ORIGINAL ARTICLE

Influence of Staphylococcus Aureus Nuclease (SNase) on Biofilm and some Virulence Factors of Pseudomonas Aeruginosa

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ABSTRACT
in this study, the presence of staphylococcus aureus was investigated from many clinical samples, and then about 14 isolates were obtained, diagnosed with all different diagnostic methods. the bacterial isolates produced for SNase were detected by the DNase agar medium,that revealed only11(78.57%) s. aureus were able to produce the enzyme. the crude activity and specific activity of SNase was 42U/ml , 231U/mg respectively.The isolate was chosen for purification by means of Precipitation with ammonium sulphate 70% saturation and ion-exchange chromatography .the purified SNase activity and specific activity was 45 U/ml and 436U/mg respectively. The purified enzyme had high efficiency to inhibit biofilm formation significantly of p. aeruginosa
Keywords: staphylococcus aureus*,* p. aeruginosa , nuclease (SNase) , biofilm

INTRODUCTION
Staphylococcus aureus is a Cocci, Gram-positive bacterium that lives on the skin, mouth, and upper respiratory tract and can be a risk factor for opportunistic infections in hospitals. (1).This bacterium has many virulence factors to subscribe the ability of s. aureus due to infection including enzymes ,cell surface protein ,toxins and many factors to assistance avoiding the innate immune defense (2).The s.aureus to produce variety of Exoenzymes (3). Nuclease is one of the important exoenzymes and it is belongs to the calss of hydrolases that degrade nucleic acid (4). The s. aureus nuclease catalyzes hydrolysis of both DNA and RNA at the 5 position of the posphodiester bond yielding a free 5- hydroxyl group and a 3- phosphor monoester(5).

MATERIAL AND METHODS
Collection of Samples: This study have collection about 44 samples from pathogenic cases as Burns, blood, med stream urine, sputum and nares swab in AL-Ramadi Teaching Hospital and AL-Fallujah Teaching Hospital, throughout the period from 11/8/2020 to 10/10/2020. The Samples were obtained by utilizing sterile container whereas Burns, nares swab were taken by sterile cotton swab. All samples were directly inoculated or from transport media (BHI broth) into plates of Mannitol salt agar (MSA) and incubation was done for 24hr at 37°C. (6)

Staphylococcus aureus Isolation and Identification :

For the purpose of isolating and diagnosing bacteria, several tests were performed :

Morphological examination was performed with Gram stain (7), Culture examination was performed by cultured bacterial colonies on the selective media Mannitol salt agar (MSA) Incubation was done for 24hr at 37°C.(8) and many biochemical exams were conducted for diagnosis bacteria including: IMVC, coagulase ,oxidase and catalase tests for diagnosing confirmed of the isolates were performed vitek-2 system.(9,10)

DNase test: All of the s. aureus isolates were cultured in trypticase soy broth (TSB) for 24hrs then, all tubes were centrifuged at 8000×g for 15 min. The filtrate was taken ,boiled for 15 min and cooled down at 4 ^{0}C .Afterward ,50µl of Filtrate was added in wells punched in DNase agar and incubated for 24hr subsequently , Hydrochloric acid (1N HCl) was added to the dish and left for 5 min . positive results are zone of clearance around the wells which were measured by ruler (11) .

P. aeruginosa Isolation and Identification: Five diagnosed P. aeruginosa isolates were obtained from postgraduate students at the Department of Biology - College of Science - University of Baghdad for the purpose of using them in studying the effect of nuclease produced from S. aureus on the biofilm formed by P. aeruginosa.

Extraction of the DN ase

The method that was described by (12) and the isolate that developed the largest of clearance on DNase agar was chosen for this experiment .The isolate was cultured in 100ml of nutrient agar at 35 ^{0}C for 24hr.on a rotary shaker at 100 rpm. A afterward, supernatant was obtained, heated in a boiling water for 20 min ,cooled down and diluted with 0.1M Tris –HCl buffer ,PH7.5 and with 0.1 M EDTA .

Partial Purification of enzyme

Preciptation with $(NH_4)_2SO_4$: The procedure that was adopted in this study was performed according to (12,13). Enzyme was precipitated by addition of $(NH_4)_2SO_4$. The product of 70% saturation that precipitated was utilized for obtaining complete Enzyme precipitation, and centrifugation for 15 min at 4^{0}C was done for separating the precipitant .

Ion exchange chromatography: The experiment was carried out according to (12,13) through using column Carboxy methyl -cellulose 2 x 15 cm that several times was washed with buffer of equilibration 0. 1 sodium acetate buffer of pH 6.5 .

Molecular weight determination: The gel filtration chromatography was used for SNase M.W purified partial determination. Enzyme was kept to flow via a glass column (1.5 x 70) cm which packed with sephadex-100, and the column was calibrated with lysozyme 14KDa, bovine serum albumin (BSA) 66 KDa and Trypsin23 KDa were protein standards.(14)

Biofilm formation assay

The procedure that was adopted in this study was performed according to (15) by the wells of sterile 96 well U shaped –bottomed polystyrene microplates .In order to investigate the effect of SNase on biofilm of p. aeruoginosa, the same protocol described previously was

followed except that an aliquot of 50µl of purified SNase was added to each well containing 50µl of an overnight TSB culture equivalent to Macfarlane standard no .0.5. None theless, 50µl of D.W was added to control wells instead of purified SNase .Thereafter, plates were incubated ,stained and quantified as it is mentioned earlier.

Statistical analysis

The data that obtained were displayed as mean ± SD and statistical significances were measured utilizing test of ANOVA (16).

RESULTS AND DISCUSSION

The Results of this study refer to clinical samples as total of 44 were taken from various cases as showed in (Table1). About only 14 isolates (31.81 %) gave morphological features and biochemical tests in relation to s.aureus, while the remaining isolates of 30 (68.19%) might related to other bacteria being pathogenic from various genera as illustrated in (Figures 1) .All colonies from primary culture were purified by subculture on blood agar and inoculated on mannitol salt agar at 37^0C for 24hr (17) ,All isolates gave positive results for the coagulase test.

The quantitative assay was done for fourteen isolates of s.aureus .The results revealed that only 11(78.57%) of s.aureus were able to produce SNase while three isolates didn't produced enzyme as illustrated in (Figures 2) .The s.aureus number 12 was chosen for SNase extraction since that it accomplished the highest zone of clearance on DNase agar.

Extraction of the enzyme SNase: The supernatant of this s.aureus that was chosen was boiled to achieve the inhibition of other enzyme activities (18) . this crude SNase activity and specific activity were 42U/ml and 231U/mg respectively. as illustrated in (Table 2).

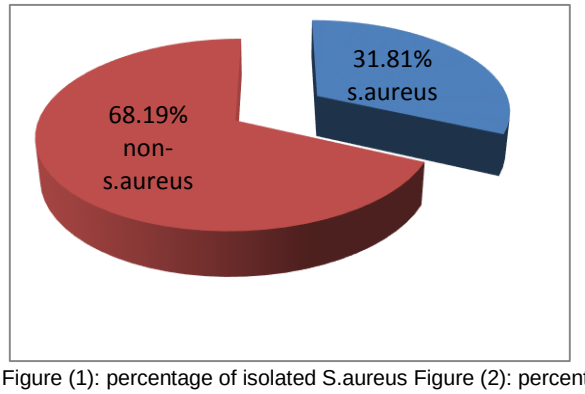
Purification of Enzyme SNase: After the extraction, the supernatant was taken for 70% ammonium sulphate precipitation .The isolates was subjected to CM cellulose column by linear gradient of NACL (0.1-1).The results show one peak for wash and other two found peaks ,the first peak of showed SNase activity in number tubes 35-37 while the second peak had on SNase activity the ibraheem and al- mathkhury (13) The researcher concluded that specific activity of SNase of s.aureus was 241.9 U/mg. as illustrated in (Figure 3).

Determination of molecular weight of SNase: The M.W was estimated by gel filtration accodrding to the molecular weight and elution volume /void volume (ve/vo), the M.W for s.aureus found about 18.1 KDa, ibraheem and al-Mathkhury (13) showed that M.W of purified SNase extracted from s aureus was 16.8 KDa by using SDS gel electrophoresis while the same enzyme purified Streptomyces by gel filtration was 19.9 KDa (19) .

Biofilm assay: The current study findings highlighted the participation of SNase purified from s.aureus in significant $P<0.05$ preventing biofilm formation by p.aeruginosa compared with untreated control .the inhibitory effect of SNase on biofilm formation is undoubtedly perceived , indicating the degradation of the structure major component of biofilm formation (i.e extracellular DNA).the results in table showed that three p.aeuroginosa isolates were have strongly biofilm , after treatment with enzyme ,they became moderately of biofilm formation, while two isolates have moderately biofilm and after treatment enzyme become weekly biofilm formation. as illustrated in (Table 4). The partially purified enzyme also showed a remarkable effect in impairing the ability of P. aeruginosa to produce a protease.

Table(1) Source and number of s.aureus

Sources	No. of samples	No .positive isolates
Nares swab	5	1
Sputum	2	0
Mid stream urine	23	8
Burn	8	3
Blood	6	2
Total	44	14



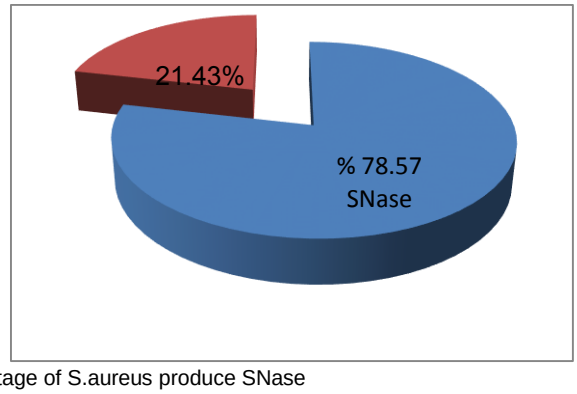


Figure (1): percentage of isolated S.aureus Figure (2): percentage of S.aureus produce SNase

Table(2) activity and specific activity of SNase

Enzyme(SNase)	activity	specific activity
Crude enzyme	42U/ml	231U/mg
Purified enzyme	45 U/ml	436U/mg

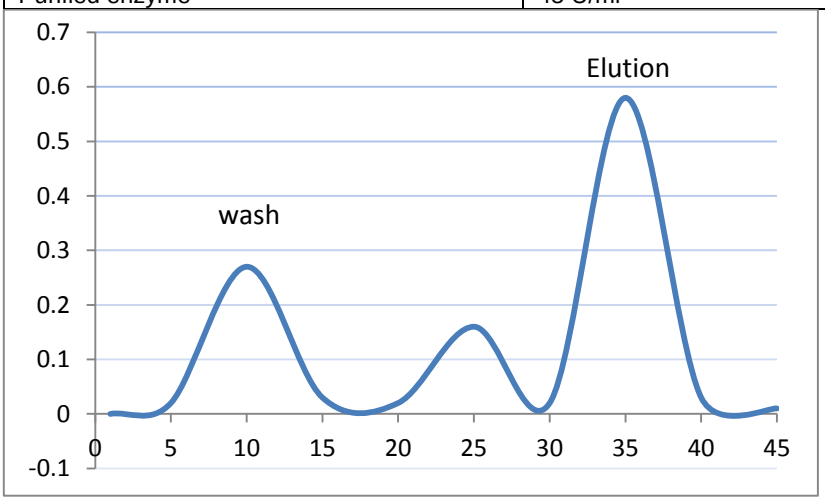


Figure (3):purification of SNase produced from s.aureus isolate by CM-cellulose ion exchange chromatography column (2 x15)equilibrated with 0.1 sodium acetate buffer.

Table(4) biofilm formation of p.aeruoginosa before and after treatment with SNase

Isolates of p.aeruginosa	Biofilm before treat	Biofilm after treat with SNase
1	Strong	Moderate
2	Strong	Moderate
3	Strong	Moderate
4	Moderate	weak
5	Moderate	Weak

CONCLUSION

This study was conducted on extracted and purification of SNase produced by local strains of s.aureus in Iraq then detection the molecular weight of enzyme and have effective on inhibition biofilm formation of p.aeruoginosa.

Conflict of interest : No conflict of interest

Funding: Self,

Ethical Clearance: This study is ethically approved by the Institutional ethical

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