ABSTRACT

Bacteria are becoming resistant day by day due the misuse and overexploitation of the antibiotics. So there is an urgent need to find some natural and novel methods to fight against the bacterial infections. As we know that honey is being used for centuries as medicinal purposes. The aim of this study was to evaluate the antibiofilm, anti-quorum sensing (QS) and dispersal activities of honey against multispecies bacterial biofilm. Honey samples of *A. dorsata* and *A. cerana* were collected, purified and tested against bacterial strains isolated from the fecal samples of obese persons, who visited Fatima memorial hospital, Lahore. In total, five bacterial strains (M4, X3, M1, T1 and T4) were isolated from obese person's feces, identified morphologically and biochemically. For identification upto species level, 16S rRNA gene sequencing was performed which indicated that five isolates, M4, X3, M1, T1 and T4 belong to *Pseudomonas aeruginosa* (MT448672), *Escherichia coli* (MT448673), *Morganella morganii* (MT448675), *Staphylococcus aureus* (MT448674) and *Klebsiella pneumonia* (MT448676) respectively. Disc diffusion method was used to determine the antibiotic susceptibility profile of five isolates against three antibiotics viz., erythromycin (20µg/ml, lincomycin (100µg/ml) and rifampicin (100µg/ml). *P. aeruginosa* showed significant ($p<0.05$) resistance against all tested antibiotics. *Escherichia coli, M. morganii* and *K. pneumoniae* showed maximum susceptibility towards erythromycin while *S. aureus* was highly susceptible towards lincomycin. In order to form multispecies biofilm, identified bacteria were grown in batch cultures by mixing equal volumes ($OD_{600}=0.1$) of 2, 3 and 5 bacterial isolates. Hence, total 11 groups (g_1-g_{11}) of multispecies biofilm were made and *P. aeruginosa* was included in each group due to its resistance against all tested antibiotics. Using crystal violet staining method, time kinetics of biofilm formation by multiple bacteria was performed over a time span of 96 hours after 24, 48, 72 and 96 hours at 37°C. Results showed that multispecies bacteria of all groups (g_1-g_{11}) produced maximum biofilm after72 hours. Minimum inhibitory concentrations (MIC) of both honey samples (sources: *A. dorsata* and *A. cerana*) were determined by testing six concentrations (2%, 5%, 8%, 11%, 14% and 17%) against multispecies biofilm. It was observed that 2% and 5% concentrations were found to be effective inhibitory

concentrations against multispecies biofilm by all groups (g_1-g_{11}). It was observed that 2% and 5% honey concentrations of *A. dorsata* was most effective against g_3, g_4, g_7, g_8, g_7, g_9, g_{10} and g_1; moderately effective against g_1 and g_5, and 5% concentration was less effective against g_2. It was observed that 2% and 5% honey concentrations of *A. cerana* was most effective against g_1 and g_3-g_1 and 5% concentration was less effective against g_2. Antibiofilm potential of *A. dorsata* and *A. cerana* honey at 2% and 5% concentrations was determined using crystal violet staining method against all multispecies biofilm groups. Both honey samples were incubated with 72 hours mature biofilm for next 24 hours at 37°C and showed significant antibiofilm activity at both concentrations against all groups (g1-g11) compared to control (erythromycin) though better results were obtained at low honey concentrations (2%) of both samples. Culture supernatant method was used to determine the anti-QS effect of *A. dorsata* and *A. cerana* honey by measuring the pyocyanin production in *P. aeruginosa,* only. For the said purpose, both honey samples at 2% and 5% concentrations were incubated with *P. aeruginosa* over a time period 96 hours. Optical densities (OD_{520}) were measured after 24, 48, 72 and 96 hours. Final concentration of pyocyanin (µg/ml) was obtained by multiplying OD with 17.072 (constant factor obtained from literature). Significant inhibition of pyocyanin production was observed after 96 hours indicating anti-QS potential of both honey samples (a constant factor). Lastly, the biofilm dispersal potential of both honey samples was evaluated using crystal violet staining method against 11 multispecies biofilm groups (g_1-g_{11}).Both honey samples at 2% and 5% honey concentrations were incubated with 72 hours mature multispecies biofilm for further 24 hours at 37°C. Best biofilm dispersal was observed at 5% concentration but 2% concentration was also effective in dispersal compared to the erythromycin (control). It is concluded that honey possess bioactive components that contribute towards antibiofilm, anti-QS and biofilm dispersal potentials against multispecies biofilm formed by *P. aeruginosa, K. pneumoniae, E. coli, M. morganii* and *S. aureus*.. More research work is needed to find out the actual components of honey which are actually responsible for the bactericidal action. This could help in developing biocidal substances without any side effects and useful in treating various multiresistant burn and wounds infections. Additionally, this study also suggest that

honey could be used as conjugant with antibiotic in preventing bacterial attachment to the medical and industrial instruments due to its antiadhesive potentials biofilm formation. This will prevent economy loss by maintaining the quality of expensive medical instruments, for example, urinary catheters, dialysis and respiratory apparatus etc.. Specific honey derived effective sprays can also be formed and used for cleaning puposes in industrial fermenters without compromising the quality of product making in these fermenters. Last but not the least this study recommends the use of honey in children as well as adults e.g. in pneumonic and asthemic condition, owing to the fact that honey consists of vitamins and simple sugars (immediate source of energy), thus having the potential to fight against pathogenic bacteria by boosting their immune systems.