POTENTIAL SKIN PATHOGENS ON SECOND HAND CLOTHES AND THE EFFECTIVENESS OF DISINFECTION METHODS

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Abstract
This study examined the pathogenic microbial levels in second hand undergarments and compared the effectiveness of disinfection methods used to reduce microbial load in the garments. Of special interest were pathogenic microbes in undergarments such as panties, bras, socks and towels which were collected from various flea markets. The study was planned following prior casual questioning of consumers to find out the most common decontamination methods used on these clothes. Clothe samples collected from the Gikomba second hand market were examined in a biomedical laboratory for evidence of high levels and types of pathogenic microbes and persistence of the pathogenic microbes that can be attributed to skin infection after decontamination procedures. Culture and biochemical methods were used for investigation. A variety of potential skin pathogens were isolated from unwashed second hand undergarments, socks and towels. Several bacteria were isolated including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA). The fungi isolated from the unwashed clothes included *Scopulariopsis brevicalis*, *Geotrichum candidum*, *Scytalidium*, *Trichophyton mentagrophytes, Rhodotorula sp.*, *Cladosporium sp., Candida tropicalis*, *Candida glabrata* and *Aspergilus flavus.* Panties and bras had the highest count of both bacteria and fungi. The mycoflora was not limited to dermatophytes such as *Trichophyton* but other fungi exist such as *Alternaria alternata* which are pathogenic. In this study, the clothes were washed with grade 2 laundry bar soap. After washing there was a reduction in the bacterial ($t12 = 9.6$, $P < 0.001$) and yeast ($t12 = 3.5$, $P > 0.005$) plate counts but therefore was a no significant reduction in mold counts ($t12 = 1.1$, $P > 0.005$). The kill time for both Jik and Savlon against the MRSA isolates from clothes was 2 minutes. The concentrations of Omo used do not kill the MRSA isolates at 10 min. The study showed that second hand clothes are frequently contaminated by several pathogenic bacteria and fungi, which remain on these clothes even after washing with ordinary bar soaps. The information obtained from this study form a basis of advising consumers, public, Ministry of Health and health workers dealing with immunocompromised patients in nursing homes and hospitals. The findings from the study also reinforce the need for appropriate disinfection and conscientious contact control precautions.

Key words: Skin infections, second hand clothes, efficacy, disinfection methods

1.0 Introduction

The second hand clothes in Kenya are known as "Mitumba", a Kiswahili word meaning bale or bundle. Mitumba are imported by businessmen/women and charitable organizations. Scabies, pediculosis, and fungal infections are possible diseases that could be transferred through used clothes (The Kurdish globe, 2008 and Bandiaga *et al*., 2008). According to the (South Australia Public Health Fact Sheet, 2009) some of the risks associated with second clothing include **;** Tinea, Impetigo ,Scabies, Body lice ,Pubic lice /crabs, Head lice and Bed bugs.

The proportion of stalls devoted to second hand clothes has increased rapidly in most African countries (Hansen, 2000). In many studies, clothes have been found to be contaminated with chemicals and biotic factors. Existing studies on pathogenic microbes on clothes include microbial flora on ties (Dixon, 2000), Laboratory coats (Srinivasan *et al*., 2008) worn by doctors and students, lanyards (Kotsanas *et al*., 2008), nurse's scrubs and gowns (Pilonetto *et al*., 2004) used in hospitals and nursing homes. Skin infections are common in Eastern Africa countries which are deprived of the most basic of care for their skin disease (Donofroi *et al*., 1994).

Microorganisms in clothes are transmissible through sharing. Fomites have been shown to aid in the transmission of pathogens from one individual to another. A common fomite is bedding, where commonly employed materials, such as cotton, act as wicks to carry pathogens far from the initial contact point with human skin, particularly when moisture is present from sweat, semen, saliva, vaginal secretions, secretions from wounds and open pimples and spilled drinks . Thus, when sheets and pillow cases are changed, the deeper lying material (mattress, mattress cover and pillow) still harbor potential pathogens of the previous user(s). Furthermore, the next user particularly when moisture is introduced onto the scene can become infected by reverse wicking; i.e., moisture can draw deep lying pathogens back toward the surface of the bedding that is in contact with the user (Roberts *et al.*, 2008).

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 while Pilonetto *et al*., 2004, detected *S. aureus*, *Klebsiella pneumoniae* and *Acinetobacter baumanii* in hospital gowns. Neck ties worn by doctors at an intensive care unit produced heavy growths of coagulase negative staphylococcus on 2/5 ties tested (Dixon, 2000). This observation reinforces the need for more care with clothing. Studies have shown that various objects, such as utensils, toys, and clothes, can serve as vehicles for transmission of *Shigella* spp. (Islam *et al*., 2001). In a study by Nelly *et al*., (2001), *Candida, Aspergillus, Mucor*, and *Fusarium* which are associated with nosocomial infections in patients survived long periods on fabrics and plastics which are routinely used in hospitals. These survival results indicate the potential for various fabrics and plastics to serve as reservoirs or vectors for fungi.

Skin related infections have grown considerably in the community. The vast majority of these infections are transmitted through skin-to-skin contact, but a smaller yet significant portion is due to shared equipment (The Mercks Online Manual, 2009). Disinfection has been shown to reduce the number pathogenic microbes in disinfected clothes.

In this study undergarments from a popular second hand market were examined. The purpose of the study was to investigate the level and type of microbial contamination present on the second hand clothes in order to assess the risk of transmission of pathogenic microorganisms. Systematic examination was also undertaken on the survival of several clinical and environmental pathogenic microbial isolates on second hand clothes after several disinfection processes. The study employed culture methods for recovery of the pathogenic microbes on the second hand undergarments.

2.0 Materials and Methods

2.1 Study Area

This study was conducted in second hand cloth market in Gikomba which is in Kamukunji constituency. Gikomba is situated off Landies Road along Quarry road in Nairobi area and is the largest second hand goods market in Kenya.

2.2 Test Materials

Three samples were collected from each of the four categories:Towels, Bras, panties and socks.

2.3 Isolation of Bactrial and Fungi

Samples were obtained from the flea markets and placed in a sterile polyethylene bag. The entire area of sample was swabbed using a sterile cotton swab immersed in sterile water. The swab was then used for culture in Mannitol salt agar for Methicillin Resistant *Staphylococcus aureus* (MRSA). The plates were incubated at 37˚C for 48 hours and 30˚C for 5 days. Mannitol salt agar plates were examined for pathogens, including Methicillin-sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus*. Total microbial counts, expressed as colony-forming units (CFU), were recorded for each plate.

The entire area of sample was swabbed using a sterile cotton swab immersed in sterile water. The swab was used for culture in Sabourands Dextrose agar plates for yeasts. The plates were incubated at 30˚C for 5 days.
Direct imprints of 2 cm^2 swatches of all the samples were cut using a sterile scissors and cultured directly onto the middle of Mycosel agar plates for recovery of yeast and molds. The plates were incubated at 30˚C for 2 weeks to 4 weeks. After incubation the colonies on the surface were counted. Mycosel agar plates were examined for molds while Sabourands Dextrose agar plates were examined for yeasts. Total microbial counts, expressed as colony-forming units (CFU), were recorded for each plate.

2.4 Survival Test

The swatches were washed using grade 2 laundry bar soap and then dried after which isolation was carried out as described previously.

2.5 Identification of Pathogens

Staphylococcus aureus ferments mannitol and grows on mannitol salt agar well. Presumptive coagulase positive colonies form colonies surrounded by yellow colour while presumptive coagulase negative colonies are surrounded by pink colour. All yellow colonies on mannitol salt agar were picked and Gram stained to observe morphology.

The catalase test was used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water. One drop of hydrogen peroxide solution was be placed on a slide. A small portion of the suspect colony was spotted onto the centre of a slide. Presence of vigorous bubbling was observed occurring within 10 seconds, a hand lens was used when necessary. For this method, a positive result (was by observation of a vigorous bubbling indicated the presence of catalase) while a negative result (was by abservance of that bubbling).

Coagulase is an enzyme produced by *Staphylococcus aureus* that converts fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates. The test used rabbit plasma that had been inoculated with a staphylococcal colony. The tube was incubated at 37 degrees Celsius for 1-1/2 hours. If negative, incubation was continued up to 24 hours. A positve test was indicated by formation of clots while negative one was by absence of clots.

Coagulase positive colonies were subjected to oxacillin disc diffusion test to detect MRSA (Methicillin resistant*Staphylococcus aureus*) strains. A suspension of the isolate was prepared to 0.5 McFarland standard, then spread evenly onto Mueller-Hinton agar in a petri dish. Disks impregnated with 1 microgram oxacillin were placed onto the surface of the agar. After incubation, a clear circular zone of no growth in the immediate vicinity of a disk indicated susceptibility to that antimicrobial. Using reference tables, the size of zone was related to the MIC and results recorded as whether the organism was susceptible (S), intermediately susceptible (I), or resistant (R) to that antibiotic.

The colonies growing on the Sabourands Dextrose Agar plates were examined, noting the colony color, shape and texture. Lactophenol cotton blue mounts were made and Gram staining was done to rule out bacteria. Using the flow chart in identification manual, the genus was determined based on microscopic morphology. All growing colonies were plated on CHROMagarCandida for preliminary identification of *Candida* species. Yellow or pink yeast colonies were identified based on morphology after preparation of Lactophenol cotton blue mounts and using the identification key (Forbes *et al*.,2002).

Identification of molds was based on the examination of macroscopic characteristics such as colonial form, surface colour, production of pigments, growth rate and textures while microscopic features such as spore type and spore bearing cells were used for identification using a general key that has been established for identification of fungi(Campbell *et al*.,1996 and Forbes *et al*.,2002).

With a wire or needle bent at a 90 degrees angle a small portion of the isolated colony was cut.The portion was picked from a point intermediate between the centre and the periphery.The portion was placed on a slide to which Lactophenol cotton blue had been added. A coverslip was placed into postion and pressure applied gently. The slide was then observed using x40 magnification(Forbes *et al*.,2002). A small portion of clear vinyl tape, adhesive side down was pressed onto surface of the colony. The tape was removed and placed onto a drop of Lactophenol cotton blue on a slide which was examined microscopically(Forbes *et al*.,2002). A small portion of the colony was inoculated into Christensen urea agar and incubated under room temperature.

Time–kill experiments against isolates of *S. aureus* were undertaken using Jik™,Savlon™,Omo™ which are common household disinfectant, antiseptic and detergent respectively. Four strains of *S. aureus* were used for the kill time studies. Concentration of the disinfectants were prepared as follows:

(i) OMO™ - Lower concentration(0.0125g/5ml),Working solution(0.025g/ml) and upper concetration (0.05g/5 ml).
(ii) JIK™ – 0.5 ml:22 ml ,1 ml:44 ml and1 ml:10 ml
(iii) SAVLON™ -1ml:20 ml,0.5 ml:10 ml and 0.25 ml:5 ml .

The concetration of the disinfectants was determined from working solution with one above the working solution and one belowthe working solution. A suspension of the isolate was prepared to 0.5 McFarland standard. Each organism time-kill assay was performed separately. The timer was started and a sterile swab inserted into the tubes containing isolate. Tryptone soy broth was streaked at At time 0 and time points 2 minute, 4 minutes, 6 minutes, 8 minutes and 10 minutes. After overnight incubation, the plates were examined for growth or absence of growth at each concentration for each disinfectant and dertegent. The kill time was read at the concentration and time required to kill the test organism.

3.0 Results

3.1 Diversity of Microflora on Clothes Samples

A great diversity of bacterial colonies growing on Mannitol Salt Agar, which is a selective medium for recovering *Staphylococcus aureus. S. aureus* ferments mannitol and is able to grow on agar containing 70-10 0g/l sodium chloride thus is able to grow well on mannitol salt agar producing yellow colonies. Upon incubation, yellow, white cream, pink colonies were observed on the agar. Yellow colonies were picked and gram stained revealing, Gram positive cocci in clusters. Catalase test was used to distinguish between catalase negative (streptococci) and catalase positive (Staphylococci). Yellow colonies producing bubbles when 3% hydrogen peroxide was added were categorized as catalase positive (Table 1).

Table 1: Summary of bacterial isolated from clothes from Mannitol Salt agar

	Gram status and cell morphology
Bras	Gram positive cocci in clusters Gram positive cocci Gram negative rods Gram positive rods
Towels	Gram positive cocci Gram positive cocci in clusters Gram positive rods Gram negative rods
Socks	Gram positive cocci Gram positive cocci in clusters Gram positive rods Gram negative rods
Panties	Gram positive cocci Gram positive cocci in clusters Gram positive rods Gram negative rods

3.2 Mannitol Fermentation

Mannitol Salt Agar plate showing 24hr growth of mannitol fermentor (yellow) and non-mannitol fermentor (pink). Mannitol fermentation is biochemical characteristic used for the presumptive identification of *S. aureus* by the production of yellow pigments on Mannitol Salt Agar. Yellow colonies are therefore indicative of presence of *S. aureus* (Plate 1).

Plate 1: Mannitol fermentation

3.3 Effects of Washing

Bacterial counts before washing were found to be 824 C.F.U for panties. Panties and socks had the highest contamination with average plate count of 673.25 and 545 respectively. Towels and bras had the lowest plate counts with 470 and 412 respectively after washing. Table 2 and Figure 2 summarize the C.F.U of the different clothes before washing and after washing.

Table 2: Effects of Washing on plate counts in different cloth samples

Sample	C.F.U Before Washing	C.F.U After Washing
1B	431	24
2B	405	66
3B	400	50
Average CFU of bras	412	46.67
1T	456	27
2T	450	255
3T	504	118
Average CFU of towels	470	133.33
1S	354	55
2S	481	19
3S	800	104
Average CFU of socks	545	59.33
1P	610	28
2P	780	44
3P	479	38
4P	824	61
Average CFU of panties	673.25	42.75

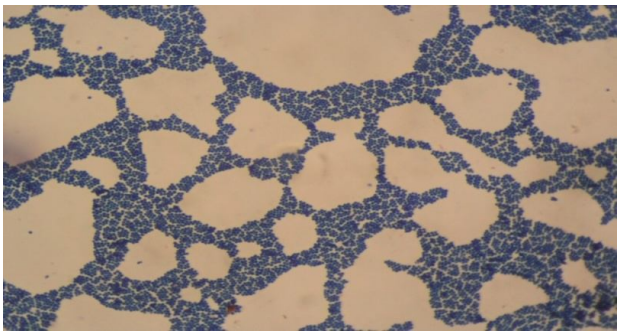

Plate 2: Photomicrograph of Gram Positive Cocci in clusters indicative of S. aureus

When the clothes were washed with laundry bar soap, socks and panties had the highest bacterial counts, followed by towels and bras (Figure 1).

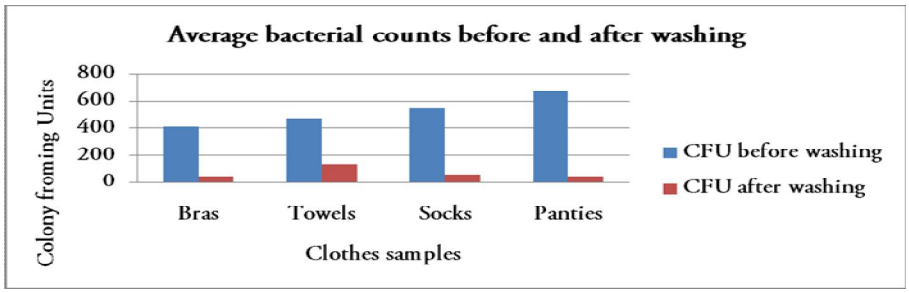

Figure 1: Average bacterial counts before and after washing

3.4 Methicillin Resistance Detection

S. aureus isolate from second hand clothes inoculated on Mueller Hinton plate, showing resistance to 1microgram Oxacilin disc (note arrow). MRSA Detection was done using the Kirby bauer disk diffusion method (Plate 3).

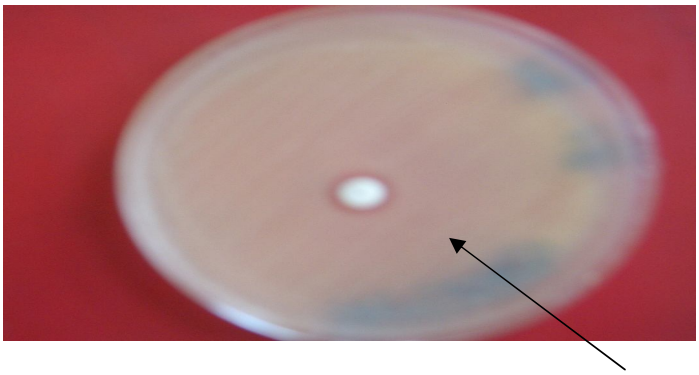

Plate 3: Methicillin Resistance Detection

Biochemical characterization revealed Coagulase positive *Staphylococcus* isolates from the second hand clothe samples. After using 1microgram Oxacilin disc 12(44.4%) of the coagulase positive *Staphylococci* isolates were found to be MSSA while 15(55.5%) were MRSA (Table 3 and Plate 3).

Table 3: Frequency of Isolation of MRSA in second hand clothes

Organism	Zone of Inhibition	Status
01	11mm	Resistant
02	10mm	Resistant
03	21mm	Sensitive
04	21mm	Sensitive
05	13mm	Intermediate
06	15mm	Intermediate
07	12mm	Intermediate
08	15mm	Intermediate
09	6mm	Resistant
10	17mm	Sensitive
11	18mm	Sensitive
12	16mm	Sensitive
13	12mm	Intermediate
14	6mm	Resistant
15	6mm	Resistant
16	11mm	Resistant
17	6mm	Resistant
18	10mm	Resistant
19	8mm	Resistant
20	16mm	Intermediate
21	8.5mm	Resistant
22	12mm	Intermediate
23	15mm	Intermediate
24	12mm	Intermediate
25	17mm	Sensitive
26	11mm	Resistant
27	6mm	Resistant

Key- Cut off point-Less than 11mm-Resistant 12-15 mm-Intermediate and >than 16 mm Sensitive while number 1-27 represents *Staphylococcus aureus* isolates.

3.5 Diversity of Fungi Iolated from Clothes

Molds isolated from second hand clothes were identified using lactophenol cotton blue mounts. These molds included *Scopularipsis brevicalis* (Plate 7), *Geotricum candicum*, *Scytalidium*, *Trichophyton mentagrophtes* (Plate 6), *Cladosporium* sp., *Paecilomyces* sp., *Altenaria alternata, Ramichhloridium mackenzei*, *Aspergilus flavus*, *and Rhizomucus pusillus* (Table 4). Table 4. Summarizes the different fungi isolated on Mycosel agar.

Table 4: Fungi isolated from the second hand clothes before and after washing

SAMPLE CODE NUMBER	COLONY FORMING UNITS	COLONIAL APPERANCE ON MYCOSEL AGAR	IDENTITY AFTER LACTOPHENOL STAINING
1P	4	Cream -colony	*Geotricum candicum*
2P	3	White powdery	*Trichopyton mentagrophtes*
		Olive green	*Cladosporium cladosporoides*
1P		Black	*Scytalidium*
3P	7	Green colony large	*Cladosporium cladosporoides*
4P	12	Sand brown colony	*Scopulariopsis brevicalis*
		Green-Blue colony with white edge	*Penicillium* sp.
1S	-	-	Negative
2S	12	Black colony with white edge ,furrows	*Penicillium* sp.
			Aspergilus sp.
3S	6	Black colony	Aspergilus sp.
1T	1	Sand brown colony	*Scopularipsis Brevicalis*
2T	7	White powdery	*Trichophyton*
3T	10	Blue green	*Penicillium*
			Aspergilus fumigatus

Yeast isolated from second hand clothes were identified using CHROMagarCandida including *Rhodotorula, Candida tropicalis* and *Candida glabrata*. Table 5 summarizes the different yeasts isolated, as well as Plates 4 and 5.

Table 5: The spectrum of yeasts isolated from second hand clothes

	C.F.U before washing	APPERANCE	IDENTITY on CHROMagarCandida	C.F.U	APPERANCE	IDENTITY CHROMagarCandida
1P	7	Pink colonies	Rhodotorula		NEGATIVE	
2P	18	White cream colonies Pink colonies	*Candida tropicalis* *Candida glabrata* *Rhodotorula* sp.	1	White cream colonies with shriveled black centre	Enlongated yeast non Candida
03P	4	Pink colony	*Rhodotorula* sp.	2	Small light pink colonies	*Rhodotorula* sp.
4P	4	White colonies	*Candida tropicalis*	1	Small colonies cream	*Candida tropicalis*
1S	16	White	*Candida tropicalis* *Candida glabrata*	4	Pink colonies	*Rhodotorula* sp.
2S	8	Pink Olive green	*Rhodotorula* sp. *Cladosporium* sp.	5	Large pink colony white	*Rhodotorula* sp. *Candida tropicalis*
3S		-		-	Negative	
1T	7	Pink colonies Olive green colonies	*Rhodotorula* sp. *Cladosporium* sp.	3	Pink colonies	*Rhodotorula* sp.
2T	15		Negative	-	Negative	
3T		-	-	-	Negative	
1B	4	Pink colonies	*Rhodotorula* sp.	2	small pink and white colonies	*Rhodotorula* sp. *Candida glabrata*
2B	10	white	Non-Candida yeast	6	Small colonies pink	*Rhodotorula* sp. *Candida tropicalis*
3B	7	Large pink colonies	*Rhodotorula* sp.	4	Large pink colonies White	*Rhodotorula* sp. *Candida glabrata*

Plate 4: Orange colonies (Rhodotorula sp.) growing on Sabourands Dextrose agar

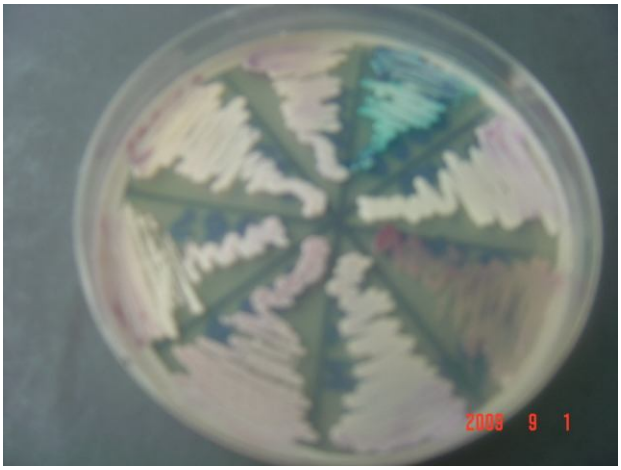

Plate 5: Colony morphology of different yeast species on CHROMagar Candida

Blue colour is usually indicative of *Candida tropicalis,* purple colour is indicative of *Candida glabrata,* pink colour is indicative of *Candida parapisilopsis* and pink colour is indicative of *Candida albicans* (Plate 5).

Plate 6: Trichophyton mentagrophytes (Table 4) isolated from towel sample

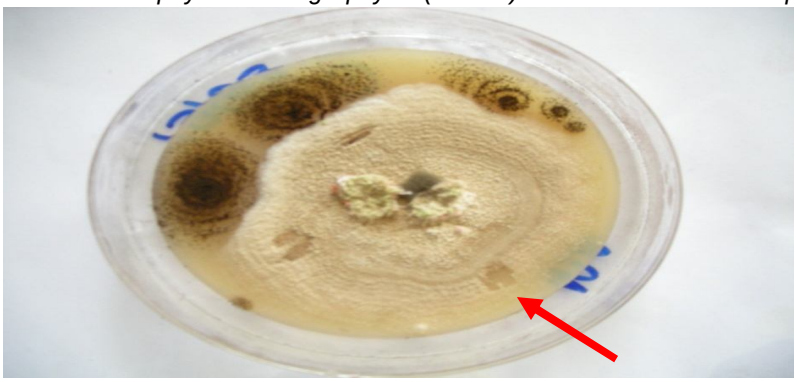

Plate 7: Scopulariopsis brevicaulis(brown colony). Note arrow

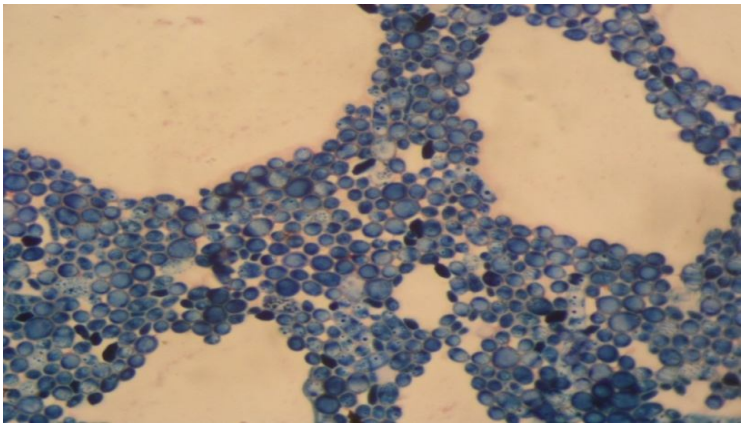

Plate 8: Photomicrograph of Candida glabrata

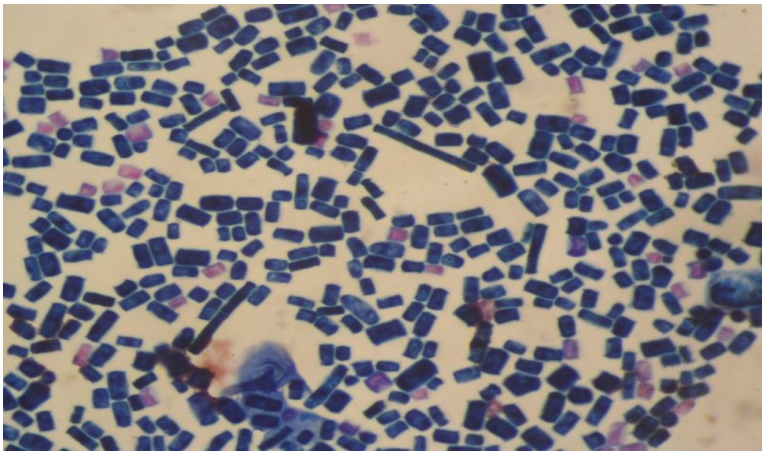

Plate 9: Photomicrograph of Geotricum sp

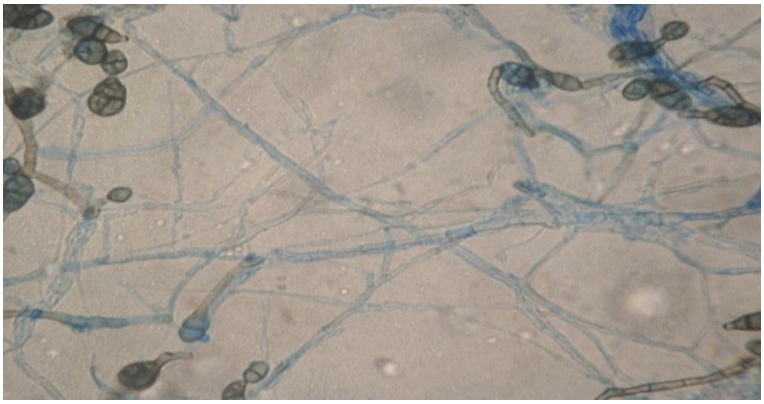

Plate 10: Photomicrograph of Altenaria alternate

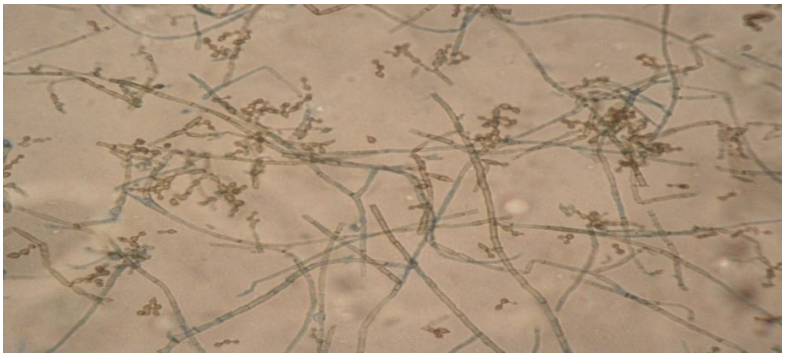

Plate 11: Photomicrograph of Cladosporium sp

Panties had the highest count of yeast with average count of 8.5 while bras had the lowest with an average count of 7 CFU as shown on Figure 2.

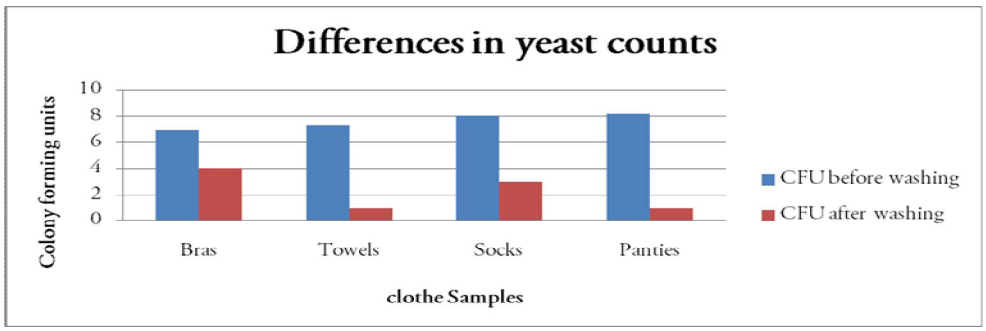

Figure 2: Differences in yeast counts before and after washing

Panties and bras had the highest count of molds with an average count of 6.5 and 6.3 respectively followed by towels and socks at 6 C.F.U per cm of the cloth before washing (Figure 3).

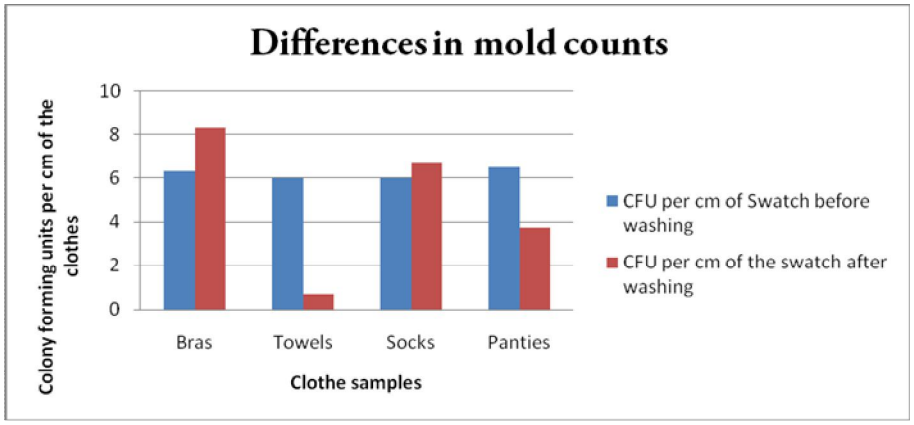

Figure 3: Differences in mold counts before and after washing

3.6 Kill Time Assay Results

The kill time of Jik was 2minutes at concentration range 1ml: 10ml (Jik: Distilled water) for all MRSA isolates tested. Growth was observed at 0minutes but no growth observed after 2 minutes (Plate 12).

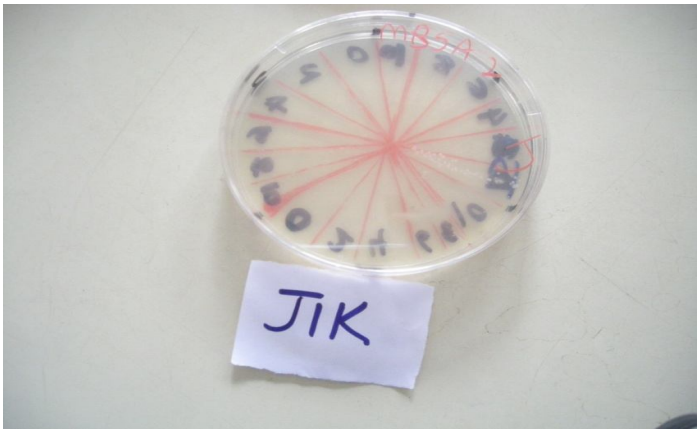

Plate 12: Determination of the Kill-Time of Sodium hypochloride(Jik™)

The kill time of Savlon was 2 minutes at 0.5 ml: 10 ml (Savlon : Distilled water) concentration for all the MRSA isolates tested. Growth was observed at 0 minutes (Note arrow) but no growth observed after 2minutes (Plate 13).

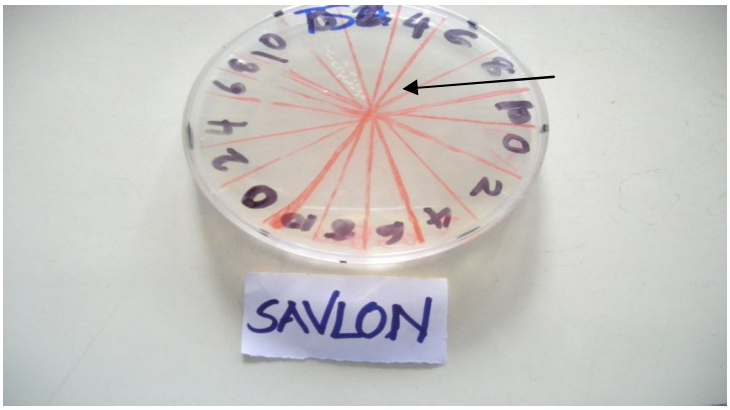

Plate 13: Determination of the Kill-Time of Savlon™

3.7 T-Test Analysis

The level of bacteria isolated reduced significantly after washing with the bar soap indicated by T-test analysis (t_{12} = 1.1, P < 0.005).

T stastic (1.1) is less than T–crtical value (2.2) therefore is no significant reduction in mold counts (t12 = 1.1, P > 0.005).

T- statistic (3.5) of differences in yeast counts before and after washing is greater than T–critical value (2.2). Therefore, was a significant reduction in yeast counts (t12 = 3.5, P < 0.005).

4.0 Discussion

This study demonstrated that second hand towels, bras, panties and socks were frequently colonized with bacteria such as *Staphylococcus aureus* including MRSA, MSSA which are of great public health concern. Detection frequencies of MRSA were noted to be higher in panties than in other clothes as shown in Figure 1. Staphylococci are widespread in the environment and can be cultured from clothing and virtually all environmental surfaces (Sattler *et al*., 2004). Because of the frequency of intimate contact with the previous owner skin and the fact that the undergarments come from people from all walks of life, it is reasonable to expect that these clothes are colonized with potentially pathogenic bacteria and fungi as demonstrated by the study. Staphylococci resist drying and can survive in dust and soil for years. They are tolerant of high temperatures; this capacity and resistance to drying allowing prolonged survival on fomites and clothing (Sattler *et al*., 2004). Coagulase-positive staphylococci

are responsible for both sporadic infections and epidemics of varying extent. It causes superficial skin lesions such as boils, styes and furuncules; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis (Todar, 2008).

Methicillin resistance in *Staphylococcus aureus* was detected by Kirby-Bauer disk diffusion method. 12(44.4%) of the coagulase positive staphylococci isolates were found to be MSSA while 15(55.5%) were MRSA (Plate 2 and Table 3). Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) cause significant morbidity and mortality (Synder *et al*., 2005). Because MRSA is resistant to all commonly prescribed beta-lactam antibiotics, these infections require treatment with alternative expensive antimicrobial drugs. Although Methicillin-resistant *Staphylococcus aureus* (MRSA) have been entrenched in hospital settings for several decades, MRSA strains have recently emerged outside the hospital, becoming known as community associated- MRSA((CA-MRSA) or superbug strains of the organism, which now account for the majority of staphylococcal infections seen in the Emergency Rescue or clinic (CDC Report,2003 and Sattler *et al*., 2004). Data from outbreaks of community-associated MRSA infection suggest that skin-skin and skin-fomite contact represent important and common alternative routes of acquisition of the skin infecting strain (Miller *et al*., 2008).

For staphylococci, the results are consistent with those of Nelly *et al*.,2000 and Loh *et al*.,2000 who reported that *S. aureus* can survive for long periods on hospital fabrics to become of epidemiological importance. In contrast, Scott and Bloomfield showed *S. aureus* surviving only 4 to 24 h on cloth; however, their inocula were low (102 CFU).

Majority of all of the clothes tested were cotton and polyester according to the clothes label. These materials are commonly used as undergarments and socks. Polyester and cotton fabrics are easily penetrated by common skin commensals. Takashima *et al*., 2004, found that polyester or acrylic fibers bound *S. aureus* and *P. aeruginosa* at high ratios (>80%), but cotton fibers bound them at low ratios (<10%). Nylon fibers bound *S. aureus* at low ratios, but *P. aeruginosa* at intermediate ratios. *Staphylococcus epidermidis* was found to adhere to fabrics much more so than *S. aureus*. The adherence of both *S. epidermidis* and *S. aureus* to fabrics increased as the content of polyester fibres in the fabrics increased. The attachment of E. coli to all fabrics was very low and was not affected by the fibre contents **(**Hsieh *et al*., 1986).

Survival of microbes on fomites is influenced by intrinsic factors which include fomite properties or microbe characteristics and extrinsic factors, including environmental temperature, humidity, etc. If these microbes remain viable on surfaces long enough to come in contact with a host, they may only need to be present in small numbers to infect the next host (Boone *et al*, 2007).

Some of the fungi isolated from the clothes include *Scopularipsis brevicalis* (Plate 7), *Geotricum candicum*(Plate 9), *Scytalidium*, *Trichophyton mentagrophtes*(Plate 6)*, Rhodotorula sp.* (Plate 4), *Cladosporium sp., Candida tropicalis*, *Candida glabrata*, *Paecilomyces, Altenaria alternata*, *Ramichhloridium mackenzei*, *Aspergilus flavus*, *Rhizomucus pusillus* (Table 4)*.* Panties and bras had the highest count of molds with an average count of 6.5 and 6.3 respectively followed by towels and socks at 6 C.F.U per cm of the cloth. This can be attributed to the high frequency of usage of the undergarments. After washing there was increase in CFU in bras and socks possibly due to dispersal of spores from one area of the clothe to a different area during washing which was then cut into swatch for isolation. Research shows that cotton fabric spreads spores of *Aspergilus* sp. better than other fabrics (Potera, 2001)

These data indicate that many of the fungi which are associated with fungal infections in patients can survive for long periods in clothes (Table 4).Several studies have been presented on the survival of yeasts on various surfaces, such as the survival study of *Aspergillus, Mucor, Fusarium, and Paecilomyces* on a variety of common hospital fabrics and plastics by Neely *et al*., 2001. Where comparisons are possible for the fungi data, my results agree with what is in the literature.

Yeast isolated included *Candida tropicalis*, *Candida glabrata, Geotricum and Rhodotorula* (Table 5). Panties had the highest count of yeast with average count of 8.5 while bras had the lowest with an average count of 7CFU. *Rhodotorula* was found to persist in the clothes even after washing the clothes with grade 2 bar soap as shown in

Table 4. Nelly et al., 2000 quotes Blaschke-Hellmessen *et al*.,1986 study of yeast resistance to dryness, who found that those species of yeast that are more common in the environment, such as *Rhodotorula* spp., were more resistant to drying than yeast species that were more common on mucous membranes, such as *C. albicans*. Several *Aspergillus* spp. and *Penicillium* sp. were isolated in this study which could have been environmental species. These fungi have diverse clinical implication and cause superficial and systemic diseases with high fatalites. These results indicate the potential for various fabrics to serve as reservoirs or vectors for fungi because the fungi tested generally remained viable on these clothes even when they were washed. People with human immunodeficiency virus (HIV) or AIDS or other immune disorders and those undergoing chemotherapy are at higher risk as well, because they have a weakened immune system.

In this study, the clothes were washed with grade 2 laundry bar soap. After washing there was a reduction in the bacterial plate counts (t_{12} = 9.6, P < 0.001) (Table 2 and Figure 2). The grade 2 laundry bar soap may contain bactericidal or bacteriostatic compounds to the microbes harbored on the clothes. The concentration of bactericidal compound within the bar soap may be less than that of antiseptics such as Savlon. Thus, it is possible that spores of fungi would not be effectively killed by the bar soap. The differences in the microbes' counts among the four clothes types and the differences in CFU before and after washing were found to be different for all the test samples as shown in Figure 1, 2 and 3. This can be attributed to the different levels of exposure to contamination before washing among the clothes samples. Fumigation may have been carried out on clothes from the country of origin. Some of the clothes are also hanged in the open in the second hand market where the microbes can be killed by ultraviolet radiation from the sun. The frequency of usage and laundering by previous user, hygiene habits and their state of health e.g. shedding of pathogens from bodily fluid by sick user can be a factor to increased number in some clothes as compared to low number in clothes from healthy donor of second hand clothes.

The results of the Kill- Time Assay using Savlon and Jik indicated that the Kill-Time for both Salvon and Jik was 2 minutes at all the three concentrations used (Jik-0.5 ml: 22 ml, 1ml: 44 ml and1ml:10ml and Savlon-1 ml: 20 ml, 0.5 ml: 10 ml and 0.25 ml: 5ml) note Figure 4 and 5. When Omo was used for time kill assay all the MRSA isolates were not killed at 10 mins for all the concentration used which are lower concentration (0.0125g/5 ml), Working solution (0.025g/ml) and Upper concentration (0.05g/5 ml). Commonly used detergents such as omo and Laundry bar soaps contain enzymes and stain removal chemicals which may have bacteriostatic properties.

Jik is household bleach which contains Sodium hypochlorite (3.85%m/v) which has bactericidal activity to microbes thus it is used widely for disinfection. Chloride Releasing Agents (CRAs) such as hypochlorite are highly active oxidizing agents and thereby destroy the cellular activity of proteins. Deleterious effects of CRAs on bacterial DNA that involve the formation of chlorinated derivatives of nucleotide bases have been described (McDonnell *et al.*, 1999). Savlon contains n-propyl alcohol, chlorhexidine gluconate 0.3 g and Cetrimide 3.0 g (2.84 % m/v) which confer antiseptic properties to Savlon. Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in hand washing and oral products but also as a disinfectant and preservative (McDonnell *et al.*, 1999). Disinfection has been shown to reduce the number of pathogenic microbes in disinfected clothes. The data on the Kill-time assay showed that savlon and Jik are suitable for laundering clothes contaminated with these MRSA isolates. Despite the improvement of medical facilities for treating skin infection, there is an urgent need to disinfect the clothes with disinfectants to prevent infection.

5.0 Conclusion and Recommendations

Variety of both MSSA and MRSA potential skin pathogens were isolated from second hand undergarments, socks and towels and other clothes. The mycoflora is not limited to dermatophytes such as *Trichophyton* but other fungi exist such as *alternaria alternata* which are pathogenic. In this study, the kill time for both Jik and Savlon against the MRSA isolates from clothes is 2minutes. The concentrations of Omo used do not kill the MRSA isolate at 10 min. Although it is not easy to establish the precise role that second hand clothes may play in transmission of skin infections, this study adds to the growing data on clothing and equipment as potential vectors. Since the clothes are very popular. Regular disinfection of clothes using antiseptic and disinfectants such as Savlon and Jik may reduce bacterial pathogen contamination.

To prevent the spread of skin pathogens from the second hand clothes, several recommendations should be considered. One, consumers should wash second hand clothing with disinfectants and strong detergents such as Savlon and Jik as this reduce the microbial load of the clothes thus preventing infections; two, consumers should approach second hand undergarments with caution since this study has demonstrated that clothes have high bacterial counts. Pathogenic fungi are also found on these clothes and can these clothes serve as potential vectors of skin infection; three, research should be carried out to show direct correlation of skin infection with actual users of these clothes; four, Kill time studies for commonly used detergents should be carried for a longer time period (more than 10 minutes) to find out whether they can kill pathogens on clothes and lastly, the gap between 0-2 minutes can be checked to find out the appropriate/real time of exposure.

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