Experimental Study of *Pseudomonas aeruginosa* Proteases Isolated from Corneal Ulcer of Iraqi Patients and Their Role in the Treatment of *Staphylococcus aureus* Keratitis

### Abstract

One-hundred and twenty samples (corneal scraping) were collected from patients diagnosed to have microbial keratitis (corneal ulcer) who attended Ibn Al-Haitham Teaching Eye Hospital from the period between May 2013 and November 2013, *Pseudomonas aeruginosa* was reported 26 (21.6%) from the total cases. All bacterial isolates were diagnosed by conventional and biochemical tests, and confirmed by Vitek 2 Compact System.

The role of proteases enzymes (Elastase (LasB), LasA, Alkaline protease and Protease IV) of *Pseudomonas aeruginosa* in the corneal ulcer was studied by using genetic and molecular biological method by real time PCR, and the results indicated that three bacterial isolates of *Pseudomonas aeruginosa* possessed elastase gene (*LasB*) (11.5%), and only one bacterial isolate of *Pseudomonas aeruginosa* harbored *LasA* protease gene (3.8%). All bacterial isolates of *Pseudomonas aeruginosa* were harbored alkaline protease gene (100%), and twenty bacterial isolates were harbored protease IV (76.9%).

The results of real-time PCR analysis indicated that four bacterial isolates of *Pseudomonas aeruginosa* (15.3%) were harbored more than one gene of different proteases enzymes (elastase, alkaline protease, and protease IV).

On the other hand our results showed that one bacterial isolates (3.8%) harbored both *LasA* protease and alkaline protease genes, and twenty bacterial isolates of *Pseudomonas aeruginosa* (76.9%) were harbored alkaline protease and protease IV genes.

The LasA protease was extracted from *Pseudomonas aeruginosa* isolate by cooling centrifuge and precipitated supernatant by ammonium sulfate at saturation (80%). The
resulted extracted crude enzyme concentration was 60 μg/ml. Then the crude enzyme was partially purified by dialysis and gel filtration chromatography by using Sephadex G-100. The concentration of partial purified enzyme reached 40μg/ml.

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Summary

The results of the experimental treatment of bacterial keratitis (in vivo) of infected eyes rabbits caused by Methicillin Sensitive *Staphylococcus aureus* showed that the efficacy of LasA protease was effective was as Lysostaphin in eradicating Methicillin Sensitive *Staphylococcus aureus* from the infected corneas after approximately 15 h after giving the drug at dose 100 μl (concentration 1μg / ml). While Vancomycin gave us very little potency in eradicating *S. aureus* from corneas in comparison with potency of LasA protease and Lysostaphin during this time but showed good potency very late approximately after 3 days of application of treatment.

The results of the experimental treatment *in vitro* (in the test tube) that is caused by Methicillin Sensitive *Staphylococcus aureus* showed that the efficacy of LasA protease was similar to that of Lysostaphin drug in the killing of Methicillin Sensitive *Staphylococcus aureus* in the bacterial broth.