	
SEA	F	TTGGAAACGGTTAAAACGAA	
	R	GAACCTTCCCATCAAAAACA	
SEB	F	TCGCATCAAACTGACAAACG	
	R	GCAGGTACTCTATAAGTGCC	
fbl	F	GTAAATAGCGAGGCACAAGC	
	R	GGTAAATCGTATCTGCCGCT	

DNA Extraction and PCR conditions

The DNA was extracted depending on the instruction of Presto Mini Gdna bacteria Kit (Geneaid, USA): Initial denaturation 94C for 3min, 94 C for 30 sec, annealing according to primer set 30 sec followed by extension 72 C for 30 sec and finally extension 72 C for 4min.

Results and discussion

Resistance phenotypes and resistance genes

Our results revealed high antibiotic resistance, 100% for both classes of Aminopenicillins, cephalosporins third generation as well as Amoxicillin/ clavulanic acid). On the other hand, isolates were sensitive to Tobramycin (100%), while other antibiotics shows higher activity against *S. aureus* isolates; Ciprofloxacin (95%); levofloxacin (86.67% sensitive isolate beside 10 % intermediate resistant). 90% of *S. aureus found* to be sensitive to trimethoprim, and a close result seen with amikacin (85%).

The percentage of resistant isolates for vancomycin antibiotic was (62.5%,) this is may be due to frequent use of vancomycin as the drug of choice for treatment of infections caused by multidrug-resistant MRSA has putatively led to selection of the isolates with reduced susceptibility to vancomycin (Shariati *et al.*, 2020).

Results as illustrated in table 2, indicates AX, AMC, CRO, CTX, PY and CFM antibiotic were absolutely having no effect on *S. aureus* isolates, wither they originated from nose or ear, since there are no significant differences between them at P >0.05 as well as the significant differences in the effectiveness of each antibiotic against *S. aureus*. One can assign this resistance for the extensive use of these antibiotics since they were the first choice in treatment of different oral infections, sinusitis and otitis media. Certainly, in appropriate use of antibiotic will definitely leads to emerging of resistant isolates, due to selective pressure applied by antibiotic, diminishing all sensitive ones.

Table (2): Percentage of antibiotic resistance for nose and ear S. aureus isolates.

Antibiotic	Ear swap						Nose swap						P value
	R		I		S		R		I		S		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
AX	25	100	0	0	0	0	35	100	0	0	0	0	0.000
AMC	25	100	0	0	0	0	30	87	0	0	5	13	0.122
AK	0	0	4.5	18	21	82	2	4	2	4	31	91	0.278
CRO	25	100	0	0	0	0	35	100	0	0	0	0	0.000
CTX	25	100	0	0	0	0	35	100	0	0	0	0	0.000
E	15	59	9	35	1	6	15	43	9	26	10	30	0.159
PY	25	100	0	0	0	0	35	100	0	0	0	0	0.000
CFM	25	100	0	0	0	0	35	100	0	0	0	0	0.000
VA	16	65	6	29	2	6	21	61	12	35	2	4	0.925
TE	16	65	6	29	3	12	18	52	9	26	8	22	0.654
TOP	0	0	0	0	25	100	0	0	0	0	35	100	0.000
TMP	2	1	0	0	23	94	5	13	0	0	30	87	0.455
TMP	6	4	0	0	19	76	14	39	0	0	21	61	0.298
CIP	0	0	1	6	24	94	2	4	0	0	33	96	0.351
LEV	0	0	4	18	21	82	2	4	2	4	31	91	0.278

Virulence genes

In order to examine these isolates genotypically, a multiplex PCR technique was applied to detect some virulence factors and antibiotic resistance for all (60) *S. aureus* isolates. The highest frequency of this study's isolates was in ***MecA*** genes 53 **(**88%) followed by ***sea*** genes 20 (33%), ***hlg*** and ***seb*** genes 18 (30%) for each one, ***erm*** genes 14 (23%) and ***luk*** genes 2 (3.3%), genes ***fbI***, ***vat*** and ***tet*** were not detected in any isolate, as shown in Table (2). Our work indicates that about 88% of *S. aureus* isolate were harboring this gene, which enables them to resist methicillin and other related drugs.

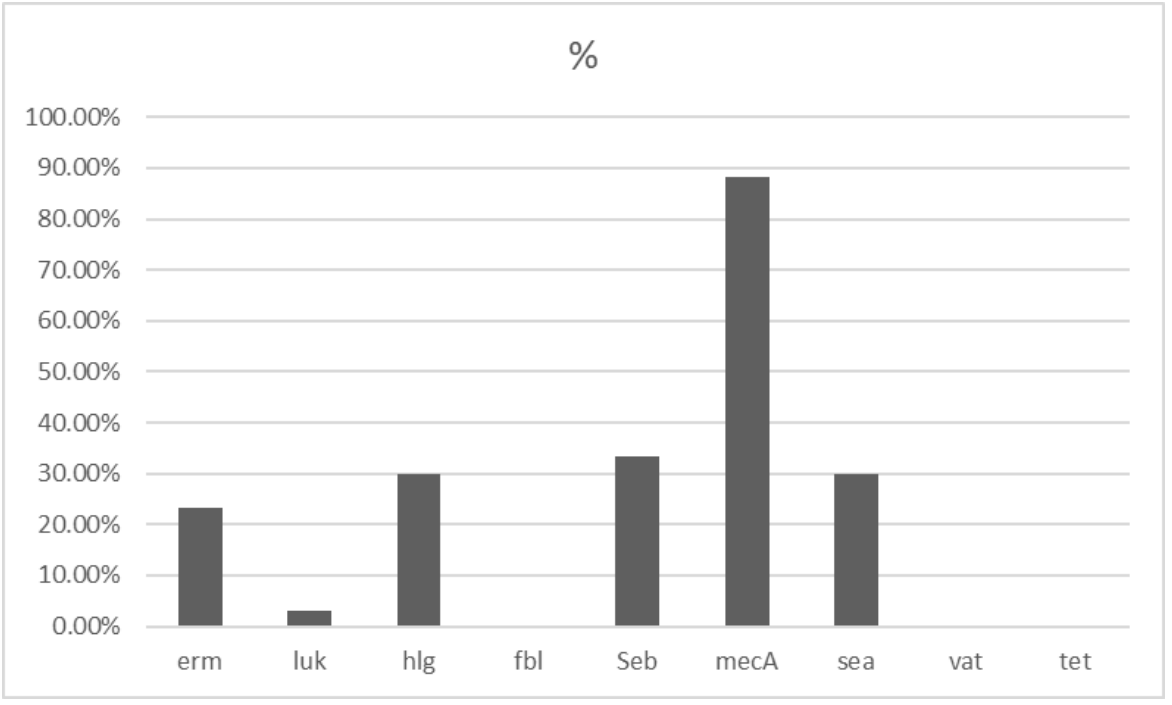

figure (2): shows virulence gene frequencies.

erm gene was detected in 14 isolates only, which is responsible to a large extent in resistance to macrolide, lincosamide, treptogramin B (MLSB). The rising frequency of methicillin resistant *Staphylococcus aureus* (MRSA) has led to an increased use of antibiotics such as macrolide, lincosamide, streptogramin B (MLSB) for the treatment of *S. aureus* infections, which in turn will leads to selection of *erm* gene.

Panton-Valentine leucocidin (PVL), which is encoded by *luk* gene, and has been touted as the cause of increased virulence in *S. aureus* (Voyich *et al.,* 2006).

PVL toxin is strongly associated with severe skin and soft tissue infections and necrotizing pneumonia. Furthermore, the ability of *S. aureus* to cause infections in immunocompetent hosts seems to be due in part to *S. aureus*

strain causing necrosis which has been linked to the lysis of neutrophils, which are

the primary target of *luk* (O"Hara *et al.*, 2008). The evidence supporting the involvement of *luk* in the pathogenicity of *S. aureus* is largely circumstantial and the cause of much debate. It has been suggested that *luk* in combination with other virulence factors is what is causing increased *S. aureus* infections. (Deip *et al.*,2008) stated that it is not *luk* alone but in conjunction with other determinants that may indeed be the driving force behind the increased virulence of *S. aureus*. Sub-typing of the *luk* gene in *S. aureus* could be a useful tool for the identification and monitoring of the dissemination of this virulence trait in *S. aureus* (Achek *et al.*, 2021). Molecular detection of *luk* gene was conducted on 60 *S. aureus* isolated, and it was seen in 2 (3%) isolates.

In terms of understanding pathogenicity, detection of virulence gene that can be exploited in laboratory studies, bio informatics analyses and population studies. Through comparative genomics we are developing a better understanding of the ability of this organism to evolve and adapt. This work recommends doing antibiotic susceptibility profile tests periodically in order restrict spreading of antimicrobial resistance. As well as, attention should be paid to the infection control and surveillance in our hospitals to reduce nosocomial infections.

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