Honey Potential as Antibiofilm, Antiquorum Sensing and Dispersal Agent against Multispecies Bacterial Biofilm

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Abstract: This study is first to test Pakistani honey bees, *Apis dorsata* and *A. cerana* honey samples as anti-biofilm, anti quorum sensing (QS) and biofilm dispersal agents honey against multispecies biofilm of bacteria (obtained from obese patients). Briefly, five previously identified isolates *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Morganella morganii* and *Klebsiella pneumoniae* (MT448672-MT448676) were selected. Antibiogram study of all five isolates was tested against three antibiotics viz., erythromycin (20 µg/mL), lincomycin (100 µg/mL) and rifampicin (100 µg/mL). In order to form multispecies biofilm, identified bacteria were grown in batch culture by mixing equal volumes (OD⁵⁹⁰nm = 0.1) of 2, 3 and 5 bacterial isolates. In total 11 groups (g₁-g₁₁) were made. Crystal violet (CV) staining method was used to evaluate the antibiofilm potential and biofilm dispersal potential of both honey samples. QS inhibition in *P. aeruginosa* was measured following culture supernatant method. Antibiogram study showed significant (*p* < 0.05) resistance by *P. aeruginosa* against tested antibiotics. *E. coli*, *M. morganii* and *K. pneumoniae* were significantly susceptible to erythromycin and *S. aureus* to lincomycin. Both honey samples at 2% and 5% concentrations showed significant (*p* < 0.05) inhibition potential of multispecies biofilm by all test groups (g₁-g₁₁). Though *A. dorsata* honey significantly inhibited biofilm formation at 2 and 5% against all groups but 2% concentration was highly significant against g₂-g₄ groups. Regarding *A. cerana* honey, 2% concentration was significantly effective against g₁, g₄-g₇ and g₉-g₁₁ groups. Both honey samples significantly inhibited QS at 2 and 5%. The 5% concentration of *A. dorsata* honey significantly dispersed biofilm by all groups compared to 2% which showed dispersal potential only by g₂ and g₃ groups. Accordingly, honey samples showed significant antibiofilm, anti-QS and biofilm dispersal potentials thus can be considered as good alternative to antibiotics.

Key words: honey potential antibiofilm, quorum sensing, antibiotic resistance, resistant isolates

1 Introduction

Bacterial biofilms are a major worldwide healthcare problem (urinary tract infections) and are associated with decreasing quality of life and significant patient morbidity¹. Bacteria in biofilm mode protect themselves from antibiotics and the patient’s immune system². Treatment of bacterial biofilm is quite complicated because of the mechanisms underlying bacterial growth as well as resistance. Multispecies biofilms have complementary metabolic strategies for obtaining nutrients and degrading host immune molecules³. Various strategies have been developed and implicated for the biofilm control, however, natural and ef-
ficient anti-biofilm forming agents are still being used widely. Bacterial cells in a biofilms can be up to a thousand times more resistant against antibiotics when compared to free living planktonic cells. Pseudomonas aeruginosa is well known for its quorum sensing (QS) mechanism in developing biofilm and it regulates QS by secreting a light green pigment known as pyocyanin. When the biofilm becomes mature, biofilm dispersal starts either actively (by external harsh conditions due to antibacterial agents) or passively (by removing complete biofilm with sharp equipment). Due to an increase resistance towards antibiotics, the need for natural antibiofilm agent like honey has generated renewed interest. No studies have identified the exact antimicrobial mechanism of honey until now, although few have reported contributing factors such as high sugar contents (~80%), minerals and vitamins, its acidity (pH 3.2–6.0), production of hydrogen peroxide due to glucose oxidase; osmotic effects, antioxidant contents and stimulation of immunity neutrophils, that contribute towards antimicrobial properties of honey include. Previously, Lu et al. and Kim and Kang, also reported antibiofilm activities of honey against P. aeruginosa and E. coli. Studies revealed that honey possesses strong anti-QS potentials at the concentration below its MIC values. Moreover, a study by Park et al., revealed that honey is also effective in dispersing mature biofilm. With all this background, the current study has been designed to investigate the antibiofilm, anti QS and biofilm dispersal potential of two honey samples obtained from honey bees, A. dorsata and A. cerana against bacterial isolates obtained from obese patients.

2 Materials and Methods

2.1 Sample collection

A. dorsata and A. cerana honey samples were collected from the farmers of Sadiq Abad, Pakistan. The beekeepers raised the bees in hives under shelter close to fresh water source but away from noise. Initially artificial nectar was provided by dissolving equal parts granulated sugar to fill quart jars. Jars with top feeder lids are invert into the holes provided by dissolving equal parts granulated sugar to fill quart jars. Jars with top feeder lids are invert into the holes.

Table 1 Possible combination of the five test strains for monitoring antibiofilm, antiQS and dispersal potential of A. dorsata and A. cerana honey samples.

|   | 1. P. aeruginosa + Morganella morganii (g1) | 2. P. aeruginosa + Klebsiella pneumoniae (g2) | 3. P. aeruginosa + Staphylococcus aureus (g3) | 4. P. aeruginosa + E. coli (g4) | 5. P. aeruginosa + Morganella morganii + Klebsiella pneumoniae (g5) | 6. P. aeruginosa + Morganella morganii + Staphylococcus aureus (g6) | 7. P. aeruginosa + Klebsiella pneumoniae + E. coli (g7) | 8. P. aeruginosa + Klebsiella pneumoniae + Staphylococcus aureus (g8) | 9. P. aeruginosa + E. coli + Morganella morganii (g9) | 10. P. aeruginosa + E. coli + Staphylococcus aureus (g10) | 11. P. aeruginosa + E. coli + Klebsiella pneumoniae + Morganella morganii + Staphylococcus aureus (g11) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | P. aeruginosa + Morganella morganii (g1) | 7 | P. aeruginosa + Klebsiella pneumoniae + E. coli (g7) |
| 2 | P. aeruginosa + Klebsiella pneumoniae (g2) | 8 | P. aeruginosa + Klebsiella pneumoniae + Staphylococcus aureus (g8) |
| 3 | P. aeruginosa + Staphylococcus aureus (g3) | 9 | P. aeruginosa + E. coli + Morganella morganii (g9) |
| 4 | P. aeruginosa + E. coli (g4) | 10 | P. aeruginosa + E. coli + Staphylococcus aureus (g10) |
| 5 | P. aeruginosa + Morganella morganii + Klebsiella pneumoniae (g5) | 11 | P. aeruginosa + E. coli + Klebsiella pneumoniae + Morganella morganii + Staphylococcus aureus (g11) |
| 6 | P. aeruginosa + Morganella morganii + Staphylococcus aureus (g6) | |

* Total 11 groups (g1-g11) were made.

2.2 Antibiogram study

Antibiotic susceptibility of the selected isolates was checked following disc diffusion assay. Three antibiotics viz., erythromycin (20 µg/mL), lincomycin (100 µg/mL) and rifampicin (100 µg/mL) were used and dilutions were made. Test strains were spread on agar plates and discs of 6 mm containing 20 µL volume of above mentioned antibiotics were placed. The plates were incubated at 37°C for 24 hours. The zone of inhibition around the discs were measured in millimeter (mm) and expressed as mean ± SE. Criteria for resistance, susceptibility and intermediate resistance or susceptible were followed according to the Trub et al. Diameter of inhibition zones less than or equal to 13 mm = resistant; diameter 14-21 mm = intermediate susceptible; diameter greater than or equal to 22 mm = susceptible were considered.

2.3 Formation of multispecies biofilm groups

Multispecies biofilms of the test strains were formed by growing isolates in batch cultures by mixing equal volumes (OD<sub>590nm</sub> = 0.1) of 2, 3 and 5 bacterial isolates. Thus, total...
11 groups (g1-g11) were made (Table 1). Antibiofilm effect of A. dorsata and A. cerana honey samples was checked against all eleven multispecies biofilm groups. P. aeruginosa was set in all the combinations because of its strong biofilm formation ability and resistance against all tested antibiotics.

2.4 Congo red agar assay (CRA) for biofilm detection

Congo red assay medium was prepared following slight modifications of method by Liaqat et al. Congo red plates were streaked with test strains groups and incubated at 37°C for 24 hours. Following 24 hours incubation, black colonies on plates indicated biofilm production.

2.5 Determination of inhibitory effects of A. dorsata and A. cerana honey on biofilm formation

Various six concentrations (2, 5, 8, 11, 14 and 17% v/v) of honey samples were prepared and tested against all bacterial groups (g1-g11) using slight modifications of CV staining method by Liaqat et al. and the rest of the biofilm quantification was done following CV staining method. Bacterial inoculum of all test groups (g1-g11) were prepared in nutrient broth and optical density (OD₆₀₀) was adjusted to 0.1 (approximately 1 x 10⁸ CFU mL⁻¹). Then 50 µL of diluted culture was transferred to the test tubes containing 3 mL fresh LB-media along with 150 µL of diluted honey samples and incubated at 37°C. After 72 hours media was discarded and test tubes were washed thrice with autoclaved distilled water, air dried and stained with 1% CV for 15-20 minutes. The stain was discarded again and test tubes were washed twice to remove the excess strain. The attached CV stain was dissolved in 33% glacial acetic acid and OD₆₀₀ was measured using UV-visible spectrophotometer. Experiment was run in triplicates. Erythromycin (20 µg/mL) was used as positive control and experiment was run in triplicate.

2.6 Biofilm formation time kinetics of test strains

Following method by Liaqat et al., biofilm time kinetics study of test groups was performed at different time intervals of 24, 48, 72 and 96 hours at 37°C. The CV staining method was used to quantify the adherent cells. Briefly, the overnight cultures of eleven test groups (g1-g11) were prepared and the OD₆₀₀ was adjusted to 0.1 (approximately 1 x 10⁸ CFU mL⁻¹). The 50 µL of diluted culture was transferred to the test tubes containing 3 mL fresh LB-media and incubated for 24, 48, 72 and 96 hours at 37°C. The experiment was run in triplicates.

2.7 Pyocyanin production time kinetics of P. aeruginosa

Culture supernatant method by Vinckx et al. with slight modifications was followed for pyocyanin measurement after various intervals of time i.e., 24, 48, 72 and 96 hours. Briefly, the overnight cultures of P. aeruginosa were prepared and OD₅₉₀ was adjusted to 0.1 (approximately 1 x 10⁸ CFU mL⁻¹). The 50 µL of diluted culture was transferred to the test tubes containing 3 mL fresh LB-media and incubated for 24, 48, 72 and 96 hours at 37°C. Bacterial cells were removed and pyocyanin was extracted and mixed with chloroform. For re-extraction of pyocyanin, 1 mL of acidified water (0.2 mol / HCl) was used. OD₂₅₀ was measured. In order to obtain the concentration of pyocyanin as µg/mL, optical densities were multiplied by a factor 17.072. The experiment was run in triplicate.

2.8 Antibiofilm quantification of A. dorsata and A. cerana honey samples

Antibiofilm effects of both honey samples were measured following CV staining method. The overnight bacterial cultures of all test groups (g1-g11) were added into test tubes containing 3 mL fresh broth and 150 µL of honey sample (2 and 5%) was used as test compound. Erythromycin (20 µg/mL), 1.4 and 3.5% fructose + sucrose solution (equivalent to 70% of 2 and 5% honey) were used as controls. Test tubes were incubated for 24 hours at 37°C. After 24 hours, antibiofilm potential of honey was tested using CV staining method as described above (determination of MIC). The experiment was run in triplicate.

2.9 Anti-QS activity of A. cerana and A. dorsata honey samples

Pyocyanin is blue color secondary metabolite and is produced by P. aeruginosa. Culture supernatant method was used for the pyocyanin measurement with slight modifications. Briefly, the overnight cultures of P. aeruginosa were prepared and the OD₅₉₀ was adjusted to 0.1 (approximately 1 x 10⁸ CFU mL⁻¹). The 50 µL of diluted culture was transferred to the test tubes containing 3 mL fresh LB-media along with 2% and 5% honey concentrations and erythromycin as positive control. Bacterial cells were removed, pyocyanin was extracted after centrifugation at 5,000 rpm and rest of the procedure was followed according to the method mentioned in section (pyocyanin production time kinetics of P. aeruginosa).

2.10 Dispersal activity of A. cerana and A. dorsata honey samples

The method by Kavita et al. with slight modifications was used to determine the biofilm dispersal potential of both honey samples against 11 multispecies biofilm groups (g1-g11). The multispecies bacterial biofilm was established up to 72 hours as mentioned in section (biofilm formation time kinetics). 2% and 5% honey concentrations were added to treat biofilms for the next 24 hours. Test tubes were washed and 1% (w/v) crystal violet was used to stain the formed biofilm. Afterwards, 1 mL of 70% ethanol was added and using spectrophotometer, absorbance was measured at 590 nm. By using following formula, biofilm
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3 Results

3.1 Antibiotic study

It was observed that P. aeruginosa was highly significantly (p<0.01) resistant against all tested antibiotics (lincomycin, erythromycin and rifampicin) as no clear zones were observed. While other isolates, M. morganii, E. coli and K. pneumoniae were significantly (p<0.05) more susceptible to erythromycin compared to other two antibiotics (inhibition zones; ZI = 30 mm, 23.6 mm and 25 mm, respectively). Among all isolates, S. aureus showed significantly highest susceptibility to lincomycin (ZI = 17 mm). Among three antibiotics, erythromycin was found to be highly effective against most of test isolates hence used as positive control in subsequent study (Fig. 1S).

3.2 Congo red agar method (CRA)

Congo red agar assay results indicated strong biofilm ca-

![Graph 1](Image)

**Fig. 1** Inhibitory effects of A. dorsata and A. cerana honey on biofilm formation of eleven multispecies biofilm test groups (g1-g11). a) A. dorsata honey significantly (p<0.05) inhibited the biofilm formation in all groups (g1-g11) at 2% and 5% concentrations. Highly significant (p<0.01) biofilm inhibition was seen at 2% and 5% concentrations against g2-g4 and g8-g11. b) A. cerana significantly (p<0.05) inhibited the biofilm formation of all multispecies biofilm groups (g1-g11) at 2% and 5% concentrations. The data was analyzed by one way ANOVA followed by Post Hoc Tukey test using SPSS software and graphs were constructed using excel (2010).

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pability by all test groups (g1-g11) as appearance of intense black colonies on all plates (Fig. 2S).

3.3 Inhibitory effects of A. dorsata and A. cerana honey on biofilm formation

Among six tested concentrations (2, 5, 8, 11, 14 and 17%) of both honey samples obtained from honey bees, A. dorsata and A. cerana, it was observed that A. dorsata honey significantly \((p < 0.05)\) inhibited the biofilm formation in all groups (g1-g11) at 2% and 5% concentrations. Highly significant \((p < 0.01)\) biofilm inhibition was seen at 2% and 5% concentrations against g2-g4 and g8-g11. While, A. cerana significantly \((p < 0.05)\) inhibited the biofilm formation of in all multispecies biofilm groups (g1-g11) at 2% and 5% concentrations (Fig. 1).

3.4 Biofilm formation time kinetics

The multispecies biofilm formation time kinetics of eleven test groups (g1-g11) was monitored using CV staining method for 24, 48, 72, 96 hours of incubation periods at 37°C. All 11 multispecies biofilm test groups showed significantly \((p < 0.05)\) high biofilm formation at 72 hours of incubation, after that there was a decline in the biofilm texture (Fig. 3S).

3.5 Pyocyanin production time kinetics of P. aeruginosa

Time kinetics for pyocyanin production of P. aeruginosa was checked by incubating for 24, 48, 72, 96 hours at 37°C. Significant \((p < 0.05)\) production of pyocyanin by P. aeruginosa was observed after 96 hours incubation period (Fig. 4S).

3.6 Antibiofilm effect of A. dorsata and A. cerana honey samples

Antibiofilm study revealed that both honey samples possesses strong multispecies biofilm inhibition potentials compared to positive control (erythromycin). A. dorsata honey showed significant \((p < 0.05)\) biofilm inhibition at 2% against all tested groups of isolates. 2% and 5% concentrations showed almost same significant \((p < 0.05)\) biofilm inhibition against g1 and g5-g11. A. cerana honey significantly \((p < 0.05)\) inhibited the biofilm formation at 2% against all the groups (g1-g11) compared to erythromycin (positive control). Overall A. cerana honey was significantly \((p < 0.05)\) effective as antibiofilm agent (Fig. 2).

3.7 Anti-QS activity of A. dorsata and A. cerana honey samples

Both honey samples showed strong anti-QS potentials against P. aeruginosa by inhibiting the production of pyocyanin. Both concentrations (2% and 5%) of A. dorsata honey significantly \((p < 0.05)\) inhibited the production of pyocyanin as compared to positive control (erythromycin). A. cerana honey was significantly \((p < 0.05)\) effective at 5% concentration as compared to 2% concentration and positive control (erythromycin). Comparison of both honey samples showed that A. cerana honey was more significantly \((p < 0.01)\) effective at high concentration (5%) while A. dorsata honey showed significant \((p < 0.05)\) results at both concentration (2% and 5%) (Fig. 3).

3.8 Biofilm dispersal activity of A. dorsata and A. cerana honey samples

A. dorsata honey strongly dispersed the multispecies biofilm. A. dorsata honey significantly \((p < 0.05)\) dispersed the multispecies biofilm of g2-g4 and g7-g11 at 5% concentration while there was no significant difference between 2% and 5% concentrations against g1, g5 and g6. A. cerana honey showed significant \((p < 0.05)\) biofilm dispersal at 5% concentrations against g2-g5 and g7-g11. In g6, both honey concentrations (2% and 5%) were significantly \((p < 0.05)\) effective compared to positive control. Best biofilm dispersal was exhibited at 5% concentration in most multispecies biofilm groups either by A. dorsata or A. cerana honey samples, however 2% concentration was also effective compared to the erythromycin (Fig. 4).

4 Discussion

Nearly, 99% bacteria on the earth live in communities known as biofilms\(^{20-22, 27}\). Bacteria attach themselves to a surface through an extracellular polymeric substance (EPS) to form biofilm\(^{26}\). Bacteria have a well-developed mechanism of communication known as quorum sensing (QS) which helps in biofilm formation\(^{27}\) e.g., pyocyanin production in P. aeruginosa\(^{29}\). Once a biofilm becomes mature, bacteria start to disperse from the biofilm\(^{30}\). Looking for a novel method to eradicate or inhibit biofilm growth has been an active area of research. This study used in vitro approach to assess antibiofilm, anti-QS and dispersal potential of two honey samples obtained bees i.e., A. cerana and A. dorsata against multispecies biofilm formed by five obese patient’s isolates.

Five bacterial isolates i.e., P. aeruginosa, E. coli, S. aureus, M. morganii, and K. pneumoniae were used to check for antibiogram study. All isolates were obtained from fecal samples of obese patients and are in agreement with data obtained by authors, who reported the presence of S. aureus, P. aeruginosa and M. morganii in the fecal samples of the persons suffering from obesity related conditions\(^{27, 28}\). Antibiotic susceptibility test was performed to check the antibiotic resistance pattern of identified isolates. Highly significant \((p < 0.01)\) resistance to antibiotics (erythromycin, lincomycin and rifampicin) was noticed in case of P. aeruginosa. Corroborate with findings by Murray et al.\(^{20}\), who reported that P. aeruginosa was resistant towards most of the antibiotics (due to its high pro-
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The increased bacterial resistance observed in this study indicated over and miss-use of the antibiotics among visiting obese patients. Using congo red agar method, appearance of intense

Fig. 2 Anti-biofilm effects of *A. dorsata* and *A. cerana* honey samples against multispecies biofilm of test groups. a) *A. dorsata* honey showed significant \((p<0.05)\) biofilm inhibition at 2% against all tested groups of isolates. 2% and 5% concentrations showed almost same significant \((p<0.05)\) biofilm inhibition against g1, g5-g11. b) *A. cerana* honey significantly \((p<0.05)\) inhibited the biofilm formation at 2% against all the groups \((g1-g11)\) compared to erythromycin (positive control). The data was analyzed by one way ANOVA followed by Post Hoc Tukey test using SPSS software and graph was constructed using excel (2010).
black colonies by all eleven multispecies test groups on media plates indicated their strong biofilm forming potential. These findings are consistent with data obtained by Liaqat et al.[19]. Biofilm time kinetics study of all multispecies test groups was performed over a time span of 96 hours. Significant ($p < 0.05$) biofilm formation was seen after 72 hours of incubation. Decline in biofilm formation after 72 hours might be due to their growth in batch cultures where nutrient deprivation and waste accumulation lead to decreased biofilm growth and its dispersal.[10]

We next determined inhibitory effects of $A.~dorsata$ and $A.~cerana$ honey on biofilm formation of all multispecies after 72 hours of incubation. Decline in biofilm formation after 72 hours might be due to their growth in batch cultures where nutrient deprivation and waste accumulation lead to decreased biofilm growth and its dispersal.[10]

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biofilm 
groups (g1-g11). It was observed that both A. dorsata and A. cerana honey at 2% and 5% concentrations significantly (p < 0.05) inhibited the multispecies biofilm formation. Previously, McLoone et al.\(^{32}\) determined the minimum inhibitory values of honey against S. aureus associated with skin wound related infection and found 2.5% as effective MIC. Similarly a study by Carnwath et al.\(^{33}\) checked antimicrobial activities of 10 honey samples against S. aureus, E. coli and P. aeruginosa using serial dilution method to produce final honey concentrations (MIC) ranging from 2% to 16%. Results demonstrated that all honey samples inhibited the growth of all the tested microorganisms, with inhibitory concentrations ranging from <2 to 6%.

Our next aim was to determine the antibiofilm effect of honey samples (sources: A. dorsata and A. cerana). It was observed that both honey samples had significant (p < 0.05) biofilm inhibitory effects against multispecies biofilm at both concentrations (2% and 5%). Though, highly significant (p < 0.01) biofilm inhibition was observed at 2% concentration in both honey samples obtained from A. dorsata and A. cerana. This might be due to the presence of high amount of hydrogen peroxide whose production is associated with honey dilution. The greater the honey is diluted, greater would its production of hydrogen peroxide\(^{34}\) and strongly it will reduce biofilm formation. However, our results are contradictory to findings by Sharahi et al.\(^{35}\), who reported that honey possessed more antibiofilm potentials at higher concentrations (25%).

Quorum sensing (QS) is important for biofilm maturation and stabilization in various pathogenic bacteria\(^{36, 37}\) (P. aeruginosa and S. aureus). Pseudomonas aeruginosa produces pyocyanin (a signaling molecule) to communicate and to initiate biofilm formation\(^{38}\). The maximum time required for pyocyanin production was between 24-48 hours incubation\(^{39}\). However, current study showed that P. aeruginosa significantly (p < 0.05) produced pyocyanin after 96 hours incubation. It was observed that 2 and 5% concentrations of A. dorsata and A. cerana honey possess significant (p < 0.05) anti-QS activities against P. aeruginosa, as noted by inhibition of pyocyanin production in this isolate. This could be explained by the fact that honey inhibits the genes (las and rhl) expression responsible for pyocyanin production in P. aeruginosa\(^{40}\). Likewise, pyocyanin inhibition might be due to inhibition of siderophore genes expression. Previously, Kronda et al.\(^{41}\) also reported that manuka honey inhibits the siderophore genes expression in P. aeruginosa. Furthermore, high sugar content of honey also plays a major role in QS inhibition by making resistance in binding pyocyanin to its cytoplasmic receptors\(^{42}\).

Honey is also effective to disperse mature biofilm and led to release of planktonic cells\(^{43}\). Hence, our last aim was to determine the biofilm dispersal potential of two honey samples (obtained from A. dorsata and A. cerana). It was observed that both A. dorsata and A. cerana honey samples were effective in dispersing multispecies biofilm. Both honey samples exhibited significant (p < 0.05) biofilm dispersal at 2 and 5% concentrations but 5% concentration was most effective as it caused highly significant (p < 0.01) multispecies biofilm dispersal of all test isolates groups. The biofilm dispersal effect of honey samples might be due to the presence of high osmotic pressure which induces the bacteria to degrade their EPS matrix, disperse and release planktonic cells. Similar results were reported by various other authors in past who described that higher concentration of honey are effective to disperse mature bacterial biofilm\(^{33, 34, 40}\).

Quality of honey is determined by its physiochemical parameters like color, pH, hydroxymethylfurfural (HMF), electrical conductivity (EC), moisture content, diastase index, Total antioxidant capacity and bioactive compounds determine the antioxidant and bioactive potential of the honey samples. Though, in Pakistan honey is consumed in many foods and herbal treatment but little information exists on physicochemical and antioxidant properties of honey from Asian bees, A. cerana and A. dorsata. For example, Kousar & Qamer\(^{44}\) observed free acidity values of 44.459, 43.169, 43.149 and 48.9 meq/kg for Nepal’s A. dorsata honey. Joshi et al.\(^{45}\) also reported that honey sampled from A. dorsata has high moisture content compared to A. cerana. Low moisture content contributes to great shelf life and best quality of honey as well as antimicrobial resistance. Kousar & Qamer\(^{46}\), who reported EC values of 0.06, 0.61, 0.48 and 0.22 mS/cm for A. dorsata honeys from four Nepal forests. Though, Joshi et al.\(^{47}\) reported 0.96 mS/cm EC values for A. dorsata honey. In ongoing study in our Lab, physicochemical and antioxidant potential of both honey samples have been studied proving A. cerana honey having higher antioxidant potential with higher phenolic, flavonoid content, ferric reducing power, and color intensity compared to A. dorsata honey.

5 Conclusion
In conclusion, the results of the current study showed that despite the lot of number of antibiotics, natural and primitive compounds, honey possess great medical importance to treat various biofilm related infections. This study is first to test Pakistani honey bees, A. cerana and A. dorsata honey samples as anti biofilm, anti QS and biofilm dispersal agents against all multispecies antibiotic resistant bacterial groups (g1-g11). Taken together, the study findings support use of honey as effective remedy against biofilm related infections such as wound healing, stomach ulcer and biofilm related problems in medical instruments *e.g.* to eradicate biofilm from urinary tract and respiratory
tract catheters. Ongoing study in lab will provide effective honey potential as adjuvants to antibiotics against multi drug resistant isolates.

Conflict of Interests
On behalf of all authors, the corresponding author states that there is no conflict of interest.

Supporting Information
This material is available free of charge via the Internet at doi: 10.5650/jos.ess21199

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