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OVERPRODUCTION OF FIBRINOLYTIC ENZYME FROM MUTANT *STAPHYLOCOCCUS AUREUS* BY CHEMICAL MUTAGENESIS

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ABSTRACT : The study design of cases are Cross-sectional study that descriptive study by internal comparison of 500 isolates collected from different provenance of human infections including 280(56%) from tonsils, 100(20%) nose, 40(8%) tumors, 17(3.4%) urine, 27(5.4%) skin and 36(7.2%) blood, the isolation and identification achieved by using Vitek2-GP and Genotypic detection PCR to confirmative identification of *S.aureus* isolated from tumors.Fibrinolytic enzyme (Staphylokinase) assay done on plasma agar plate and Casein agar plate to determine fibrinolysis, caseinolysis on medium , the result exhibit 260 (56%) isolates possess production of staphylokinase, inclusive throat 100 (38%), Blood 36(15%), Tumors 38(10%), Skin 27(23%) and Nose 59(23%) production staphylokinase; 180 (69%) isolates of Vancomycin resistance *S.aureus*(VRSA) and Methicillin resistance *S.aureus* (MRSA) produces Staphylokinase. The chemical mutagenesis was achieved viaexposition VRSA *S.aureus* and MRSA *S.aureus* to different doses of chemical mutagen including Hydroxylamine(HA) in (10,20,30) mg ; Acridine Orange (AO) in (30,40,50,60) mg and Ethylemethansulfonate (EMS) in (0.01, 0.02, 0.03, 0.04, 0.001, 0.002)ml, results of mutagenesis on viability with increase production of staphylokinase were of Hydroxylamine in 10mg with viability 85, as well Acridine Orange (AO) give rise to increase production of Staphylokinase in 30 mg with viability 75 and Ethylemethansulfonate (EMS) in 0.001ml, 0002 ml with viability 73.

Study chemical mutagenesis as well one after exposition*S.aureus* (VRSA) and *S.aureus* (MRSA) to mutagen turn into sensitive to VancomycinrenownedVancomycin Sensitive *S.aureus* (VSSA) and sensitive to Methicillin renownedMethicilline Sensitive *S.aureus* (MSSA)howevere, continue possess higher productivity of Staphylokinase(fibrinolytic enzyme).

Key words : Mutage, mutagen, genotoxicity, thrombolytic enzyme, acridine orange, ethylemethansulfunate, hydroxyleamine.

INTRODUCTION

Staphylococcus aureus is human pathogen give rise to skin infection and tissue infection, passionate abscess, wound infections, sepsis, endocarditis, septic arthritis and osteomyelitis (Shagufta *et al*, 2014).

Virulence factors of *S. aureus* are chromosomally encoded (intrinsic resistance) inclusive capsules and endotoxin, others reluctance from mobile genetic elements (Extrinsic resistance) like plasmids and bacteriophages reason horizontal gene transfer that transform bacteria from harmless into risky pathogens (Bubeck *et al*, 2008). Methicillin-Resistant *S. aureus* (MRSA) bacteria hold acquired gene grant resistance to methicillin antibiotics till other beta-lactam antibiotics of hospital-related strains (Lee *et al,* 2003).

Staphylokinase (SAK) extracellular protein consist of 136 amino acid produced during the late exponential growth phase of *S. aureus* (Moussa *et al*, 2012).

Staphylokinase known thrombolytic enzyme and proteolytic enzyme act to dissolve fibrin that help bacteria in spreading and causing damage to tissue by interact with plasminogen converted into plasmin (proteolytic enzyme), which hydrolyses fibrin clots, subsequently inhibiting phagocytosis (Chen *et al*, 2013).

Mutation is inheritance change in DNA sequence that impact or not impact on the phenotype of organism. Procedure ofevents mutationcalled mutagenesis and the agent promote mutations called mutagen, the organisms that transfer mutation into progeny called mutants. The chemical mutagensthat not integrated into DNA, but changeable a base that rise specific mispairing inclusive alkylating agents that are ethyl methanesulfonate (EMS) and nitrosoguanidine (NG) that add up to alkyl groups to many positions on all four bases (Jeremy *et al*, 2010).

MATERIALS AND METHODS

Isolation and identification of *S. aureus*

The isolation of *S. aureus* were 500 specimens from different provenance of humans possessed from hospitals

Table 1 : Primers utilizing for Genetic analysis of *16srRNA* gene for identification of *S.aureus* isolated from tumors.

Specific primer	Primers of sak gene encoded for thrombolytic enzyme	References
Forward primer	5'GGAATTCAAAGGAATTGACGGGGGC -3'	Ali *et al* (2014)
Reverse primer	5'CCAGGCCCGGGAACGTATTCAC-3'	

in Baghdad in 2018 to 2019, all samples were cultivated in Nutrient broth, Nutrient agar medium and MannitolSalt agar medium incubated at 37^0C for 24 hr. according to Lemaire *et al* (2008) the gathering of specimens were from blood, tonsils, nose, urine, skin (Acne) and tumors.

The identification of *S. aureus* achieved by Vitek2-GP and by Genotypic revelation PCR to confirmative identification of tumor isolates, the Vitek2 system was advanced for affirmation the identification of bacterial isolates. All the isolates identified by using Vitek2-GP cardspecific for Gram Positive bacteria, taken 1ml of bacterial suspension from bacterial culture then centrifuged at 8000 rpm for 5 minutes, the pellet re-suspended in 0.3 ml of normal saline and the turbidity compared with MacCfrland solution, the card position into Vitek-2 apparatus for identification of *S.aureus*, the results were revealed after 6 hr. The genotypic revelation PCR as wellutilized for the affirm the identification of *S. aureus* secluded from tumors by using Go Taq Master Mix Kit with primers showed in Table 1 (Biomeruex, 2010).

Phenotypic of fibrinolytic enzyme (Staphylokinase) production

Plasma agar plate assay

The medium was intended according to Pulicherla *et al* (2011) by adding human plasma at 56°C for 20 minutes to nutrient agar medium in a percentage of 20% of the total volume, subsequently media was mixed gently and put inpetriplates, preserved for time so as to solidification. The plasma agar utilized to detect production of staphylokinaseor thrombolytic enzyme activity for all bacterial isolates performed by inoculating 5ml of nutrient broth medium into 50ìl of fresh culture incubated at 37^oC plasma agar plates incubated at 37^oC for 24 hours, the positive result by formalization of zone of hydrolysis (fibrinolysis) nearly wells found on plasma agar plate.

Casein agar assay (Skimmed milk agar assay)

The medium milk agar used in order that reveal for staphylokinase due to the medium possess low fat milk prepared 78.5 ml of distilled water sterilized by autoclave, subsequently cooled by dissolve nutrient agar in 12.5 ml of unsaturated skimmed milk A bacterial strain of pure bacterial isolates was notice in Nutrient broth for 18 hr. at 37°C and achieved in the center of the milk agar accomplished 5mm by utilizing Cork borer. 0.1 ml taken by micropipette placed in the pore of Casein agar plate, plasma agar plate incubated for 24-18 hours at 37°C the inhibition zone nearly the hole were metrical and noticed with transparent areas around the hole (Sneath *et al*, 1986).

Chemical mutagenesis

The chemical mutagenesis achieved by utilizing Hydroxylamine (HA), Acridine orange(AO) and Ethylmethanesulfonate (EMS) with some modification of procedures.

Mutagenesis of *S. aureus* (VRSA) and (MRSA) according to Kodym *et al* (2003) with some modification. The mutagens, hydroxylamine, acridine orange and ethyl methane sulfonate were utilizing to enhance mutation into Vancomycin Resistance *S. aureus* (VRSA) and Methicillin Resistance *S. aureus* (MRSA). The hydroxylamine mutagens used indifferent mutagen (dose of mutagen) at concentrations of 10, 20, 30 mg, as well Acridine orange mutagen used in different mutagen (dose of mutagen) at concentrations (10, 20, 30, 40, 50, 60) mg and Ethylemethansulfonate (EMS) in different mutagen (0.01, 0.02, 0.03, 0.04, 0.001, 0.002) ml, which is most suitable for chemical mutagenesis of bacteria in order that enhance mutation inoculated into Muller Hinton Agar, incubated at 37°C for 24 hr.

RESULTS AND DISCUSSION

Isolation and identification of *S. aureus*

The study design of cases are cross-sectional study by internal comparison of 500 isolates of *S.aureus*were gathering from hospitals in Baghdad. All samples were cultivated in nutrient broth and nutrient agar medium at 37^0C for 24 hours taken 100 ìl from the dilution that pervasion on mannitol salt agar medium (selective medium) incubated at 37^0C for 24 hr. to choose *S. aureus* according to Lemaire (2008).

The results of seclusion from different provenance of human are 280(56%) tonsils, 100(20%) nose, 40(8%) tumors, 17(3.4%) urine, 27(5.4%) blood and 36(7.2%) skin (Acne) showed in Table 1 and Fig. 1, the isolation and identification achieved by Vitek2-GP exhibit in Table 2 and Genotypic detection PCR of *16srRNA* gene to affirmitive identification of *S. aureus* secluded from tumors, exhibit in Fig. 1.

The pathogenicity of *S. aureus* infections regarding

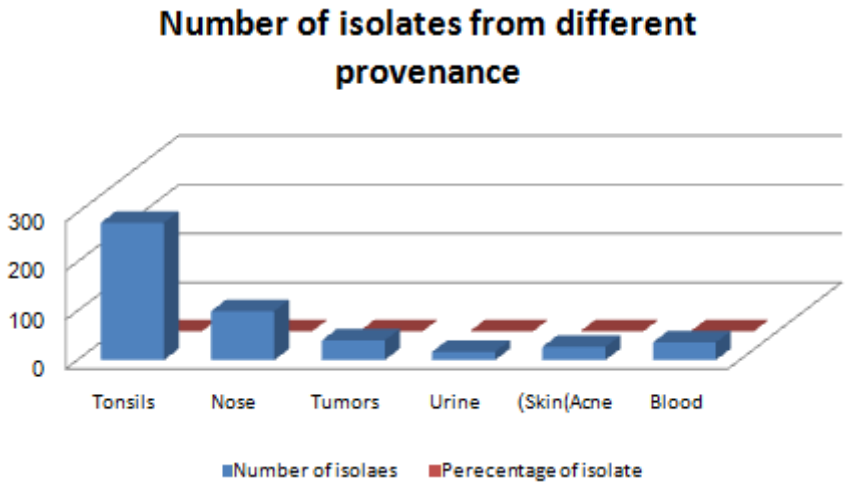

Fig. 1 : Prevalence of *S.aureus* from different provenance with percentage of isolates.

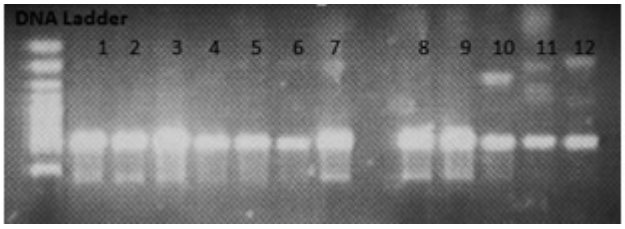

Fig. 2 : PCR product of *16srRNA* (Ribosomal RNA of 30s Subunit) Agarose gel electrophoresis with size (479 bp~ 500 bp) compared with DNA Ladder to confirmative the identification of (VRSA), (MRSA) *S.aureus* from Tumors.

to some of virulence factor inclusive protein A and extracellular proteins like staphylokinase, coagulase, hemolysin, enterotoxins, toxic-shock syndrome (TSS) toxin, exfoliatins and Panton-Valentine leukocidin (PVL) have roles in invasive infections to cutaneous abscesses and severe necrotic skin infections to promote its colonization cause and infection (Gopal *et al*, 2015).

Results of identification *S. aureus* of bacterial isolates by Vitek2-GP are positive for biochemical test arginine dihydrolase 1, alpha-glucosidase, phosphatase, Leucinearylamidase, D-maltose, bacitracin resistance, D-mannitol, growth in 6.5% NaCl and optochin resistance but they were negative for urease, D-sorbitol, D-ribose, novobiocin resistance, arginindihydrolyase 2, D-xylose and cyclodextrin, the results grant positive of all isolates of *S. aureus*.

Genotypic procedure for identification Vancomycin Resistance *S. aureus* (VRSA) and Methicillin Resistance *S. aureus* (MRSA) by Conventional PCR to affirmative identification of *S. aureus* relying on a housekeeping gene (*16s rRNA*), the results revealed that the all isolates from tumors 40 isolates (8%) contain *16s rRNA* gene (house-keeping gene). The Genotypic revelation of *S. aureus* to affirmative identification of bacteria by using Conventional PCR of *16s rRNA* gene with size479 bp compared with DNA Ladder exhibited in Fig. 2.

Genotypic identification *S. aureus* by Polymerase Chain Reaction (PCR)

Results exhibit all *S. aureus* isolated from tumors possess gene *16srRNA* (Ribosomal RNA of 30s Subunit). Primers of *16srRNA* used in this study showed in Table 1.

As an alternate way for bacterial identification is metagenomic sequencing analysis for the diagnosis of bacterial infections (Didelot *et al*, 2012) applied the sequence *16SrRNA* ribosomal RNA genes as molecular marker for bacterial classification by utilizing Next Generation Sequencing (NGS) is robust technology for identifying bacteria that grant delicate classification due to rapid detection of pathogen with more accuracy and sensitivity (Leggett *et al*, 2017; Sanderson *et al*, 2017; Mitsuhashi *et al*, 2017; Shin *et al*, 2018).

Amplification of *16S rRNA* genesequences (~479 bp) is the utmost commonly utilizing method for identifying and classifying bacteria, inclusive staphylococci (Mohammed *et al*, 2007; Ghebremedhin *et al*, 2008).

The genotypic process for identification of bacteria was preferable than phenotypic due to that PCR technique presently is a golden standard tool to confirm bacteria with included *S. aureus* (Macarthur, 2009). The rapid diagnostic of pathogen in the clinical the specimen important to progress care of the patient and therapy with control of the diseases (Croxatto *et al*, 2012).

Genetic identification methods based to identification bacteria by revelation of of*16SrRNA* genes (30s subunit of *S. aureus*) to permit the identification of many bacterial pathogens, the process is remain work-intensive for diagnostic laboratories that preferable than phenotypic criterion (Patel *et al*, 1987).

Table 2 : Identification of *S. aureus* by Vitek2-GP have different biochemical test (64 biochemical test).

Biochemical test	*S. aureus*
D-Amygdalin (AMY)	-
Phosphatidylinositol Phospholipase(PIPL)	-
D-Xylose (dXYL)	-
Argydrolase1 (ADH1)	+
Beta-Galactosidase (BGAL)	-
Alpha-Glucosidase (AGLU)	+
Ala-Phe-Pro Arylamidase (APPA)	-
Cyclodextrin (CDEX)	-
L-Aspartate Arylamidase(AspA)	-
Beta- Galactopyranosidase (BGAR)	-
Alpha-Mannosidase (AMAN)	-
Phosphatase (PHOS)	+
Leucine Arylamidase (LeuA)	-
L-Proline Arylamidase (ProA)	-
Beta-Glucuronidase (BGURr)	-
Alpha-Galactosidase (AGAL)	-
L-Pyrrolidonyl-Arylamidase (PyrA)	+
Beta-Glucurgnidase (BGUR)	-
Alanine Arylamidase (AlaA)	-
Tyrosine Arylamidase (TyrA)	-
D-Ribose (dRIB)	-
L-Lactatealkalinization (lLATk)	+
Lactose (LAC)	-
N-Acetyl-D-Glucosamine (NAG)	+
D-Maltose (dMAL)	+
Bacitracin Resistance (BACI)	+
Novobiocin Resistance (NOVO)	+
Growth in 6.5% NaCl	+
D-Mannitol (dMAN)	+
D-Mannose (dMNE)	+
Methyl –B-D-Glucopyranoside (MBdG)	+
Pullulan (PUL)	-
D-Raffinose (dRAF)	-
O/129 Resistance (comp.vibrio)(O129R)	+
Salicin (SAL)	-
Saccharose/Sucrose (SAC)	+
D-Trehalose (dTRE)	+
Arginine Dihydrolase 2 (ADH2s)	-
Optochin Resistance (OPTO)	+
Polymyxin B Resistance (POLY B)	+
D-Sorbitol (dSOR)	-
D-Galactose (dGAL)	+
Urease (URE)	-

Negative (-), Positive(+).

Phenotypic detection of fibrinolytic enzyme (Staphylokinase) production

The production of fibrinolytic enzyme (Staphylokinase) achieved on plasma agar plate and Casein agar plate to limit fibrinolysis and caseinolysis areas hydrolysis areas of plasma in the medium, result of phenotypic exhibit 260 (56%) isolates possess production of staphylokinase, inclusive tonsils 100 (38%), Skin(Acne) 36(14%), Tumors 38(15%), Blood 27(10%) and Nose 59(23%) that produce staphylokinase; 180 (69%) isolates from expressed staphylokinase are resistance to Vancomycin and Methicillin (Vancomycin resistance *S.aureus* (VRSA) and Methicillin resistance *S. aureus* (MRSA)) showed in Table 3 and Figs. 3, 4.

Production of thrombolytic enzyme (fibrinolytic enzyme) on plasma agar medium and Casein agar medium showed 260 (56%) isolates from which 180 isolates (69%) have the expression of staphylokinase resistance expressed by VRSA, MRSA *S. aureus* as well resistance for Vancomycin, Methicillin, Imepinem, Erythromycin and Azithromycin.

Results of phenotypic detection of staphylokinase (thrombolytic enzyme) of 180 (VRSA) and (MRSA) have expression are 96(53%) tonsils, 36(20%) tumors, 30(17%) Blood and 18(10%) Skin(Acne) showed in Table 6, Fig. 7 before chemical mutagenesis.

Thrombolytic therapy is substantial in clot specific that grant maximum patency in a short time with fewer side effects inclusive minimal bleeding hazard (Pulicherla *et al*, 2011).

Thrombotic disturbanceone of the major effectiveness in human death by causes thrombosis. Therapy of thrombosis by Staphylokinase, plasminogen activator and urokinase widely utilized as thrombolytic agents, staphylokinase plays major role in fibrinolysis (Rother *et al*, 2013). Staphylokinase utilized for therapy of myocardial infarction and peripheral thrombosis due to profibrinolytic properties (Sweta *et al*, 2006).

Chemical Mutagenesis of Vancomycin Resistance *S. aureus* (VRSA) and Methicillin Resistance *S.aureus* (MRSA)

Results the chemical mutagenesis exhibit when exposition VRSA *S. aureus* and MRSA *S. aureus* to different mutage (doses of chemical mutagen) inclusive Hydroxylamine (HA) in (10, 20, 30) mg.; Acridine Orange (AO) in (30, 40, 50, 60) mg and Ethylemethansulfonate (EMS) in (0.01, 0,02, 0.03, 0.04, 0.001, 0.002) were resultsthe mutagenesis on viability of VSSA and MSSA have overproduction (increase production) of staphylokinase when utilizing Hydroxylamine mutagen in 10mg with viability 85, as well Acridine Orange (AO) causes overproduction of Staphylokinase in 30 mg with viability 75 and Ethylemethansulfonate (EMS) in 0.001ml, 0002 ml with viability 73, the results exhibit in Tables 4, 5 and Figs. 4, 5, 6.

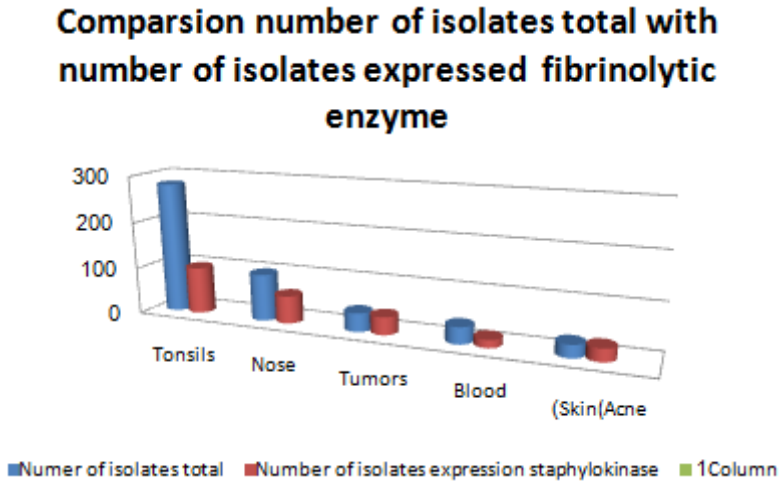

Fig. 3 : Comparison number of isolates production fibrinolytic enzyme phenotypic on plasma agar and casein agar medium with total of isolates from different provenance.

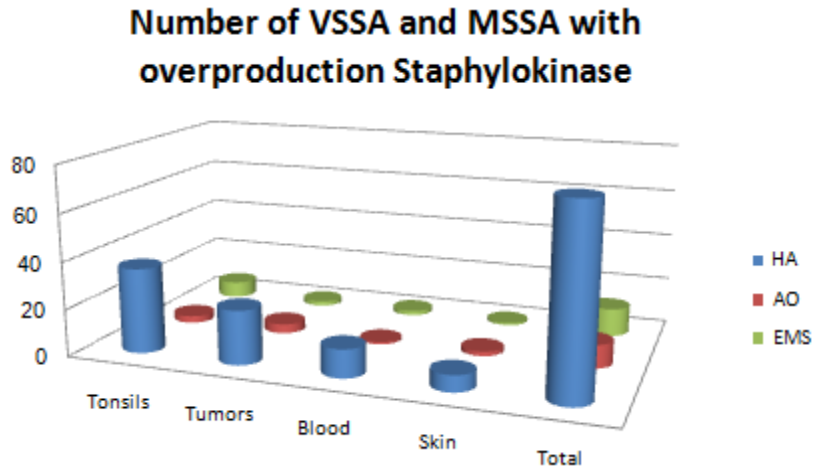

Fig. 4 : Number of mutant isolates possession overproduction of fibrinolytic enzyme (Staphylokinase) of Vancomycin sensitive *S.aureus* (VSSA) and Methicillin Sensitive *S.aureus* (MSSA) exposed to Hydroxyleamine (HA), Acridine Orange (AO) and Ethylemethanesulfonate (EMS) chemical mutagen.

Table 3 : Fibrinoytic enzyme (Staphylokinase) productionof 180 of (VRSA) Vancomycin Resistance *S.aureus* and (MRSA) Methicillin Resistance *S.aureus*before the chemical mutagenesis, as well resistance forImipenem, Erythromycin, Cloxacillin and Azithromycin.

	Sources of isolates sample	**Number of isolates**	**Perecentage of isolates**
1	Tonsils	96	53%
2	Tumors	36	20%
3	Blood	30	17%
4	Skin (Acne)	18	10%
	Total	180	100%

Results the comparsion of production staphylokinase of number of Vancomycin resistance *S.aureus* (VRSA) before exposition to chemical mutagenesis from 180 isolate were resistance to Methicillin and Vancomycin that production staphylokinase (fibrinolytic enzyme) were from tonsils 96 isolates (53%), tumors 36(20%), blood 30 isolates (17%) and skin 18 isolates (10%) have production of thrombolytic enzyme when study phenotypic on plasma agar and casein agar medium of MRSA and VRSA, either results of the production of staphylokinase were overproduction of staphylokinase (fibrinolytic enzyme) from 100 isolates (56%) Vancomycin sensitive *S.aureus* (VSSA) and Methicillin Sensitive *S.aureus* (MSSA) that sensitive to Methicillin and Vancomycin due to chemical mutagenesis of tonsils 46 isolates (46%), tumors 29 isolates (29%), blood 15 isolates (15%) and skin (Acne) 10 isolates (10%) have overproduction give rise to transform Vancomycin Resistance *S. aureus* (VRSA) into Vancomycin Sensitive *S. aureus* (VSSA) and convert Methicillin Resistance *S. aureus* (MRSA) into Methicillin Sensitive *S.aureus* (MSSA) turn off into mutant non-virulent that transformed from virulent into non virulent.

Results of chemical mutagenesis showed in Table 8 and Fig. 7 by Hydroxyleamine (HA), Acridine Orange (AO) and Ethylemethanesulfonate (EMS) when exposition to mutant isolates of Vancomycin Resistance

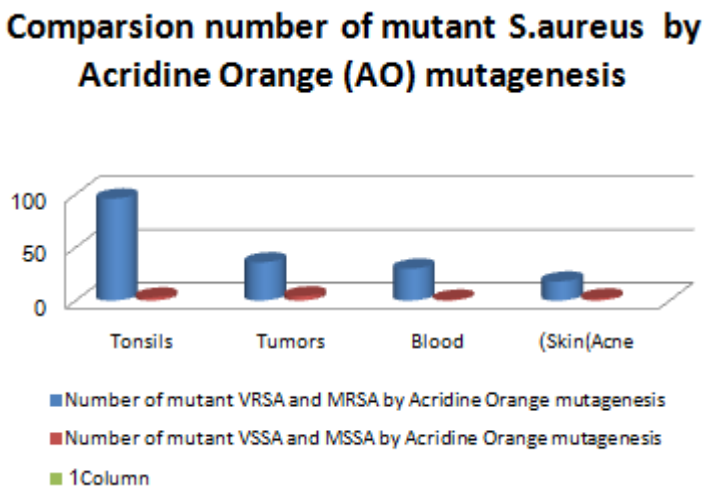

Fig. 5 : Comparison number of mutant isolates after chemical mutagenesis by Acridine Orange(AO) of VRSA, MRSA and VSSA, MSSA turn off sensitive to Vancomycine and Methicillin.

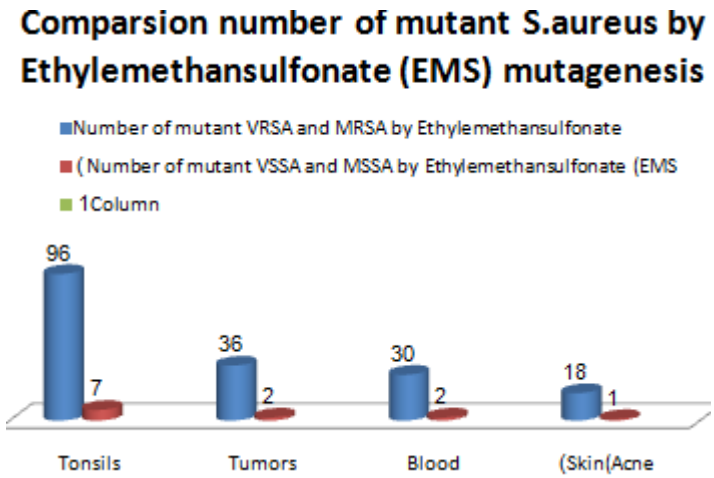

Fig. 6 : Comparison number of mutant isolates after chemical mutagenesis by Ethylemethanesulphonate (EMS) of VRSA, MRSA and VSSA, MSSA turn off sensitive to Vancomycine and Methicillin.

Table 4 : Comparison of number of VRSA and MRSA isolates before chemical mutagenesis and VSSA and MSSA after mutagenesis with increase production of staphylokinase (fibrinolytic enzyme).

	Provenance of isolates (Specimens) isolates	Number of VRSA and MRSA isolates before mutagenesis	Percentage of VSRSA and MRSAisolates before mutagenesis	Number of VSSA and MSSA after mutagenesis	Percentage of VSSA and MSSAisolates after mutagenesis
1	Tonsils	96	53%	46	46%
3	Tumors	36	20%	29	29%
4	Blood	30	17%	15	15%
5	Skin (Acne)	18	10%	10	10%
Total		180	100%	100	100%

S.aureus (VRSA) and mutant Methicillin Resistance *S.aureus* (MRSA) give rise to mutation in *sak* gene encoded to staphylokinase that leading to overproduction of fibrinolytic enzyme, inclusive 126 isolates have overproduction of thrombolytic enzyme, 100 isolates (55%) have overproduction of staphylokinase with sensitive to Vancomycin and Methicillin that leading to transform from resistance Vancomycin of *S. aureus* (VRSA) into sensitive Vancomycin *S. aureus* (VSSA) and transform from Resistance Methicillin of *S. aureus* (MRSA) into Sensitive to Methicillin *S. aureus* (MSSA), these isolates (VSSA) and (MSSA) utilized to complete the other experiments because turn off into mutant non virulent isolates with high expression (overproduction) of *S. aureus* that utilized in medical applications.

Results in Table 9 revealed number of mutant isolates after chemical mutagenesis by Hydroxyleamine (HA), Acridine Orange (AO) and Ethylemethanesulfonate

Table 5 : Comparison of number of mutant isolates after chemical mutagenesis turn into sensitive to Vancomycine and Methicillin.

	Number of mutant isolates after chemical mutagenesis						Number of mutant isolates before chemical mutagenesis	
	Sensitive *S.aureus* Vancomycin and sensitive to Methicillin (MSSA),(VSSA)					Resistance *S.aureus* (MRSA), (VRSA)	Resistance *S.aureus* (MRSA),(VRSA)	
No.	**Sources**	**HA**	**AO**	**EMS**	**Total VSSA and MSSA**	**Number of isolates production staphylokinase of mutant *S. aureus***	**Number of isolates Resistance Methicillin and Vancomycin**	**Number of isolates Before mutagenesis**
1	Tosils	36(46%)	3(30%)	7(58%)	46(46%)	96(53%)	108(41%)	280(56%)
2	Tumors	23(29%)	4(40%)	2(17%)	29(29%)	36(20%)	36(14%)	40(8%)
3	Blood	12(16%)	1(10%)	2(17%)	10(10%)	18(10%)	27(10%)	27(5%)
4	Skin (Acne)	7(9%)	2(20%)	1(8%)	15(15%)	30(17%)	30(12%)	36(7%)
	Nose	—	—	—	—	—	59 (23%)	59 (23%)
Total		78	10	12	100	180	260	500

(EMS) turn off sensitive to Vancomycin (VSSA) and Sensitive to Methicillin (MSSA) including 46 isolates (46%) from tonsils, 29 (29%) from tumors, 15(15%) from blood and 10(10%) from skin(Acne) become sensitive to vancomycin and Methicillin with overproduction of staphylokinase (fibrinolytic enzyme) compared with number of isolates before mutagenesis 500 isolates inclusive 280(56%) isolates from tonsils, 40(8%)from tumors, 36 (7.2%) from blood and 27 isolates (5.4%) from skin (Acne) from 500 isolates (Wild type) of *S.aureus*.

The results of number of *S. aureus* not exposition to chemical mutagenesis, but expressed staphylokinase were 260 isolates including 108 isolates from tonsils, 36 isolates from tumors, 30 isolates from blood and 27 isolates from skin (Acne) comared with number of isolates of (VSSA), (MSSA) have overproduction of staphylokinase from mutant Vancomycin Sensitive *S. aureus* and from mutant Methicillin Sensitive *S. aureus* of 100 mutant isolates (non-virulent isolates) were 46 isolates from tonsils, 29 isolates from tumors, 10 isolates from skin and 15 isolates from blood from total isolates 100 isolates.

Compartion with 180 isolates have overproduction of staphylokinase, but inclusive mutant isolates sensitive and resistance of Vancomycin, Methicillin after chemical mutagenesis were number isolates 96 isolates from tonsils, 36 isolates from tumors, 30 isolates from blood and 18 isolates from skin (Acne).

Results of chemical mutagenesis in Fig. 8, Table 9 showed comparison number of mutant isolates of VRSA, MRSA after exposition to Hydroxyleamine (HA) compared with 78 isolates the number of VSSA, MSSA with overproduction of staphylokinase were 36 isolates from tonsils, 23 from tumors, 12 from blood and 7 from skin (Acne), these result grant higher number of mutant VSSA, MSSA were from tonsils, subsequently tumors, then blood and skin respectively that expressed overproduction of staphylokinase from mutant non virulent *S. aureus*.

Results of chemical mutagenesis in Fig. 9, Table 9 showed comparison number of mutant VRSA, MRSA after exposition to Acridine Orange (AO) compared with 10 isolates the number of VSSA, MSSA with overproduction of staphylokinase were 3 isolates from tonsils, 4 isolates from tumors, 1 isolates from blood and 2 isolates from skin (Acne) of these result showed higher number of mutant VSSA, MSSA were from firstly tumors, subsequently tonsils, then skin and blood respectively that expressed overproduction of staphylokinase from mutant non virulent *S. aureus*.

Results of chemical mutagenesis in Fig. 10, Table 9 showed comparison number of mutant VRSA, MRSA after exposition to Ethylemethnsulfonate (EMS) compared with 12 isolates the number of VSSA, MSSA with overproduction of staphylokinase were 7 isolates from tonsils, 2 isolates from tumors, 2 isolates from bloodand 1 isolates from skin (Acne), these result showed higher number of mutant VSSA, MSSA were from firstly tonsils, subsequently blood, then tumors and skin respectively that expressed overproduction of staphylokinase from mutant non virulent *S. aureus*.

The best mutagen that cause mutation in *sak* gene encoded for staphylokinase with overproduction of fibrinolytic enzyme of non-virulent VSSA, MSSA are Hydroxyleamine chemical mutagen that enhance increase production of staphylokinase.

Phenotypic detection of Staphylokinase after chemical mutagenesis

Staphylokinase possess up affinity for fibrin that its thrombolytic agent leading to tissue damage, its help bacteria to spreading through human (Biomeruex, 2010).

Results exhibit increase hydrolysis zone area nearly well on plasma agar and nearly casein agar medium give rise to overproduction of expression of fibrinolytic enzyme (Staphylokinase) when exposition Methicillin Resistance *S. aureus* and Vancomycin Resistance *S. aureus* to Hydroxyleamine (HA) 10mg, Acridine Orange (AO) 30mg and Ethylemethansulphonate (EMS) 0.001, 0.002 ml, the diameter of fibrinolysis and caseinolysis (hydrolysis zone) are different from diameter of hydrolysis area before chemical mutagenesis to another, overproduction of staphylokinase occassion mutation occurs in *sak* gene encoded for staphylokinase that leading to increase hydrolysis zone from 0-20mm into 10-40mm of Vancomycin Sensitive *S. aureus* (VSSA) and Methicillin Sensitive *S.aureus* (MSSA), thrombolytic enzyme (Staphylokinase) acts to hydrolysis human plasma in plasma agar and hydrolysis casein protein in casein agar leading to formation fibrinolysis and caseinolysis zone (hydrolysis zone) nearly the wells.

Staphylokinase has very substantial activity as a thrombolytic agent created from *S.aureus* in order that its activity and cheap cost as compared to else thrombolytic agent. Overproduction of staphylokinase achieved after exposition *S. aureus* to random mutagenesis by UV radiance (Mohanasrinivasan *et al*, 2014).

Sak gene with a molecular size 420 bp encoded for staphylokinase possess pretty clot hydrolysis specificity than tissues plasminogen activator, its produce from native *S. aureus* but a major hazard in protein production by pathogenic bacteria, but Cloning of *sak* gene in non - pathogenic host low hazard, safe, low cost and effective therapeutic protein fabrication (Pulicherla *et al*, 2011).

This study exhibit that the PCR is a particular and effective procedure for classifying and identifying *S. aureus* isolated from tumors and display overproduction of staphylokinase when occurrence chemical mutagenesis.

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