Summary

Pseudomonas aeruginosa is an opportunistic pathogen which has the ability to invade human body and cause acute and chronic infections such as pneumonia, cystic fibrosis, urinary tract infections, bone and joint infections, bacteremia, corneal infections, conjunctival erythema, skin and soft tissue infections, and other infections. *P. aeruginosa* has several factors that promote the progression of infection and its capacity to resist the antibiotics. The medical importance of this bacterium stems from its capacity to develop mechanisms of resistance to antibiotics, leading to hospital-acquired infections as well as rising the morbidity and mortality compared to other bacterial infections. Increasing prevalence and severity of multi-drug resistant (MDR) *P. aeruginosa* strains require further searching for novel antibacterial strategies. Phage therapy represents a promising approach in treatment strategies for bacterial infections, where bacteriophages exhibit several advantages that make them the better therapeutic solution to antibiotic-resistant infections.

This study concerns the investigation of the bacteriophages and their components efficacy against multi-drug resistant (MDR) *P. aeruginosa* strains PAR21 and PAR50 isolated from diabetic foot ulcer patients, aiming to define the nature of the interaction between the phage and bacteria by immunochemical analyses of surface polysaccharides and proteins to reveal the nature of phage protein receptors on bacterial cell, as well as study how the phage proteins are affecting the bacteria and their impact in synergizing with antibiotics.

Evaluation of the activity of phages and their proteins has been achieved by spot assay, zymography, spectrophotometric assay and Scanning Electron Microscopy at Low Voltage. The phage proteins PA-PP1 and PA-PP2 were isolated from their phages by 0.1 N HCl and then purified twice using Toyopearl HW-55S column with 0.06 M phosphate buffer. The purified proteins were identified using comparative analysis of peptides masses (NCBI, UniProt databases) and then by comparative analysis of peptide sequences. Resorufin-labeled casein was used as the substrate to determine the proteolytic activity of this protein. Bacterial exopolysaccharide (EPS) and outer membrane proteins (Opr) were targeted to define the phage receptor on *P. aeruginosa* strains. EPS for each strain (PAR21 and PAR50) was extracted by three methods,

namely with phenol, trichloroacetic acid (TCA), and PBS extraction and then purified using DEAE-Sephadex A-25 followed by Toyopearl HW-55S column. The purified EPS was subjected to structural analysis using GLC-MS and NMR spectroscopy as well as the immunological assay ELISA. The chemical interaction between phage protein and EPS was analyzed to reveal the existence of phage protein receptor in EPS employing the estimation of reducing sugar by Nelson-Somogyi method. In addition, zymography of phage protein loaded on 12.5%, acrylamide with 0.1% bacterial EPS was done. To uncover the phage protein impact on Opr, each bacterial strain was treated with phage protein and then Opr were isolated using SDS extraction method followed by loading them on 12.5% acrylamide gel have been performed and compared them with Opr that were isolated from untreated strains. The Opr that was targeted by phage proteins was identified using comparative analysis of peptides masses (NCBI, UniProt databases). Piperacillin, ticarcillin-clavulanic acid, ceftriaxone, amikacin, gentamicin and tobramycin discs were used against *P. aeruginosa* strains treated and untreated with phage proteins to evaluate the antibiotic action in synergizing with phage proteins.

Two phages showed activity against *P. aeruginosa* PAR50 strain without any impact on *P. aeruginosa* PAR21 strain. Based on the results obtained with the spot assay, zymography, spectrophotometric assay and SEM-LV, two proteins PA-PP1 and PA-PP2 isolated from both phages showed high efficacy against *P. aeruginosa* PAR50 strain. There were no significant differences observed among both proteins with respect to their effect on the bacteria. The comparative analysis of both, peptides masses and peptide sequences of protein have evidenced that these proteins belong to serine protease family. These results were corroborated by the interaction of each protein with resorufin-labeled casein, which revealed the high ability of the phage protein to utilize the casein as substrate. This, in turn, supports the results of chemical estimation and zymography of the interaction between bacterial EPS and phage proteins which showed that phage proteins have no ability to use EPS as a receptor.

Structural analysis of bacterial EPS by GLC-MS and NMR spectroscopy showed the existing of three main structures: structure I as appears in PAR21-PS5, PAR50-PS1 and PAR50-PS2 samples, structure II exists in PAR21-PS4, whereas structure III is the most common in the PAR21-EPS and appears in PAR21-PS2 and PAR21-PS3 samples.

- Structure I is linear polysaccharide with a repeating unit consisting of two monosaccharides;

$$\alpha\text{- D-Gal}p\text{-}[(1\rightarrow3)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow]_n$$

- The structure II is a linear polymer of β-1,3-linked d-galactopyranose;

$$[\rightarrow3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow]n$$

- The structure III is a branched heteropolysaccharide with a repeating unit consisting of five sugar residues;

$$\begin{array}{l}[\rightarrow3)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow3)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow3)\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow3)\text{-}\beta\text{-D-Rha}p\text{-}(1\rightarrow]n \\ \qquad\qquad\qquad\qquad\quad 2 \\ \qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\quad \alpha\text{-D-Man}p\end{array}$$

This variation in exopolysaccharides appeared clearer in their immunoreactivity assayed with anti-PAR21 and anti-PAR50 antibodies generated in rabbit using whole cells, where the ELISA showed that PAR21 EPS was recognized only with anti-PAR21, likewise, PAR50 EPS interacted only with anti-PAR50. The comparison in SDS-PAGE of Opr isolated from the *P. aeruginosa* PAR50 strain treated with PA-PP proteins and those from untreated strain showed the disappearance of one protein band on the OM proteins pattern of treated one. This protein was identified by comparative analysis of peptides masses as porin protein. The combination of phage proteins and antibiotics against *P. aeruginosa* PAR50 demonstrated a clear change in the antibiotic efficiency, where piperacillin was more efficient against *P. aeruginosa* PAR50 when applied with phage protein.

In this work, two phage proteins PA-PP1 and PA-PP2 are demonstrated which have therapeutic efficacy against multi-drug resistant *P. aeruginosa* PAR50 strain. The phage receptor on the surface of this bacterial strain has appeared as one of the OM porin proteins. This protein exists in multidrug efflux systems MexAB and MexXY which plays important role in antibiotic resistance, thereby the utilizing this protein by phage leads to changes the efflux pump mechanism and cause increased sensitivity to several antibiotics. Furthermore, the findings obtained from Scanning Electron Microscopy indicate the fatal effects of phage proteins on the bacterial cell, suggesting further roles of these proteins on bacteria.