Antibacterial effects of antibiotics and cell-free preparations of probiotics against *Staphylococcus aureus* and *Staphylococcus epidermidis* associated with conjunctivitis

Sara Mohamed a, Mohamed N. Elmohamady b, Sohier Abdelrahman c, Mahmoud M. Amer a, Ahmed G. Abdelhamid a,*

a Botany and Microbiology Department, Faculty of Science, Benha University, Benha 13511, Egypt
b Ophthalmology Department, Faculty of Medicine, Benha University, Benha 13511, Egypt
c Clinical pathology Department, Faculty of Medicine, Benha University, Benha 13511, Egypt

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**A B S T R A C T**

Conjunctivitis, caused by bacterial infections, represents health concern and diagnosis of the disease is pivotal for the proper selection of the treatment. The main causes of bacterial conjunctivitis vary in different countries. The current study investigated the common bacterial causes of bacterial conjunctivitis from eye clinics’ attendants and evaluated the effectiveness of different therapeutic approaches. Eye swabs from patients, diagnosed with conjunctivitis, were assessed microbiologically and the isolated bacteria were identified using the standard biochemical identification and sequencing of the 16S rRNA gene. Antibiotics’ susceptibility of the conjunctivitis-associated bacterial pathogens was evaluated against nineteen broad-spectrum antibiotics. In the meanwhile, cell-free preparations from probiotic *Lactobacillus* and *Bifidobacterium* strains were used to evaluate their antagonistic activities. Findings from this study showed that out of 52 specimen, 17 eye swabs from patients with conjunctivitis were bacterial culture-positive. The identity of the bacterial species, using the biochemical identification system, was *Staphylococcus aureus* (4 isolates) and *S. epidermidis* (13 isolates). *Staphylococcus* spp. showed susceptibility to linezolid, vancomycin, novobiocin, and fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin and levofloxacin). However, isolates from the two *Staphylococcus* spp. expressed resistance to penicillin G, oxacillin, and cephalaxin. As alternatives to antibiotics, the growth of *Staphylococcus* spp., including isolates with antibiotic resistance, was inhibited by cell-free preparations of the 4 probiotic *Lactobacillus* and the 2 *Bifidobacterium* strains. These findings provide evidence that topical antibiotics such as fluoroquinolones are still effective antimicrobial agents against staphylococci associated with conjunctivitis whereas probiotic preparations could be promising for further research to pave the way for their therapeutic applications against ophthalmic diseases.

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**1. Introduction**

Bacterial conjunctivitis (BC) is a common type of infectious conjunctivitis, and affects all human age groups (Tarabishy and Jeng, 2008). The disease is caused by inflammation of the conjunctiva through one or more bacterial species resulting in pus formation. Bacterial infection may occur due to exposure to external bacteria or invasion by internal bacteria that is transported by blood stream (Muluye et al., 2014). The immediate treatment of BC is important in serious eye infection because it may cause loss of vision and affect the cornea (Tesfaye et al., 2013). The common bacterial pathogens that cause conjunctivitis are *Staphylococcus aureus, Streptococcus pneumoniae*, *Haemophilus influenzae* and Coagulase-negative *Staphylococcus* (Karpecki et al., 2010; Ung et al., 2020). As said, Gram-positive bacteria are the most prevalent and frequently diagnosed in eye infections. However, the leading type of bacteria is different among several parts of the world. For example, in the USA, *S. aureus* is the leading cause of BC in adults whereas *S.
*pneumoniae* and *H. influenzae* are common causes of the disease in children (Cronau et al., 2010). Being an underexplored research area in Egypt, the current work was conducted to identify the bacterial causes of conjunctivitis in Egyptian clinical settings and to decipher whether the isolated bacterial pathogens exhibited resistance to the commonly-used antibiotics.

The rise of bacterial resistance to antibiotics that are commonly used to treat BC, imposes safety concerns. Resistance to several classes of ophthalmic antibiotic preparations such as aminoglycosides (e.g. Tobramycin), polymyxin B combinations, macrolides (e.g. Azithromycin) and fluoroquinolones (e.g. moxifloxacin) has been reported (Alexandakis, 2000; Ashell et al., 2008; Ohnsman et al., 2007). The development of bacterial resistance is impacted by uncontrolled antibiotics use, inadequate bactericidal concentrations of antibiotics at the site of action, limited adherence to regimens of prescribed antibiotics and the undesirable adverse events (Karpecki et al., 2010). Therefore, the development of successful therapy of BC necessitates finding more efficacious antimicrobial alternatives. Probiotics are beneficial bacteria that confer health benefits to human body when administered in adequate amounts (Foligné et al., 2013). Of their health benefits, the antagonistic characteristics, antivirulence activities, immune-modulatory properties and strengthening mucosal gut barrier are well-documented traits and desirable features of probiotics (Bayoumi and Griffiths, 2012; Patel et al., 2014; Tejero-Sariñena et al., 2012; Abdelhamid et al., 2018). Because of being rarely explored in BC treatment, probiotics served as candidates, in this study, to unravel their antagonistic properties against causal pathogens associated with BC.

In this work, we aimed, (1) to identify the bacterial causes of conjunctivitis from an Egyptian clinical setting, (2) evaluate the antimicrobial efficacy of a variety of antibiotics, and (3) assess the inhibitory effect of probiotics against the causal pathogens. To our knowledge, very limited literature evaluated the use of probiotics to combat pathogenic bacteria isolated from patients with conjunctivitis.

### 2. Material and methods

#### 2.1. Collection of specimens

Swab samples were obtained from 52 patients who were clinically diagnosed with ocular infections at ophthalmology Center at Benha University hospital, and eye care centers in the period between 2017 and 2018 as shown in Fig. 1 (The sampling region is indicated by red arrow on the map). Cases of mucopurulent conjunctivitis were included while cases with history of topical or systemic antibiotic administration one week from presentation or with history of other ocular diseases or surgery were excluded. A patient information checklist including the patient’s age, gender or symptoms was prepared. Oral and written consents from participants were obtained and the study was performed according to declaration of Helsinki and approved by Benha University ethics committee. Ten control samples were collected from volunteers that have no symptoms of conjunctivitis disease. These samples were collected by sterile swabs following aseptic precautions. Briefly, sterile cotton-tipped swabs moistened in sterile normal saline solution were used for collection of specimens without using any anesthetics. Specimens were collected from 1) The lower fornix conjunctiva and palpebral conjunctiva of upper lid by, gently turning and rolling the swab back and forth, and 2) The lid margin in some of the cases. Specimens were taken, cautiously avoiding contamination, from both eyes in some cases and from the more inflamed eye in most cases. Swabs were streaked on blood agar and mannitol salt agar media under aerobic conditions at 37 °C for isolation of *Staphylococcus* strains. Bacteria were identified by standard and automated biochemical tests.

#### 2.2. Identification of the bacterial isolates causing conjunctivitis

##### 2.2.1. Biochemical identification of the *Staphylococcus* spp.

Typical colonies of staphylococci (beta or alpha-hemolytic) on blood agar (Oxoid, Hampshire, England) or mannitol salt agar (Oxoid; Pink to yellow colonies) were streaked for several consecutive times on nutrient agar for purification. The colonies were then isolated and checked by microscopic examination using Gram’s stain. The schematic identification of *Staphylococcus* spp. isolates was determined as described previously (Kawamura et al., 1998; Kloos and Schleifer, 1975) but with modifications. Briefly, colony and cell morphologies were determined using blood agar and Gram-stain, respectively. The following phenotypic and conventional tests were performed: catalase test (H₂O₂; 3%); tube coagulase test (Rabbit plasma; Oxoid); DNase test (DNase test agar, Oxoid); Mannitol fermentation (Mannitol salt agar, Oxoid). For confirming the identity of *Staphylococcus* spp. isolates, VITEK2 system (bioMerieux, Inc, Hazelwood, Mo.), a rapid and automated biochemical identification system, was used according to the manufacturer instructions. The biochemical results were analyzed using the VITEK2 database to identify the bacteria.

##### 2.2.2. Molecular identification of *Staphylococcus* spp. using 16S rRNA gene sequencing

The identification of selected *S. epidermidis* and *S. aureus* strains, was confirmed by 16S rRNA gene sequencing and further analysis of the sequences against NCBI database was performed using BLASTn tool. Briefly, the genomic DNA of two isolates, namely *S. epidermidis*-29 and *S. aureus*-35, representing the two species isolated in this study, was extracted using QIAamp DNA Mini Kit (Qiagen; Valencia CA, Catalogue no.51304) according to the manufacturer instructions. The genomic DNA was used as a template for amplifying the 16S rRNA gene using Eudraigal GT PCR master mix (Takara; catalogue no. RR310A) in a total 25 μL reaction mixture. The amplification conditions for *S. aureus* were performed as the following: initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 1 min (denaturation), 51.5 °C for 30 s (annealing), and 72 °C for 1 min (extension), and final extension at 72 °C for 10 min. The same conditions were used for *S. epidermidis* except that the annealing occurred at 60 °C for 1 min. The PCR products were separated on 1.5% agarose gel electrophoresis and purified using QIAquick PCR Product extraction kit (Qiagen) before sequencing using Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). The 16S rRNA gene sequences were analyzed for sequence homology against GenBank using BLASTn. The phylogenetic tree was constructed, by including the closest blast results to each of the query sequences of the two isolated staphylococci, using NCBI Distance tree online tool (Neighbor Joining tree method).

#### 2.3. Antibiotic susceptibility profiling

Bacterial pathogens, causing conjunctivitis, were investigated for their susceptibility to antibiotics using standard disc diffusion method (Bauer et al., 1966; Hudzicki, 2009). Schematic representation of the procedures used for the current study is summarized in Fig. 2. Briefly, single colony from each isolate was inoculated into 5 mL nutrient broth, incubated for 24 h at 37 °C before the turbidity for each culture was equally adjusted to 0.5 McFarland standard solution. Sterile swabs were used to evenly spread the turbidity-adjusted cultures on the surface of Muller Hinton (MH) agar. Two plates were used for each strain while antibiotic discs were applied to the surface at constant distances. Nineteen
different broad-spectrum antibiotics were used. The plates were incubated at 37 °C for 24 h. The inhibition zones were measured with a millimeter ruler including the diameter of disc. Inhibition zones (mm) on MH agar were measured for each antibiotic against each bacterial isolate. Results were interpreted according to the Clinical & Laboratory Standards Institute (CLSI, 2011) guidelines.

2.4. Antibacterial activities of probiotics

2.4.1. Preparation of the cell-free spent medium (CFSM) of probiotics

Six strains of probiotics belonging to the genera Lactobacillus (L. acidophilus EMCC 1324, L. helveticus EMCC 1654, L. plantarum EMCC 1027 and L. rhamnosus EMCC 1105) and Bifidobacterium (B. longum EMCC 1547 and B. bifidum EMCC 1334) were grown in Man-Rogosa-Sharpe (MRS, Oxoid, Hampshire, England) agar medium for 24 h at 37 °C with 5% CO2. A single colony from each strain was transferred into MRS broth, and incubated for 24 h at 37 °C with 5% CO2 for the preparation of the cell-free spent medium (CFSM) as described by Abdelhamid et al. (2019, 2018). Briefly; overnight cultures of the six probiotic strains grown in MRS broth were diluted 1:100 with fresh MRS medium and allowed to grow under same conditions to an optical density600nm of 1.6 (~1 × 10^8 cells/ml), before the cells were removed using centrifugation at 6000g at 4 °C for 10 min. The supernatant was filter-sterilized with 0.2 μm –pore-size filter and referred to as CFSM. The CFSM of all probiotic strains was stored at –20 °C until use for further assays.
2.4.2. Antibacterial activity of the probiotic CFSM

One hundred microlitres from overnight cultures of Staphylococcus spp., grown in nutrient broth until cell density of 8 log CFU/mL, were mixed with 20 mL of molten MH agar, before pouring into petri dishes. After solidification of the MH agar, wells with a diameter of 10 mm were punched aseptically with a sterile micropipette tip, and a volume (100 Âµl) of the probiotic CFSM is dispensed into each well. Staphylococcal cultures without or with treatment of vancomycin (30 µg/disc) served as negative and positive control, respectively. The plates are incubated at 37 °C for 24 h. The probiotic suspension diffuses in the medium and inhibits the growth of the inoculated bacterial pathogens. Inhibition zone was measured in millimeters as mean ± SD of all zones obtained from S. aureus or S. epidermidis isolates against each probiotic strain and the experiment was performed twice.

3. Results

3.1. Staphylococcus spp. are associated with conjunctivitis

Fifty-two specimens were collected from different age groups (less than 2 years (n = 7), 2–18 years (n = 24), and ≥18 years (n = 21)), gender (male (n = 21), and female (n = 31)) or eye symptoms (yellowish discoloration (n = 8), white discoloration (n = 35), redness (n = 32), and burning (n = 27)). The rate of culture-positive samples was not significant between the groups (Fisher’s exact test; P > 0.05). Seventeen specimens, out of 52, from patients with conjunctivitis showed bacterial growth on blood agar and mannitol salt agar. Cultural and biochemical characteristics were used for identification of the bacterial isolates that were obtained from patients with culture-positive BC. Using gram staining, the 17 bacterial isolates were Gram-positive cocci. The colony characteristics, on nutrient or mannitol salt agar, showed that the colonies were round whereas the microscopic examination revealed the bacterial cells as grape-like clusters and non-motile. Biochemical characteristics demonstrated that the 17 isolates were categorized into two different species of the Staphylococcus genus. Of the isolated bacteria, 4 isolates were S. aureus and 13 were S. epidermidis. The identity of S. aureus and S. epidermidis isolates was confirmed using VITEK 2 identification cards for Gram-positive bacteria. The biochemical profile of the isolates matched accurately S. aureus and S. epidermidis found in VITEK database.

The 16S rRNA-based identification confirmed that the two staphylococcal isolates, selected for sequencing in this study, shared closest similarity with S. epidermidis or S. aureus strains, publicly available, in the NCBI GenBank. The two isolated Staphylococcus strains were deposited in NCBI GenBank with accession numbers of MT193675 or MT193624 for S. aureus EG-BC1 (originally designated as S. aureus-35) or S. epidermidis EG-BC1 (originally designated as S. epidermidis-29), respectively. The phylogenetic trees, constructed with 10 or more Staphylococcus spp. strains including S. aureus EG-BC1 or S. epidermidis EG-BC1, indicated that the latter two staphylococcal isolates clustered with high similarity with their closely-related strains (Fig. 3). These findings coincide with the biochemical identification of the isolated staphylococci herein.

3.2. Susceptibility of Staphylococcus spp. to different antibiotics

S. epidermidis was the most common bacterial species, isolated in this study, in association with conjunctivitis. All Staphylococcus spp. were susceptible to vancomycin, novobiocin, and linezolid while most of the isolates were susceptible to gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, and clindamycin. All Staphylococcus spp. were resistant to oxacillin, penicillin G, and cephalaxin.

Data shown in Table 1 distinguish the efficacy of the tested antibiotics against S. aureus isolates. The four S. aureus were resistance to oxacillin, penicillin-G, cephalaxin, while were sensitive to linezolid, gentamicin, vancomycin, norfloxacin, levofloxacin, ofloxacin, gatifloxacin and novobiocin. Approximately, 75% of the isolates were sensitive to ciprofloxacin, rifampin, and clindamycin.

Table 2 showed that all S. epidermidis isolates were resistant to oxacillin, penicillin G, and cephalaxin while 11 isolates, out of 13, were resistant to azithromycin and 10 isolates conferred resistance to cefoxitin. On the other hand, all S. epidermidis isolates were sensitive to ofloxacin, linezolid, vancomycin, and novobiocin. Twelve S. epidermidis isolates were sensitive to norfloxacin, rifampin, clindamycin, and levofloxacin. Ten to 11 isolates of S. epidermidis were sensitive to ciprofloxacin, trimethoprim-sulfamethoxazole, and gatifloxacin. Collectively, fluoroquinolones such as norfloxacin, levofloxacin, gatifloxacin, and ciprofloxacin were effective antagonistic agents against both Staphylococcus spp. associated with conjunctivitis in this study.

3.3. Antibacterial activity of the probiotic CFSM

Cell-free preparations of probiotic Lactobacillus and Bifidobacterium strains, grown in MRS medium, showed promising growth inhibition of all S. aureus or S. epidermidis isolates, even though against those with antibiotic resistant pattern. Data shown in Table 3 demonstrate that all probiotic strains were efficient to inhibit the growth of Staphylococcus spp. associated with conjunctivitis. Moreover, the results indicate that the highest antibacterial activities were obvious for Lactobacillus acidophilus EMCC 1324, Bifidobacterium bifidum (Tissier 1900) EMCC 1334, and L. rhamnosus EMCC 1105 against S. aureus and epidermidis isolates (Table 3). Additionally, S. aureus was more sensitive to the inhibitory CFSM of probiotics than S. epidermidis (P < 0.001; Table 3). These findings suggest that probiotics could be promising antimicrobial alternatives against pathogenic Staphylococcus spp. associated with conjunctivitis.

4. Discussion

Conjunctivitis imposes a great social and economic burden on human health. Conjunctivitis from bacterial infections can be attributed to dysbiosis of native conjunctival microflora or direct transmission from infected individuals (Mannis and Plotnik, 2006). Additionally, immunosuppression, trauma, disrupting the epithelial barrier, and compromised production of tears may prompt people to BC (Varu et al., 2019). It is important to differentiate BC from other causes of conjunctivitis because this facilitates the proper selection of the antibiotic treatment.

In this study, S. epidermidis and S. aureus were associated with BC from the selected patients attending eye care units. In Egypt, there are limited studies aimed to profile the microbiological causes of conjunctivitis. Hashish et al., (2018) found S. aureus, S. pneumoniae, and Pseudomonas aeruginosa to be the commonly isolated microorganisms from patients with infantile BC. Similar to our results, Shaker et al., (2016) found Staphylococcus spp. and P. aeruginosa to be the dominant species from patient with conjunctivitis. BC, due to S. aureus or S. epidermidis, could be effectively treated with antibiotics to reduce the duration of the disease. Although the current study enriched the information about the bacterial causes of conjunctivitis in northern Egypt, further studies should expand to include diverse regions to gain insights about the prevalence of the disease, antibiotic resistance patterns of the...
Fig. 3. Neighbor-joining trees showing the phylogenetic relationships between two isolated staphylococci, namely *S. epidermidis* EG-BC1 (panel A; indicated in bold) and *S. aureus* EG-BC1 (panel B; indicated in bold) and the closest *S. epidermidis* and *S. aureus* strains publicly available on NCBI GenBank. The NCBI BLASTn tool was used to find the 16S rRNA sequence similarities among the presented staphylococcal strains.

### Table 1
Susceptibility differences of *S. aureus* isolates against different antibiotics.

| Antibiotic                        | Conc. (μg/ disc) | Resistant (R) No. | % | Intermediate (I) No. | % | Susceptible (S) No. | % |
|-----------------------------------|------------------|-------------------|---|----------------------|---|---------------------|---|
| Linezolid                         | 30               | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Vancomycin                        | 30               | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Rifampin                          | 5                | 1                 | 25% | 0                    | 0.0% | 3                    | 75% |
| Ciprofloxacin                     | 5                | 1                 | 25% | 0                    | 0.0% | 3                    | 75% |
| Erythromycin                      | 15               | 2                 | 50% | 2                    | 50% | 0                    | 0.0% |
| Penicillin-G                      | 10               | 4                 | 100% | 0                    | 0.0% | 4                    | 100% |
| Gentamicin                        | 10               | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Tobramycin                        | 10               | 1                 | 25% | 1                    | 25% | 2                    | 50% |
| Cephalexin                        | 30               | 4                 | 100% | 0                    | 0.0% | 0                    | 0.0% |
| Norfloxacin                       | 10               | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Trimethoprim - sulfamethoxazole   | 25               | 1                 | 25% | 1                    | 25% | 2                    | 50% |
| Azithromycin                      | 15               | 1                 | 25% | 2                    | 50% | 1                    | 25% |
| Oxacillin                         | 1                | 4                 | 100% | 0                    | 0.0% | 0                    | 0.0% |
| Cefoxitin                         | 30               | 2                 | 50% | 0                    | 0.0% | 2                    | 50% |
| Clindamycin                       | 2                | 0                 | 0.0% | 1                    | 25% | 3                    | 75% |
| Ofloxacin                         | 5                | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Levofloxacin                      | 5                | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Gatifloxacin                      | 10               | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |
| Novobiocin                        | 5                | 0                 | 0.0% | 0                    | 0.0% | 4                    | 100% |

* expressed as percent in reference to all *S. aureus* isolates per each antibiotic studied.

Denotes for number of *S. aureus* isolates.
The use of antibiotics in treatment of BC is thought to be effective in patients with positive results of bacterial cultures. According to a systematic review, antibiotics increased clinical and microbial cure rate in patients with culture-positive BC, whereas they improved microbial cure rate in patients with suspected BC (Eppling, 2010). Broad spectrum antibiotics seem to have effectiveness in treatment of BC. In this study, most Staphylococcus spp. were sensitive to fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin and levofloxacin), linezolid, vancomycin, and novobiocin. Previous study indicated that topical fluoroquinolones, used to treat BC, were found to be safe and effective (Hutnik and Mohammad-Shahi, 2010). There is no best choice for topical antibiotics for BC treatment, but cost, availability and side effects are important factors (Høvding, 2008). These considerations apply to Egyptian settings for patients with BC. Staphylococcus spp., isolated in this study, showed resistance to oxacillin, penicillin G, and cephalaxin. Resistance to penicillin and oxacillin by S. aureus, was reported to be due to lower dose of treatment used (Lim et al., 2018). Despite their effectiveness in the treatment of BC, antibiotics have some limitations such as the increased bacterial resistance to the prescribed antibiotics because of the widespread use, improper adherence of patients to the prescribed regime or the low concentration of the antibiotic at the infection site (Karpecki et al., 2010). These concerns encourage the search for antimicrobial alternatives for future applications.

Probiotics possess health benefits, to human, which include enhancing immune system (Ljungh and Wadström, 2006), competitively excluding microbial pathogens (Amalaradjou and Bhunia, 2012), treatment of gastrointestinal and diarrheal diseases (Guandalini, 2011; Rolfe, 2000), and reduction of traveler's diarrhoea incidence (Hilton et al., 1997). In this study, six strains of probiotic Lactobacillus and Bifidobacterium spp. inhibited the growth of all S. aureus and S. epidermidis isolates. The mode of action of the probiotic cell-free supernatants, used in this work, could be attributed to the secreted antimicrobial peptides, namely bacteriocins, or the lowered pH due to lactic acid production by the probiotic strains. Negi et al., (2018) found that 116 isolates of Lactobacillus spp. inhibited S. aureus and attributed the antimicrobial activity of the cell-free supernatants from these isolates to the presence of an antimicrobial protein (19 kDa). On the other hand, Hor and Liong, (2014) indicated that cell-free extracts of lactic acid bacteria and bifidobacteria contained lactic acid, hydrogen peroxide, acetic acid, and diacetyl, and these antimicrobial compounds inhibited S. aureus growth. These findings augment the effectiveness of probiotics against Staphylococcus spp. and possibly support the assumption that they could be promising candidates for the treatment of BC.

The use of probiotics in treatment of ocular inflammations has received attention recently and research findings support their health benefits in reducing clinical signs or symptoms associated with ophthalmic diseases or improving host immune responses. The current advances in exploiting probiotics in therapeutic applications against eye diseases were summarized in Table 4. However, the sufficient scientific evidence about their safety and efficacy against eye infections is needed by conducting clinical trials (phase 2) prior to their commercial use as drugs against ophthalmologic diseases. Silva et al. (2020) emphasized that more bioguided studies are needed to address the existing gaps on safety, clinical efficacy, and the antimicrobial mechanisms of probiotics in human. The safety characteristics, host adherence capabilities, and even the broad-spectrum antimicrobial activities of the probiotic strains, used in this work, were well-characterized previously.

| Table 2 | Susceptibility differences of S. epidermidis isolates against different antibiotics. |
|---------|-----------------------------------------------|
| Antibiotic | Conc. (μg/ disc) | Resistant (R) | Intermediate (I) | Susceptible (S) |
|          | No. * | % | No. * | % | No. * | % |
| Linezolid | 30   | 0  | 0.0% | 0   | 0.0% | 13  | 100% |
| Vancomycin | 30   | 0  | 0.0% | 0   | 0.0% | 13  | 100% |
| Rifampin | 5    | 1  | 7.7% | 0   | 0.0% | 12  | 92.3% |
| Ciprofloxacin | 1    | 2  | 15.4% | 1   | 7.7% | 10  | 76.9% |
| Erythromycin | 15   | 7  | 53.8% | 1   | 7.7% | 5   | 38.5% |
| Penicillin-G | 10   | 13 | 100% | 0   | 0.0% | 0   | 0.0% |
| Gentamicin | 10   | 3  | 23.1% | 1   | 7.7% | 9   | 69.2% |
| Tobramycin | 10   | 3  | 23.1% | 1   | 7.7% | 9   | 69.2% |
| Cephalexin | 30   | 13 | 100% | 0   | 0.0% | 0   | 0.0% |
| Norfloxacin | 10   | 1  | 7.7% | 0   | 0.0% | 12  | 92.3% |
| Trimethoprim - sulmamethoxazole | 25   | 1  | 7.7% | 2   | 15.4% | 10  | 76.9% |
| Azithromycin | 15   | 11 | 84.6% | 0   | 0.0% | 2   | 15.4% |
| Oxacillin | 1    | 13 | 100% | 0   | 0.0% | 0   | 0.0% |
| Cefoxitin | 30   | 10 | 76.9% | 0   | 0.0% | 3   | 23.1% |
| Clindamycin | 2    | 1  | 7.7% | 0   | 0.0% | 12  | 92.3% |
| Ofloxacin | 5    | 0  | 0.0% | 0   | 0.0% | 13  | 100% |
| Levofloxacin | 5    | 1  | 7.7% | 0   | 0.0% | 12  | 92.3% |
| Gatifloxacin | 10   | 2  | 15.4% | 0   | 0.0% | 11  | 84.6% |
| Novobiocin | 5    | 0  | 0.0% | 0   | 0.0% | 13  | 100% |

* expressed as percent in reference to all S. epidermidis isolates per each antibiotic studied.

* Denotes for number of S. epidermidis isolates.

| Table 3 | Antimicrobial activity of cell-free preparation of probiotic Lactobacillus and Bifidobacterium strains against the Staphylococcus spp. associated with BC. |
|---------|------------------------------------------------------------------------------------------------|
| Probiotic strain | S. aureus | S. epidermidis |
|          | Inhibition zone (mm) |          |          |
| Lactobacillus acidophilus EMCC 1324 | 17.5 ± 3.51 | 14.0 ± 1.29 |
| Bifidobacterium longum Reuter 1963AL | 14.25 ± 1.5 | 12.69 ± 1.44 |
| EMCC 1547 | 16.0 ± 1.15 | 13.54 ± 1.71 |
| Bifidobacterium bifidum (Tissier 1900) | 16.0 ± 1.15 | 13.54 ± 1.71 |
| EMCC 1334 | 14.75 ± 1.5 | 12.69 ± 1.44 |
| Lactobacillus plantarum EMCC 1027 | 14.75 ± 1.5 | 12.69 ± 1.44 |
| Lactobacillus helveticus EMCC 1654 | 14.5 ± 2.52 | 13.08 ± 1.98 |
| Lactobacillus haminous EMCC 1105 | 15.0 ± 1.83 | 13.0 ± 1.73 |
| P value | 0.0008

* The effect of cell-free preparations of probiotics against the two groups of Staphylococcus species is compared using Student’s t-test with the P < 0.05 is significant.
to influence the work reported in this paper.

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Table 4 Summary of advances in implementation of probiotics in prevention or treatment of some ophthalmic diseases.

| Probiotic species | Disease | Application | Outcome | References |
|-------------------|---------|-------------|---------|------------|
| L. acidophilus, L. rhamnosus, L. helveticus, L. plantarum, B. bifidum, and B. longum | Bacterial conjunctivitis | In vitro application of cell-free supernatants of probiotics against staphylococci (the cause of the disease) | Inhibition of Staphylococcus growth | This study |
| L. acidophilus | Keratoconjunctivitis | Probiotic eye drops (4 times daily for 4 weeks) | Improvement of clinical symptoms | (Iovieno et al., 2008) |
| L. rhamnosus | Chlamydia-related disease | - Administration via conjunctiva in mice - Administered as combination of Chlamydia trachomatis polymorphic membrane protein (as vaccine) + L. rhamnosus (adjuvant) | Enhanced stimulation of specific cellular and humoral immune responses | (Inoue et al., 2016) |
| Escherichia coli Nissle 1917 | Ocular surface diseases | Bacterial ghosts (empty bacterial envelopes) were internalized into human conjunctival epithelial cell line and in vivo by guinea pig conjunctival epithelial cells | - Successful internalization of bacterial ghosts into human conjunctival cells and uptake into the eye of guinea pig conjunctival epithelial cells | (Stein et al., 2013) |
| Probiotic mixture (L. casei, L. acidophilus, L. reuteri, B. bifidum, and S. thermophilus) | Autoimmunity of dry eye | Oral administration for 3 weeks after induction of autoimmune in mice | Clinical manifestations (Retinal inflammation and ocular staining scores) were attenuated in the probiotic group | (Kim et al., 2017) |
| Combination of probiotics (L. acidophilus, S. thermophilus, L. plantarum, L. hammerso, B. lactis) and vitamins (B1, B2, B6, and niacin) | Dry eye disease | Consumption of the probiotic-vitamins mix as one capsule daily for 28 days | Decrease signs and symptoms of dry eye disease | (Chisari et al., 2016) |
| Combination of fish oil, zinc, Y-aminobutanoic acid, vitamin C, lactoferin, vitamin E, lutein, and the probiotic Enterococcus faecium WB1000 | Dry eye disease | Dietary supplement once per day for 8 weeks | Significant improvement of clinical symptoms at weeks 4 and 8 | (Kawashima et al., 2016) |
| E. faecium and Saccharomyces boulardii | Dry eye syndrome | Treatment with probiotic mixture + substitute tear | Reducing dry eye syndrome | (Chisari et al., 2018) |

(Abdelhamid et al., 2019, 2018). The anti-Staphylococcus activity along with the probiotic traits of these strains emphasize their potential applications in medicine and encourage further research for their use in drug design.

5. Conclusion

S. epidermidis and S. aureus caused bacterial conjunctivitis, as shown in the current work, and the isolated bacteria showed susceptibility to topical antibiotics such as fluoroquinolones. Cell-free preparation of probiotics, particularly L. acidophilus EMCC 1324, exerted antibacterial activity against some antibiotic-resistant Staphylococcus strains and could be exploited for further applications albeit with adequate evidence about their safety.

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Author contributions

S.M. contributed experimental design, data acquisition and analysis, and drafting the manuscript; A.G.A. contributed to experimental design, data analysis and interpretation, and drafting/revising the manuscript; M.N.E. helped in experimental design, data interpretation and revising the manuscript; S.A. helped in experimental design, technical lab work and data acquisition; M. M.A. helped data interpretation and revising the work. All authors approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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