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Research Article

Biochemical Characterization of Bacterial Pathogens from Corneal Eye Infections and their Control via Chemotherapy, Phytotherapy and Apitherapy

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Abstract | The present study was conducted to isolate and characterize pathogenic bacteria from eye infections. The samples were taken from corneal eye infections. The collection of samples was done from "Layton Rehmat Eye Hospital, Township Lahore". Isolated bacterial pathogens were identified on the basis of morphological and various biochemical tests. Morphologcal tests included different staining procedures includng Gram's staining and Endospore staining. Identification of pathogenic bacterial isolates was confirmed by growing on selective medium. Antibacterial activity of different compounds was observed against the pathogenic bacterial isolates. Minimum Inhibitory Concentration (MIC) test of different kinds of honey was performed against the isolated bacterial pathogens. The antibacterial activity of leaf extract of *Azadirachta indica* (Neem Plant) was also checked against the pathogenic bacterial isolates. The bacterial pathogens isolated from eye infections were identified as *Staphylococcus sp., Streptococcus sp., Neisseria sp., and Bacillus sp.* These bacteria commonly caused corneal infections. All the pathogenic bacterial isolates showed sensitivity against all the compounds except *Streptococcus sp.* (BS1) and it showed resistance only against 3-Nitro-aniline (SAHC-11). Bacterial isolate *Staphylococcus sp.* (BS4c) showed maximum sensitivity against methyl-amine (SAH-5) with zone of inhibition of about 16mm in diameter while the bacterial isolate *Staphylococcus sp.* (BS6) showed minimum sensitivity against Propyl-diamine (SAH-16) and Ethylamine cinnamaldehyde derivative (SAHC-7) compounds with 1mm diameter of zone of inhibition under optimum growth conditions *i.e.*, optimum pH and temperature. Antibacterial activity of leaf extract of *Azadirachta indica* (Neem Plant) was also checked. Methanol is used as a standard against which all the pathogenic bacterial isolates showed resistance. The minimum sensitivity showed by *Staphylococcus sp.* (BS4C) with the zone of inhibition of about 2mm in diameter while the maximum sensitivity showed by *Staphylococcus* sp. (BS6) with zone of inhibition of 8mm. MIC test of four different kinds of honey was also performed. Different honeys showed different results. For all the isolated bacterial pathogens, Pak honey inhibited the growth at 1ml concentration, Local honey at 3ml concentration, Marhaba's honey at 4ml concentration, and Young's honey inhibited the growth at the concentration of 5ml.

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1. Introduction

Eye infections such as Conjunctivitis (pinkish or red eye), blepharitis, Stye, Cellulitis, Keratitis, Corneal Ulcer and trachoma are spreading commonly in the global world. Eye infections occur when harmful microorganisms penetrate in any part of the eyeball or surrounding area of an eye. The most common opportunistic pathogens *Staphylococcus aureus,Staphylococcusepidermidis* and *Pseudomonas aeruginosa* are responsible for a broad spectrum of eye infections in children. The epidemiology of ocular infections is important because it provides information about the behaviour of different microorganisms in the eye. These data help to understand the cause and frequency of infections, as well as to identify resistant strains that can infect the eye (Verdayes *et al*., 2006).

Ocular bacterial infections are universally treated with antibiotics, which can eliminate the organism but cannot reverse the damage caused by bacterial products already present. The three very common causes of bacterial keratitis-*Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pneumoniae*, all produce proteins that directly or indirectly cause damage to the cornea that can result in reduced vision despite antibiotic treatment (Marquart and O'Callaghan, 2013). Some antibiotics *viz*., Gentamycin, Erythromycin, Tetracycline, and Ampicillin are commonly used for ocular infections. Resistance appears to be even greater in ocular infections caused by Gram-positive organisms. Adverse drug effects such as punctuate epithelial keratitis have been encountered in some patients (Kwapong *et al*., 2013). Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey (Mandal, 2011).

The use of traditional medicine to treat infection has been practiced since the origin of mankind, and honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments. The natural honey has found broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. The *Leptospermum scoparium* honey has been reported to have an inhibitory effect on around 60 species of bacteria (Mandal, 2011). The Difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. Herbal medicine is becoming increasingly popular in both developing and developed countries . Man objectves of study were;

1. to isolate and screen pathogenic bacteria
2. to study effect of different antibiotics
3. to study antibacteral effect of honey; synthetic compounds and neem

2. Materials and Methods

2.1 Sample collection

Samples of corneal eye infections were obtained using sterilized culture sticks from Layton Rehmat eye hospital, Township Lahore, Pakistan. These samples were brought to the laboratory and later spread on nutrient agar plates (Oxide CMOO3).

2.2 Isolation of microbes

Nutrient agar was used as a primary growth medium. The autoclaved medium was poured into the Petri plates in the laminar air cabinet and then allowed to solidify. After solidification, spreading of samples was done and incubated at 37°C for overnight. The bacterial colonies were appeared and then these colonies were picked and streaked on another nutrient agar plates and incubated again at 37°C for overnight. Colonies were picked, cultured using nutrient broth (Oxoid; CM1) and glycerol stocks (60%) were made to store at -20 °C.

2.3 Morphological and biochemical characterization of bacterial strains

Selected bacterial strains were initially characterized by observing cell morphology, Gram's and endospore, staining protocols. A thorough biochemical investigation was carried out *via* various biochemical tests including catalase test, citrate test, urease test, H2S test, carbohydrate fermentation, litmus paper test, MR-VP test etc, and by using differential and selective media.

2.4 Plant collection and its extraction

Arial part (leaf) of *Azadirachta indica* (Neem) was collected. Methanolic extracts of plants were prepared by dissolving 60 g of plant in 360 ml methanol then placed in shaker for 24 hrs. The extracts were filtered and concentrated on rotary vacuum evaporator (R/201B/II) for 3 to 4 hrs. The concentrated extracts were finally soaked in methanol in the ratio 1:6 and used for antibacterial activity.

2.5 Antibiogram assay by agar well diffusion method

To assess antibiotic sensitivity test, the agar well diffusion method was used (Figure 1). Various antibiotics were used against bacterial strains. Nutrient agar and Nutrient Broth Media were used for bacterial culture. The overnight prepared culture was mixed with freshly prepared nutrient agar medium (NAM) at 45 °C and was poured into the sterilized Petri dishes. All Petri dishes were kept at room temperature in laminar flow for solidification. After solidification, three wells were made on each agar plate with a flamed, cooled cylinder of 5 mm diameter and sterile needle was used for the removal of agar plugs. The volume (50 µl) of antibiotics was separately dispensed into each well of prepared plates. Methanol was used as negative control. Before each experiment, the optimal density (OD) of bacterial growth 107 colony forming units (cfu)/ml was measured through spectrophotometer at 600 nm (Seeley *et al.*, 1981). The inhibitory effect was recorded in millimetre (mm) by measuring the diameter of the zone of growth inhibition after 24–48 h. The growth inhibition was recorded as (0) for no sensitivity, (1–10) for low sensitivity, (11–20) for moderate, and (21-above) for high sensitivity (Figures 1 and 2).

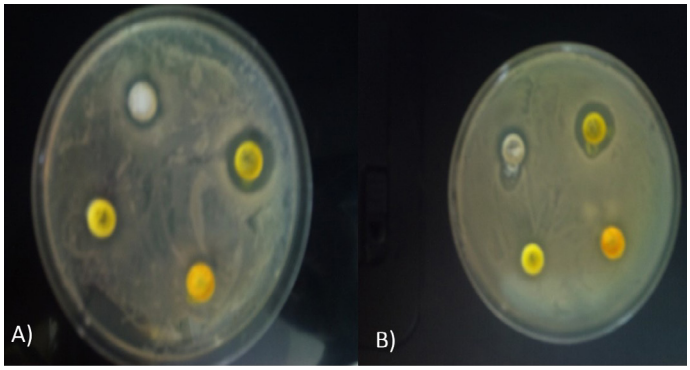

Figure 1: (A and B). Antibacterial activity of compounds against pathogenic bacterial isolates.

2.6 MICs of different honeys on pathogenic bacterial strains

Four types of honeys such as Young's Honey, Marhaba's Honey, Local Honey and Pak- Honey were used. Minimum inhibitory concentration was measured using agar well diffusion method as discussed above. Honey concentrations were prepared as follows: 25 ml of nutrient agar was mixed with different volumes (1-5 ml) of honey samples, mixed well and used against bacterial strains.

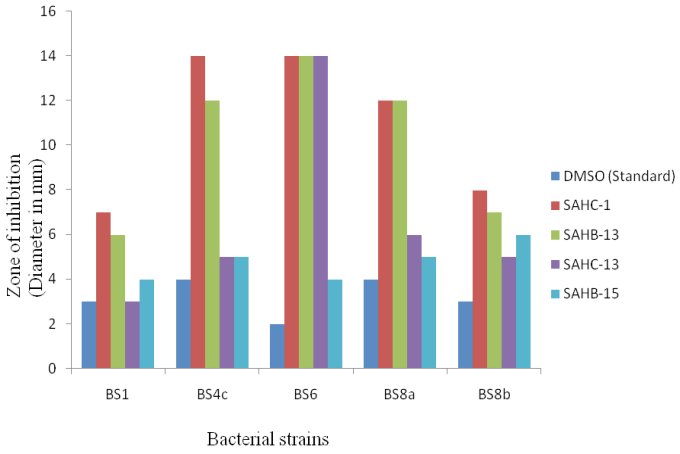

Figure 2: Antibacterial activity of compounds against different pathogenic bacterial isolates; *BS1: Streptococcus sp.; BS4c: Staphylococcus sp.; BS6: Staphylococcus sp.; BS8a: Neisseria sp.; BS8b: Bacillus sp.; DMSO: Dimethyl- sulphoxide; SAHC-1: Aniline; SAHB-13: 4- amino benzoic acid; SAHC-13: Amino benzoic acid; SAHB-15: 22- amino thiophenol.*

2.7 Antibacterial activity of azadirachta indica

Antibacterial activity of medicinal plant was measured using agar well diffusion method as discussed above .

3. Results

In the present study, different bacteria were isolated from eye infection samples. The bacteria isolated from eye infections were later identified as *Streptococcus sp.*, *Staphylococcus sp.*, *Neisseria sp.*, and *Bacillus sp.*

3.1 Bacterial strain 1 (BS1)

The bacterial strain 1 (BS1) was identified as *Streptococcus* sp. (Table 1). It is gram-positive coccus bacteria. Every human once in life must get infected with streptococcus sp. It gives positive result only for MR-VP, Litmus and glucose fermentation tests while Catalase, Urease, Citrate, H2S production, Sucrose and Lactose fermentation tests were negative (Table 1). It shows no motility and no endospores were formed. *Streptococcus sp.* showed resistance only against 3-nitro-aniline and also showed maximum sensitivity against aniline with zone of inhibition 12 mm (Table 2).

Table 1: Morphological and biochemical characterization of bacteria isolated from eye infection samples.

	BS1	BS4C	BS6	BS8a	BS8b
Gram's staining	+	+	+	-	+
Endospore staining	-	-	-	-	+
Shape	Cocci	Cocci	Cocci	Cocci	Bacilli
Citrate	-	-	-	-	-
MR-VP test	+	+	+	+	+
Urease	-	-	-	-	-
Catalase	-	+	+	+	+
H_2S	-	-	-	-	-
Glucose	+	+	+	+	+
Sucrose	-	-	-	-	-
Litmus	Curd formation+- Clear Zone (Stormy fermentation)	Curd formation + Clear Zone (Stormy fermentation)	Curd formation + Clear Zone (Stormy fermentation)	Curd formation + Clear Zone (Stormy fermentation)	Curd formation+ Clear Zone (Stormy fermentation)
MacCkonkey agar test	-	-	-	-	-
Blood agar test	α hemolysis	α hemolysis	α hemolysis	α hemolysis	α hemolysis

MIC test was also performed for all bacterial strains by using four different honeys. For BS1, MIC of young's honey was 5 ml, Marhaba's honey was 4 ml, Local honey was 3 ml, and Pak honey was 1 ml (Table 3). Moreover, the antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract was also observed against all the bacterial strains. BS1 showed resistance against methanol (standard). It showed sensitivity against the extract with zone of inhibition 4 mm (Figure 1 and Table 4).

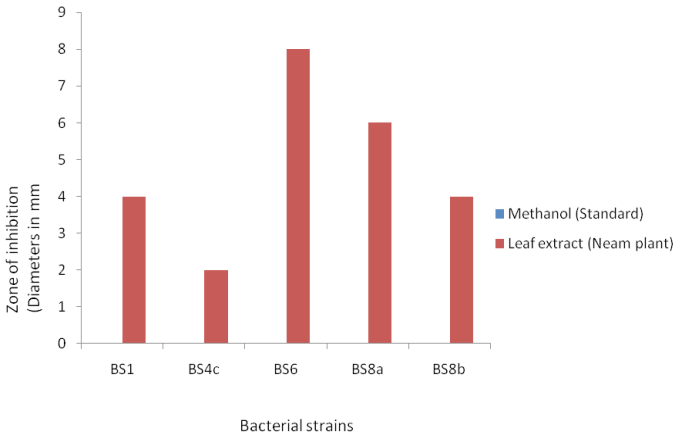

Figure 3: Antibacterial activity of *Azadirachta indica* (neem plant) leaf extract against Pathogenic bacterial isolates. *BS1: Streptococcus sp.; BS4c: Staphylococcus sp.; BS6: Staphylococcus sp.; BS8a: Neisseria sp.*

3.2 Bacterial strain 2 (BS4c)

This bacterial strain was identified as *Staphylococcus* sp. It is also gram-positive coccus bacteria. It is a common disease-causing bacteria. It gives a positive result for catalase, MR-VP, Litmus milk, glucose and lactose fermentation test. The antibacterial resistance of strain BS4C was observed. *Staphylococcus* sp. showed no resistance against any antibiotic but showed minimum sensitivity against o-toludinebenzaldehyde derivative with zone of inhibition 2mm. This bacterium showed maximum sensitivity against methyl-amine and zone of inhibition is 16mm. MIC test was also performed for all bacterial strains by using four different honeys. For B4c, MIC of young's honey was 5ml, Marhaba's honey was 4ml, Local honey was 3ml, and Pak honey was 1ml. Moreover, the antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract was also observed against all the bacterial strains. BS4c showed resistance against methanol (standard). It showed minimum sensitivity against the extract with zone of inhibition 2mm.

3.3 Bacterial strain 3 (BS6)

This bacterial strain was identified as *Staphylococcus sp.* It is gram-positive coccus bacteria. Every human once in life must get infected with the staphylococcus causing minor or major infection. Its catalase, MR-VP, litmus milk, glucose and sucrose fermentation tests were positive. It showed no motility and no formation of endospores. *Staphylococcus sp.* showed minimum sensitivity against ethyl-amine cinnamaldehyde derivative and propyl-diamine with zone of inhibition 1mm and showed maximum sensitivity against 2-amino thiophenol with zone of inhibition 15 mm. MIC test was also performed for all bacterial strains by using four different honeys. For BS6, MIC

Table 2: sensitivity of antibiotics against bacteria isolated from eye infection samples.

Bacteria → Antibiotics ↓	Zone of inhibition (mm) M±SD				
	BS1	BS4C	BS6	BS8a	BS8b
DMSO (Standard)	(3.5±0.41)	(4.0±0.35)	(2.5±0.41)	(4.0±0.35)	(3.0±0.35)
SAHC-I (123mg/ ml)	(7.0±0.35)	(14.0±0.35)	(14.0±0.36)	(12.0±0.33)	(8.0±0.25)
SAHB-13 (107mg/ ml)	(6.0±0.5)	(12.0±0.05)	(14.0±0.15)	(12.0±0.5)	(7.0±0.5)
SAHC-13 (129mg/ ml)	(3.0±0.37)	(5.0±0.36)	(14.0±0.01)	(6.5±0.41)	(4.0±0.05)
SAHB-15 (117mg/ ml)	(4.0±0.25)	(5.0±0.35)	(4.0±0.36)	(5.0±0.33)	(6.0±0.27)
SAHC-10 (100mg/ ml)	(3.3±0.05)	(6.2±0.07)	(7.0±0.15)	(4.0±0.17)	(6.0±0.25)
SAHC-15 (176mg/ ml)	(7.3±0.05)	(10.0±0.5)	(15.0±0.35)	(12.0±0.36)	(8.5±0.41)
SAHC-9 (17mg/ ml)	(3.0±0.25)	(5.0±0.05)	(6.0±0.01)	(5.0±0.27)	(4.0±0.5)
SAHC-12 (52mg/ ml)	(4.0±0.1)	(4.0±0.25)	(3.0±0.37)	(4.0±0.1)	(4.0±0.15)
SAHC-14 (6.7mg/ ml)	(6.0±0.3)	(4.0±0.41)	(2.0±0.35)	(5.0±0.03)	(7.0±0.25)
SAH-16 (13.8mg/ ml)	(4.0±0.1)	(6.0±0.15)	(1.0±0.15)	(7.0±0.05)	(5.0±0.1)
SAHB-1 (150mg/ ml)	(2.0±0.35)	(4.0±0.33)	(4.0±0.25)	(4.0±0.1)	(3.0±0.5)
SAHC-11 (50mg/ ml)	(0.0±0.0)	(6.0±0.15)	(6.0±0.1)	(5.0±0.32)	(2.0±0.25)
SAHB-10 (110 mg/ ml)	(8.0±0.05)	(10.0±0.5)	(10.0±0.35)	(12.0±0.30)	(7.0±0.25)
SAHB-8 (62.5mg/ ml)	(5.0±0.05)	(8.0±0.25)	(8.0±0.37)	(9.0±0.1)	(6.5±0.41)
SAHC-17 (128mg/ ml)	(4.0±0.25)	(14.0±0.01)	(14.0±0.2)	(13.0±0.05)	(5.0±0.26)
SAHC-4 (100mg/ ml)	(4.0±0.3)	(7.0±0.16)	(3.0±0.25)	(6.0±0.5)	(3.0±0.05)
SAHS-3 (100mg/ ml)	(2.0±0.37)	(4.0±0.38)	(3.0±0.24)	(5.0±0.17)	(2.0±0.26)
SAHB-2 (100mg/ ml)	(2.0±0.21)	(2.0±0.33)	(2.0±0.25)	(3.0±0.36)	(2.0±0.1)
SAHC-7 (100mg/ ml)	(3.0±0.35)	(6.0±0.1)	(1.0±0.05)	(2.0±0.26)	(4.0±0.5)
SAHB-4 (100mg/ ml)	(3.3±0.27)	(11.0±0.15)	(2.0±0.05)	(10.0±0.15)	(4.0±0.05)
SAHV-(100mg/ ml)	(4.0±0.35)	(8.0±0.37)	(2.0±0.25)	(5.0±0.36)	(7.0±0.3)
SAHS-2 (100mg/ ml)	(2.0±0.05)	(10.0±0.25)	(3.66±0.07)	(3.0±0.35)	(8.0±0.26)
SAHS-4 (100mg/ ml)	(4.0±0.15)	(6.0±0.1)	(4.0±0.27)	(5.6±0.33)	(6.0±0.36)
SAHB-3 (100mg/ ml)	(5.0±0.15)	(9.0±0.35)	(6.0±0.25)	(7.0±0.05)	(6.0±0.15)
SAH-5 (100mg/ ml)	(8.0±0.05)	(16.0±0.05)	(9.0±0.15)	(14.0±0.15)	(7.0±0.25)
SAHS-7 (100mg/ ml)	(3.0±0.05)	(14.0±0.05)	(4.0±0.35)	(12.0±0.27)	(2.0±0.1)
SAH-1 (100mg/ ml)	(12.0±0.15)	(15.0±0.5)	(8.5±0.43)	(10.0±0.27)	(12.0±0.05)
SAHV-7 (100mg/ ml)	(6.0±3.33)	(6.0±0.24)	(4.0±0.32)	(5.0±0.15)	(4.0±0.1)
SAHB-6 (100mg/ ml)	(6.0±0.37)	(10.0±0.04)	(4.0±0.26)	(8.0±0.33)	(5.0±0.37)
SAHV-2 (100mg/ ml)	(3.0±0.35)	(6.0±0.26)	(3.0±0.38)	(5.0±0.40)	(4.0±0.17)
SAHB-7 (100mg/ ml)	(2.0±0.01)	(8.0±0.05)	(4.0±0.25)	(7.0±0.35)	(3.0±0.36)
SAHS-8 (100mg/ ml)	(5.0±0.33)	(7.0±0.15)	(14.0±0.25)	(8.0±0.27)	(4.0±0.32)
SAHC-3 (100mg/ ml)	(2.0±0.35)	(5.0±0.18)	(3.0±0.36)	(5.0±0.1)	(3.0±0.05)

DMSO: Dimethyl- sulphoxide; SAHC-1: Aniline; SAHB-13: 4- amino benzoic acid; SAHC-13: Amino benzoic acid; SAHB-15: 22- amino thiophenol; SAHC-10: 2- nitro aniline; SAHC-15: 2-amino thiophenol; SAHC-9: cyclo- hexyl amine; SAHC-12: 3- amino benzoic acid; SAHC-14: 4- chloro- aniline; SAH-16: propyl- diamine; SAHB-1: Aniline; SAHC-11: 3- nitro- aniline; SAHB-10: 2- nitro-aniline; SAHB-8: Benzyl- amine; SAHC-17: ortho-amino phenol; SAHC-4: p-toluidine cinnamaldehyde derivative; SAHS-3: m-toluidine salisylaldehyde derivative; SAHB-2: o-toluidine benzaldehyde derivative; SAHC-7: ethyl amine cinnamaldehyde derivative; SAHB-4: p-toluidine benzaldehyde derivative; SAHV-4: p-toluidine vanillin derivative; SAHS-2: o-toluidine salisylaldehyde derivative; SAHS-4: p-toluidine salisylaldehyde derivative; SAHV-3: m-toluidine vanillin derivative; SAH-5: methyl amine; SAHS-7: ethyl amine salisylaldehyde derivative; SAH-1: aniline; SAHV7: ethyl amine vanillin derivative; SAHB-6: propyl amine benzaldehyde derivative; SAHV-2: o-toluidine vanillin derivative; SAHB-7: ethyl amine benzaldehyde derivative; SAHS-8: benzylaminesalisylaldehyde derivative; SAHC-3: m-toluidine cinnamaldehyde derivative.

Table 3: Sensitivity of MICs of various honeys against bacteria isolated from eye infection samples.

Young's honey (ml/25 ml agar)					
Bacteria↓	**1**	**2**	**3**	**4**	**5**
BS1	High growth	High growth	Slight growth	Very slight growth	No growth
BS4C	High growth	High growth	Slight growth	Very slight growth	No growth
BS6	High growth	High growth	Slight growth	Very slight growth	No growth
BS8a	High growth	High growth	Slight growth	Very slight growth	No growth
BS8b	High growth	High growth	Slight growth	Very slight growth	No growth
Marhaba's honey (ml/25 ml agar)					
Bacteria↓	**1**	**2**	**3**	**4**	**5**
BS1	High growth	Slight growth	Very slight growth	No growth	No growth
BS4C	High growth	Slight growth	Very slight growth	No growth	No growth
BS6	High growth	Slight growth	Very slight growth	No growth	No growth
BS8a	High growth	Slight growth	Very slight growth	No growth	No growth
BS8b	High growth	Slight growth	Very slight growth	No growth	No growth
Local honey (ml/25 ml agar)					
Bacteria↓	**1**	**2**	**3**	**4**	**5**
BS1	Slight growth	Very slight growth	No growth	No growth	No growth
BS4C	Slight growth	Very slight growth	No growth	No growth	No growth
BS6	Slight growth	Very slight growth	No growth	No growth	No growth
BS8a	Slight growth	Very slight growth	No growth	No growth	No growth
BS8b	Slight growth	Very slight growth	No growth	No growth	No growth
Pak honey (ml/25 ml agar)					
Bacteria↓	**1**	**2**	**3**	**4**	**5**
BS1	No growth	No growth	No growth	No growth	No growth
BS4C	No growth	No growth	No growth	No growth	No growth
BS6	No growth	No growth	No growth	No growth	No growth
BS8a	No growth	No growth	No growth	No growth	No growth
BS8b	No growth	No growth	No growth	No growth	No growth

Table 4: Sensitivity of *Azadirachtaindica* leaf extracts against bacteria isolated from eye infection samples.

Extract → Bacteria ↓	**Zone of inhibition (mm) M±SD**	
	Methanol	**Leaf extract**
BS1	(0.0±0.0)	(4.0±0.35)
BS4C	(0.0±0.0)	(2.0±0.76)
BS6	(0.0±0.0)	(8.0±0.76)
BS8a	(0.0±0.0)	(6.0±0.35)
BS8b	(0.0±0.0)	(4.0±0.35)

of young's honey was 5ml, Marhaba's honey was 4ml, Local honey was 3ml, and Pak honey was 1ml. Furthermore, the antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract was also observed against all the bacterial strains. *Staphylococcus sp.* showed resistance only against methanol (standard) and showed maximum sensitivity against the extract with zone of inhibition 8mm in diameter.

3.4 Bacterial strain 4 (BS8a)

This bacterial strain was identified as *Neisseria sp.* It is gram negative coccus bacterium. It gives positive result for Catalase, MR-VP, litmus milk, glucose, and lactose fermentation tests. It shows no H_2S production, citrate, urease, and Sucrose fermentation tests. The antibacterial resistance of the strain BS8a was observed. *Niesseria* sp. showed maximum sensitivity against methyl-amine with zone of inhibition 14mm and showed minimum sensitivity against ethyl-amine cinnamaldehyde derivative with zone of inhibition 2mm. MIC test was also performed for all bacterial strains by using four different honeys. For BS8a, MIC of young's honey was 5ml, Marhaba's honey was 4ml, Local honey was 3ml, and Pak honey was 1ml. Furthermore, the antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract was also

observed against all the bacterial strains. *Neisseria* sp. showed resistance only against methanol (standard) and showed sensitivity against the extract with zone of inhibition 6mm in diameter.

3.5 Bacterial strain5 (BS8b)

This bacterial strain was identified as *Bacillus* sp. It is gram positive bacillus bacteria. Its Catalase, MR-VP, litmus milk, glucose, and lactose fermentation tests were positive. It shows no motility but give endospores positive test. The tests which were negative include urease, citrate H_2S production, and sucrose fermentation test. *Bacillus sp.* showed maximum sensitivity against aniline with zone of inhibition 12mm and showed minimum resistance against ethyl-amine salisylaldehyde derivative, o-toludinebenzaldehyde, m-toluidine salisylaldehyde derivative with zone of inhibition 2mm. MIC test was also performed for all bacterial strains by using four different honeys. For BS8b, MIC of young's honey was 5ml, Marhaba's honey was 4ml, Local honey was 3ml, and Pak honey was 1ml. Furthermore, the antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract was also observed against all the bacterial strains. *Bacillus sp.* showed resistance only against methanol (standard) and showed sensitivity against the extract with zone of inhibition 4mm in diameter .

4. Discussion

Honey is a by-product of flower nectar and the upper aero-digestive tract of the honey bee, which is concentrated through a dehydration process inside the bee hive. Honey has a very complex chemical composition that varies depending on the botanical source. It has been used both as food and medicine since ancient times. Human use of honey is traced to some 8000 years ago as depicted by Stone Age paintings. In addition to important role of natural honey in the traditional medicine, during the past few decades, it was subjected to laboratory and clinical investigations by several research groups and it has found a place in modern medicine. Honey has been reported to have an inhibitory effect on around 60 species of bacteria, some species of fungi and viruses. Antioxidant capacity of honey is important in many disease conditions and is due to a wide range of compounds including phenolics, peptides, organic acids, enzymes, and Maillard reaction products. Honey has also been used in some gastrointestinal, cardiovascular, inflammatory and neoplastic states. This review covers the composition, physico-chemical properties and the most important uses of natural honey in human diseases (Oskouei *et al.*, 2013).

To identify the incidence of infectious keratitis in a population based in southern England. A retrospective review between January 1997 and December 2003 and a prospective study between January and December 2006 were undertaken at the eye casualty department of Queen Alexandra Hospital (QAH), Portsmouth, UK to identify the incidence of infectious keratitis. QAH is a tertiary teaching hospital that serves Portsmouth and the catchment area of the county of Hampshire with an average population of 489,391 in the 7 year period of the retrospective study and 499100 in the one year prospective study. Infectious keratitis occurred in 1,786 patients in the retrospective study with an average of 255 patients per year and in 201 patients in the one year prospective study. The annual incidence of infectious keratitis was 52.1 and 40.3 per 100,000 persons in the retrospective and prospective studies respectively. The rate of bacterial and viral ulcers was lower in the prospective study than the average of either ulcer type in the retrospective study. A significant trend over time was found in the retrospective study that was mainly made by bacterial rather than viral ulcers. The rate of viral ulcers showed initial steady increase between 1997 and 2000 followed by continuous decline over the next three years of the retrospective study and maintained in the prospective study. Despite widely accepted views of the predominance of viral keratitis in the developed countries, these are decreasing in the population studied and contact lens-related bacterial corneal ulcers are more frequent than viral ulcers (Ibrahim *et al.*, 2012).

The antibacterial activity of local Sidr and Mountain Saudi honeys against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* were evaluated. Disc diffusion method, gel diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were used in this investigation. The findings indicated that both honey samples had growth inhibitory effect and all tested gram negative bacteria were sensitive to 40-80% concentrations. Increasing the honey concentration significantly ($P \leq 0.05$) increased the inhibition of growth of the tested bacteria. Sidr honey was more potent than

Mountain honey in producing the inhibitory growth effect as an antibacterial agent. Sidr and Mountain honeys in different concentrations were more effective against *E.coli* than other bacteria. MIC of the two honey samples was 20 mg/mL while the MIC of *K. pneumoniae* was 40 mg/mL in the case of Mountain honey. The MBC of the two honey samples was 40 mg/mL and the MBC of *A. baumannii* that valued was 20 mg/mL. Sidr and Mountain Saudi honeys could potentially be used as therapeutic agents against bacterial infection particularly to the tested microorganisms.(Alqurashi *et al*., 2013).

In the present research work the aim was to identify and characterize various bacteria isolated from corneal eye infections. During the course of research work different samples of patients suffering from different diseases of eye infections have been collected and observed which are identified as: Corneal infections and Corneal ulcer. Different bacterial species belonging to the genus *Streptococcus*sp. *Staphylococcus* sp. *Neisseria*sp. *and Bacillus* sp. have been isolated from the above mentioned diseases. Then checked the minimum inhibitory concentration (MIC) of different honeys, antibiotics resistance of many antibiotics and antibacterial activity of *Azadirachta indica* (Neem plant) leaf extract on these bacteria that were isolated from corneal eye infections.

In order to analyze the physical characteristics of aerobic and anaerobic bacteria that have been isolated from eye infections, different morphological tests were performed in the laboratory. These tests included Gram Staining and Endospore Staining. To check the motility of these isolated bacterial species specific Motility Agar Test was performed. The end result of these tests was gathered and tabulated in the form of tables. On the basis of the result that was obtained from the above mentioned tests, we were able to identify the shape, size, motility, sporulating and non-sporulating, gram-positive and gram-negative bacteria.

Mostly gram-negative strains of bacteria were isolated as compared to gram-positive strains. On average basis, non-spore forming and non-motile bacteria were identified. Different biochemical tests were performed in the laboratory to analyze the biochemical characteristics of these isolated bacterial species. These tests include Blood Agar test, MR-VP test, Catalase test, Carbohydrate fermentation test, Litmus milk test, , MacConkey Agar test, Urease test, Hydrogen sulphide production test and Citrate test. The results were compiled and tabulated in the form of tables. The effect honey of and different antibiotics on the isolated bacterial species was also observed during research work. Mostly Catalase positive strains of bacterial isolates were observed. Identification of all these bacterial strains was done with the help of Bergey's Manual.

The bacteria that identified from bacterial strain 1 (BS 1) are *Streptococuss* sp.Streptococci are Gram-positive cocci in the family Streptococcaceae. They often occur in pairs or chains, especially in fluids. Many members of the genus *Streptococcus* are pathogenic for humans and animals. The genus *Streptococcus* includes important pathogens and commensals of mucosal membranes of the upper respiratory tract and, for some species, the intestines. Streptococci are generally strong fermenters of carbohydrates, resulting in the production of lactic acid, a property used in the dairy industry. Most are facultative anaerobes, but peptostreptococci are obligate anaerobes. Streptococci do not produce spores and are non-motile. They are catalase-negative. Its minimum inhibitory concentration showed by pak honey and the maximum inhibitory concentration showed by young's honey. It showed resistance only against 3-nitro-anilin and showed maximum sensitivity against aniline. When checked it's *Azadirachta indica* (Neem plant) leaf extract resistance, it showed resistance against methanol (standard) and minimum sensitivity is observed against the extract.

The bacteria that identified from bacterial strain 4c (BS 4c) are *Staphylococcus sp. Staphylococcus* bacterial cells are usually spherical, about 0.5-1.5µm in diameter, occurring singly or in irregular clusters. They are Chemoorganotrophic, with both respiratory and fermentative metabolism (Madigan *et al*., 2000). Colonies are usually opaque and may be white or cream and sometimes yellow to orange. Cytochromes are present in these bacteria. Nitrate is often reduced to nitrite (Gunasekaran, 2007). They usually grow with 10% NaCl. The optimum temperature is 30-37°C. Its minimum inhibitory concentration showed by Pak honey and the maximum inhibitory concentration showed by young's honey. It showed minimum sensitivity against o-toludinebenzaldehyde derivative and maximum sensitivity against methyl-amine. When checked it's *Azadirachta indica* (Neem plant) leaf extract resistance, it showed resistance against

methanol (standard) and sensitivity is observed against the extract.

The bacteria that identified from bacterial strain 6 (BS 6) are *Staphylococcus sp. Staphylococcus* bacterial cells are usually spherical, about 0.5-1.5µm in diameter, occurring singly or in irregular clusters. They are Chemoorganotrophic, with both respiratory and fermentative metabolism (Madigan *et al.*, 2000). Colonies are usually opaque and may be white or cream and sometimes yellow to orange. Cytochromes are present in these bacteria. Nitrate is often reduced to nitrite (Gunasekaran, 2007). They usually grow with 10% NaCl. The optimum temperature is 30-37°C. Its minimum inhibitory concentration showed by pak honey and the maximum inhibitory concentration showed by young's honey. It showed minimum sensitivity against o-toludinebenzaldehyde derivative and maximum sensitivity against methyl-amine. When checked it's *Azadirachta indica* (Neem plant) leaf extract resistance, it showed resistance against methanol (standard) and sensitivity is observed against the extract.

The bacteria that identified from bacterial strain 8a (BS 8a) are *Neisseria* sp. They are obligate human pathogens with no other natural host. They are Gram-negative cocci, 0.6-1.0µm in diameter, occurring singly but more often in pairs with adjacent sides flattened. They are non-motile and flagella are absent. Some species produce a greenish-yellow caretenoid pigment and may be nutritionally fastidious and haemolytic. The optimum growth temperature is 35°C-37°C. Its minimum inhibitory concentration was showed by pak honey and the maximum inhibitory concentration showed by young's honey. It showed maximum sensitivity against methyl-amine and showed minimum sensitivity against ethyl-amine cinnamaldehyde derivative. When checked its*Azadirachta indica* (Neem plant) leaf extract resistance, it showed resistance against methanol (standard) and maximum sensitivity is observed against the extract.

Conclusion

The bacteria that identified from bacterial strain 8b (BS 8b) are *Bacillus sp.* They are Gram-positive, aerobic, rod-shaped endospore-forming bacteria of the Genus *Bacillus* are the most widely represented organisms in the soil. Due to their ability to form spores and withstand a range of variable environmental conditions, *Bacillus* spp. adapt easily to diverse habitats. Several Bacilli may be linked to opportunistic infections, *e.g* in post-surgical wounds, cancer patients, or immunocompromised individuals. Pathogenicity among *Bacillus* spp. is however mainly a feature of organisms belonging to the *B. cereus* group, a subgroup of the *B. subtilis* group (group II) within the *Bacillus* genus and which are commonly found in the environment. It showed minimum inhibitory concentration by pak honey and the maximum inhibitory concentration by young's honey. It showed maximum sensitivity against aniline with and minimum resistance against ethyl-amine salisylaldehyde derivative, o-toludinebenzaldehyde, m-toluidine salisylaldehyde derivative. When checked it's *Azadirachta indica* (Neem plant) leaf extract resistance, it showed resistance against methanol (standard) and sensitivity is observed against the extract .

From current study it can be concluded that new and innovatve techniques must be adapted against common pathogenic bacteria isolated from air sources. Because of increasng antibiotic resistance of bacterial pathogens new agents like plants honey and synthetic compounds can be used as antbacterial agents.

Consent

As per international standard or university standard, patient's consent has been collected and preserved by the authors.

Competing Interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration between all authors. NMA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. IL and BM managed the analyses of the study. AA and SA managed the literature searches. All authors read and approved the final manuscript.

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