Microbiological Analysis Of Bacteria And Fungi From Socks Of School Children And Antibacterial Activity Of Herbal Foot Disinfectant Spray

Summera Rafiq, Mohammed Rafeeq Shaimah Yasin, Fatima Raja and SK. Jasmine Shahina

Abstract: A sock is an item of clothing worn on the feet and often covering the ankle or some part of the calf. The foot is among the highest producer of sweat in the body as it can produce over 0.12 of perspiration per day. Socks help to absorb this perspiration. However, this is a good environment for the growth of microorganisms including pathogenic bacteria and fungi especially in contact with the human skin which provides them with moisture, warmth and nutrients. A total of 60 samples were collected from the socks of school children and were processed, from which 64 bacteria and 38 fungi were isolated and identified. The prevalence of bacterial isolates were as follows- Staphylococcus aureus, CoNS, Klebsiella pneumoniae, Pseudomonas aeruginosa, Alcaligenes faecalis, Enterobacter aerogenes, Serratia marcescens, E. coli and Citrobacter freundii. Our study also showed the presence of gram positive rods like Bacillus polymyxa, Bacillus steareothermophilus, Bacillus firmus, Bacillus cereus and Bacillus pumilus. Among the fungi our study showed the presence of Yeasts, Rhizopus spp, Mucor, Penicillium spp, Aspergillus flavus, Aspergillus niger, Rhizoctonia solani, Aspergillus fumigatus, Fusarium oxysporum and Lichthemia corymbifera. Antibacterial activity of herbal foot disinfectant spray was performed and it exhibited bactericidal activity by producing a zone of inhibition of 17mm in size.

Keywords: Socks, Bacteria, Fungi, Foot infection, Sweat, Foot disinfectant, Hygiene.

INTRODUCTION:
Sock is a Latin word derived from the Latin “soccus”, a term which is used to describe a “light, low-heeled shoe” [1]. Due to culture and socio-economic advancements, socks have become inevitable accessories. Most number of sweat glands is found in foot than any other part of the body. Wearing shoes and socks can prevent sweat from evaporating or being absorbed and this favours the growth of both bacteria and fungi. The sweat along with the bacteria cause bad odour, leading to bromodosis (smelly feet). Sweat is colourless and odourless, but it creates a suitable environment for certain bacteria to grow and produce bad odour substances. As part of the human flora these bacteria are naturally present on our skin. Closed-toe shoes causes more smell than the open shoes [2]. Odour inside the shoe can increase due to accumulation of dead skin cells, dirt and oils over the time [3]. Foot have a rich bacterial flora most of which is not normally pathogenic if the feet are in good health [4]. Lifestyle factors can expose the feet to higher risks of contamination by certain bacteria. Most of the school children do not wash their socks regularly even at an interval of at least 3-7 days. Some children are forced to wear wet socks due to lack of time to dry them. Wet and dirty socks are colonized by microorganisms. Nylon which is a non-absorbent causes the perspiration to remain in contact with the feet favouring bacterial growth. Since there was a dearth in this area of research, this study was undertaken to detect various fungal and bacterial isolates from the socks worn by school children as this will reflect their personal hygiene and reinforce the importance of maintaining hygiene in one’s life.

MATERIALS AND METHOD:

Collection of data:
All the school children from whom the samples were collected were given a simple questionnaire about their socks hygiene. The questionnaires were collected after proper consent from their parents and teachers. It had two sections which included general information and other specific questions about their usage of socks.

Sample collection and Processing:
Sixty samples were collected with the help of sterile swabs from the socks of school going children belonging to the age group of 10 to 12 years. The swabs were moistened with nutrient broth and normal saline to facilitate proper swabbing from the surface of the socks. The samples were transported to the laboratory for further processing.

Isolation of bacteria:
Thirty samples with nutrient broth were incubated at 37°C for 24 hrs. They were inoculated on to nutrient agar, mannitol salt agar, blood agar and Mac conkey agar. The plates were incubated at 37°C for 24 hrs. Next day the colonies were picked up and gram staining, motility, catalase and oxidase were done. The bacterial isolates were identified as per the standard protocols.

Isolation of fungi:
The fungal isolation was done with the other thirty samples with normal saline. They were inoculated on to Sabouraud dextrose agar and the plates were incubated at 25°C to 35°C for a week. Identification of the fungus was done as per standard methods. Antibacterial activity of herbal foot disinfectant spray by disc diffusion method:
Antibacterial activity of herbal foot disinfectant spray was performed as per the standard protocol of Kirby-
Bauer's disc diffusion method[5] Muller-Hinton agar (MHA) was prepared and poured into sterile petriplates aseptically and was allowed to solidify. Standard suspension of the test isolates was made and the turbidity was matched to Mac Farland standard 0.5. The lawn of the test bacterial strain was done with the help of sterile cotton swab. Sterile disc was saturated with the foot disinfectant spray. Discs were placed onto MHA with sterile forceps. The test plates were incubated aerobically at 37°C for 24 hrs. After incubation, the results were recorded as the presence or absence of inhibition zone. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the disc.

RESULTS:

Analysis of the questionnaire:
A total of thirty questionnaires were collected, of which 15 were from girls and the other 15 were from the boys. It was observed that most of them used cotton socks, which was regularly washed. On an average they had at least three pairs of socks which were used in turns. Socks of the boys were more contaminated when compared to girls, as they had more physical activity. None of them used any kind of antiseptic for washing their socks. It was also found that not much attention was given towards the washing of their shoes regularly.

Prevalence of bacterial isolates from socks:
A total of 30 samples were collected from the socks of school going children and were processed, of which 64 bacteria were isolated and identified. Out the 64 isolates, 17 were found to be gram positive cocci in clusters (27%) followed by 22 were gram negative rods (34%) and 25 were gram positive rods (39%). (Fig 1)

Prevalence of gram positive cocci:
Among 17 gram positive cocci in clusters, 7 (41%) were found to be Staphylococcus aureus and 10 (59%) were found to be Coagulase negative Staphylococci (CoNS).

Prevalence of gram negative rods:
Among 22 gram negative rods, 6 (27%) were found to be Klebsiella pneumoniae, 3 (14%) Pseudomonas aeruginosa, 3 (14%) Alcaligenes faecalis, 3 (14%) Enterobacter aerogenes, 3 (14%) Serratia marcescens, 2 (9%) E.coli and 1 (4%) Citrobacter freundii. (Table 1)

Prevalence of gram positive rods:
Among 25 gram positive rods, 7 (28%) were found to be Bacillus polymyxa, followed by 11 (44%) Bacillus stearothermophilus, 3 (12%) Bacillus firmus, 3 (12%) Bacillus cereus and 1 (4%) Bacillus pumilus. (Table 2).

Prevalence of fungal isolates from socks:
A total of 30 samples were collected from the socks of school children and were processed, of which 38 fungi were isolated and identified. Out of 38 isolates, 13 (34%) were found to be Yeasts followed by 7 (18%) Rhizopus spp, 5 (13%) Mucor, 3 (8%) Penicillium spp, 3 (8%) Aspergillus flavus, 2 (5%) Aspergillus niger, 2 (5%) Rhizoctonia solani, 1(3%) Aspergillus fumigatus, 1(3%) Fusarium oxysporum and 1(3%) Lichtheimia corymbifera. (Table 3)

Antibacterial activity of herbal foot disinfectant spray by disc diffusion method:
Antibacterial activity of herbal foot disinfectant spray was performed as per the standard procedure of Kirby- Bauer's

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the bacteria</th>
<th>Percentage of the bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus polymyxa</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus stearothermophilus</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus firmus</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus cereus</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>5</td>
<td>Bacillus pumilus</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

![Fig 1:Bacterial isolates from socks](image)

![Table 1: Prevalence of gram negative bacteria in socks (n =22)](data)

![Table 2: Prevalence of gram positive rods in socks (n =25)](data)

![Table 3: Prevalence of fungi in socks (n=38)](data)
disc diffusion method and it exhibited bactericidal activity by producing a zone of inhibition of 17mm in size.

DISCUSSION:
A critical barrier between the human body and its outer environment is human skin. It prevents loss of moisture and restricts the entry of pathogenic organism [6]. Different microbial communities are harboured on skin as it is an excellent ecosystem [7]. Depending on the environmental conditions specific to distinct region of the skin, these microbial communities are distributed across the human skin and live in physiologically diverse and topographically distinct niches [8], [9]. Generally the normal microflora coexists asymptptomatically with host, but these can cause infection whenever the host immune system gets compromised or skin is damaged and microorganisms causing such infection are said to be opportunistic pathogens. The most common colonizers of the skin in mammals and birds are the genus Staphylococcus. Sweat glands and mucous membranes surrounding body openings are harboured with large number of Staphylococci. In our study, 17 gram positive cocci in clusters were isolated of which, 7 (41%) were found to be Staphylococcus aureus and 10 (59%) were found to be Coagulase negative Staphylococci (CoNS). This was found to be in agreement with Kloo and Schleifer, 1986[10]. Wet socks when worn are prone to bacterial and fungal infections [11]. Sweat produced by the body acts as a nutrient which favours bacterial multiplication and gives rise to odour, socks, and shoes a strong odour [3]. The infections and proliferation of bacteria and fungi are influenced by microclimate, temperature, humidity, physical activity, lifestyle [12], [13] and on individual predisposition factors. In our study 22 gram negative rods and 25 gram positive rods were isolated. Of which 6 (27%) were found to be Klebsiella pneumoniae, followed by 4 (18%) Pseudomonas aeruginosa, 3 (14%) Alcaligenes faecalis, 3 (14%) Enterobacter aerogenes, 3 (14%) Serratia marcescens, 2 (9%) E.coli and 1 (4%) Citrobacter freundii, 7 (28%) were found to be Bacillus polymyxa, followed by 11 (44%) Bacillus stearothermophilus, 3 (12%) Bacillus firmus, 3 (12%) Bacillus cereus and 1 (4%) Bacillus pumilus. This was found to be in agreement with Li et al., 2011[14] and Messina, 2012 [15]. The most common infection of people who wear shoes and socks is the fungal infections of foot skin as there is no proper circulation of air [16]. In such individuals there is initial itching between the toes and the development of small vesicles that rupture and discharge thin fluid. The skin of the toe web becomes macerated and peels and cracks appear. Peeling and cracking of the skin become the principal manifestations, accompanied by pain and pruritus when the infections become chronic [17], [18]. In our study a total of 30 samples were collected from the socks of school going children and were processed, of which 38 fungi were isolated and identified. Of the 38 isolates, 13 (34%) were found to be Yeasts followed by 7 (18%) Rhizopus spp, 5 (13%) Mucor, 3 (8%) Penicillium spp, 3 (8%) Aspergillus flavus, 2 (5%) Aspergillus niger, 2 (5%) Rhizoctonia solani, 1 (3%) Aspergillus fumigatus, 1 (3%) Fusarium oxysporum and 1 (3%) Lichtheimia corymbifera. This was found to be in agreement with the studies done by Romano et al., 2001[19] and Ninomyia, 2000[20]. Antibacterial activity of herbal foot disinfectant spray was performed as per the standard procedure of Kirby- Bauer’s disc diffusion method and it exhibited bactericidal activity by producing a zone of inhibition of 17mm in size. The spray was found to be effective due to active components like Azadirachta indica, Mentha pipetita oil, citrus oil and ethanol. Presently, there is very little information available on the inhibitory effects of herbal foot disinfectant sprays and our study shows promising antimicrobial activity of the spray tested.

CONCLUSION:
Limited research has been done on socks as a possible source of microbial contamination, despite the likelihood that socks are rarely completely clean. Contamination is not always sufficient to determine an infection but remains a possible source, especially if the feet have lesions. Our study showed that school children’s socks harboured microorganisms such as bacteria and fungi. These microorganisms could have been isolated due to poor personal hygiene, like wearing the same socks for over a week without washing or inappropriate washing or wearing wet socks. These organisms isolated can be pathogenic, especially to children. Our study showed promising results of the herbal foot disinfectant spray, which can be used as an effective foot sanitizer. Therefore, good hygiene is certainly the first step towards healthy feet.

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REFERENCES:


