



## **Microbial Analysis of Second Hand Children Socks Sold in Some Selected Markets in Makurdi Metropolis, Nigeria**

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### **Authors' contributions**

*This work is in collaboration with all the authors. Author GMG designed and supervised the study, Author JOA wrote the protocol and first manuscript. Author COA helped in literature research and drafting of the final manuscript. All the authors were involved in the experiments, findings and analyses. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Second hand children socks were analysed for microbial contaminants. A total number of 50 samples were collected from three major markets in Makurdi metropolis namely; Wadata, Wurukum and Modern markets and were transported immediately to the laboratory in the Department of Biological Sciences, University of Agriculture Makurdi for analysis. *Staphylococcus aureus*, *Streptococcus spp* and *Bacillus spp* were the bacteria isolated and *Aspergillus spp*, *Penicillium spp*, *Mucor spp*, *Candida albicans* and *Yeast cell* were the fungi species isolated. *Staphylococcus aureus* occurred more with frequency of 21 (75.0%) and *Bacillus spp* occurred least with frequency of 1 (3.5%), while *Streptococcus spp* had a frequency of 6 (21.4%). In respect to the various markets, study shows that Wadata market has the highest number of bacteria with frequency of 15 among the three different suspected bacteria, followed by Wurukum market with frequency of 9 and Modern market with frequency of 4. *Aspergillus spp* has the highest number of occurrence with frequency of 15 (60%) followed by *Mucor spp* with the frequency of 6 (24%). The least are

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*Penicillium spp* and *Yeast cell* with frequency of 1 and 4% each, *Candida albicans* had frequency of 2 (% 8). Frequency of fungi was highest in Wadata market with a frequency of 9, while Wurukum and Modern markets had equal number of occurrence with frequency of 6 each. High microbial loads were observed in this study with respect to second hand children socks. Therefore, disinfecting of second hand children socks is highly recommended before use, to avoid infections from these organisms.

**Keywords:** Second hand; socks; children; microbial; Makurdi.

## 1. INTRODUCTION

Second hand clothes are clothing items that have been previously used by one person prior to the present user [1]. Examples are used socks, shirts, undergarments, skirts, household linens such as pillow cases, towels, bedding, curtains, table cloths, etc [1]. The origins of second hand clothing industries dates as far back as 1834 where the most technologically advanced were the production of “shoddy” clothe in the North of England. These were made from rags to wool, cotton, and indeed all fibres (except silk) which became the staple in that year [2]. The second hand clothing trade in colonial Africa began in the immediate past World War 1 period with an influx of surplus military uniforms shipped by used clothing dealers in Europe and from production areas in the United States [3]. The post-independence period throughout much of sub Saharan Africa was characterized by an increased emphasis on domestic production of textiles and finished apparel for local consumption [4]. This led to the ban of the importation of second hand clothing in Kenya, and elsewhere throughout most of sub-Saharan Africa, as import substitution policies were adopted to strengthen domestic manufacture. Despite the ban, second hand clothing began entering Kenya in the 1970s and 1980s as a consequence of regional political crises [4].

The number of stalls devoted to second hand clothing has increased rapidly in most African countries [3]. They are imported by business men/women and charitable organizations [5]. The world’s largest exporters of used clothing are the United States of America (USA), United Kingdom (UK), Germany and Netherlands, while the worlds’ largest importers of used clothing are the sub-Saharan countries, receiving over 25% of global second hand clothing exports [6]. The international recirculation of used clothing is not as straight forward as simply the export from richer to poorer nations; specific markets present more demand for particular items, for example Japan imports a considerable percentage of the

world’s trade in used designer denim jeans and sneakers. In these ways the state of the second hand clothes trade could be understood as diversifying in economic potential and enjoying a favourable shift in its industrial, public, and cultural profiles [2]. Many philanthropic groups in United States have been involved in collecting and donating clothes to the poor since the end of the nineteenth century [3]. Many of these charitable organizations such as the Salvation Army, Oxfam, St. Vincent de Paul, Amvets, etc. introduced store sales in the 1950s which helped in the collection and resale of these second hand clothing items, thereby providing capital for their benevolent course [7]. According to Oxfam, more than 70% of the clothes donated globally end up in Africa [8].

The second hand clothing trade has expanded hugely in both its economic power and global scope, more than doubling worldwide between 1991 and 2004 [3]. Globally, the wholesale used clothing trade is valued at more than \$2.8bn [9]. Second hand clothing items, due to antecedents and wide spread use pose a major health risks to the public [10]. This fact prompted the Rwanda Bureau Standard to ban second hand undergarments in Rwanda [11]. Bloomfield documented the ability of various types of clothing items to retain bacteria and fungi for varying lengths of time [12]. The spread of HIV and Hepatitis Viruses by contact of contaminated materials has created increased pressure for protection of person with functional clothing; also, all articles of apparel and home textiles are susceptible to problems of hygiene in normal daily use. For example, socks, sport wears and working clothes. Diseases that may be transmitted via contact are candidiasis, hepatitis A, B and C as well as skin infections such as ringworm and scabies could also be transmitted through these popular clothing items [13]. Some other risks associated with second hand clothing include: Tinea, impetigo, body lice/crabs, head lice and bed bugs [14]. Socks are items of clothing worn on the feet and often covering the ankle and some part of the calf, typically knitted

from wax, cotton, or nylon (Concise English Dictionary). A major role of socks is to absorb perspiration; the foot produces more sweat per square inch of the skin than any part of the body, as it can produce over 0.25 pints (0.12L) of perspiration per day. Socks help to absorb this sweat and draw it to areas where air can evaporate the perspiration [15]. Second hand children's socks have become increasingly popular in Nigeria, as these are cheaper and easily available to low income families who cannot afford brand new ones. Even families that can afford brand new socks tend to patronize second hand ones as many believe that second hand clothing are of higher quality and long lasting than brand new ones [16]. Microorganisms in clothes are transmissible through sharing. Fomites have been shown to aid in the transmission of pathogens from one individual to another [5]. Secondhand clothes as well as other second hand clothing have an inherent ability to retain and transmit microorganism from the first user to the final user [10]. In a study by Nelly et al., 2000, the data indicated that *Staphylococci* and *Enterococci* can survive for extended periods of time on materials commonly worn by patients and health care workers and on various other fabrics in the hospital environment. When such clothing items are sold as secondhand goods, these microorganisms remain in the deeper lying materials of the clothing, the next user particularly when moisture is introduced onto the scene can become infected by reverse wicking; that is, moisture can draw deep lying pathogens back toward the surface of the bedding that is in contact with the user according to Roberts et al., 2008. Therefore, making secondhand children socks sometimes unsafe and an easy way of contacting some diseases that are transmitted through contact such as candidiasis, hepatitis A, B and C as well as skin infections such as scabies, ringworm could be transmitted through these popular clothing items [13]. The way these items of clothing are handled in the markets place by traders and buyers also exposes them to contamination, as different people touch them with sweaty, dirty hands, and traders spread them most times on the bare floor, or on dirty sacks and papers for sale [16].

In recognition of the importance of socks in various aspects of children's lives, for example its use in sports, in cold weather, as fashion apparel, as part of school uniforms, etc. The study was necessary to show the public the risks that secondhand socks pose to children's health

if not properly washed and disinfected before use. Research is important in the microbial analysis of secondhand children's socks due to the popularity and wide spread use of second hand clothing in Nigeria, bringing to light their inherent ability to retain and transmit microorganisms in them, even after a period of time. The study is designed to enlighten the public on the dangers of some diseases or infections that could be contacted from secondhand children's socks which can either be gotten from the first user to the present user or was contaminated in the market place.

### **1.1 Aim of the Study**

The aim of the study is to carry out the microbial analysis of secondhand children socks to find the microorganisms present in them.

## **2. MATERIALS AND METHODS**

### **2.1 Study Area**

This study was conducted on samples collected from three selected markets across Makurdi metropolis. Makurdi town is the capital of Benue State of Nigeria, situated in central Nigeria along the Benue River, with a population of about 292,664 people. The distance from Makurdi to the major city of Lagos is about 585 km.

### **2.2 Sample Collection**

The study was conducted on a total of thirty (30) samples of second hand children socks. Ten (10) samples were collected from each of the selected markets (Modern market, Wadata market and Wurukum market). Each sample was collected separately and packaged in a sterile polythene bag to avoid cross contamination. The samples were each swabbed entirely with a sterile cotton swab immersed in normal saline. The swab sticks were transported to the laboratory at the Department of Biological Sciences, University of Agriculture, Makurdi, Benue State.

### **2.3 Microbiological Analysis**

#### **2.3.1 Media preparation**

The culture media used were Nutrient agar, Blood agar and sabouraud dextrose agar. The procedures used in the media preparation was according to the standard prescribed by the manufacturers.

### **2.3.2 Inoculation/culturing**

The swabbed sticks were each inoculated and grown on blood agar which was incubated at 37°C for 24 hours. For fungi culture, the same was done on sabouraud dextrose agar and incubated at 23°C room temperature for 3-5 days. The growths in colonies were then counted macroscopically [17,10].

### **2.3.3 Identification of bacteria**

The bacteria were identified based on their morphological and biochemical characteristics.

### **2.3.4 Morphological identification**

The colonies were observed based on their morphology such as color, shape, appearance, size.

### **2.3.5 Gram staining procedure**

- Smears of the specimen were made on a clean glass slide with the aid of sterilized wire loop. The smears were fixed by passing the slides over a flame of a bunsen burner.
- The smears were flooded with crystal violet until the slides are covered and stained for a minute
- The slides were gently rinsed with tap water and drained, and thereafter covered with Lugol's iodine and allowed to stand for one minute. Slides were quickly rinsed with tap water and drained.
- About 95% alcohol was added to smears for 30 seconds or until stain no longer washes off if slides were held at an angle and quickly rinsed with tap water and drained.
- Smears were covered with safranin and stained for 30 seconds and rinse with tap water and drain.
- Smears were allowed to air dry and viewed under oil immersion lens (X100).

## **2.4 Biochemical Analysis**

All biochemical tests were done according to the methods of Cheesbrough [17].

### **2.4.1 Catalase test**

The colony of test organism were mixed on a drop of hydrogen peroxide where Production of gas bubbles (effervescence) is an indication of a positive result while a negative result had no gas bubbles.

### **2.4.2 Coagulase test**

The colony of test organism were mixed into a drop of plasma, mixed gently and clumping of the same sample was observed within 10 seconds indicating a positive test while a negative test shows no clumping of the sample.

### **2.4.3 Indole test**

The test organism was inoculated into 3mls of sterile peptone water in a bijou bottle and incubated aerobically at 37°C for 48 hours. Indole was tested by the addition of 0.5 ml of kovac's reagent to broth culture and allowed to stand for 5 mins, bright pink colours at the top of the broth indicate a positive result while yellow layer at the top of the broth indicates a negative result.

### **2.4.4 Oxidase test**

A filter paper was flooded with oxidase reagent and the test organisms were smeared spot on the filter paper. The emergence of purple colour shows a positive result otherwise negative.

### **2.4.5 Motility test**

A drop of the colony suspension was placed on a slide and cover with a cover slip it was sealed with a molten petroleum jelly and inverted. The preparation was examined microscopically for motile organisms using 10x and 40x.

### **2.4.6 Fungal identification**

The growths on the sabouraud dextrose agar plates were observed morphologically for color, size of growth, pigment production. Lactophenol cotton blue test was carried out for identification of mould.

### **2.4.7 Lactophenol cotton blue test**

A drop of methanol was placed on a clean slide and a portion of fungi growth was cut and was tested in the methanol. A drop of lactophenol cotton blue was added, a cover slip was then placed on it gently to which it was observed under microscope with x40 objectives. The picture seen was compared with an identification chart [17].

## **2.5 Data Analysis**

Data was analyzed using descriptive statistics (SPSS version 20).

### 3. RESULTS AND DISCUSSION

A total of thirty (30) second-hand children socks samples were swabbed and cultured on blood agar and nutrient agar from three major markets in Makurdi metropolis namely; Wadata, Wurukum and Mordem market. The cultured plates from the different localities were further analysed by observing the morphological characteristics, Gram staining, percentage of bacteria and fungi, average colony count and other biochemical tests and the following results were obtained as shown in the Tables 1, 2, 3,4,5 and 6.

Table 1 shows the morphological characteristics of the isolated bacteria and also the colony characteristics of each microorganisms namely; *Staphylococcus aureus* with shiny Yellowish, and spherical, *Streptococcus spp* with Cream gold, shiny, smooth, spherical and *Bacillus spp* with creamy, rough edge.

Table 2 shows the morphological characteristics of fungi with their colony characteristics and their microscopic appearance; the colony characteristics of *Aspergillus spp* is black in colour, large mass of colony, *Penicillium spp* has velvety, fuzzy, green, dark in colour, *Mucor spp* has Cotton, fuzzy, greyish white in colour, *Candida albicans* has Creamy colony and *Yeast cell* has Moist, shiny whitish colony.

Table 3 shows the biochemical characteristics of Gram positive and Gram negative microorganisms isolated from the samples of second hand children socks and also the various tests which were carried out such as catalase test, coagulase test and oxidase test. The suspected organisms are *Streptococcus spp*, *Staphylococcus aureus* and *Bacillus spp*.

Table 4 shows the frequencies and percentages of bacteria isolated from the second hand children socks from the various markets and also it shows the market with the highest number of organisms present in the samples of second hand children socks collected from there, *Staphylococcus aureus* occurred more with frequency of 21 and percentage of 75.0 and *Bacillus spp* occurred least with frequency of 1 and percentage of 3.5 while *Streptococcus spp* is in between *Staphylococcus aureus* and *Bacillus spp* with frequency of 6 and percentage of 21.4.

Table 5 shows the frequencies and percentages of fungi isolates from the samples of second hand children socks from the various markets. *Aspergillus spp* has the highest number of occurrence with the frequency of 15 and percentage of 60% followed by *Mucor spp* with the frequency of 6 and percentage of 24%. The least is *Penicillium spp* and *Yeast cell* frequency of 1 and percentage of 4%, *Candida albicans* is in-between *Mucor spp* and *Yeast cell* with frequency of 2 and percentage of 8%.

**Table 1. Morphological characteristics of bacteria isolated**

S/no	Colony characteristics	Probable organism
1	Shiny yellowish, & spherical	<i>Staphylococcus aureus</i>
2	Creamy with rough edge.	<i>Bacillus spp</i>
3	Cream gold, shiny, smooth, spherical.	<i>Streptococcus spp</i>

**Table 2. Morphological characteristics of fungi isolated**

S/no	Colony characteristics	Microscopic appearance	Probable organism
1	Black in colour, large mass of colony	Conidiospores aseptate with hyphae	<i>Aspergillus spp</i>
2	Velvety, fuzzy, green, dark in colour	Green spores with whitish hyphae	<i>Penicillium spp</i>
3	Cotton thick , greyish white colour, black at maturity	Non-septate hyphae	<i>Mucor spp</i>
4	Creamy colony	Ova, spherical in shape, transparent	<i>Candida albicans</i>
5	Moist, shiny whitish colony	Ova, spherical shape	<i>Yeast cell</i>

**Table 3. Biochemical characteristics of gram positive and negative organisms isolated from second hand children socks**

S/no	Shape	Gram reaction	Catalyst	Coagulase test	Oxidase test	Suspected organism
1	Coci	+	-	+	-	<i>Streptococcus spp</i>
2	Coci	+	+	+	-	<i>Staphylococcus aureus</i>
3	Coci,	+	+	+	-	<i>Bacillus sp</i>

**Table 4. The frequencies of isolated bacteria found on second hand children socks**

S/no	Organism	Mdm	Wdm	Wrm	Total frequency	Percentage (%)
1	<i>Staphylococcus aureus</i>	2	12	7	21	75.0
2	<i>Bacillus species</i>	1	0	0	1	3.5
3	<i>Streptococci species</i>	1	3	2	6	21.4
Total		4	15	9	28	100.0

Key: Mdm = Modern market, Wdm = Wadata market, Wrm = Wurukum market

**Table 5. The frequencies of isolated fungi found on second hand children socks**

S/no	Organisms	Mdm	Wdm	Wrm	Total frequency	Percentage
1	<i>Aspergillus spp</i>	3	9	3	15	60.0
2	<i>Penicillium spp</i>	0	1	0	1	4.0
3	<i>Mucor spp</i>	3	2	1	6	24.0
4	<i>Candida albicans</i>	0	1	1	2	8.0
5	<i>Yeast cell</i>	0	0	1	1	4.0
Total		6	13	6	25	100.0

Key: Mdm = Modern market, Wdm = Wadata market, Wrm = Wurukum market

Table 6 explains the numbers of colonies of bacteria isolates and their colony forming unit per mil. The organisms were *Staphylococcus aureus* with colonies of 66, *Bacillus species* with 12 colonies and *Streptococci species* with colonies of 8.

**Table 6. Numbers of colonies of isolated bacteria and their percentages**

Organisms	Colonies	Percentage
<i>Staphylococcus aureus</i>	66	76.7
<i>Bacillus species</i>	12	13.9
<i>Streptococci species</i>	8	9.3
Total	86	100.0

This research showed that second hand children socks are colonized with bacteria and fungi. A total of fifty (50) second-hand children socks samples were examined from three major markets in Makurdi metropolis namely; Wadata, Wurukum and Mordem markets. Three bacteria were identified namely; *Staphylococcus aureus*, *Streptococci spp* and *Bacillus spp* and five fungi species were also identified namely; *Aspergillus spp*, *Penicillium spp*, *Mucor spp*, *Candida albicans* and *Yeast cell*. *Staphylococcus aureus* occurred more with frequency of 21 and percentage of 75.0 and *Bacillus spp* occurred least with frequency of 1 and percentage of 3.5 while *Streptococcus spp* is in between *Staphylococcus aureus* and *Bacillus spp* with frequency of 6 and percentage of 21.4. In respect to the various markets, Wadata market has the highest number of bacteria with frequency of 15 among the three different suspected bacteria followed by Wurukum market with frequency of 9 and Modern market with frequency of 4. The data shows that *Staphylococcus aureus* showed the highest prevalence rate among bacteria isolated.

*Staphylococcus aureus* may occur commonly in our environment, it can be cultured from clothes and virtually all environmental surfaces [11]. It is transmitted through several methods such as direct contact with objects that are contaminated by the bacteria. Approximately 30% of healthy humans carry *Staphylococcus aureus* in their nose, back of the throat and on their skin. *Staphylococcus aureus* is considered opportunistic as it takes advantage of broken skin or other entry sites to cause infection. In immunocompromised persons, this bacteria may be life threatening. It may lead to pyogenic (abscessing) infections of the skin, eyes and genital tract [18]. This study is similar with the recent study by Agbulu et al., (2015), it was shown that second hand pants, bras, and children wears were colonized bacteria, *Bacillus spp* and the fungi *Penicillium species* and *Candida albicans*. The study showed that *Bacillus spp* has the ability to withstand environmental temperature, and also has a unique cell structure "endospore" with ability to remain dormant for years and resistant to damaging conditions such as heat, desiccation, toxic chemicals and ultraviolet radiation. This enables *Bacillus spp* to survive the time of duration from when the second hand clothes are sorted and packaged into bails and reach the required destination. *Streptococci spp* are of different types, with each group causing different types of infections [19]. Group A strep are found on the surface of the skin and inside the throat, they are a common cause of infection in children and adults. Strep A infection such as impetigo causes sores, blisters and crust to develop on the skin, according to NHSchoices.

The bacteria isolated (*Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococci spp*) are

associated with bacterial infections. They are mostly opportunistic, and harmful to immunocompromised persons. For fungal, *Aspergillus spp* has the highest number of occurrence with the frequency of 15 and percentage of 60 followed by *Mucor spp* with a frequency of 6 and percentage of 24. The least were *Penicillium spp* and *Yeast cell* with frequency of 1 and percentage of 4 each, *Candida albican* had frequency of 2 and percentage of 8. Frequency of fungi was highest in Wadata market with a frequency of 9, while Wurukum and Modern markets had equal number of occurrence with frequency of 6 each. *Aspergillus spp* are primarily saprophytic fungi, occurring commonly in soils and on other organic and inorganic substrates. *Aspergillus spp* are thermotolerant, and capable of growth in temperatures ranging from 12°C to over 50°C which has contributed to their success as wide ranging opportunistic pathogens in humans [20]. They are a common mould that causes the infection *Aspergillosis*. Environmental conditions could potentially facilitate the build-up of *Aspergillus* populations increasing the risk of infection according to Foley et al., 2014. People breathe in *Aspergillus* spores every day. According to a study by Potera (2001) cotton fabrics spread more of *Aspergillus spp* better than other fabrics; socks are commonly produced with cotton fabric. The research agrees with this study. *Penicillium spp.* is a genus of moulds found everywhere world-wide. *Penicillium* causes food spoilage and are common contaminants on various substances. The spores can trigger allergic reactions in individuals sensitive to mould. Some species of *Penicillium* can also infect immunocompromised individuals. For example, *Penicillium marneffeii* is pathogenic particularly in patients with AIDS [21]. *Penicillium spp* rarely cause disease but following skin damage, may proliferate within the dermis and cause ulcerative lesions [15]). *Mucor spp* is a filamentous fungus with several species, few of which grow well at 37°C (body temperature) and thereby able to infect humans. It may also be opportunistic. *Candida albicans* causes *Candidiasis*; it is a common inhabitant of the skin, mouth, gastrointestinal tract, and vagina. It also causes infections among infant, pregnant women, diabetics [22]. *Candida albicans* also causes onychomycosis which is a fungal infection of the nail. *Yeast cell* grows mainly in moist environments. Second hand children socks are mostly packed into air tight sacks in bails which

provide room for *Yeast cell* to grow faster [22]. This findings agrees with that of Sharifzada 2011 who isolated *Candida albicans* and related it to ringworm infection among children. The survival of these microorganisms for varying lengths of time is attributed to the ability of various types of clothing items to retain them [12]. This work is also in line with those of Roberts et al. 2008 and Nelly et al., 2000 who isolated some microorganisms from second hand beddings and clothes of patients and health workers respectively.

#### 4. CONCLUSION

This study showed that second hand children socks harbour microorganisms such as bacteria and fungi. Strains of fungi and bacteria were isolated from second hand children socks and the bacteria are *Staphylococcus aureus*, *Streptococci spp*, and *Bacillus spp* while the fungi are *Aspergillus spp*, *Penicillium spp*, *Mucor spp*, *Yeast cell* and *Candida albicans*. These microorganisms isolated can be pathogenic, especially to children. The data shows that Wadata market had the highest percentages of isolates in both bacteria and fungi, therefore pose greater risk to the public.

In the course of this study, second hand children socks have been shown to harbour pathogenic microorganisms. The following control measures are recommended:

1. The use of second hand children socks in children especially in infants should be avoided if possible.
2. In a situation where consumers cannot avoid the use of these clothing item due to their living standard, second hand children socks should be washed properly with strong detergents and if possible disinfected before use.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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