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RESEARCH ARTICLE

Antibacterial Efficiency of Quaternary Ammonium Silane-Coated Shoe Insoles Using the Sol-Gel Technique

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Abstract

Objective: Foot microbial infections are a common concern, particularly among individuals with diabetes, athletes, and those with compromised immune systems. Traditional methods for managing these infections include topical treatments and oral antibiotics. Diabetes mellitus patients, sportsmen and women, postoperative foot/ankle patients, and people who wear shoes for longer periods require extra attention due to the increased risk of microbial infection. The microenvironment of footwear, including its humidity, temperature, and aeration, supports the growth of pathogenic or opportunistic microorganisms. However, these approaches can not provide continuous protection against emerging infectious diseases and can not prevent antibiotic-resistant infections. Antimicrobial-incorporated shoe insoles present a novel and promising solution for preventing and managing foot microbial infections through their sustained release, which ensures long-term antimicrobial activity and reduces the risk of infection recurrence. These insoles are embedded with antimicrobial agents, like silver nanoparticles, copper ions, or organic antimicrobial compounds that provide continuous protection against a broad spectrum of microorganisms (bacteria, fungi and viruses). The occurrence of common foot infections like athlete's foot, fungal nail infections, and bacterial infections associated with diabetic foot ulcers can also be mitigated.

Materials and Methods: This study was conducted to examine the possibility and efficacy of the application of antibacterial quaternary ammonium silane compound-treated shoe insole materials in the mitigation of foot microbial infections. EN ISO 20645:2004, AATCC 147-2019, ISO 16187:2013, and fluorescence staining (CTC/DAPI) test methods were used in this study to analyze the antibacterial efficacy of the tested insole foam against *Klebsiella pneumoniae, Escherichia coli*, and *Staphylococcus aureus*.

Results: Antimic®[(3-(trimethoxysilyl)-propyl, cocodimethylammonium chloride] compound-incorporated shoe insoles resulted in total bacteria reduction (92-96%) after 24-hour contact time, without triggering a viable but non-culturable (VBNC) state in the tested bacteria.

Conclusion: Antimic® compound-incorporated shoe insoles represent a significant advancement in the prevention and management of foot microbial infections, providing a continuous, effective, and convenient method for maintaining good foot health.

Keywords: Shoe insoles, antibacterial activity, foot, footwear, functional materials

Introduction

Shoes, like most footwear, protect the feet and legs against various risks that would otherwise be detrimental to the person. The morbidity of disorders related to foot and ankle skin infections is influenced by the quality of footwear (Hsu & Hsu, 2012). Therefore, foot infections are common, although their frequency differs according to the various conditions. Foot and footwear hygiene is an important aspect whose negligence affects a variety of shoe wearers (Messina *et al*., 2015; Nguyen *et al*., 2023).

Patients with diabetes mellitus need extra attention due to the increased risk of developing diabetic foot infections (Li *et al*., 2011). A diabetic foot infection in patients with diabetes refers to a bone or soft tissue infection mostly due to peripheral arterial disease or neuropathy leading to a lack of sensation in the legs. Blood flow is also affected by diabetes, leading to longer healing times for sores, cuts, and wounds, subsequently leading to the development of ulcers or tissue death due to blood inaccessibility. Forming foot ulcers in patients with diabetes predispose them to foot infection, leading to polymicrobial foot infections. Microorganisms like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus anthracis*, and *Bacillus thuringiensis* (Li *et al*., 2011; Matheson *et al*., 2021; Steglińska *et al*., 2019) are among the species isolated from shoes.

Sportsmen and women are also affected by foot infections due to the nature and vigorous nature of sports, which lead to foot sweating and an increased risk of leg injuries. A study in some European countries (spring 1997-1998) found that 50% of participants had foot disease, whereas 66.7% had superficial fungal infections (Caputo *et al*., 2001). However, children were largely affected compared with the other age groups.

The importance of the feet and legs to the body in carrying and sustaining body weight necessitates hygienically and ergonomically appropriate shoes to avoid foot and leg injuries or worsening of already wounded feet. The presence of wounds on the foot, such as foot cuts and abrasions, blisters that could burst, ulcers, chronic wounds, and punctures caused by sharp objects, increases the susceptibility of the foot to infection by microorganisms. The incidence and morbidity of shoe and insole foot infections are influenced by the presence of sweat/moisture, improper foot wound hygiene, weakened immune system, and environmental exposure (e.g. walking barefooted) (Hsu & Hsu, 2012).

Shoes and shoe insoles are conducive environments for microbial growth due to the favorable humidity/moisture that is provided by sweat and the warmth created during wear. Thus, proper disinfection is paramount to mitigate infections by foot pathogens. Being reservoirs of different microorganisms, frequent cleaning of shoe insoles reduces the microbial load on the insole (Messina *et al*., 2015).

Antimicrobial components whose effects on microorganisms are persistent for extended periods (Akpınar *et al*., 2024) would be suitable for application on shoe insoles. This antimicrobial application method can help to readily eliminate microorganisms during the initial microbial attachment to shoe insoles, thus reducing the possibility of persistent colonization and biofilm formation. Regular sanitization of infected shoes, textiles, and socks is important for preventing infection (Gupta *et al*., 2022), re-infection, and cross-infection of footwear or mutual users. Footwear sanitization approaches used so far have involved the use of formaldehyde fumigation, foot powder application, silver-light irradiation, ozone application, and UV irradiation (Gupta & Versteeg, 2019).

The use of topical, oral, or combined topical and oral treatments for foot infections is an important strategy for mitigating foot infections. Factors like the severity of the foot infection, previous antibiotic response (Kimiran Erdem *et al*., 2007), and local antimicrobial sensitivity should be considered. Mild infections might require oral or topical antibiotic therapy, whereas severe infections might require intravenous antibiotic therapy (Matheson *et al.*, 2021).

The use of antimicrobial-treated shoe insoles and other footwear alongside oral or topical antimicrobial treatment for foot wounds and infections could be a promising strategy in foot infection management. However, deep infections require debridement of dead tissue from the wound before antimicrobial treatment (Bengalorkar, 2011).

In our study, it was aimed to evaluate the antibacterial effect of shoe insoles treated with quaternary ammonium silane [Antimic®] compound using the sol-gel technique. The antibacterial activity of the produced insoles was evaluated using qualitative and quantitative methods against *Staphylococcus aureus, Klebsiella pneumoniae*, and *Escherichia coli* bacteria selected as representative organisms.

Materials and Methods

The footwear products used in this study were made of foam and used in shoe insoles. Antimicrobial Antimic® compound was incorporated into insole materials using the sol-gel technique.

Since the insole material was porous, slightly modified qualitative AATCC 147-2019 and semi-quantitative EN ISO 20645:2004 methods were used in this study. ISO 16187:2013 test and fluorescence staining methods were used for quantitative evaluation. *Staphylococcus aureus, K. pneumoniae,* and *E. coli* bacteria were used as representative organisms.

Testing Antibacterial Activity of Samples

1. AATCC 147-2019 Test Method **(Antibacterial Activity of Textile Materials: Parallel Streak Method)**

The AATCC 147-2019 method used in this study was used to qualitatively determine the bacteriostatic and antibacterial activity of the samples.

Footwear samples, antibacterial-treated (test sample) and untreated (control sample), were prepared in a rectangular shape with dimensions of 25 × 50 mm using sterile forceps, scissors, scalpel, and mold. Suspensions of test organisms were prepared individually by adding 1.0 ± 0.1 ml of the 24-hour liquid bacterial culture to a sterile test tube containing 9.0 ± 0.1 ml of sterile water.

A loopful of the bacterial inoculum was taken with a 4 mm diameter loop and 5 lines were marked on the surface of the sterile agar plate. The lines were prepared in the center of the Petri dish, approximately 60 mm long, with a 10 mm gap between them. During the line inoculations, bacterial culture samples were collected only once with a loop, and care was taken to ensure that there was no damage or tear to the plate surface.

Sterile insole test and control samples were placed transversely to the 5 lines of the inoculated bacteria, ensuring full contact of the samples with the inoculated bacterial lines. Sterile glass coupons were then placed on the samples to prevent them from curling during incubation and contact with the test organism. Petri plates were incubated at 37 ± 2°C for 18-24 hours.

At the end of the incubation period, Petri plates were evaluated for the presence of interruptions (inhibition zones) in the bacterial inoculum lines and a clear growth inhibition zone around the edges of the sample (AATCC, 2019). The inhibition zone formed along an inoculum line on one side of the tested sample was calculated using the following formula:

$$W = \frac{T - D}{2}$$

(W: Inhibition zone- mm, T: Sum of sample and inhibition zone- mm, D: Test sample- mm)

The size of the inhibition zone was not used to quantitatively evaluate antibacterial activity, however, the presence of the inhibition zone and the absence of bacterial growth under the sample indicated the presence of antimicrobial activity in the test sample. For acceptable antibacterial activity, no bacterial colonies should be present below the sample at the contact area (AATCC 147-2019).

2. EN ISO 20645:2004 Test Method **(Textile Fabrics - Determination of Antimicrobial Activity - Agar Diffusion Plate Test Method)**

The EN ISO 20645:2004 standard allowed the semi-quantitative determination of antibacterial activity in samples. Antibacterial-treated test samples and untreated control samples were prepared in circular forms with 25 ± 5 mm diameters using sterile forceps, scissors, a scalpel, and a mold. Suspensions of the test organisms were prepared by transferring the 24 hour bacterial culture prepared from the lyophilized bacterial strain to the solid medium.

In this test, the samples were placed between two agar layers, enabling analysis of both sides of the samples. Although the lower agar layer did not contain bacteria, the upper layer was inoculated with the selected test bacteria. The lower layer contained 10 ± 0.1 ml of nutrient agar poured onto the Petri dish. However, the upper layer contained a mixture of 1 ml of the bacterial suspension (1.5×10^8 cfu (colony forming unit)/ml) and nutrient agar, which was mixed with bacteria in the molten form at 45˚C before solidifying. 5 ml of agar solution containing bacteria was poured onto the samples that were placed with sterile forceps on the lower agar layer. After solidifying the upper agar layer, the Petri dishes were incubated at 37 ± 2°C for 18-24 hours. Both test and control samples were evaluated after the required incubation period for the presence of a clear growth or inhibition zone around the edges of the sample and growth below the sample (ISO 20645: 2004; Kimiran Erdem & Sanli Yurudu, 2008).

The inhibition zone formed around the tested sample was calculated using the following formula:

$$H = \frac{(D - d)}{2}$$

(H: Inhibition zone- mm, D: Sum of sample and inhibition zone- mm, d: Test sample- mm).

After measuring the inhibition zone, the samples were lifted using sterile forceps to evaluate the presence of growth below the sample. Evaluation was performed according to Table 1:

Table 1. EN ISO 20645:2004 test method evaluation criteria (ISO 20645:2004).

Inhibition zone (mm)	Growth[a]	Evaluation
> 1	No growth	Good Effect
1-0	No growth	
0	No growth	
0	Weak growth	Limited Effect
0	Mild growth	Insufficient Effect
0	Intensense growth	

[a]Growth under the sample

3. ISO 16187:2013 (Footwear and footwear components — Test method to assess antibacterial activity) Test Method

A quantitative static challenge method (ISO 16187:2013) was used to determine the percentage reduction in the test bacterial load of footwear and its components. The study involved samples with dimensions of 25 × 25 × 1 mm for both the Antimic® compound-treated test samples and control samples. Each sample was placed in a sterile glass flask, followed by the addition of 1 ml of a 5.0×10^5 cfu/ml bacterial concentration on each sample and incubation for specific contact times. 1- and 24-hour contact times were used in this study. At the end of the contact times, test and control samples were collected, and 20 ml of soybean casein lecithin polysorbate (SCDLP) neutralizing medium was added to each sample, and the sample was incubated for 5 minutes. Followed by ten-fold serial dilution with a sterile 0.85% NaCl aqueous solution. 100 µl of each diluted bacterial solution was spread out and cultured on tryptic soy agar (TSA, Oxoid) plates. The surviving bacterial colonies on the plates were enumerated after a 24-hour incubation at 37°C (ISO 16187:2013). The percentage reduction in bacterial counts was measured using the following equation:

$$R(\%) = \frac{C - T}{C} x100$$

where R is the bacterial percentage reduction, and C and T are the average number of colonies of three control (C) samples and three test (T) samples after 1- and 24- hour contact times, respectively, cfu/ml.

4. Fluorescence staining (CTC/DAPI) Test Method

This method was used to determine whether the bacteria died from the effect of the antibacterial agent. The method is based on the use of fluorescent dyes that allow the differentiation of dead and living organisms, and samples are examined under an epifluorescence microscope. This study assessed the number of actively respiring bacteria in comparison with the total number of bacteria after a contact time of applying the tested bacteria to both antimicrobial-treated (test) and untreated (control) footwear samples.

In this test, 5-Cyano-2,3-ditolyltetrazolium chloride (CTC) was used as the indicator of bacteria (living bacteria) with active electron transport systems (ETS). This staining was followed by 4′,6-diamidino-2-phenylindole (DAPI) staining, which was used to indicate the total number of cells present by staining the DNA of all cells present. Samples were then filtered using black polycarbonate membranes and observed under an epifluorescence microscope (Sanli Yurudu & Kimiran, 2015).

Bacterial percentage reduction was measured using the following equation:

$$\text{Percentage Reduction (\%)} = \frac{\text{Live Cell Count of the Control Sample} - \text{Live Cell Count of the Experiment Sample}}{\text{Live Cell Count of the Control Sample}} \text{x100}$$

Results

Results of qualitative antibacterial activity tests are presented in Tables 2 and 3.

AATCC 147 standard demonstrates bacteriostatic activity via the diffusion of an antibacterial agent through agar. According to this test method, the antibacterial compound-treated material should be compared with the untreated corresponding material, and for acceptable antibacterial activity, there must be no bacterial growth under the treated material. Antimic®-treated shoe insole samples exhibit acceptable antibacterial effects against *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 4352, and *E. coli* ATCC 11229 bacteria (Table 2).

Table 2. Antibacterial efficiency of Antimic®-treated shoe insole foam according to AATCC 147-2019 standard test method.

Samples	*S. aureus* ATCC 6538	*K. pneumoniae* ATCC 4352	*E. coli* ATCC 11229
Antimic®-treated shoe insole	0 mm and No growth below sample[b]	0 mm and No growth below sample[b]	0 mm and No growth below sample[b]
Untreated insole (Control)	No zone, Intense growth[b]	No zone, Intense growth[b]	No zone, Intense growth[b]

[a] Acceptable antibacterial effect, [b] No Antibacterial effect

Similar results were obtained using the semi-quantitative EN ISO 20645:2004 standard test method for shoe insoles (Table 3). According to this method, the insoles exhibited good antibacterial activity against representative bacteria.

Table 3. Antibacterial efficiency of Antimic®-treated shoe insole foam according to EN ISO 20645:2004 standard test method.

Samples	*S. aureus* ATCC 6538	*K. pneumoniae* ATCC 4352	*E. coli* ATCC 11229
Antimic®-treated shoe insole	0 mm and No growth below sample[1]	0 mm and No growth below sample[1]	0 mm and No growth below sample[1]
Untreated insole (Control)	0 mm and Intense growth[0]	0 mm and Intense growth[0]	0 mm and Intense growth[0]

[1] Good effect, [0] No effect

Quantitative and semi-quantitative tests indicated that the compound diffused poorly in the agar medium. However, it was determined that there was intense growth below the samples of the control group, whereas it was observed that the lower part of the treated insole samples inhibited bacterial growth by creating a barrier effect.

Results of quantitative antibacterial activity tests performed against Gram-positive and Gram-negative representative bacteria are presented in Table 4. Under static conditions, the bactericidal rates of the antibacterial shoe insole samples against *S. aureus*, *K. pneumoniae*, and *E. coli* after 24-hour contact times were 96.59%, 95.77%, and 92.87, respectively (Table 4).

Table 5 presents the results of the antibacterial activity of the insole samples according to fluorescence staining. This method, which is used to detect dead and viable microorganisms when evaluated simultaneously with colony counting, also allows the detection of live but non-culturable forms (VBNC) that bacteria use as a survival strategy. As shown in Figure 1 (a-f), which includes representative fluorescence microscopy images, live cells are shown in red; dead cells are shown in blue. Antimic® (3-(trimethoxysilyl)-propyl, cocodimethylammonium chloride) compound-incorporated shoe insoles reduced live bacteria by 94.97%, 92.00%, and 91.15% for *S. aureus*, *K. pneumoniae*, and *E. coli* after 24-hour contact times, respectively (Table 5). When evaluated together with the quantitative culture method, it was determined that the Antimic® shoe insole did not trigger the VBNC status of the tested bacteria (Table 5, Fig. 1).

Table 5. Antibacterial activity results of the Antimic®-treated shoe insole foams after 1- and 24-hour contact time according to fluorescence staining test.

Samples	Bacteria	Contact Time (h)	Total Cell Count	Live Cell Count	Percent Reduction (%)
Antimic® Treated Foam	*S. aureus*	**1**	4.09×10^5	1.89×10^5	**50.13**
		24	3.26×10^7	1.18×10^6	**94.97**
	K. pneumoniae	**1**	3.61×10^5	1.90×10^5	**43.95**
		24	4.09×10^7	2.28×10^6	**92.00**
	E. coli	**1**	3.08×10^5	1.75×10^5	**39.02**
		24	3.74×10^7	2.76×10^6	**91.15**
Control (untreated) Foam	*S. aureus*	**1**	3.81×10^5	3.79×10^5	-
		24	2.44×10^7	2.35×10^7	-
	K. pneumoniae	**1**	3.47×10^5	3.39×10^5	-
		24	3.01×10^7	2.85×10^7	-
	E. coli	**1**	2.98×10^5	2.87×10^5	-
		24	3.28×10^7	3.12×10^7	-

Discussion

This study demonstrated the distinctive action of antimicrobial-incorporated footwear components (insoles) in decreasing the bacterial load on the test substances. The qualitative test (EN ISO 20645:2004) revealed the considerate action of Antimic®-treated shoe insole samples against the tested bacteria, with the inhibition of bacterial growth below the tested samples and

Table 4. Antibacterial activity results of the Antimic®-treated shoe insole foams after 1- and 24-hour contact time, according to the ISO 16187:2013 standard test method.

Samples	*S. aureus* ATCC 6538		*K. pneumoniae* ATCC 4352		*E. coli* ATCC 11229	
Contact times (h)	**1**	**24**	**1**	**24**	**1**	**24**
Control	3.77×10^5	2.23×10^7	3.23×10^5	2.65×10^7	2.79×10^5	3.06×10^7
Antimic® Treated Insole	1.78×10^5	7.6×10^5	1.73×10^5	1.12×10^6	1.65×10^5	2.18×10^6
Percent Reduction (%)	52.78	96.59	46.43	95.77	40.86	92.87

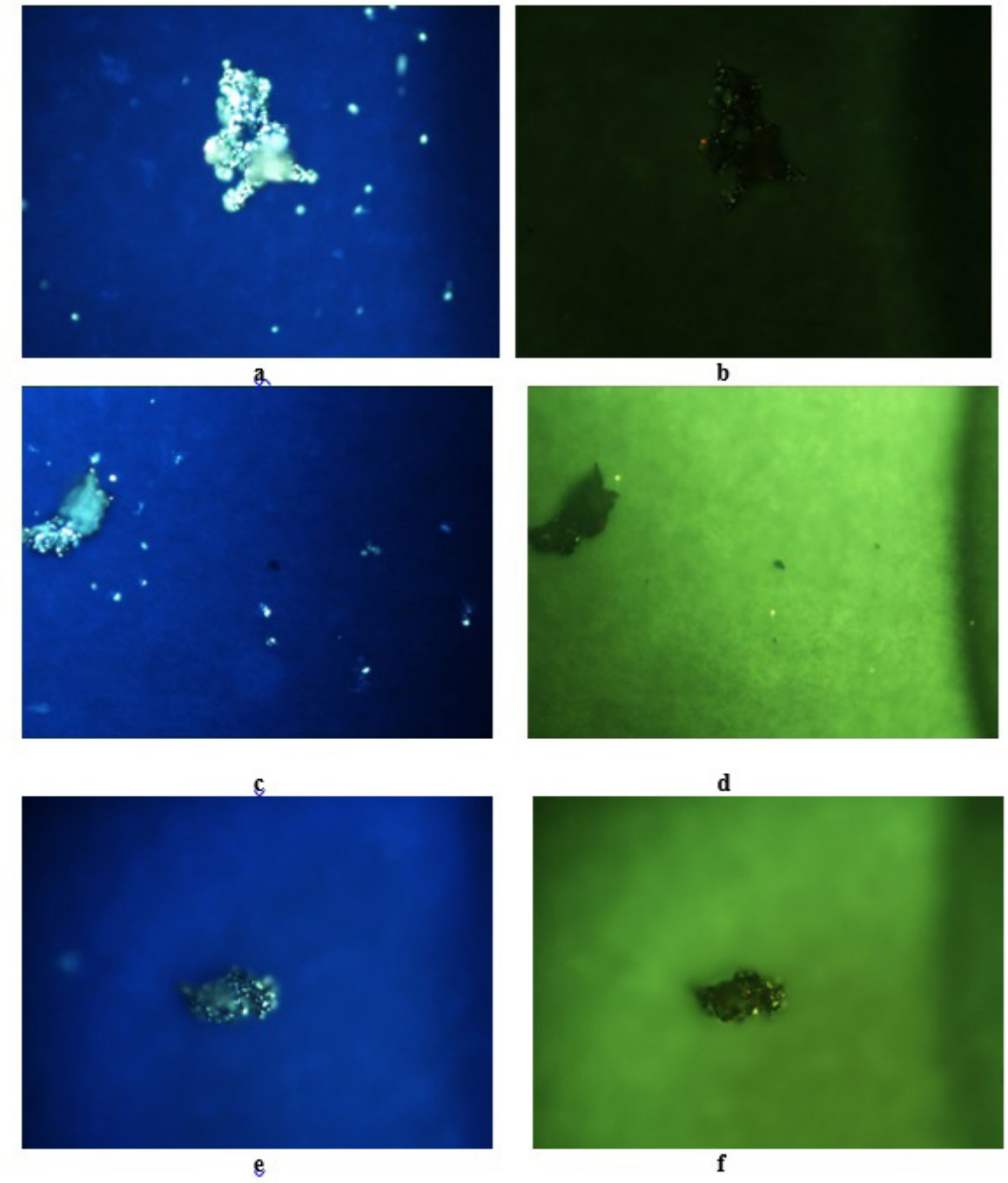

Figure 1. Representative photographs obtained in the evaluation of the antibacterial activity of insole samples according to the fluorescent staining method. a. *S. aureus* DAPI staining, b. *S. aureus* CTC staining; c. *K. pneumoniae* DAPI staining, d. *K. pneumoniae* CTC staining; e. *E. coli* DAPI staining, f. *E. coli* CTC staining.

no significant zone was observed. However, the quantitative test (ISO 16187:2013) revealed high percentage reductions in the bacterial loads of *S. aureus*, *K. pneumonia*, *and E. coli in* treated insole samples by 96.59%, 95.77%, and 92.87%, respectively after a 24-hour contact time.

Fluorescent staining (DAPI-CTC) of treated foam samples showed a significant decrease in the viable cell counts of *K. pneumonia*, *E. coli,* and *S. aureus* by 92.00%, 91.15%, and 94.97% respectively after a 24-hour contact time.

Modern footwear materials should have properties like the ability to withstand abrasion, wear, humidity, and temperature variations. Novel antimicrobial footwear technologies, including nanotechnologies and microencapsulation processes, can change the challenges met by wearing various types of footwear and the different

materials used to manufacture them. A previous study indicated that casual shoes provide the most conducive bacterial growth conditions, followed by running and perforated shoes (Miao *et al*., 2021). This would have been due to aeration leading to the ability of the shoe to maintain or get rid of excess moisture and temperature, which facilitate bacteria growth. A different study observed that leather shoes harbored microorganisms, including fungi, whereas shoe dampness increased the risk of microbial growth. However, regular dehydration and the use of antimicrobials can mitigate foot infections (Kenneth *et al*., 2017). The applications of this study would therefore be of importance if used in various footwear like insole materials like leather whose nature would otherwise encourage microbial growth and colonization. A study by Vo *et al*., (2020) investigated shoe insoles coated with copper oxide-zinc oxide (CuO-ZnO) nanocomposites and demonstrated their antibacterial effects against *E. coli*, *Bacillus cereus*, *S. aureus*, *Salmonella* spp., and *Pseudomonas aeruginosa*. This was in accordance with our study in which a different antimicrobial was used. However, in their study, the antibacterial activity of the shoe insole samples was remarkably reduced when they were soaked and washed with a rubbing action (Vo *et al*., 2020). This demonstrates the limitations involved in the use of antimicrobial-treated insole products, including the wearing of materials due to the continuous rubbing of the feet during movement (Vo *et al*., 2020). Moreover, the continuous washing of footwear with water and soap would reduce the efficacy of some insole-incorporated antimicrobial products (Vo *et al*., 2020). Therefore, it is important to replace insole footwear after a specific period or to perform repeated treatment of the same insole material with antimicrobial products to achieve the required antimicrobial effect against shoe insole-related microorganisms.

In a similar study by Lu *et al*., (2018), using the antimicrobial nanotechnology compound Bio-Kil, treated socks showed significant reductions in the *S. aureus* and *E. coli* bacterial counts after a 0-, 8-, and 48-hour contact time compared with untreated samples. It was observed that the Bio-Kil-treated socks from diabetic patients and healthy individuals showed significant reductions in bacterial counts compared with untreated samples after a 40-hour contact time (Lu *et al*., 2018). Our study, in accordance with Lu *et al*., (2018), recognized the possibility of the use of insole-incorporated antimicrobial components and other footwear components to prevent microbial colonization of insole footwear. Additionally, they can help eliminate microorganisms in diabetic and other foot wounds, including post-surgical foot or ankle wounds (Lu *et al*., 2018). Leather shoes have good water-absorbing properties, contrary to most athletic shoes made from synthetic materials. The poor water absorption of shoes encourages the accumulation of moisture from the perspiring feet, thereby creating favorable growth environments for microorganisms that lead to the pathogenic invasion of the foot skin (Gnanasundaram *et al*., 2013). Therefore, modified antimicrobial insole materials are a desirable choice for mitigating foot microbial infections (Gnanasundaram *et al*., 2013). The continuous antimicrobial activity of the treated shoe insoles can help eliminate bad shoe odors caused by the activities of certain bacteria (Messina *et al*., 2015).

On the other hand, our study focused only on some bacteria and did not examine the effect of our shoes with antimicrobial insoles against fungi. Therefore, this study provides insight for further studies on the antifungal activities of shoe insoles and foam.

Similar to our study, Sanchez-Navarro *et al.* (2013) found silver nanoparticles (AgNPs) and silver (AgNPs) @silica nanocomposites treated insole leather materials to have significant antimicrobial effects against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *K. pneumoniae*) bacteria using the agar diffusion method (Sanchez-Navarro *et al*. 2013).

Similar to Sanchez-Navarro *et al*. (2013), TiN-Ag antimicrobial coating of leather by Marques *et al.* (2022) showed great antimicrobial activity with >2 log reductions according to the Japanese Industrial Standard Z 2801:2000 (JIS Z 2801:2000) when tested against *Candida parapsilosis*, *Trichophyton mentagrophytes*, *S. aureus*, and *E. coli*. This was in accordance with our study in which the antibacterial activity of the Antimic®-treated shoe insole foams showed higher percentage reductions of tested bacteria (96.59%, 95.77%, and 92.87% for *S. aureus*, *K. pneumonia*, *E. coli* respectively) using the ISO 16187:2013 test method.

Based on the results of the study, using the Antimic®-treated shoe insole foams can prevent cross-contamination and foot microbial infections by acting as a suggestive alternative with strong antibacterial properties. The compounds used in this study have a quaternary ammonium structure and contain silane groups. The quaternary region of the compound neutralizes microorganisms by affecting the cell membrane, whereas the silane groups ensure that the chemical is retained on the surface. The compound (Antimic®) used in our study has important advantages by its nature: i) as being easily applied uniformly to a variety of materials like fabrics, plastics, and synthetic fibers; ii)

not creating any toxicity risks in long-term exposure since it does not release particles that are toxic to the environment and humans, iii) the immobilization and regeneration of the compound on applied material. These properties may be highly desirable for possible applications in materials science and healthcare, where the compound's ability to pass through biological membranes and other barriers is needed.

Conclusion

Antimic® compound-incorporated shoe insole products in this study exhibited significant antibacterial activity against the selected bacteria, with acceptable percentage reductions in the bacterial loads on test surfaces. This approach represents a significant advancement in the prevention and management of foot microbial infections, providing a continuous, effective, and convenient method for maintaining good foot health.

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