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Thesis Title	Experimental Study of Pseudomonas aeruginosa Proteases Isolated from Corneal Ulcer of Iraqi Patients and Their Role in the Treatment of Staphylococcus aureus Keratitis
Year	2015
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, <i>Pseudomonas aeruginosa</i> 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 <i>Pseudomonas</i> <i>aeruginosa</i> 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 <i>Pseudomonas</i> <i>aeruginosa</i> possessed elastase gene ( <i>LasB</i> ) (11.5%), and only one bacterial isolate of <i>Pseudomonas aeruginosa</i> harbored <i>LasA</i> protease gene ( 3.8%). All bacterial isolates of <i>Pseudomonas aeruginosa</i> were harbored <i>alkaline protease</i> gene (100%), and twenty bacterial isolates were harbored <i>protease IV</i> (76.9%). The results of real - time PCR analysis indicated that four bacterial isolates of <i>Pseudomonas aeruginosa</i> (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 <i>LasA</i> protease and <i>alkaline</i> <i>protease</i> IV genes. The LasA protease was extracted from <i>Pseudomonas</i> <i>aeruginosa</i> isolate by cooling centrifuge and precipitated supernatant by ammonium sulfate at saturation (80%). The

resulted extracted crude enzyme concentration was 60  $\mu$ g/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 40 $\mu$ g/ml. III

## 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  $\mu$ l ( concentration 1 $\mu$ g / 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.