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Original Article

Effect of monodispersed silver ions in cotton socks induces antimicrobial activity

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ABSTRACT

The developments of antimicrobial agents are extensively applicable in the health care settings by which the environmental, personal and patient care has been successful. Among the antimicrobial metal salts, silver has been explored for long years with its wide microbicidal effect. The main objective of this study is to understand the in vitro antimicrobial activity of the microbes especially bacteria with the formulation of inexpensive products including against antibiotic resistant strains. The *in vitro* analysis of the impending efficiency of a silver impregnated cotton socks as an antimicrobial agent was evaluated in this study. Silver nitrate (10^{-3}mM) dispensed cotton socks significantly reduced the bacterial load of the socks flora among the subjects included. The bacterial load is directly proportional to number of days and inversely proportional to the concentration of silver nitrate. *Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas* species were isolated. As a result, we observed the observable microbicidal effect of silver ions against the bacterial isolates including Methicillin resistant *Staphylococcus aureus* isolated from post operative wound cases.

Keywords: Monodispersion, silver nitrate, bacterial isolates.

INTRODUCTION

Traditionally, silver ions are determined as very good antimicrobial metal agent that have broad spectrum activities [1,2,3,4]. This phenomena is well applicable to various critical and emergency care by which the patients of burn wounds and post operative wound infections. The formulation of anti-infective agents is now a current and hot topic of interest due to the emergence of multi drug resistant bacterial and fungal strains as super bugs. The pharmacological investigations of silver ions against various microorganisms are not studied much thus this investigation may provide some baseline information regarding the anti-infective textiles. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol groups [5]. The interaction of silver ions with thiol groups in enzymes and proteins play an important role in the performance of antimicrobial action against infectious agents[5,6]. Traditionally, it is indentified the direct application of silver solution over wounds provide the surface interactive mechanism by which the infection rate get reduced. Literature clearly indicated that silver, silver ions and silver based nano compounds could provide safer alternative to conventional antibiotics and antimicrobial agents which threaten the health care settings by failure in patient care with antimicrobial resistance [4,5,7,8].

Due to outbreak of various infectious diseases and it's spreading among the communities, the researchers designing newer products daily to meet out such hectic situations. By reviewing various traditional system of practice, the silver coins that maintain the shelf life of milk, water and other liquids. The important mechanism of action of silver is to shrink the cell and gets dehydrated [9]. Current days, the pharmaceutical industry searching the new, novel and effective antimicrobial products with less production cost and to serve the human kind with low cost antimicrobial substances. This may overcome the mutated infectious agents that have been emerged leads to multi organ dysfunction to multi organ failure that tend to life threatening conditions [10].

The awareness about the general sanitation, communicable disease transmission and personal care has led to the development of antimicrobial textiles to protect wearers against the personal

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hygiene and spread of bacterial and other diseases rather than to protect the quality and durability of the textiles [11]. For male grooming and business executives, shoes and socks have become necessary accessories due to socio economic advancements and business achievements. Due to heavy load of work and laziness, the bachelors and students staying in the hostels do not wash their socks regularly. Most of the time, hostel students and representatives use their used socks daily and also in some situations, they too share their socks.

An interesting observation published in The Hindu, 2007 stated even though people wash their socks regularly, they sometimes forced to wear wet socks due to lack of time to dry them. Wetness, dirtiness and sweat provide shelter for micro organisms to grow and multiply which leads to bad odour, rashes, athlete's foot, etc. In diabetic patients, small cut injuries may become a foot ulcer due to the habit of wearing unhygienic socks. This induced us to design the protocol to dispense monodispertive silver solutions and prove the efficacy of silver nitrate impregnated commercial socks. No proven reports published regarding bacteriological status of socks and microbiological studies of silver coated socks. Therefore, the present investigation was undertaking to prepare silver nitrate solution of 10^{-3}mM, to determine the effect against skin flora and also to detect the shelf life of antimicrobial socks in days.

MATERIALS AND METHODS

After getting informed consent from the participants and approval from the Institutional Ethical Committee, this work was carried out. The study was conducted in the rural teaching hospital. After explaining the study methodology, swabs were taken from the unused and used socks for three days from ten medical students who are attending clinical postings. The inclusion criteria explained to the students are to wear the cotton socks continuously for three days and should not share the socks with their friends. Students who have ulcers, skin lesions, rashes and dermatological infections in their foot and students who take antibiotics regularly were excluded from the study.
Swabs were taken from the socks of participants on day 0, 1, 2 and 3. New cotton socks were dipped in 10^{-3}mM silver nitrate solution and dried in hot air oven at 50^{o}C until get complete dry. The same study group was instructed to wear the socks and similarly, swabs will be taken on day 0, 1, 2 and 3. The presence of bacteria was quantitatively and qualitatively determined by microbial culture methods. The effect, shelf-life, hygiene of socks were determined by colony count versus time/day. The bacterial load and hygienic practices among the subjects were compared. Antibiotic sensitivity was performed on Muller-Hinton agar using Kirby-Bauer disk diffusion technique.

RESULTS AND DISCUSSION

In this investigation, swabs were collected from the socks of all the ten subjects before and after usage. As a preliminary study, the swabs from the fresh socks (non silver nitrate dispensed) were considered as control and processed bacteriologically. 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. An interesting observation was noted that from samples LPS 2, 3, 5, 6 and 10, colonies of *Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa* were grown on all the three days.

The isolates of *Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas* species were found in 1, 1 and 2 samples respectively. The specification of the isolates among the subjects included will be studied further so that the exact isolation and its determination will be determined. The detailed isolation of bacterial members was depicted in Figure 1.

The main investigation of this study is to identify the antibacterial potential of 10^{-3}mM silver nitrate which is dispensed in the cotton socks. Further, the microbiological procedures were followed as mentioned above. The antibiotic sensitivity pattern was also performed. Swabs taken from the cotton socks dipped in the silver nitrate solution in the concentration of 10^{-3}mM 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. LPS 3, 5, 8 and 10). Overall, in the sample number LPS 3 and 5, the colonies of *Pseudomonas* were identified in the day 3 sample. Further, *Staphylococcus aureus* and *Proteus mirabilis* were identified in sample 8 and 10 respectively. The number of samples showed the growth of bacteria before and after exposed to 10^{-3}M silver nitrate was depicted in Figure 2.

Interestingly, the isolates *Pseudomonas* species showed maximum resistant to various antibiotics. The effect of 10^{-3}mM silver nitrate solution controlled the bacterial growth in six subjects whereas the samples LPS 3, 5, 7 and 8 the growth of *Pseudomonas* was abundant in the day 3 that proved the *Pseudomonas* is resistant to silver nitrate. Further, antibiotic sensitive strains of

Staphylococcus aureus and *Proteus mirabilis* were isolated and these strains excluded from the antimicrobial activity of silver socks. From this study, we learnt that the concentration of 10^{-3}mM silver nitrate solution have wide bactericidal activity. Further, study required to understand the effect of silver nitrate in lowest concentrations.

All the *Pseudomonas* isolated in the study were subjected to antibiotic sensitivity pattern analysis using battery of 14 antibiotics including cefoperazone/sulbactam (CFS), levofloxacin (Le), azithromycin (AZM), doxycycline (Do), gentamycin (G), cefixime (CFM), ceftazidime (CAZ), cefotaxime (CTX), piperacillin/tazobactam (PiT), cotrimoxazole (Cot), ciprofloxacin (Cip), ceftrioxone (CTR), ampicillin (Amp) and amoxicillin clavulinic acid (Amc). The results of sensitivity pattern showed maximum sensitive to CFS (35mm), CTX (30mm) and PiT (30mm). The *Pseudomonas* showed resistance to other antibiotics was depicted in Figure 3. There was a significant reduction in the bacterial load after exposed to silver nitrate solution and the difference was statistically significant ($P<0.01$).

This study was mainly designed to find out the efficacy of silver nitrate solution in known concentration impregnated in the cotton socks. In this study, the bacteria present in the used socks consistently increased when day increases. The *Pseudomonas* was isolated as a resistant bacteria was quite interesting. The impregnation of silver nitrate solution in the socks control and inhibits the growth of bacteria and act as topical anti-microbial agent [12].

Some studies suggested the network of cotton fabric may provide maximum absorption of silver nitrate and is an effective method for the preparation of antibacterial fabrics [11]. The importance of silver ions that enter into cell complex with DNA leads to formulate effective antimicrobial dressing[13]. This methodology is likely to be of significant clinical benefit due to convenient, safe and economic means of preparing antimicrobial fabrics. 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 [2,10,14].

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 solution 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. Further, this research work is planned to continue for the identification of newer antimicrobial socks for various infections state patients including burns, leprosy etc.

Figure 1: Details of bacterial isolates

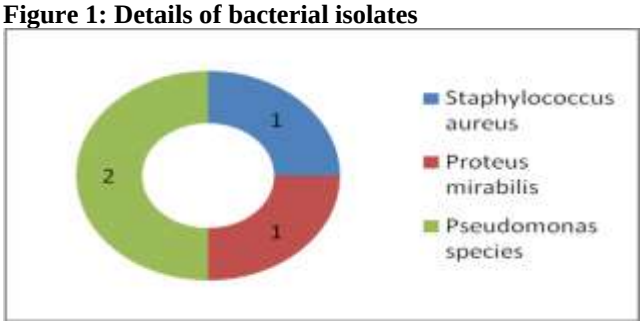


Figure 2: Samples showed bacterial growth Vs. 10^{-3}M $AgNO_3$ Solution

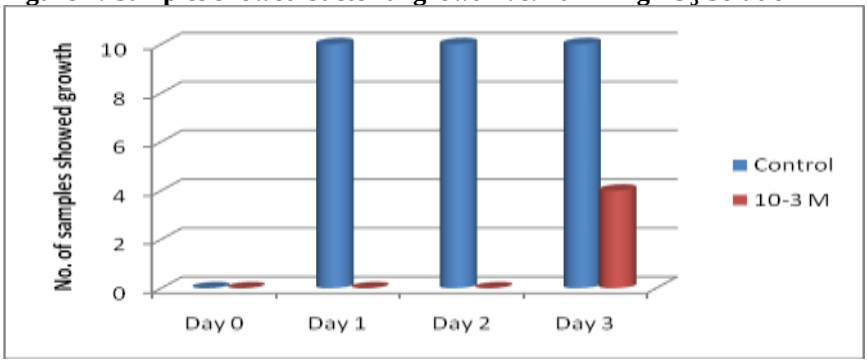
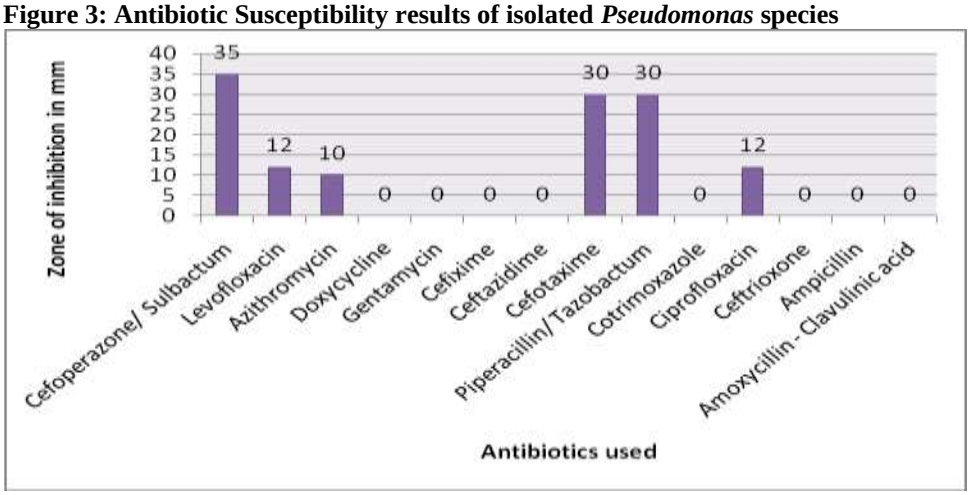


Figure 3: Antibiotic Susceptibility results of isolated *Pseudomonas* species



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