

A Microbial Survey of Second Hand Clothe Samples Collected from Baghdad Market

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Abstract

Second hand represent one of the sources of illness due to environmentally related disease and injury. Used clothes are sold all over the country since they are very cheap compared with the new ones. The current study included collecting samples from different used clothing markets in Baghdad and comparing the efficiency of detergents used to reduce microbial load in the garments. The samples were adult's clothes, babies' clothes, and fabric toys. Isolation and identification of bacteria with CFU were done to determine which cloth samples carry the highest bacterial contamination and dominant pathogenic microbes associated with skin infection. Culture and biochemical methods were used for the identification. Samples were washed using two types of detergents (soap, granulated detergent) to investigate the efficiency of these detergents in reducing bacterial contamination in such clothes. The results showed that the dominant isolate bacteria in adult clothes were *Staphylococcus* sp. and *Enterobacteriace* sp. while *Enterobacteriace* sp., *Bacillus* sp. in baby clothes and *Bacillus* sp. in fabric toys. The results of CFU found to be the highest in baby's clothes (4×10^6), fabric toys (2.2×10^4), adult clothes (20×10^2) respectively. Results showed that the isolated fungi were *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus*. After washing with detergents including soap and granulated detergent, the results showed that the granulated detergent is more effective in reducing bacterial and fungal contamination than soap in all samples. This study concluded that babies' cloths are vulnmrable to contaminating with bacteria and fungi then fabric toys followed by adult clothes. It's recommended to wash second hand clothes using other methods to remove both bacterial and fungal contamination such as disinfectants then ironing. [DOI: [10.22401/JNUS.20.3.19](https://doi.org/10.22401/JNUS.20.3.19)]

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Introduction

Second hand clothes (SHC) are clothing items that have previously been used by one person earlier to the present user. Most of the used clothes, including sweaters, coats, shirts, underwear, socks, boots, shoes, toys and sandals. These clothes are imported commonly from the Asia and West. the largest exporter of second hand clothes in the world are United state America (U.S.A) follow by Germany, the United Kingdom (U.K) and Netherland while the largest importers of second hand clothing are Sub- Sahara African, South East Asia and Eastern Europe [1].

The used clothes in Iraq are known as bale means a bundle of different kinds of clothes which come from different world source or called (Langah) means used clothes in the Kurdish Language. Bale are imported by businessmen/women and charitable organizations. Bale enters to Iraq since 1850 in (Al-Bab Al Sharqi) which represent the largest

market of bale in Baghdad. Clothes are sold all over the capital, on hand carts and in shops.

The (South Australia Public Health Fact Sheet, 2009) mentioned some of the risks associated with used clothing consist of; Tinea, Impetigo, Scabies, Body lice, Pubic lice/crabs, Head lice and Bed bugs and fungal infections are possible diseases that could be transported through used clothes. Used clothes have high health risks and they may spread skin diseases [2].

In numerous studies, clothes have been found to be infected with chemicals and biotic factors [3]. Used clothes have been shown to aid in the transmission of pathogens from one individual to another, sickness the person that wear the cloth had before (his or her) death could most likely be transmitted to the consumer, especially if it is a disease that can be transmitted to the new consumer through body fluids like sweat. "so must fabric types (wool, nylon, and cotton, act as wicks to carry

pathogens far away from the original contact position with human skin, mainly when moisture is present from sweat, saliva, secretions from open pimples, wounds and spilled drinks [4].

In a research carried out by Nelly the records referred that *Enterococci* and *Staphylococci* can survive for extended periods of time on materials commonly wear by patients and health care workers and on various other fabrics in the hospital environment [5] while another study refers that neckties worn by doctors at an intensive care unit produced heavy growths of coagulase negative *Staphylococcus* on 2/5 ties tested [3]. Studies have revealed that a variety of objects, such as tools, toys, and clothes, can serve as vehicles for the transmission of *Shigella* sp. In the community, the skin infections have grown significantly and the majority of these infections are transmitted through, shared equipment also due to contact skin-to-skin [6]. In a study conducted 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 [5].

Detergents are essential to personal and public health. Through their ability to loosen and remove dirt from a surface, they contribute to good personal hygiene; reduce the presence of germs that cause infectious diseases. Antimicrobial agents Kill or inhibit the growth of microorganisms that cause diseases.

Detergents and soaps found in the home can be grouped into four general Categories: personal cleaning, cloth cleaning, dishwashing and household cleaning. Within each category are different product types formulated with ingredients selected to perform a broad cleaning function as well as to deliver properties specific to that product [7]. A detergent is a chemical compound it is an effective cleaning product because it contains one or more surfactants. The surfactants used in detergents can be engineered to perform well under a variety of conditions. These days detergents are made from a multiplicity of petrochemical chemicals (derived from fats

and oils) other chemicals (such as sulfur trioxide, sulphuric acid, and ethylene oxide) and alkalis [8].

Antimicrobial agents also called disinfectants or sanitizers such detergents, are either microbiocidal (kill microorganisms) or microbiostatic (inhibit the growth of microorganisms). Detergents can work in a number of ways. Some interfere with the formation of the microbial cell walls or cell membranes, usually having a microbiocidal effect. Some interfere with the synthesis of microbial proteins, nucleic acids, or essential metabolites. For examples, Quaternary ammonium chlorides and alcohols may be used as antimicrobial agents [9]

The previous study indicated that soaps can remove 65 to 85% bacteria from human skin [10]. A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or kill them. The number of chemicals in enormous, probably at least 10000 with 1000 commonly used in the hospitals and homes. These chemicals exist as solids, liquids, and gases such as granulated detergent and soaps which are used to reduce or destroy microbes [11].

Although fats and oils are general ingredients of soaps but some chemical compounds are added to enhance the antibacterial activities of soaps [12] Transient bacteria are deposited on the skin surface from environmental sources such as used clothes causing skin infections. Examples of such bacteria are *Pseudomonas aeruginosa* [13] and *Staphylococcus aureus* [14].

The aim of the study was to investigate bacteria and fungi contamination in the second hand clothes and assess the risk of transmission of pathogenic microorganisms. Also, assess the efficiency of two kinds of detergents to remove this bacterial contamination.

Materials and Methods

Collection of samples

Samples were collected from three used cloth markets, including Baghdad algededa, Al Dora, Al Binook district markets. These Markets represent the largest second hand clothes in Baghdad. All samples were kept in sterile plastic bags and transferred to the

laboratory for bacterial isolation and identification.

Ready to use media

Nutrient agar and MacConkey agar were prepared according to the instruction of manufacturing company. Three samples were collected from each of the following categories: adult's clothes, baby's clothes, and fabric toys.

Isolation method

The samples which are obtained from the markets using swab were inoculated to isolate bacteria, for adults clothes a swab from the entire area (under arm, neck) of the sample was swabbed using a sterile cotton swab immersed in sterile water while baby clothes were swabbed in areas of (chest, foot). Different swabs were taken from fabric toys. Samples suspension was prepared by adding the swabs to 4.5ml D.W. in a sterile tube and mixed well, then inoculated into Nutrient agar and MacConkey agar using ABC streaking method for isolation of G-ve and G+ve bacteria then incubated at 37°C for 24 hrs[18].

Total bacterial count (CFU/ml)

Samples (10 ml) were aseptically pipetted into a sterile Erlenmeyer flask and diluted by adding 90 ml of peptone water pH 7.2, then series dilutions were done (10^{-2} - 10^{-8}). 0.1 ml of each dilution was spread on nutrient agar and incubated at 37 °C for 24 hours with three replicate [15]. At the end of the incubation period, the colonies on the Petri plates were counted using digital colony counter. The numbers of colonies were estimated as CFU/ml according to the below equation:

$$CFU /ml = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume plated in ml}}$$

The same procedure was done before and after samples washing.

Isolation of Fungi

The entire area of the 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 cm² swatches of all the samples were cut using 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[16]. Appearance of isolated fungi were calculated according to [17].

Inoculum preparation

The samples were washed with two types of detergents the Neon-trademark as soap detergent and the granulated detergents of Al-wazeer- trademark. Each sample of (Adult clothes. baby's clothes, fabric toys) were cut into two pieces, one of them was washed with soap detergent and the other washed with granulated detergent. The Temperature of washing water was 50°C. All samples were dried before isolation. Swabbs were taken from each pairs of pieces and presented a primary procedure to isolate bacteria.

Identification of bacterial isolates

Isolated bacteria were identified by using Bergey's Manual of Determinative Bacteriology, on the basis of physiological, morphological and biochemical properties [18].

Morphological characterization

Colonies developed on nutrient agar, then classified according to their shape, color, odor, and their margin.

Microscopic examination

Microscopic examination was done for the gram-stained slides in order to characterize gram-positive bacteria from others.

Results and Discussion

Incidence of bacteria on Clothes Samples

Bacterial growth exhibited a mixture of colonies in the Nutrient and MaCconkey Agar, which represent a selective medium for recovering G-ve and G+ ve bacteria. This latter work included the preparation of ferment mannitol medium which sustains bacteria growth on agar containing sodium chloride (70-100g/l), therefore, it's able to grow on mannitol salt agar to produce yellow colonies. During incubation, colonies with white cream

color observed on the agar also yellow colonies refer to Gram-positive cocci in clusters. Catalase test was used to distinguish between catalase negative (*Streptococci*) and catalase positive (*Staphylococcus aureus*). Yellow colonies showed bubbles after adding hydrogen peroxide 3% and categorized as catalase positive Table (1).

Table (1)

Summary of bacteria isolated from different samples in nutrient agar.

Sample type	Gram status and cell morphology
adult's clothes	Gram positive cocci in cluster Gram positive cocci
baby's clothes	Gram positive cocci in cluster Gram positive bacillus
fabric toys	Gram positive bacillus

Table (2)

Colonies morphology, gram stain, and biochemical tests results.

Microorganism Characteristics	<i>Staphylococcus aureus</i>	<i>Bacillus sp.</i>	<i>Enterobacter sp.</i>
Colony color	gold	creamy	creamy
Cell shape	cluster	short rod	rod
Gram stain	+	-	-
Catalase test	+	+	+
Oxidase test	-	+	-
Methyl red test	-	-	-
Voges – Proskauer test	-	-	+
Nitrate reduction test	+	+	+
Citrate utilization test	-	+	+
Urease production test	-	-	+
Indole production test	-	-	-
Motility test	-	+	+
H ₂ S production test	+	-	+

(+) = *positive result.* (-) = *negative result*

The results of this study showed that used clothes such as adults and baby's clothes as well as fabric toys were colonized frequently with bacteria such as *Staphylococcus aureus*, *Bacillus*, and *Enterobacteriaceae* which have great public health interest. In the environment, *Staphylococci* are widespread and can be found on clothing as well as environmental surfaces [4]. A previous study referred that *Staphylococci* can survive for years in dust and soil particles, also it can resist the dryness conditions and tolerant high

Biochemical characteristic and identification of bacterial isolates

The biochemical tests were performed to identify the selected bacterial isolates. On the basis of various biochemical tests, results showed that the bacterial isolates were *Bacillus sp.*, *Staphylococcus sp.*, and *Enterobacter sp.* The details of the gram staining and biochemical tests are given in Table (2).

temperature, such bacterial characteristics allows it to survived on fabric and clothes for a long time and most of clothes and undergarments frequency contact with the previous owner skin, expect to colonize with potentially pathogenic bacteria [4]. For *Staphylococci sp.*, our results come agree with those of Nelly *et al*[5] and Loh *et al* [19] who reported that *S. aureus* can survive for long periods on hospital fabrics to become epidemiological.

The existing *Staphylococci* sp. is considered as the main cause of both sporadic infections and epidemics of varying scope. It causes superficial skin lesions such as boils, pneumonia, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis [20].

In the current study greater part of tested samples were made of polyester (fabric type) as it mentioned in clothes label. The baby clothes and toys made of this type of fabric. Takashima *et al* refer that polyester and cotton fabrics are colonized easily by ordinary skin bacteria. Also, they found that polyester or acrylic fibers bound *Staph. aureus* and *P. aeruginosa* at high ratios (>80%), but *P. aeruginosa* at intermediate ratios, but cotton fibers bound them at low ratios (<10%). Nylon fibers bound *Staph. aureus* at low ratios, [21]. There are two factors that control the survival of microbes in fabrics, the first is microbe characteristics and fabric properties while the second is environmental factors such as temperature, humidity, etc. If these microbes stay viable on surfaces long enough to come

into contact with a host, they may only need to be present in small numbers to infect the next host [4].

Viable count of bacterial isolates

Results of the current study recorded high viable counts in the N-agar plates in all cases, which were reported in the order of 10^6 per sample. Compare to before and after washing treated samples, the viable counts were found to be higher in the case of before washing treated with detergent samples. In the case of Adults clothes samples, the viable counts were in the order of 10^3 per piece, and in the case of Baby's clothes, it was 10^6 per piece, while in the case of Fabric toys it was 10^4 per piece before washing.

The samples were washed with granulated detergent showed that the viable counts of bacterial isolates were the lowest compared to soap. The details are as presented in Table (3) and Table (4).

Table (3)
Effects of washing with granulated detergent on plate counts in different sample.

Sample	C F U before washing	C F U after washing
A1	23×10^2	2×10^1
A2	26×10^2	2×10^1
A3	12×10^2	1×10^1
Average CFU of Adults clothes	20.3×10^2	1.6×10^1
B1	3×10^4	1×10^2
B2	13×10^4	1×10^2
B3	15×10^4	1×10^2
Average CFU of Baby's clothes	10×10^4	1×10^2
F1	40×10^3	2×10^1
F2	15×10^3	1×10^1
F3	30×10^3	1×10^1
Average CFU of Fabric toys	28.3×10^3	1.3×10^1

Table (4)
Effects of washing with soap detergent on plate counts in different samples.

Samples category	CFU before washing	CFU after washing
A1	2×10^3	1×10^1
A2	3.7×10^3	1×10^1
A3	1.5×10^3	1×10^1
Average CFU of Adults clothes	2.4×10^3	1×10^1
B1	3×10^6	2×10^1
B2	4×10^6	1×10^1
B3	5×10^6	1×10^1
Average CFU of Baby's clothes	4×10^6	1.5×10^1
F1	2.6×10^4	1×10^1
F2	1.2×10^4	1×10^1
F3	3×10^4	2×10^1
Average CFU of Fabric toys	2.2×10^4	1.3×10^1

**Effects of Washing
Granulated detergent**

From the results shown in Fig.(1), the bacterial counts before washing with granulated detergents were 20.3×10^2 C.F.U in adult's clothes. Baby's clothes had the highest average plate count of 10×10^4 while fabric toys recorded C.F.U 28.3×10^3 . After washing

with granulated detergent fabric toys recorded the lowest plate counts with 1.3×10^1 and 1.6×10^1 for adult's clothes C.F.U while Baby's clothes were found to be the highest record 1×10^2 . Table (3) and Fig.(2) summarize the C.F.U of the different samples before and after washing with granulated detergents.

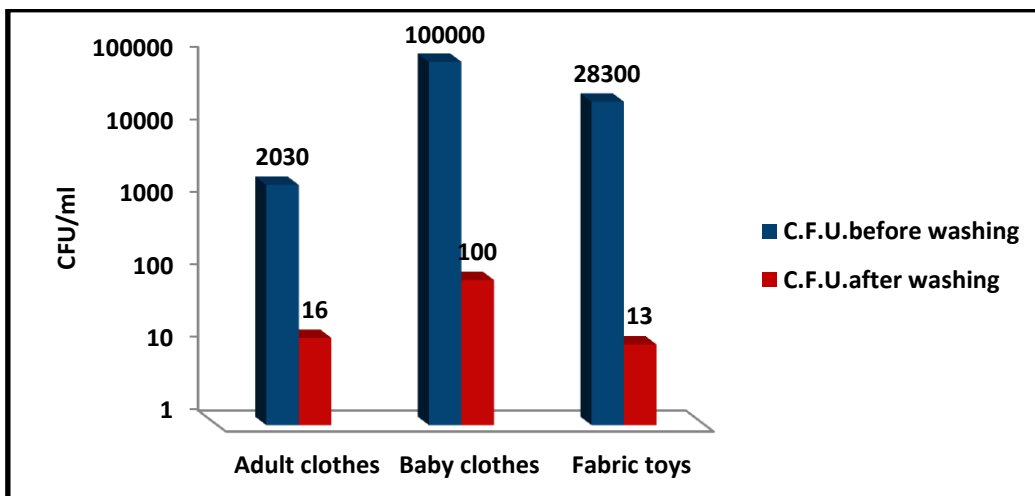


Fig.(1): Mean bacterial counts before and after washing with granulated detergent.

Soap detergent

Results showed that bacterial counts before washing with soap detergent were 2.4×10^3 C.F.U in adult's clothes. Baby's clothes and Fabric toys had the highest contamination with mean plate counts of 4×10^6 and 2.2×10^4 respectively. After washing with detergent, adult's clothes recorded the lowest plate counts with 1×10^1 and 1.3×10^1 C.F.U for Fabric toys, while baby's clothes were 1.5×10^1 . Table (4) and Fig.(2) summarize the C.F.U of the different samples before washing and after washing with soap detergent.

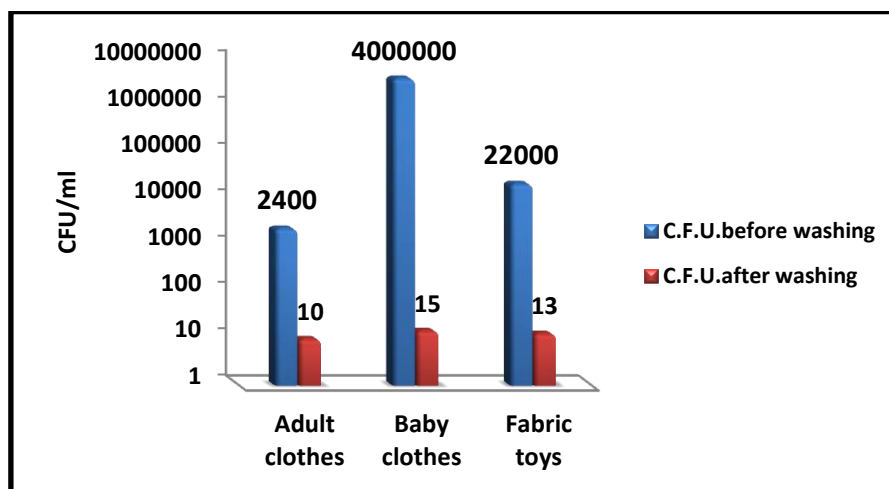


Fig.(2): Average bacterial counts before and after washing with soap detergent.

In the recent study, the clothes that washed with soap showed a reduction in the bacterial plate counts Table (3) and Fig.(2) but this reduction is less than that found in samples washed with granulated detergent. The granulated detergent may contain bactericidal or bacteriostatic compounds that contribute to inhibiting microbe's growth in used clothes, and the concentration of the bactericidal compound in soap may be less than that of granulated detergent. The differences in the microbes' counts among the three samples types and the differences in CFU before and after washing as shown in Fig. (1, 2) could be attributed to different reasons such as, Fumigation that carried out on clothes from the country of origin or some of the clothes are hanged in the open in the used clothes market where the microbes can be killed by ultraviolet radiation of the sun or the frequency of usage and laundering by the previous user, as well as hygiene habits and their state for e.g. sick user could be considered as a source to increase

microbes number in some clothes as compared to the lower number of microbe in healthy users used clothes[1].

Diversity of Fungi Isolated from Clothes

Molds isolated from second-hand clothes were identified using lacto phenol cotton blue mounts. These molds included *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus*. Table 5 shows that baby clothes recorded the highest incidence of fungi (100%) among other used clothes categories, while adult clothes recorded 66.6% followed by fabric toys samples which recorded 50%. A previous study by potera. 2001 referred that some fungi species were isolated from the clothes used frequently by different individuals, such as *Rhizopus* and *Aspergillus flavus* these common fungus has long been known to cause a potentially deadly threat of infections for hospital patients with injured or impaired immune systems [22].

Table (5)
Isolated fungi sp. and their incidence percent from used clothes categories.

Clothes category	Number of samples that appeared growth	incidence percent %	Fungi Isolate number	Isolating fungi sp.
Fabric toys	2	50	30	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>
Baby's clothes	3	100	49	<i>Aspergillus niger</i> <i>Aspergillus flavus</i>
Adult clothes	2	66.6	223	<i>Rhizopus</i> <i>Aspergillus niger</i> <i>Aspergillus flavus</i>

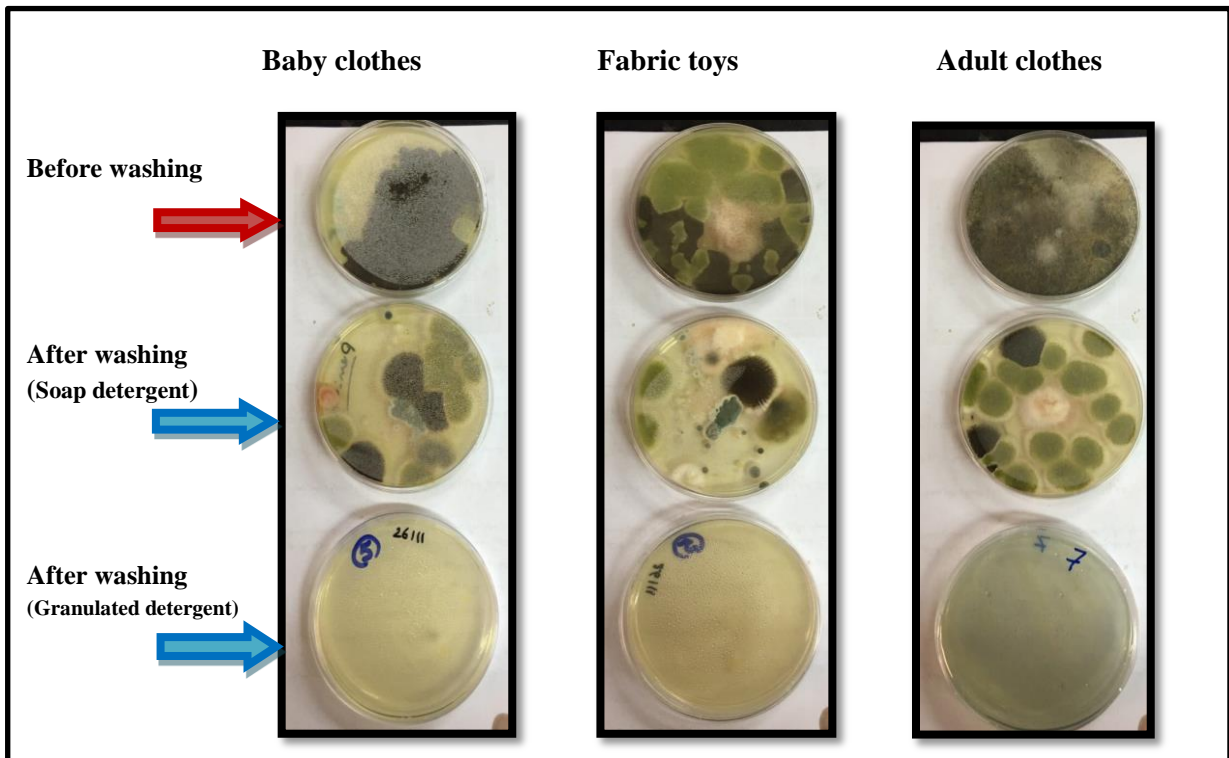


Fig.(3): Fungi species colonies before and after washing with soap and granulated detergent.

Table (6)
Spectrum of fungi isolated from used clothes categories.

Clothes category	Isolating fungi sp.	C.F.U Before washing	C.F.U after washing with detergent	
			Soap	granulated
Fabric toys	<i>Aspergillus niger</i>	20	4	1
	<i>Aspergillus flavus</i>	10	8	1
Baby's clothes	<i>Aspergillus niger</i>	22	2	0
	<i>Aspergillus flavus</i>	27	7	0
Adult clothes	<i>Rhizopus</i>	200	0	0
	<i>Aspergillus niger</i>	3	2	0
	<i>Aspergillus flavus</i>	2	14	0

Table (6) refers to both adult and baby clothes which recorded the highest count of fungi CFU respectively, followed by fabric toys per cm of the cloth. This can be attributed to the high frequency of usage of these clothes. After washing with soap, there was a decrease in CFU except adult's clothes which showed increasing CFU after washing with soap. A previous study showed that cotton fabric spread spores of *Aspergillus* sp. more than other fabrics [22].

Granulated detergent appears to be more effective than soap in reducing fungi CFU these data indicated that many of fungi which are associated with fungal infections in patients could survive for long periods in clothes even if washed with detergent. Neely *et al* [5] 2001 indicated in their study the survival of yeasts on various surfaces, such as the survival of *Aspergillus* in common hospital fabrics and plastics. The results of current study agrees with the reference [5] conclusions.

Conclusion

Purchasing of second hand clothes is considered as one of the reasons for skin pathogens transmission among users especially when it is used without washing, also washing clothes with a granulated detergent is more effective on removing bacterial contamination than ordinary soap (Neon soap). Children clothes should be cleaned carefully before use since they are colonized with bacterial colonies even after washing with detergents. This study recommends that besides washing of second hand clothes with detergents it's preferred to use other methods to remove bacterial contamination like antiseptics. In addition to washing clothes by using hot water and then ironing on both sides.

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