International Journal of Innovation Sciences and Research
Vol.4, No, 3, pp. 120-123 March- 2015

Available online at
http://www.ijisr.com

RESEARCH ARTICLE

INTERACTION OF SILVER NITRATE WITH COMMERCIALLY AVAILABLE COTTON SOCKS; RELATIONSHIP TO THE ANTIBACTERIAL ACTION OF SILVER IONS

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Accepted 21st February, 2015; Published Online 31st March, 2015

ABSTRACT

Silver nitrate (10^{-3}M) coated cotton socks significantly reduced the bacterial load of the socks flora among the medical students. The bacterial load is directly proportional to number of days and inversely proportional to the concentration of silver nitrate. *Pseudomonas* species was isolated from four samples in day 3. Other samples showed significant colonies of ASBs at low concentration on third day which was statistically significant. This study reported that 10^{-3}M silver nitrate solution coated cotton socks were very effective and having bacterial isolates rarely. The strength of the study are rigid criteria with standard protocol adopted to select subjects and concentrations of silver nitrate, completion of study among all subjects without dropout and good laboratory practices adopted to process the samples.

Key Words: Silver Nitrate, 10^{-3}M, Cotton Socks, Antibacterial Efficacy

INTRODUCTION

Shoes and Shocks have become inevitable accessories of male grooming due to culture and socio economic advancements. Bachelors and students especially hostellers do not wash their socks regularly at an interval of at least three to seven days. Moreover hostel students share socks among themselves (Lakshika *et al.,* 2014). Some students even though they wash their socks regularly, they are forced to wear wet socks due to lack of time to dry them. Wet and Dirty socks are colonized by micro-organisms. Sweat also provides a medium for the growth and multiplication of microorganisms on wearing socks repeatedly (Katsutoshi *et al.,* 2006; Kanlayavattanakul and Lourith, 2011). This causes bad odor, rashes, itchy skin, Athlete's foot, fungal and bacterial infections on foot are common among these people (Tanaka *et al.,* 2006). These infections increase the severity of puncture wound and cut injuries. In diabetic patients, small cut injuries may become a diabetic foot ulcer due to the habit of wearing dirty socks. The key reason for foot odor and bacterial colonization is foot sweat. Sweat itself is neutral in aroma, but it creates a beneficial environment for certain bacteria to grow and produce horrific stinking substances (Kanlayavattanakul and Lourith, 2011).

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These bacteria are naturally present on our skin as part of the human flora (though exponentially more smell is created when closed toe shoes are worn). The front part of the foot is where it produces the most sweat (Katsutoshi *et al.,* 2006). Therefore, more stink is created with factors causing more sweating, such as wearing closed toe shoes for many hours. Socks generally do not cause foot odor on their own, but when worn along with shoes, socks can help to trap the hair on the feet, especially on the toes, may contribute to the odor's concentration by adding increased exterior area in which the bacteria can flourish. Wearing various socks (cotton socks, nylon stockings and wool socks), showed variance in the bacterial colonization (Watanabe *et al.,* 2000).

The mechanism of the antibacterial action of silver ions is closely related to their interaction with thiol groups (Fuhrmann and Rothstein, 1968), although other target sites remain a possibility (Liau *et al.,* 1997). This principle induced to take this concern and prove the effectiveness of silver nitrate soaked with commercial socks for improving the shelf life. Some reports published by the same group of authors with small test samples. Therefore, the present study was undertaken to analyze the antibacterial effect of silver nitrate coated commercial cotton socks that effective against skin flora.

MATERIALS AND METHODS

After getting prior consent and institutional ethical clearance, ten medical students with the habit of wearing shoes regularly were selected as a study group. They were explained about the proposed work. The study group was instructed not to walk with bare socks foot or wash their socks during the study period. Swabs were taken from their socks on the 0th and 3rd day. New cotton socks were washed with double distilled water and then dipped in 10^{-3}M silver nitrate solution and dried in shade. The same study group was instructed to wear it and similarly, swabs were taken on the 0^{th} and 3rd day. The same procedure was repeated with the same study group using 10^{-4}, 10^{-5} and 10^{-6}M silver nitrate impregnated socks subsequently at weekly intervals.

The presence of microbes was quantitatively and qualitatively estimated by microbial culture methods. The effect, shelf-life, hygiene of socks were determined by colony count versus time. The microbial load and hygienic practices among the subjects included and compared. The major implication of this study was helping us to improve our knowledge of bacteriological status of socks, knowing the usefulness of silver impregnated socks, minimizing microbial carriage and may pave way for further studies for the doctors and nurses. This preliminary study was helped us to improve foot hygiene. Further, Antibiotic sensitivity was performed on Muller-Hinton agar using Kirby-Bauer disk diffusion technique.

RESULTS

As a result, very less number of colonies was observed in blood agar (BA), MacConkey agar (MA) and nutrient agar (NA) at 0^{th} day. Whereas, in the end of day 1, day 2 and day 3, more than 300 colonies (too numerous to count-TNTC) were observed and recorded. An interesting observation was noted that from samples 2,3,5,6 and 10, colonies of *Pseudomonas* were grown on all the three days. The number of samples showed the growth of bacteria before and after exposure to silver nitrate was depicted in Figure 1.

This study was to identify the antibacterial potential of various concentrations of silver nitrate which are dispensed in the cotton socks. Meanwhile, the antibiotic sensitivity pattern was also performed. The swabs taken from the cotton socks dipped in the silver nitrate solution in the concentration of 10^{-3}M showed no growth (too less to count-TLTC) on day 0, day 1 and day 2 in BA, MA and NA. Whereas on the day 3, colonies were observed in four samples (sample no. 3, 5, 8 and 10). Overall, in the sample no. 3 and 5 the colonies of *Pseudomonas* were identified in the day 3 sample. Simultaneously, 10^{-4}M concentration showed no growth (TLTC) on day 0 and day 1 in BA, MA and NA. On the day 2, the colonies of *Pseudomonas* were observed in the sample no 2. On the day 3, all the samples showed more than 300 colonies (TNTC). Colonies of *Pseudomonas* were identified in the sample no 2, 3 and 5. Similarly, in concentration 10^{-5}M silver nitrate solution showed no growth (TLTC) on the day 0 and day 1 in BA, MA and NA. More than 300 colonies (TNTC) were identified on day 2 and 3. The colonies of *Pseudomonas* were identified in the sample no 2 and 10 on day 2 while the sample no 3 and 5 also showed the growth of *Pseudomonas* on day 3. The detailed observations of the bacterial growth from the cotton socks dipped in various concentrations of silver nitrate solution are depicted in Table 1. The attractive remarks in the study were the growth of *Pseudomonas* and aerobic spore forming bacteria (ASB). In the sample 3 and 5, the *Pseudomonas* growth was profuse in the day 3 of all the concentrations.

In the day 2 and 3, along with the sample no 3 and 5, sample no 2 and 10 showed *Pseudomonas* growth. Hence, this analysis proved that the *Pseudomonas* is highly resistant to silver nitrate. In the concentration 10^{-3}M and 10^{-4}M, the growth was observed on the day 3. Further, 10^{-3}M silver nitrate solution controlled the bacterial growth in six subjects. From this study, we concluded that 10^{-3}M silver nitrate concentration have wide antibacterial activity than the lowest concentrations. All the *Pseudomonas* isolated in the investigation were subjected to antimicrobial susceptibility test using series of 14 antibiotics including amoxicillin clavulinic acid (Amc), ampicillin (Amp), azithromycin (AZM), cefixime (CFM), cefoperazone/ sulbactam (CFS), cefotaxime (CTX), ceftazidime (CAZ), ceftrioxone (CTR), ciprofloxacin (Cip), cotrimoxazole (Cot), doxycycline (Do), gentamycin (G), levofloxacin (Le) and piperacillin/tazobactam (PiT). The results of sensitivity pattern showed maximum sensitive to CFS (35mm), CTX (30mm) and PiT (30mm).

Table 1. Bacterial growth of various concentrations of silver nitrate solutions dipped socks

Sample No.	Concentration of silver nitrate vs Number of bacterial colonies in the study days												
	Control	10^{-3} silver nitrate				10^{-4} silver nitrate				10^{-5} silver nitrate			
		Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3
1	TNTC	NG	NG	NG	NG	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
2	TNTC	NG	NG	NG	NG	NG	NG	TNTC	TNTC	NG	NG	TNTC	TNTC
3	TNTC	NG	NG	NG	TNTC	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
4	TNTC	NG	NG	NG	NG	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
5	TNTC	NG	NG	NG	TNTC	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
6	TNTC	NG	NG	NG	NG	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
7	TNTC	NG	NG	NG	NG	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
8	TNTC	NG	NG	NG	TNTC	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
9	TNTC	NG	NG	NG	NG	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC
10	TNTC	NG	NG	NG	TNTC	NG	NG	NG	TNTC	NG	NG	TNTC	TNTC

[TNTC: Too numerous to count; NG: No growth]

The *Pseudomonas* showed resistance to other antibiotics listed (Fig. 2). There was a significant reduction in the bacterial load after exposed to silver nitrate solution and the difference was statistically significant ($P<0.01$).

solutions

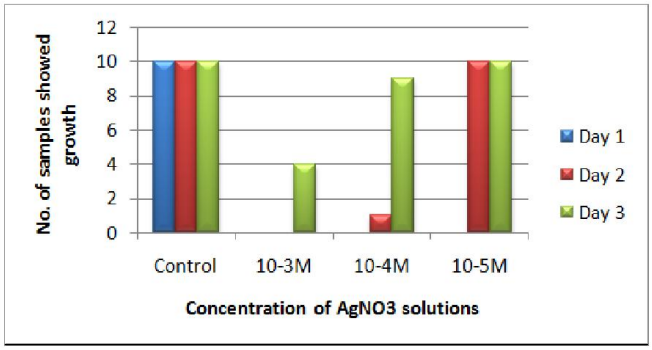

Figure 1 Number of samples showed bacterial growth vs concentration of silver nitrate solutions

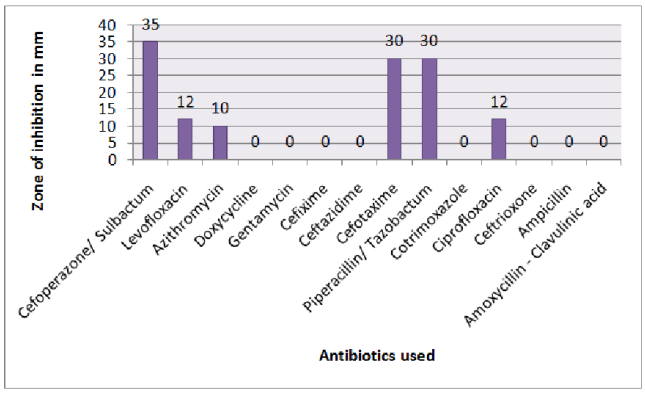

Figure 2. Antibiotic Susceptibility results of isolated *Pseudomonas* species

DISCUSSION AND CONCLUSION

This investigation was mainly intended to find out the effectiveness of silver nitrate solution in diverse concentrations infused in the cotton socks. In this study, the bacteria present in the used socks reduced consistently when the concentrations increased; whereas, the bacterial load got increased when days increased. The *Pseudomonas* was isolated as a resistant bacteria was quite interesting, observable and novel. The impregnation of silver nitrate solution in the sock's control and inhibits the growth of bacteria and act as topical antimicrobial agent (Percivala *et al.,* 2005; Prabhu *et al.,* 2014). The network of cotton fabric may provide them maximum absorption of silver nitrate and is an effective method for the preparation of antibacterial fabrics. The exploration of the importance of silver ions that enter cells complex with DNA leads to formulate effective antimicrobial dressing (Gupta *et al.,* 2008; Fox and Modak, 1994). This methodology is likely to be of significant clinical benefit due to convenient, safe and economic means of preparing antimicrobial fabrics. The problems associated with the use of silver compounds may be replaced by the formulation of silver coated fabrics (Deitch *et al.,* 1983). The reduction of bacterial count recorded in this investigation is quite interesting and no visible bacterial growth was observed. As a result, this silver based fabric formulation have been applied to a wide range of products such as burn and traumatic wound dressings, etc., (Feng *et al.,* 2000; Ansari *et al.,* 2011; Hussmann *et al.,* 2013).

This antimicrobial socks formulated using silver nitrate solution will be useful for the bachelors and the students who are staying in the hostels and mansions. In our study, the bacterial isolates after exposure to silver nitrate solutions got reduced and also found extension of shelf life for the usage of individuals who did not wash their socks regularly. As per our study to be concerned, we found the shelf life was about 3 days. However, further studies required to find out the extension of the days. The major implications of this study are silver nitrate (10^{-3}M) coated cotton socks significantly reduced the bacterial load of the socks flora among the medical students.

The bacterial load is directly proportional to number of days and inversely proportional to the concentration of silver nitrate. *Pseudomonas* species was isolated from four samples in day 3, other samples showed significant colonies of ASBs at low concentration on third day which was statistically significant and this study reported that 10^{-3}M silver nitrate solution coated cotton socks were very effective and having bacterial isolates rarely. Further study required to identify the newer antimicrobial socks for various patients more prone for infections including burns, leprosy, diabetic food, immune compromised cases etc.

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