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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, cephalexin, 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 um 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

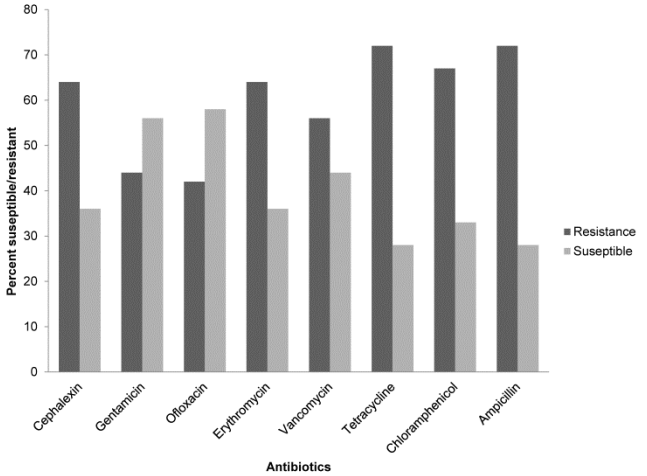
The antibiotic resistance/susceptibility profile of *S. epidermidis* isolates revealed that most of the isolates were resistant to one or more of the tested antibiotics (Figure 1). Most of the isolates were resistant to erythromycin, ampicillin, tetracycline, and chloramphenicol (Figure 1), while gentamycin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

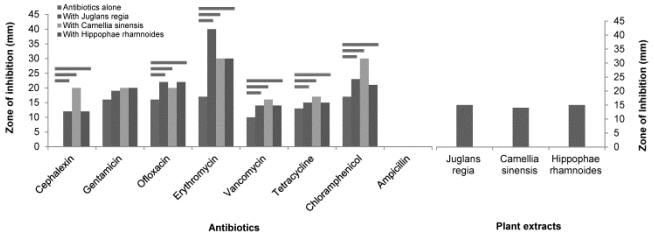
These aqueous extracts together with antibiotics exhibited appreciable synergistic activity (Figure 2).

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

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 ($p < 0.05$).



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, 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 x 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.

References

1. Singhai M, Malik A, Shahid M, Malik MA, Goyal R (2012) A study on device-related infections with special reference to biofilm production and antibiotic resistance. J Glob Infect Dis 4: 193-198.
2. Otto M (2013) Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Ann Rev Med 64: 175-188.
3. Bispo PJ, Hofling-Lima AL, Pignatari AC (2014) Characterization of Ocular Methicillin-Resistant Staphylococcus epidermidis belonging predominantly to the Clonal Complex 2 Subcluster II. J Clin Microbiol 52: 1412-1417.
4. Mack D, Davies AP, Harris LG, Jeeves R, Pascoe B, Knobloch JK, Rohde H, Wilkinson TS (2013) Staphylococcus epidermidis in biomaterial-associated infections. In Moriarty TF, Zaat SA, Busscher HJ, editors. Biomaterials Associated Infection. New York: Springer. 25-56.
5. David MZ, Daum RS (2010) Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23: 616-687.
6. Elsahn AF, Yildiz EH, Jungkind DL, Abdalla YF, Erdurmus M, Cremona FA, Rapuano CJ, Hammersmith KM, Cohen EJ (2010) In vitro susceptibility patterns of methicillin-resistant Staphylococcus aureus and coagulase-negative Staphylococcus corneal isolates to antibiotics. Cornea 29: 1131-1135.
7. Guidry CA, Mansfield SA, Sawyer RG, Cook CH (2014) Resistant pathogens, fungi, and viruses. Surg Clin North Am 94: 1195-1218.
8. Wang LF, Li JL, Ma WH, Li JY (2014) Drug resistance analysis of 1914 bacterial strains isolated from burn patients. Genet Mol Res 13: 9727-9734.
9. Fey PD, Olson ME (2010) Current concepts in biofilm formation of Staphylococcus epidermidis. Future Microbiol 5: 917-933.
10. Zegaer BH, Ioannidis A, Babis GC, Ioannidou V, Kossyvakis A, Bersimis S, Papaparaskevas J, Petinaki E, Pliatsika P, Chatzipanagiotou S (2015) Detection of Bacteria Bearing Resistant Biofilm Forms, by Using the Universal and Specific PCR is Still Unhelpful in the Diagnosis of Periprosthetic Joint Infections. Front Med (Lausanne) 1: 30.
11. Abidi SH, Ahmed K, Sherwani SK, Bibi N, Kazmi SU (2014) Detection of Mycobacterium Smegmatis Biofilm and its Control by Natural Agents. Int J Curr Microbiol App Sci 3: 801-812.
12. Abidi SH, Ahmed K, Sherwani SK, Kazmi SU (2014) Reduction and removal of Pseudomonas aeruginosa biofilm by natural agents. Int J Chem Pharm Sci 5: 28-34.
13. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU (2013) Drug resistance profile and biofilm forming potential of Pseudomonas aeruginosa isolated from contact lenses in Karachi-Pakistan. BMC Ophthalmol 13: 57.
14. Vandevelde NM, Tulkens PM, Van Bambeke F (2013) Antibiotic activity against naive and induced Streptococcus pneumoniae biofilms in an in vitro pharmacodynamic model. Antimicrob Agents Chemother 58: 1348-1358.
15. Qiao LD, Chen S, Yang Y, Zhang K, Zheng B, Guo HF, Yang B, Niu YJ, Wang Y, Shi BK, Yang WM, Zhao XK, Gao XF, Chen M, Tian Y (2013) Characteristics of urinary tract infection pathogens and their in vitro susceptibility to

antimicrobial agents in China: data from a multicenter study. BMJ Open 3: e004152.
16. Chhabra S, Kunimoto DY, Kazi L, Regillo CD, Ho AC, Belmont J, Maguire J, Vander J, Brown GC (2006) Endophthalmitis after open globe injury: microbiologic spectrum and susceptibilities of isolates. Am J Ophthalmol 142: 852-854.
17. Vedantham V, Nirmalan PK, Ramasamy K, Prakash K, Namperumalsamy P (2006) Clinico-microbiological profile and visual outcomes of post-traumatic endophthalmitis at a tertiary eye care center in South India. Indian J Ophthalmol 54: 5-10.
18. Upadhyay NK, Kumar MS, Gupta A (2010) Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (Hippophae rhamnoides L.) leaves. Food Chem Toxicol 48: 3443-3448.
19. Yogendra Kumar MS, Tirpude RJ, Maheshwari DT, Bansal A, Misra K (2013) Antioxidant and antimicrobial properties of phenolic rich fraction of Seabuckthorn (Hippophae rhamnoides L.) leaves in vitro. Food Chem 141: 3443-3450.
20. Qadan F, Thewaini AJ, Ali DA, Afifi R, Elkhawad A, Matalka KZ (2005) The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. Am J Chin Med 33: 197-204.
21. Rather MA, Dar BA, Dar MY, Wani BA, Shah WA, Bhat BA, Ganai BA, Bhat KA, Anand R, Qurishi MA (2012) Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of Juglans regia L. and its constituents. Phytomedicine 19: 1185-1190.
22. Rasheed A, Shama SN, Joy JM, Reddy BS, Roja C (2012) Formulation and evaluation of herbal anti-acne moisturizer. Pak J Pharm Sci 25: 867-870.
23. Sharma A, Gupta S, Sarethy IP, Dang S, Gabrani R (2012) Green tea extract: possible mechanism and antibacterial activity on skin pathogens. Food Chem 135: 672-675.
24. Lee JH, Shim JS, Lee JS, Kim JK, Yang IS, Chung MS, Kim KH (2006) Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (Camellia sinensis). J Agric Food Chem 54: 8717-8723.
25. Lee JH, Shim JS, Chung MS, Lim ST, Kim KH (2009) In vitro anti-adhesive activity of green tea extract against pathogen adhesion. Phytother Res 23: 460-466.
26. Handzlik J, Matys A, Kieć-Kononowicz K (2013) Recent Advances in Multi-Drug Resistance (MDR) Efflux Pump Inhibitors of Gram-Positive Bacteria S. aureus. Antibiotics 2: 28-45.

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