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Antimicrobial sensitivity for *Staphylococcus aureus* produce Staphylococcal fibrinolysin (Staphylokinase) isolated from Nose

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Abstract

The aim of this study in order to study sensitivity for antimicrobial agents of production staphylokinase by phenotypic detection. In this descriptive study design 100 *S. aureus* isolates were collected from nose and identified from Baghdad hospitals by Vitek$_2$-GP system.

Antimicrobial susceptibility test achieve for 14 antibiotics include Met R (100%) , S (0%) ; Vancomycin R (92%), S (8%) ; Imipenem R (92%), S (8%) ; Erythromycin R (30%), S (60%) ; Cloxacillin R (30%), S (60%) ; Azithromycin R (53%), S (47%) ; Ceftazidine R (10%), S (90%) ; Ceftriaxone R (3%), S (97%) ; Cephalexin R (3%), S (97%); Norfloxacin R (0%), S (100%) ; FOX R (0%), S (100%) ; Gentamycin R (0%), S (100%) ; Oxacillin R (0%), S (100%); Clindamycin R (0%), S (100%).

Production Staphylokinase by phenotypic detection on plasma agar plate achieve for 100 *S. aureus* isolated from nose, results showed an increasing lysis zone around the well on plasma and casein agar medium. *S. aureus* isolated from nose produce Staphylococcal fibrinolysin, there are many isolates of *S. aureus* resistance for many types of antibiotics isolated from nose.

Key words: Fibrinolytic enzyme, Expression, Antibiotics.

Introduction

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations causing skin and tissue infection, deep abscess, wound infections, sepsis, endocarditis, septic arthritis and osteomyelitis [1, 2].

Staphylokinase consist of 136 amino acid aids to dissolve blood clotting throughout altering plasminogen to plasmin. The kinase also has proteolytic acitivity which helps to degrade the fibrin clot, a major constituent of thrombus [3].

Virulent Methicillin-Resistant *S. aureus* (MRSA) wide spread in community and hospital environments [4] is a genuine threat to public health [5] that causes multiple human infections include bacteremia and abscesses [6].

In severe *S. aureus* disease, the infections caused by number of different factors operating together in the pathogenic process. Survival of *S. aureus* in the host is important for pathogenesis, it may be protected by a polysaccharide capsule that inhibits opsonization by complement and escapes from phagocytosis [7]. It may also secrete cytolytic toxins and enzymes the cleaving tissues [8].

MRSA resistance to β-lactam antibiotics by inhibitors to cell wall and by ribosomal inhibitors for aminoglycosides group, tetracycline and macrolide antibiotics [9, 10] others mechanisms for resistance to antibiotics by producing modifying enzymes, alteration in target site of antibiotics and efflux pumps that prevent accumulation of antibiotics within bacteria [11].

Methedolgy

Study design

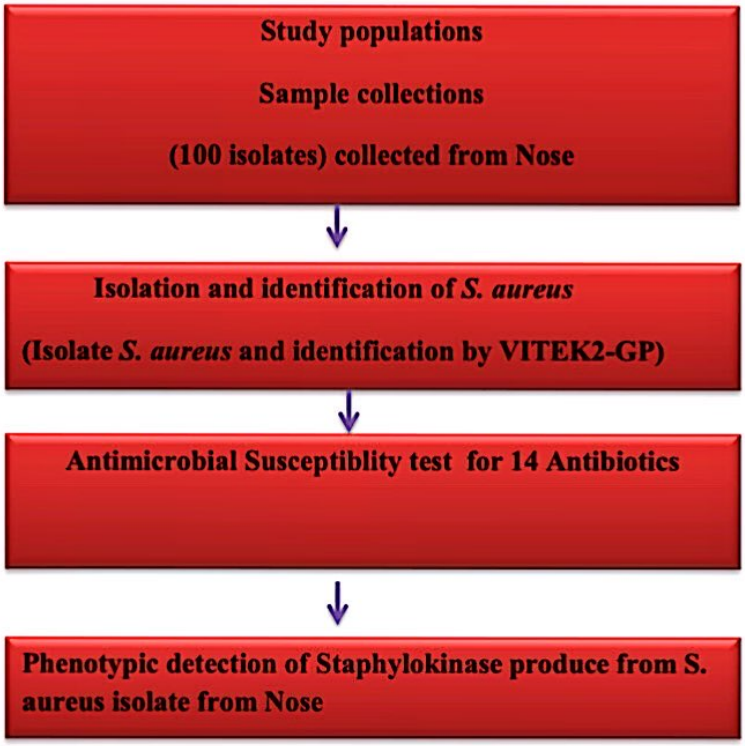


Figure (1): Scheme for Study design of this research.

Study populations

Selection criteria and sampling method for all was done, then strains were cultivated in nutrient broth, nutrient agar medium and mannitol salt agar medium, incubated at 37 ^{0}C for 24 hrs. according to [12]. Sample selection and isolation of *S. aureus* for 100 isolates from nose. Identification of *S. aureus* was achieved by Vitek2-GP with specific card for Gram positive bacteria [13].

Identification of *Staphylococcus aureus by* Vitek2 GP

The Gram positive (GP) card was used for the automated identification of most significant. Gram-positive bacteria. The GP identification card is based on established biochemical methods and newly developed substrates. There are 64 biochemical tests including carbon source utilization, enzymatic activities and antibiotics resistance [16, 17].

Antimicrobial susceptibility test

Antimicrobial susceptibility test was done by Kirby pour disc diffusion method of antibiotic discs for 14 antibiotics as shown in table 1 and compared with the recommendation of CLSI [15].

Table 1 :The antibiotic discs code and concentrations.

NO.	Antibiotics	Code	Concentration (µg/ disk)	Dimeter of inhibition zone around antibiotic discs in CLSI			Company (origin)
				R	I	S	
1	Azithromycin	AZM	15	≤13	14-17	≥18	Bioanalyse (Turkey)
2	Cefoxitin	Fox	30	≤14	15-17	≥18	
3	Cephalexin	CP	30	≤13	14-17	≥18	
4	Clindamycin	CC	2	≤14	15-20	≥21	
5	Cloxacillin	CX	1	≤21	…..	≥14	
6	Ceftazidine	CAZ	30	≤17	18-20	≥21	
7	Ceftriaxone	CRO	30	≤22	23-25	≥26	
8	Erythromycin	E	15	≤13	14-22	≥23	
9	Gentamycin	CN	30	≤12	13-14	≥15	
10	Imipenem	IMP	10	≤19	20-22	≥23	
11	Methicillin	M	5	≤ 28	….	≥29	
12	Norfloxacin	NOR	10	≤12	13-16	≥17	
13	Oxacillin	OX	10	≤ 21	……	≥ 22	
14	Vancomycin	VA	30	≤17	15-17	≥16	

R: Resistance S: Sensitive I: Intermediate

Antimicrobial susceptiblity test screening by Kirby- Baur methods

Kirby-Baur method was used as described by [15] to carry out the antibiotic susceptibility test for 14 different antibiotics as follows:

1. Bacterial suspension was prepared by picking up (4-5) isolated colonies from the original culture and suspended in a test tube containing 5ml of normal saline to produce a bacterial suspension of moderate turbidity which compared with McFarland (standard turbidity solution).

2. A portion taken from bacterial suspension was transferred and spread on muller hinton agar using sterile cotton swab.

3. The antimicrobial discs were placed on the agar using a sterile forceps. 4. The plates were incubated at 37C° for 24 hrs.

5. Inhibition zones around the discs were measured by millimeter (mm).

Plasma agar medium

The medium was prepared by adding human plasma into sterilized nutrient agar medium at 56°C for 20 minutes then mixed gently, poured into petriplates and leave for solidification [14].

Phenotypic assay for Staphylococcal fibrinolysin (Staphylokinase)

Semi-quantitative screening of Staphylokinase on plasma agar plate assay

The plasma agar was used to detect the production of staphylokinase or fibrinolytic enzyme activity for all isolates (100) performed by 20% added 20 ml human plasma into

100 ml nutrient broth incubated at 37°C for 24 hrs., the positive result was the formation zone around wells on plasma agar plate [18].

Screening of Staphylokinase on skimmed milk agar plate assay in casein agar assay. The skimmed milk agar medium was used in order to detect staphylokinase. It was prepared by mixing 78.5 ml distilled water, sterilized by autoclave with 12.5 ml of unsaturated skimmed milk, added into the center of milk agar, a hole (5mm) was made using cork borer and 0.1 ml was taken 100µl suspension of bacterial growth from cultivated nutrient broth was put in the hole of casein agar plate, incubated for 18 hrs. at 37°C. The inhibition zone around the well was measured and noticed with transparent areas around the well [19].

Results and discussions

Study design

Study design in this research Cross-sectional in descriptive of 100 isolates.

Isolation and identification of *Staphylococcus aureus*

100 *S. aureus* isolates were collected and identified from different Baghdad hospitals by Vitek$_2$-GP system.

Figure (2): Growth of *S. aureus* isolated from nose on Nutrient agar.

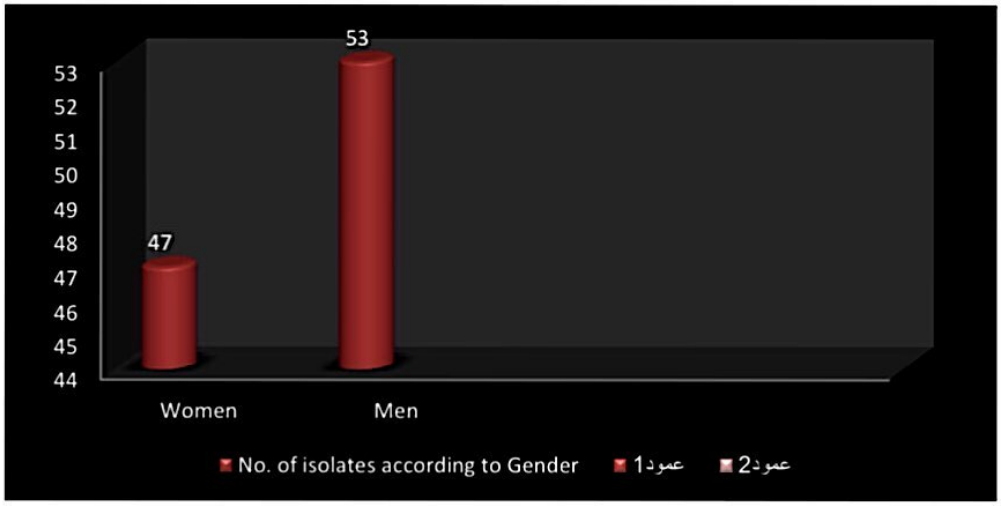
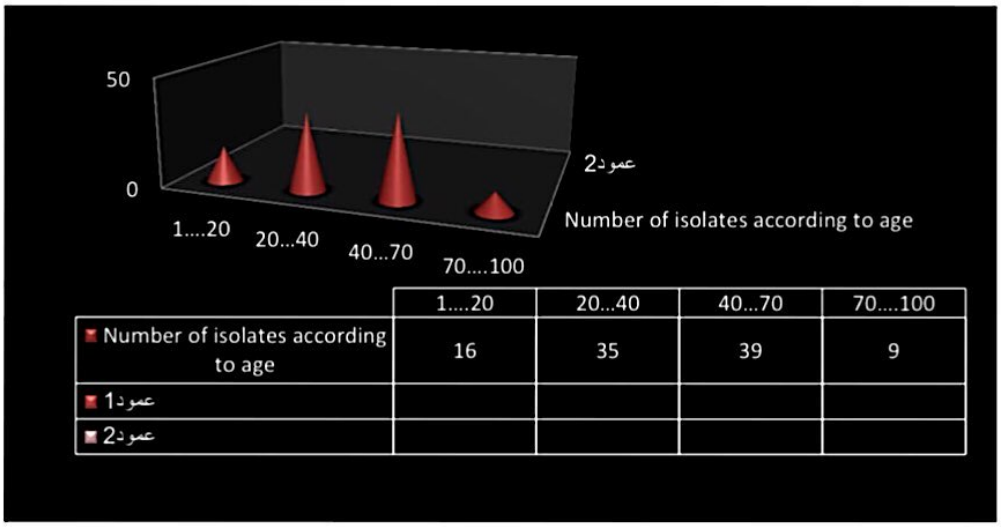


Figure (3): A- Number of isolates according to Gender.



	1....20	20...40	40...70	70....100
Number of isolates according to age	16	35	39	9
عمود1				
عمود2				

Figure (3):B- Number of isolates according to Age.

The results in figure (2) represents growth of *S. aureus* isolates from nose on Nutrient agar and identification for 100 isolates by Vitek2 GP in *S. aureus* and figure (3) A- number of isolates according to gender, results in this figure men more isolates collected from women

are 47% and 53% from men; in figure (3) B- number of isolates according to age, results in this figure showed 1-20 year collect 16 isolates, 20-40 year collect 35 isolates, 40-70 year collect 39 isolates and 70-100 year collect 9 isolates, more isolates collected 39 isolates from 40-70 years.

Antimicrobial susceptibility test for *S. aureus*

Antimicrobial sensitivity test achieve in order to determine each isolates resistance or sensitive for antibiotics.

Figure (4): Sensitivity and resistance of antibiotics on Muller Hinton agar (MHA) for *S. aureus* isolated from nose.

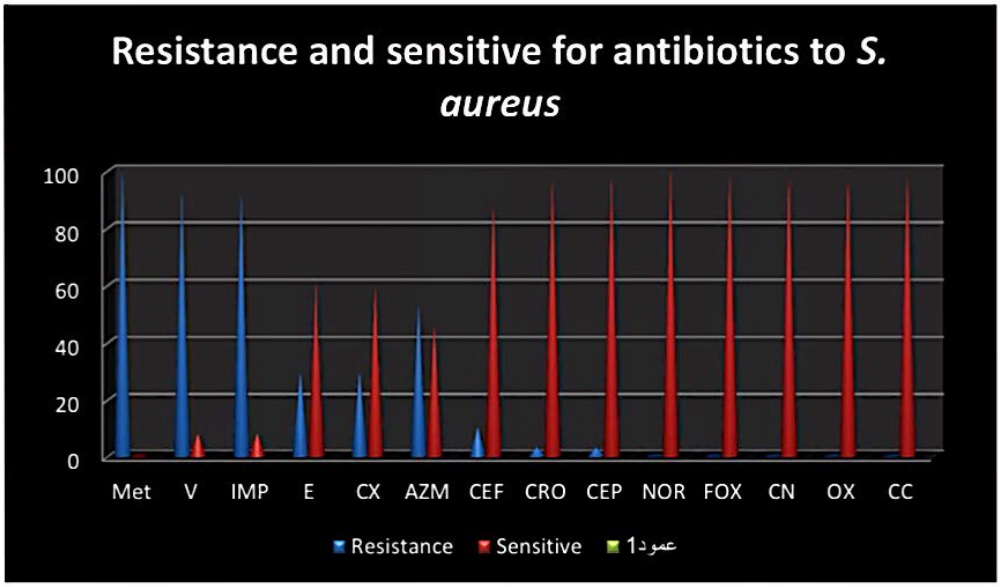


Figure (5): Resistance and sensitive for antibiotics to *S. aureus*.

The results of antimicrobial susceptibility test for 100 *S. aureus* isolates against 14 antibiotics were determined CLSI (2020) Antimicrobial susceptibility test achieve for 14 antibiotics include Metionine Met R (100%) , S (0%) ; Vancomycin R (92%), S (8%) ; Imipenem R (92%), S (8%) ; Erythromycin R (30%), S (60%) ; Cloxacillin R (30%), S (60%) ; Azithromycin R (53%), S (47%) ; Ceftazidine R (10%), S (90%) ; Ceftriaxone R (3%), S (97%) ; Cephalexin R (3%), S (97%); Norfloxacin R (0%), S (100%) ; FOX R (0%), S (100%) ; Gentamycin R (0%), S (100%) ; Oxacillin R (0%), S (100%); Clindamycin R (0%), S (100%). as shown in figure (4) and figure (5).

A previous study by [20] who examined antimicrobial agents for many 100 isolates resistant to cloxacillin (100%), cefoxitin (86%), cephalexin (51%), lincomycin (26%), Azithromycine (24),trimethoprim (23%), rifampicin (22%), gentamycin (18%), vancomycin (17%), clindamycin (13%), levofloxacine (13%) and teicoplanin (4%).

Phenotypic detection of Staphylokinase

Produce Staphylokinase	Nationalty	Swabs	Age	Gender	No.
SAK+	Iraqi	Nose	73	Woman	1
SAK+	Iraqi	Nose	12	Woman	2

Staphylokinase possess high affinity for fibrin clots since its thrombolytic agent leads to damage tissue, helps bacterial invasiveness through human body to cause infection [21].

Figure (6): Production Staphylokinae on plasma agar plate added human plasma with hydrolysis zone around well.

Table (1): Information of Nose isolates from *S. aureus*.

SAK+	Iraqi	Nose	32	Woman	3
SAK+	Iraqi	Nose	33	Man	4
SAK+	Iraqi	Nose	65	Woman	5
SAK+	Iraqi	Nose	76	Woman	6
SAK+	Iraqi	Nose	98	Man	7
SAK+	Iraqi	Nose	35	Woman	8
SAK+	Iraqi	Nose	65	Woman	9
SAK+	Iraqi	Nose	43	Woman	10
SAK+	Iraqi	Nose	43	Man	11
SAK+	Iraqi	Nose	21	Man	12
SAK+	Iraqi	Nose	31	Woman	13
SAK+	Iraqi	Nose	27	Man	14
SAK+	Iraqi	Nose	12	Man	15
SAK+	Iraqi	Nose	64	Man	16
SAK+	Iraqi	Nose	20	Woman	17
SAK+	Iraqi	Nose	40	Man	18
SAK+	Iraqi	Nose	32	Woman	19
SAK+	Iraqi	Nose	13	Man	20
SAK+	Iraqi	Nose	65	Man	21
SAK+	Iraqi	Nose	65	Woman	22
SAK+	Iraqi	Nose	76	Man	23
SAK+	Iraqi	Nose	58	Man	24
SAK+	Iraqi	Nose	32	Man	25
SAK+	Iraqi	Nose	40	Woman	26
SAK+	Iraqi	Nose	14	Man	27
SAK+	Iraqi	Nose	70	Woman	28
SAK+	Iraqi	Nose	91	Man	29
SAK+	Iraqi	Nose	72	Woman	30
SAK+	Iraqi	Nose	23	Man	31

SAK+	Iraqi	Nose	14	Woman	32
SAK+	Iraqi	Nose	27	Man	33
SAK+	Iraqi	Nose	36	Man	34
SAK+	Iraqi	Nose	39	Man	35
SAK+	Iraqi	Nose	34	Woman	36
SAK+	Iraqi	Nose	31	Man	37
SAK+	Iraqi	Nose	22	Man	38
SAK+	Iraqi	Nose	10	Woman	39
SAK+	Iraqi	Nose	40	Woman	40
SAK+	Iraqi	Nose	30	Woman	41
SAK+	Iraqi	Nose	8	Woman	42
SAK+	Iraqi	Nose	87	Woman	43
SAK+	Iraqi	Nose	27	Woman	44
SAK+	Iraqi	Nose	43	Man	45
SAK+	Iraqi	Nose	26	Man	46
SAK+	Iraqi	Nose	54	Man	47
SAK+	Iraqi	Nose	19	Man	48
SAK+	Iraqi	Nose	43	Man	49
SAK+	Iraqi	Nose	31	Man	50
SAK+	Iraqi	Nose	40	Woman	51
SAK+	Iraqi	Nose	28	Woman	52
SAK+	Iraqi	Nose	73	Woman	53
SAK+	Iraqi	Nose	6	Woman	54
SAK+	Iraqi	Nose	55	Man	55
SAK+	Iraqi	Nose	40	Woman	56
SAK+	Iraqi	Nose	32	Man	57
SAK+	Iraqi	Nose	53	Woman	58
SAK+	Iraqi	Nose	57	Man	59
	Iraqi	Nose	15	Woman	60

	Iraqi	Nose	63	Woman	61
	Iraqi	Nose	31	Man	62
	Iraqi	Nose	23	Woman	63
	Iraqi	Nose	17	Man	64
	Iraqi	Nose	54	Woman	65
	Iraqi	Nose	15	Man	66
	Iraqi	Nose	65	Woman	67
	Iraqi	Nose	65	Woman	68
	Iraqi	Nose	30	Man	69
	Iraqi	Nose	29	Man	70
	Iraqi	Nose	64	Man	71
	Iraqi	Nose	51	Man	72
	Iraqi	Nose	65	Man	73
	Iraqi	Nose	34	Man	74
	Iraqi	Nose	65	Woman	75
	Iraqi	Nose	19	Man	76
	Iraqi	Nose	22	Man	77
	Iraqi	Nose	54	Woman	78
	Iraqi	Nose	21	Man	79
	Iraqi	Nose	31	Man	80
	Iraqi	Nose	32	Woman	81
	Iraqi	Nose	45	Man	82
	Iraqi	Nose	44	Man	83
	Iraqi	Nose	64	Woman	84
	Iraqi	Nose	62	Man	85
	Iraqi	Nose	12	Woman	86
	Iraqi	Nose	59	Man	87
	Iraqi	Nose	21	Man	88
	Iraqi	Nose	58	Woman	89

	Iraqi	Nose	45	Man	90
	Iraqi	Nose	39	Man	91
	Iraqi	Nose	34	Woman	92
	Iraqi	Nose	23	Woman	93
	Iraqi	Nose	76	Man	94
	Iraqi	Nose	45	Woman	95
	Iraqi	Nose	69	Man	96
	Iraqi	Nose	30	Woman	97
	Iraqi	Nose	28	Man	98
	Iraqi	Nose	29	Woman	99
	Iraqi	Nose	39	Woman	100
				100(20%)	Total

Results in figure (6) showed an increasing lysis zone around the well on plasma and casein agar medium indicating production of Staphylococcal fibrinolysin enzyme (staphylokinase) and table (1) showed 59 isolates produce Staphylokinase from *S. aureus* isolated from nose.

Study by [22] *S. aureus* for the production of Staphylokinase (Fibrinolytic activity) screening on human plasma agar medium which revealed all bacterial isolates were able to production staphylokinase at variable degrees by *S. aureus* isolated from nose infection with efficient production of staphylokinase at diameter zone of hydrolysis (36 mm) around well on plasma agar medium compared with other isolates.

Conclusions

1- *S. aureus* isolated from nose produce Staphylococcal fibrinolysin (Staphylokinase).
2- There are resistance of many types of antibiotics for *S. aureus* isolated from nose.

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