ABSTRACT

Biofilm is preferential mode of life that protects the microorganisms from harsh external environment. These microorganisms produce a hydrated extra polymeric substance (EPS) in biofilm mode. These biofilms serve many functions for microorganism, hence increase their resistance against antibiotics and many others antimicrobial agents. To overcome increased microbial resistance, there is a dire need to find the alternative solutions. Honey has great clinical importance for the treatment of many infectious diseases. Honey is likely to be used as medicine because it has antibacterial properties as it is highly acidic and has high sugar content that produces an osmotic effect and thus prevent the growth of microorganisms. Other reason for the honey to be used as medicine is the presence of hydrogen peroxide that has a strong antibacterial activity. Additionally, certain enzymes are also responsible for antibacterial activity of honey. This study was aimed to investigate the antibiofilm, anti-quorum sensing (QS) and biofilm dispersal potential of honey obtained from *Apis cerana* and *Apis dorsata*. Among various concentrations tested, effective minimum inhibitory concentrations (MIC) were observed to be 2% and 5%, hence used in subsequent study to achieve the aforementioned aim. In total, five isolates (M4, X3, T1, T4 and M1) were isolated from obese patients visiting Fatima Memorial Hospital, Lahore. Morphological, biochemical characterization and 16S rRNA gene sequencing revealed that isolates M4, X3, T1, T4 and M1 belong to *Pseudomonas aeruginosa* (MT448672), *Escherichia coli* (MT448673), *Morganella morganii* (MT448674) *Staphylococcus aureus* (MT448675) and *Klebsiella pneumoniae* (MT448676), respectively. Antibiotic susceptibility of all strains was checked using antibiotics viz., erythromycin (20 µg/ml), rifampicin (100 µg/ml) and lincomycin (100 µg/ml) following disc diffusion assay. All the strains were highly susceptible to antibiotic erythromycin that was used as positive control except for *P. aeruginosa* that was resistant against all used antibiotics. The biofilm forming ability of monospecies bacterial strains was checked in borosilicate (BS) glass tubes using crystal violet assay at 24h, 48h, 72h and 96h at 37°C. The highest biofilm forming ability was observed in all bacterial strains after 72h ($p<0.05$). Antibiofilm potential of both honey (Sources: *A. cerana* and *A. dorsata*) at 2% and 5% concentrations against monospecies

biofilm of five bacterial strains (*P. aeruginosa, E. coli, M morganii, S. aureus* and *K pneumoniae*)using crystal violet method.Highly significant decrease ($p<0.001$) was observed in monospecies biofilm forming ability of the bacterial strains at both concentrations(2% and 5%) of honey from two sources(*A. cerana* and *A. dorsata*). Regarding anti QSpotential, it was observed in *P. aeruginosa* only using pyocyanin inhibition method via supernatant culture method. *P. aeruginosa*produces pyocyanin, which is controlled/regulated by QS. Actually, two QSsystems;*rhl* and *las* are responsible for the production of biofilm, pyocyanin production and different virulence factors in *P. aeruginosa*. These systems are controlled by two signaling molecules N-acyl-homoserine lactone. Results showed that both 2% and 5% concentrations of honey (from *A. cerana, A. dorsata*) have anti QS effect on pyocyanin production by *P. aeruginosa*. Highly significant ($p<0.001$) decrease in the pyocyanin production by *P. aeruginosa* was caused by 2% concentration of honey (from both sources, *A. cerana, A. dorsata*). Biofilm dispersal is a synchronized and controlled process, in which sessile biofilm cells changes into free living, planktonic bacterial cells. This study examined the action of 2% and 5% honey concentration (*A. cerana, A. dorsata*) on the dispersal of biofilm.So as established previously via biofilm time kinetic study, biofilm was grown upto 72 hours, treated with 2% and 5% concentrations of both honeysfor the next 24 hours at 37°C. Honey (of *A. cerana*) at 2% concentration caused a highly significant ($p<0.001$) biofilm dispersal formed by all test strains. While, 5% concentration of *A. dorsata*honey strongly dispersed *P. aeruginosa* biofilm compared to 2% which was goodin dispersing biofilm by other bacterial strains(*E. coli, M morganii, S. aureus* and *K. pneumoniae*).This study concluded that honey from*A. cerana* and*A. dorsata* has antibiofilm, anti QS and biofilm dispersal potential against pathogenic microbes as well as their biofilms. It is recommended that honey could be used as adjuvants to antibiotics for the treatment of biofilm and biofilm related infections. However, further in-depth research is needed to know the bioactive compounds conferring anti-biofilm activity to honey.