

Original Article

Synergy between antibiotics and natural agents results in increased antimicrobial activity against *Staphylococcus epidermidis*

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Abstract

Introduction: *Staphylococcus epidermidis* is one of the most frequent causes of biofilm-associated infections on indwelling medical devices. With the emergence of methicillin-resistant *S. epidermidis* (MRSE), there is an urgent need to discover novel active agents against a range of Gram-positive pathogens. We screened the clinical isolates of *S. epidermidis* for susceptibility/resistance against commonly prescribed antibiotics. Furthermore, we tested some natural agents alone and in combination with antibiotics to find possible synergistic antimicrobial effects.

Methodology: *S. epidermidis* clinical isolates were screened for susceptibility/resistance against vancomycin, erythromycin, tetracycline, chloramphenicol, ampicillin, ofloxacin, cephalexin, and gentamicin using the Kirby-Bauer disk diffusion method. The antimicrobial potential of *Camellia sinensis*, *Juglans regia*, and *Hippophae rhamnoides* alone and in combination with antibiotics were examined using the disk diffusion method, where the antimicrobial potential activity was measured in terms of formation of zones of inhibition.

Results: Most *S. epidermidis* isolates were found to be resistant to one or more antibiotics. Gentamycin and ofloxacin were found to be the most effective antibiotics against *S. epidermidis* isolates. Extracts of *Hippophae rhamnoides*, *Juglans regia*, and *Camellia sinensis* were found to be equally effective against *S. epidermidis* isolates. In combination with antibiotics, these extracts exhibited appreciable synergistic activity; the highest synergistic activity was observed with erythromycin and cephalexin. In the case of cephalexin, a reversion in resistance was observed.

Conclusions: The plant extracts used in the study exhibited additive and synergistic antibacterial activity against *S. epidermidis*, hence providing an effective alternative to deal with the problem of multidrug resistance.

Key words: biofilm; *Staphylococcus epidermidis*; synergistic antimicrobial effects; plant extracts.

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Introduction

The growing use of indwelling medical devices such as intravascular catheters, artificial heart valves, and orthopedic implants has resulted in a significant increase in the number of device-related infections [1]. Staphylococci, above all *Staphylococcus aureus* and *Staphylococcus epidermidis*, are the most frequent causes of biofilm-associated infections on indwelling medical devices, with *S. epidermidis* representing up to 75% of all clinical isolates in some cases [2-4].

S. epidermidis that has developed the same bacterial resistance as *S. aureus* and is known as methicillin-resistant *S. epidermidis* (MRSE). In recent years, the prevalence of multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) strains exhibiting resistance to other antibiotics such as aminoglycosides, minocycline, and fluoroquinolones

has also increased [5-7]. Some studies have found the drug resistance rates for *S. epidermidis* to be higher than 90% of all samples tested [8].

One of the major mechanisms that enable *S. epidermidis* to cause serious health problems and develop antibiotic resistance is its ability to adhere to surfaces and grow a biofilm [9,10].

The rapid increase in the prevalence of multidrug-resistant Gram-positive bacteria has created an urgent need to discover novel active agents against a range of Gram-positive pathogens. In this study, we screened the clinical isolates of *S. epidermidis* from Pakistan for susceptibility/resistance against commonly prescribed antibiotics for these infections. Additionally, we analyzed some natural agents previously shown to exhibit antimicrobial and antibiofilm activity against *Pseudomonas* and *Mycobacterium* species [11,12], for

their antimicrobial activity against *S. epidermidis*. These natural agents were tested alone and in combination with antibiotics to observe possible synergistic antimicrobial effects.

Methodology

S. epidermidis isolates and antibiogram development

Thirty-six *S. epidermidis* isolates, obtained from pus/wounds (IIDRL-SEP/W-1 to IIDRL-SEP/W-36) or blood infections (IIDRL-SEB-1 to IIDRL-SEB-10) were used in this study. The isolates were screened for susceptibility/resistance against eight commonly prescribed antibiotics (vancomycin, erythromycin, tetracycline, chloramphenicol, ampicillin, ofloxacin, cephalixin, and gentamicin) using the Kirby-Bauer disk diffusion method as described previously [13]. Commercially available standard antibiotic disks of standardized concentrations (Oxoid, Basingstoke, UK) were used in antibiotic resistance/susceptibility testing.

Antimicrobial activity of plant extracts

Aqueous plant extracts (5%) were prepared by soaking 2.5 g dried berries, dried barks, and dried leaves of *Hippophae rhamnoides*, *Juglans regia*, and *Camellia sinensis*, respectively, in 50 mL of autoclaved distilled water and boiling for three minutes, thrice, with two-minute intervals between each boiling time. Subsequently, the extract or supernatant was collected in a separate 50 mL tube and centrifuged for 15 minutes at 5,000 rpm. The clear supernatant obtained was collected and filter sterilized using a 0.2 µm filter (micropore filters), and was stored at -20°C. For this study, extracts no older than one week were used; otherwise, fresh extracts were prepared.

The antimicrobial potential of *Camellia sinensis*, *Juglans regia*, and *Hippophae rhamnoides* aqueous extracts (5%) against *S. epidermidis* isolate IIDRL-SEP/W-10 and *S. epidermidis* strain ATCC 12228 was examined using the disk diffusion method as described previously [11].

Evaluation of synergistic activity of plant extracts with antibiotics

Synergistic activity of aqueous extracts of *Camellia sinensis*, *Juglans regia*, and *Hippophae rhamnoides* and the eight above-mentioned antibiotics was examined using the disk diffusion method. Briefly, each plant extract was incorporated into a separate Müller-Hinton agar (MHA) plate to achieve a final concentration of 1 mg/mL. Next, a lawn of 0.5 McFarland matched IIDRL-SEP/W-10 culture was

prepared on MHA plates, using sterile cotton swabs. Commercial antibiotic disks (Oxoid) were placed at appropriate distances on the lawn, and plates were incubated for 24–48 hours at 37°C. The synergistic activity was measured in terms of formation of zones of inhibition in millimeters. The difference between antimicrobial activities of antibiotics alone and in combination with natural agents was tested using one-way analysis of variance (ANOVA) with Tukey's multiple correction [14], using Graphpad software.

Results

Antibiotic resistance/susceptibility profile of *S. epidermidis* isolates

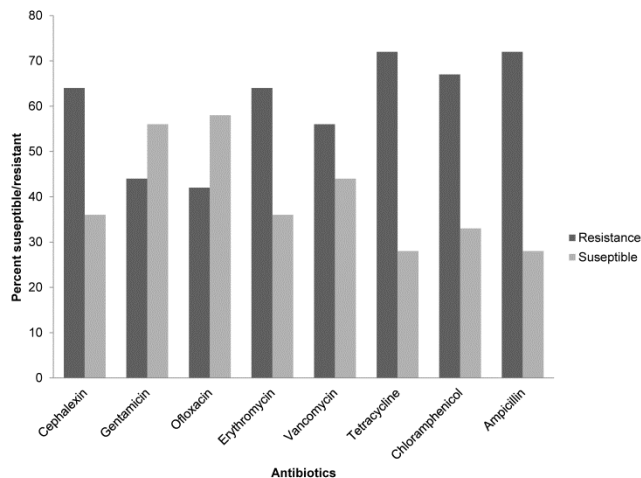
The antibiotic resistance/susceptibility profile of *S. epidermidis* isolates revealed that most of the isolates were resistant to one or more of the tested antibiotics (Figure 1). Most of the isolates were resistant to erythromycin, ampicillin, tetracycline, and chloramphenicol (Figure 1), while gentamycin and ofloxacin were found to be the most effective antibiotics (Figure 1).

Synergistic activity of plant extracts in combination with antibiotics against *S. epidermidis*

Aqueous extracts of *Hippophae rhamnoides*, *Juglans regia*, and *Camellia sinensis* were found to be equally effective against *S. epidermidis* isolate IIDRL-SEP/W-10 (Figure 2, grey bars) as well as against *S. epidermidis* reference strain ATCC 12228 (data not shown).

These aqueous extracts together with antibiotics exhibited appreciable synergistic activity (Figure 2).

Figure 1. Antibiogram for *Staphylococcus epidermidis* isolates. Antibiotic resistance or susceptibility profile was developed by testing *Staphylococcus epidermidis* isolates against eight commonly prescribed antibiotics.



The highest activity was observed for erythromycin and cephalixin, where > 50% increase in antimicrobial activity was observed as compared to antibiotics alone ($p < 0.001$; Figure 2). In the case of cephalixin, a reversion in resistance was observed, where an isolate previously resistant to cephalixin alone was found to be susceptible to a cephalixin-plant extract combination (Figure 2). However, no change in susceptibility was observed for ampicillin.

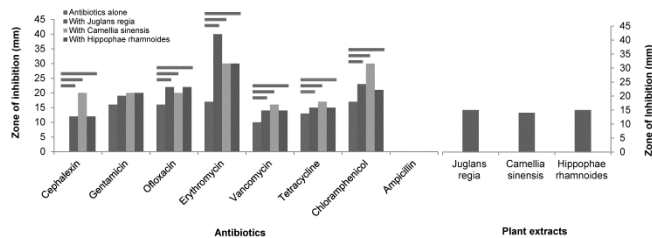
Discussion

Our study showed that the majority of *S. epidermidis* isolates were resistant to one or more tested antibiotics, which supports earlier studies showing a high rate of resistance in *S. epidermidis* against quinolones and a moderate resistance rate to second-generation and third-generation cephalosporins [15]. In our study, we found gentamycin and ofloxacin to be the most effective antibiotics against our *S. epidermidis* isolates. This information can be helpful for practicing ophthalmologists and dermatologists because *S. epidermidis* has been the most common causative pathogen, responsible for endophthalmitis associated with intraocular foreign bodies [16,17], acne, and burn-associated infections.

Our report appears to be the first to report synergistic antibacterial effect of *Hippophae rhamnoides* and antibiotics against *S. epidermidis*. *Hippophae rhamnoides* is known to have antibacterial and antioxidant effects, which are mediated by phenols and flavonoid contents. [18]. Phenolic rich fraction of *Hippophae rhamnoides* has been found to contain gallic acid, rutin, quercetin-3-galactoside, quercetin-3-glucoside, myricetin, quercetin, kaempferol, and isorhamnetin. These constituents have also proven to have potent antibacterial and antioxidant activity against *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* [19].

We also found that the addition of *Juglans regia* to the antibiotics resulted in increased activity against *S. epidermidis*. Significant synergistic effects were observed when erythromycin was combined with the plant extracts (Figure 2). It has been shown that *Juglans regia* leaf extracts have an inhibitory effects on the acne lesions caused by *S. epidermidis*, possibly because of their anti-inflammatory properties [20]. *Juglans regia* contains α -pinene, β -pinene, β -caryophyllene, germacrene D, and limonene. These constituents are known to exhibit broad-spectrum antibacterial activity against all the bacterial strains,

Figure 2. Antimicrobial activity of natural agents alone and in combination with antibiotics. Graph shows antimicrobial activity of plant extracts alone, and in combination with eight antibiotics against *S. epidermidis* isolate IIDRL-SEP/W-10. Grey lines over the bars represent significant difference between the antimicrobial activities of antibiotics alone, and in combination with natural agents ($p < 0.05$).



especially against Gram-positive bacteria, including *S. epidermidis* [21].

Our study is in agreement with earlier studies showing strong antimicrobial potential of *Camellia sinensis* against *S. epidermidis* [22,23]. *Camellia sinensis* is known for its therapeutic properties, especially anti-inflammatory and anti-microbial. Although the anti-microbial properties of *Camellia sinensis* are known, its role against bacterial strains involved in skin infections is not well understood. The main active ingredient of *Camellia sinensis* is an acidic polysaccharide CS-F2, a pectin-type polysaccharide with a molecular weight of approximately 8.0×10^4 Da, which has been characterized by its anti-adhesive effects against pathogenic bacteria, most notably against *Helicobacter pylori*, *Propionibacterium acnes*, and *S. aureus*. Interestingly, CS-F2 showed no inhibitory effects against *S. epidermidis* [24], because *Camellia sinensis* has been shown to exert a selective anti-adhesive effect against certain pathogenic bacteria with no adverse effects against beneficial or commensal bacteria [25].

The other possible mechanism by which these natural agents might be exerting their antimicrobial effect or synergistic effect with antibiotics is through inhibition of bacterial efflux pumps [26]. Bacteria use efflux pumps as a self-defense mechanism to remove antibiotics from the cell. The activity through efflux pumps results in sub-lethal drug concentrations at the active site that often predisposes the organism to the development of high-level resistance against antimicrobial agents [26]. Several studies have reported the crucial role of phytochemistry in the search for effective efflux pumps inhibitors (EPIs), especially against Gram-positive bacteria such as *S. aureus* and *S. epidermidis*. The activity of plant-derived EPIs is attributed to their chemical properties,

including flavones, isoflavones, acylated glycosides, porphyrin phaeophorbide A, or kaempferol rhamnoside [26]. The structures of the plant-derived EPIs have been exploited to produce several synthetic EPIs exhibiting inhibitory activity at micrometer concentrations [26].

Conclusions

Antibiotics have been effective in treating infectious diseases, but resistance to these drugs has led to the emergence of new, and the re-emergence of old, infectious diseases. One strategy employed to overcome these resistance mechanisms is the use of combination of drugs, such as beta-lactams together with beta-lactamase inhibitors. We have shown that plant extracts have exhibited additive and synergistic antibacterial activity against *Staphylococcus epidermidis*. Hence, this provides an effective alternate way to deal with the problem of multidrug resistance.

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Authors' contributions

SHA was responsible for study design and analysis; SHA; SHA, KA, and SKS performed the experiments; SHA and KA wrote the manuscript; SUK supervised the work. All authors read and approved the final manuscript.

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