Advances in Environmental Biology, 11(2) February 2017, Pages: 12-16

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Effect of Hyaluronidase enzyme which produced by *Staphylococcus aureus* on killing *Klebsiella pneumoniae* in serum

[1]Khawlah Jebur Khalaf and [2]Luma Saleem Hachim

[1,2]Department of Biology, College of Science, AL-Mustansiryia University, Baghdad /Iraq.

Address For Correspondence:
Khawlah Jebur Khalaf, Department of Biology, College of Science, AL-Mustansiryia University, Baghdad /Iraq.

Received 18 December 2016; Accepted 12 February 2017; Available online 26 February 2017

ABSTRACT

Background: Hyaluronidase is part of extracellular bacterial structure that degrade hyaluronic acid, hyaluronidases event by β elimination of the β-1,4 glycosidic bond in hyaluronic acid . Hyaluronidase regarded as one of bacterial virulence factor Hyaluronidase can aid in organisms penetration into tissues rich in hyaluronic acid such as cartilage, synovial fluid and skin so are important drug diffusion agents. **Objective :** Hyaluronidase enzyme partial purified was obtained from *Staphylococcus aureus* which isolated from wound source, and examined Effect on killing Bacteria in serum by used *Klebsiella pneumoniae* . **Results**: illustrate that the hyaluronidase enzyme with two concentration (20, 40 mg/ml) give a higher percentage for killing *Klebsiella pneumoniae* in serum (83.6 %, 92.04 %) respectively. **Conclusion :** Hyaluronidase enzyme decrease on Bacterial survival in serum.

KEYWORDS: Hyaluronidase, killing Bacteria, *Klebsiella pneumoniae*, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a gram-positive, non-motile, not producing spores ubiquitous bacteria that responsible for human and animal infections [1]. These groups of microorganisms colonize skin, hair, nose and throat of people and animals [2]. They have various virulence factors subscribe to the ability of *S. aureus* to cause infection enzymes, cell-surface proteins, toxins, factors that assistance the *S. aureus* to avoid the innate immune defense [3] and antibiotic resistance mediate existence of the *S. aureus* and tissue infestation at the site of infection Moreover, certain toxins cause specific disease entities [4] *Staphylococcus aureus* produce enzymes capable of degrading hyaluronate. Hyaluronidase is part of extracellular bacterial structure that degrade hyaluronic acid , hyaluronidases event by β elimination of the β-1,4 glycosidic bond in hyaluronic acid[5]. The degradation of hyaluronic acid (hyaluronan, HA) by bacterial hyaluronidases. *Klebsiella pneumoniae* is a pathogenic bacteria, Gram negative and capsulated [6]. Bacterial capsules are surface include different installation that are able of animating the steward defenses and prompting steward immune responses. Whereas the capsule is extracellular polysaccharides [7], it's the components of capsule is the hyaluronic acid and colanic acid [8]. Blood is structures of two parts: liquid transporter appellation plasma and a cellular component include of white blood cells, red blood cells and platelets [9].The bactericidal impact of normal human serum functional significant role in steward defense versus bacterial infection. The complement system is a pivotal created of the immune system in keeping the steward from infection via many pathogenic agents [10]. Purpose of the present study was to determine the role of hyaluronidase in killing *klebsiella pneumoniae* in serum.

MATERIALS AND METHODS

Isolation of bacteria:

Bacteria isolated as pure colonies on Mannitol salt agar, Blood agar and, MacConkey agar, Eosin methylene blue agar then bacterial isolates were inspected microscopically via using Grams stain technique and symmetry tests including cultural, physiological and morphological characteristics of all bacterial isolates were performed [11].

Detection of Hyaluronidase:
Plate method:

Brain heart serum albumin (BHSA) agar inoculation with *staphylococcus aureus*10^8×1. The plate was then incubated at 37° C for 24 hrs. After incubation, the plat was submerged for (2 N) acetic acid for (10) minutes. Diameters of the hydrolyzed or clear zone (production of hyaluronidase) by the isolates were measured in mm [12].

Enzyme Purification [13]:

staphylococcus aureus Grow thin tryptic soy broth overnight in 37° C, then It was centrifuged on(8,000 X g)for used cool centrifuge for(30)minutes. Take the supernatant containing extracellular hyaluronidase. concentration the hyaluronidase by ethanol precipitation. The supernatant was cooled with dry ice and Mixing with ethanol (95%) to get the final concentration 33% ethanol, then centrifuged on (8,000 X g),used cooling centrifuge for (30)minutes. After take the precipitate which contained most of the enzyme activity was suspended in 10 ml of sodium phosphate buffer (pH 6.0) for 0.05 M NaCl. The suspension was dialyzed against the phosphate buffer.

Assay of Enzyme:

Detected activity of partial purified enzyme by plate method assay [12] with well diffusion agar [14] and determination of hyaluronidase. By zone hydrolyzed**.**

Effect of Hyaluronidase enzyme on killing klebsiella pneumoniae in serum. [15]:

Single colony of *Klebsiella Pneumoniae* grown on nutrient agar for (18-24) hrs. was transferred to tubes contain on 5 ml of the nutrient broth by sterilized loops, shaking the tubes then compared turbidity with McFarland Standard tube 0.5 which is equivalent to 1.5 ×10^8 cell/ml. the same method was repeated for three times one tube without enzyme and serum, one tube without enzyme and the two other tubes with presence of enzyme. Groups that contained enzyme the first one was labeled by adding 40 mg /ml concentration of enzyme and the second one with 20 mg /ml concentration. The two groups of tubes were incubated aerobically for 24 hr. at 37°C.

Tubes after incubation centrifuged on (6,000 X g) for (10) minutes. The precipitate containing cells were taken and neglect the supernatant, washing the cells with a phosphate buffered saline (PBS) and again centrifuged on (6,000 X g) until (10) minutes taken the precipitate containing cells. Solved the cells with phosphate buffered saline (PBS) so have the number of cells ^{8}10 × 1.5 cell/ml and do a serial dilution. After that taken 250 µl from cells solution without enzyme mixed with 750 µl from serum as the control. Also mixed cells solution contains different concentration of enzyme (40mg/ml, 20mg/ml) with serum. Then incubated for (30) minutes at 37°C. After that take 0.1 ml from the broth contain cells solution and serum, the separately by spreader on nutrient agar and It incubated aerobically until (24) hr. in 37°C. Next day, the colonies were counted and the inhibition activity of was evaluated and calculated percent reduction of bacteria using the following equation described as [16].

Rate % = (A-B/A)*100 Which

A =No. of colony in control (*K. pneumoniae* and serum). B=No. of colony in treatment (hyaluronidase with *K . pneumoniae* and serum).

R= percentage of reduction of *K .pneumoniae* colony.

RESULT AND DISCUSSION

Extraction of Hyaluronidase Enzyme:

After we do sequential action steps, we got a partial enzyme purity,take the bacteria growth of overnight broth culture with hyaluronic acid to produce the Hyaluronidase the enzyme degradation of hyaluronic acid (HA, hyaluronan), used centrifuge, Take the supernatant containing extracellular hyaluronidase. Mixing the ethanol with supernatant to get the final concentration 33% ethanol, the purpose of used Ethanol precipitation was an effective concentration and purification step.

Advances in Environmental Biology, 11(2) February 2017, Pages: 12-16

Hyaluronidase activity was recovered by elution with 0.2 M NaCl buffer. After that put the extraction in Dialysis bag, Dialysis bag is the secession of smaller molecules of larger molecules in solution by eclectic diffusion during semi permeable membrane. Thus, a dialysis membrane with a 14KDa molecular-weight cutoff (MWCO) because it is closest to the desired molecular weight of hyaluronidase [17]. The Put suspension in glass dish and then incubated at 37°C for purpose drying the hyaluronidase. After that prepared the stock of Hyaluronidase concentration (100 mg/ml) by dissolving 1 g. of powdered Hyaluronidase to 10 ml D.W.

Then detected activity of partial purified enzyme by plate method assay [12] with well diffusion agar [14] and determination of hyaluronidase. We found it was very active with zone hydrolyzed (16mm) fig (1).

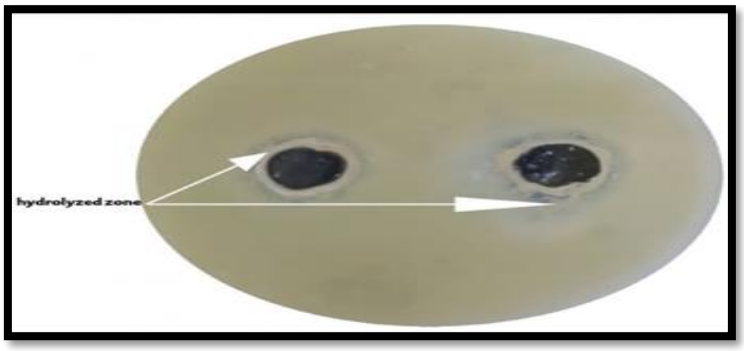

Fig. 1: Assay of partial purified hyaluronidase enzyme produced by *S. aureus*

Effect of Hyaluronidase Enzyme on killing Klebsiella pneumoniae in serum:

Effect of Hyaluronidase enzyme on killing Bacteria in serum which examined by used *Klebsiella pneumoniae*. Results illustrate that the hyaluronidase enzyme with two concentration (20, 40 mg/ml) when mixed with serum give a higher percentage for killing bacteria (*Klebsiella pneumoniae*) in serum (83.6%,92.04 %) respectively when compared with control (*K .pneumonia* with serum only).(Fig 2).

The results were comparable to the results of [18] and found that the bactericidal impact of normal human serum function a significant role in steward defense versus bacterial infection. The serum contain the complement system, the complement system is a pivotal created of the immune system in keeping the steward from infection via many pathogenic agents [10]. It's now clear that stimulate of complement via bacteria can happen via the classical or alternative pathway; the former usually requires for complement is stimulate confession of the membrane attack complex (MAC) which formation of terminal or late acting complement proteins (C5b-9) complexes [19].

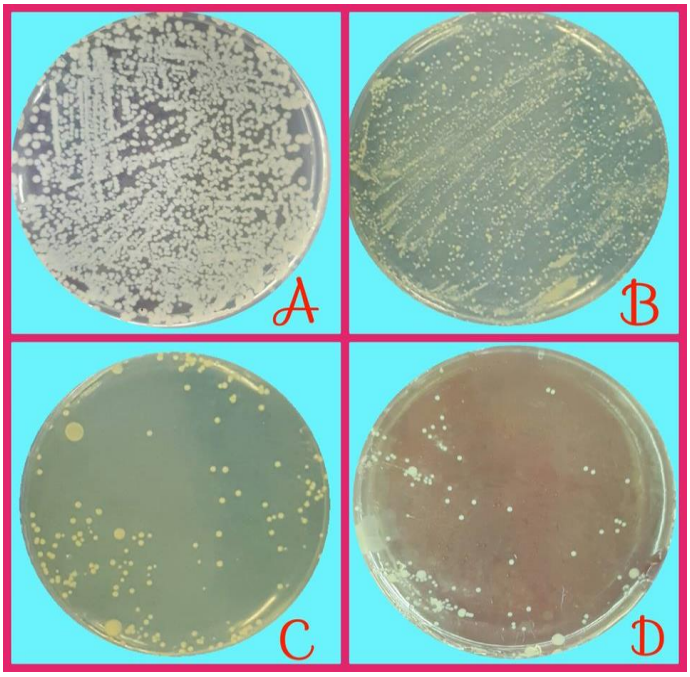

Fig. 2: Effected Hyaluronidase enzyme on killing *Klebsiella pneumoniae* in serum
A: Bacteria without serum C: Bacteria with enzyme (20mg/ml) and serum.
B: Bacteria with serum (control). D: Bacteria with enzyme (40mg/ml) and serum.

Gram-positive bacteria have heavy cell walls which prevents C5b-9 complexes to transfuse them and harm their cytoplasmic membranes. the crossing of "molecules" of differ molecular weights via C5b-9 pores as a consequence of inner membrane harm participate to the killing of Gram-negative bacteria via promote osmotic power and or reaction with cytoplasmic proteins[20].

The capsule may action in concert with other cell-surface compositions, as O antigens, to allow impedance to complement mediated death of ten times, it is a specific combination of cell-surface compositions that are administrator for giving a rise degree of impedance to complement mediated death [21]. Via providing a permeability bar to complement complex, the capsule might visor underlying cell-surface compositions which would otherwise be strong stimulate of the alternative pathway [22] and the degradation of hyaluronic acid (hyaluronan, HA) by hyaluronidase .the capsular polysaccharide, which is composed of hyaluronic acid [23].

Conclusion:

In the present study, we can conclude that, Hyaluronidase enzyme decrease on Bacterial survival in serum. Through the degradation of hyaluronic acid in capsule by hyaluronidase, leading to increases the effectiveness of the complement in serum .its use to treatment of the pathogenic bacteria causing bacteremia .

ACKNOWLEDGMENTS

We Special thanks to the Dr. Ikbal Khider Al-Joofy who gave us a scientific support.

REFERENCES

[1] Miller, L.G. and B.A. Diep, 2008. Clinical practice: Colonization, for mites, and virulence: Rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Journal Clinical Infect. Dis., 46: 752-760.

[2] Nair, R., B.M. Hanson, K. Kondratowicz, A. Dorjpurev, T.C., 2013. Antimicrobial resistance and molecular epidemiology of *Staphylococcus aureus* from Ulaanbaatar, Mongolia. Journal PubMed, 67(179): 19-24.

[3] Zecconi, A. and F. Scali, 2013. *Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases. Journal PubMed, 150(1-2): 12-22.

[4] Bien, J., O. Sokolova and P. Bozko, 2011. Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Pro inflammatory Response . Journal of Pathogens, 7(9): 629-641.

[5] Karla, C.F.B., A.W. Gisele, G.A. Fernanda and C.A. Eliane, 2015. Arthropod venom hyaluronidases: biochemical properties and potential applications in medicine and biotechnology. Journal of Venomous Animals and Toxins including Tropical Diseases, 10(1): 127-31.

[6] Keynan, Y. and E. Rubinstein, 2007 ."The changing face of *Klebsiella pneumoniae* infections in the community" International Journal of Antimicrobial Agents, 30(5): 385-389.

[7] Merino, S. and J.M. Tomás, 2015. Bacterial Capsules and Evasion of Immune Responses. Journal of Wiley Online Library, 4, DOI: 10.1002/9780470015902

[8] Gottesman, S. and V. Stout, 1991. Regulation of capsular polysaccharide synthesis in Escherichia coli 12. Mol. Microbial, 5: 1599-1606.

[9] Psychogios, N., D.D. Hau, J. Peng, C. Guo, R. Mandal, S. Bouatra, L. Sinelnikov, R. Krishnamurthy, R. Eisner, B. Gautam, N. Young, J. Xia, C. Knox, E. Dong, H. P. Paul, Z. Hollander, T.L. Pedersen, S.R. Smith, F. Bamforth, R. Greiner, B McManus, J.W. Newman, T. Good friend and D.S. Wishart, 2011. The Human Serum Metabolomics. Journal of PLoS ONE, 6(2): 1690-16957.

[10] Nesargikar, P.N., B. Spiller and R. Chavez, 2012. The complement system: history, pathways, cascade and inhibitors. Europa Journal Microbial Immune (Bp), 2(2): 103-111.

[11] Benson, H.J., 2002. Microbiological Applications: Laboratory Manual in General Microbiology, 8thed.McGraw-Hill company, Boston, USA.

[12] Samantha, J.K., G. Andrew, D.J. Allen, G.D. Christopher and M. Adrian, 2004. Distribution, Genetic Diversity, and Variable Expression of the Gene Encoding Hyaluronate Lyase within the *Streptococcus* sues Population Journal OF bacteriology, p: 4740-4747.

[13] Tam, Y.C. and E.C. Chan, 1985 .Purification and characterization of hyaluronidase from oral *Peptostreptococcus* species. Infect. Immun, 47: 508-513.

[14] Gupta, U., Radramma, E.R. Rati and R. Joseph, 1998.Nutrition Quality of lactic acid fermented bitter gourd and fenugreek leaves International Journal Food Science .and Nuter, 94(2): 101-108.

[15] Lin, T.H., S. Huang, C.C. Wu, H. Liu, T.R. Jinn, Y. Chen and C.T. Lin, 2013.Inhibition of *Klebsilla pneumonia* growth and capsular polysaccharide biosynthesis by *Fructusmume*. 8(7): 135-41.

[16] Lai, Y., H. Peng and H. Chang, 2003. "RmpA2, an activator of capsule biosynthesis in *Klebsiella pneumoniae* CG43, regulates K2 cps gene expression at the transcriptional level," Journal of Bacteriology, 185(3): 788-800.
[17] Reed, R., 2007. Practical Skills in Bimolecular Sciences, 3rd ed. Essex: Pearson Education Limited. p. 379. ISBN 978-0-13-239115-3.
[18] Sunitha, K., P. Suresh, M.S. Santhosh, M. Hemshekhar, R.M. Thushara, G.K. Marathe, C. Thirunavukkarasu, K. Kemparaju, M.S. Kumar and K.S. Girish, 2013. Inhibition of hyaluronidase by N-acetyl cysteine and glutathione: role of thiol group in hyaluronan protection. International Journal of Microbiology Micromole, 55: 39-46.
[19] Rabbee, C., M. Shahriar, M.A. Bhuyian, Rishikesh and A. Islam, 2013. Evaluation of Bactericidal Action of Serum Collected from Paratyphoid Patients and Normal Human against *Salmonella* Paratyphoid. Dhaka University Journal Pharm. Science, 12(1): 71-75.
[20] Bloch, E.F., S.M. Pinkett, S. Campbell, S. Baskin, S. Dillahunt, S. Peters, S. Lucas, D. Evans, C. Johnson, T. Everett and Y. Kanaan, 2011. New mechanism for complement killing of gram negative bacteria Journal Academic of Microbiology, 5(23): 3936-3941
[21] Cross, A.S., 1990. The biologic significance of bacterial encapsulation .Curr Top Microbiology Immunoll, 50: 87-98.
[22] Roberts, I.S., 1995. Bacterial polysaccharides in sickness and in health. Microbiology, 141: 2023-2031.
[23] Mark, E.H., J.H. Morgan and J.R. Anna, 2009. Genotypic and Phenotypic Assessment of Hyaluronidase among Type Strains of a Select Group of Staphylococcal Species, International Journal of Microbiology. Article ID 614371, 8.