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# Isolation And Identification Of Potentially Pathogenic Bacteria From Second Hand Items Of Flea Market Of District Haripur, Pakistan

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**Abstract**

Secondhand is one of the sources of illness owing to disease and harm caused by the environment. Used clothing is sold all around the country since it is much cheaper than new clothing. The current investigation involved collecting samples from various used clothing market places in Haripur, Pakistan, and comparing the efficacy of detergent used to reduce microbial load in the garments, as well as checking antibiotic susceptibility for the isolated bacterial strains. Adult and baby clothing, fabric toys, underwear, bags, and bed covers were among the samples. The identification was done using culture and biochemical methods. In adult garments, Staphylococcus aureus was the most common isolate bacteria, followed by Bacillus subtilus in baby clothes and fabric toys. The highest levels of CFU were identified in baby garments (4x106), fabric toys (2.2x104), and adult clothes (20x102), respectively. Furthermore, these bacteria have shown resistance to the majority of antibiotics. These resistant strains may cause serious sickness in those who buy used goods. PCR was done to confirm the presence mec A gene responsible for Methicillin resistance.

**Key words;** CFU: Colony Finding Unit; Granulated Detergents; Second Hand Clothes.

## 1. Introduction

Many human diseases, particularly sexually transmitted diseases, which are transmitted through body fluids (sweat, blood, urine, vaginal discharge, and so on),(Rowley, 1998) have been recognized as a major public health problem for a number of years, and the causative organisms implicated in many of these diseases have been isolated in the clothing of people with these diseases(Busby, 2012). Infections include vaginal and skin candidiasis, scabies, ringworm, body lice, chicken pox, gonorrhea, syphilis, and hepatitis (types A, B, C, D, and G) can be spread through clothing worn by infected people.

Secondhand clothing (SHC) refers to clothing that has been worn previously. Shirts, coats, sweaters, under garments, socks, sandals, toys and bed covers are among the most commonly worn items(Al-easawi & Emran, 2017). These garments are imported often from the West and Asia. The United States of America (USA) is the world's largest exporter of secondhand clothing, followed by Germany, the United Kingdom (UK), and the Netherland, while Sub-Saharan Africa, Southeast Asia, and Eastern Europe are the world's major importers(Bash, 2015).

In Pakistan, worn garments are referred to as bale, which refers to a bundle of various types of clothing sourced from various parts of the world, or as (Landa), which refers to used clothing. These secondhand garments are sold in open markets known as flea markets or landa markets, as well as on hand carts. Because of their good quality and low prices, a large percentage of buyers began to patronize them because SHC are less expensive and of higher quality than new clothing on the market(Wetengere, 2018). According to a research most of the Gram positive bacteria can servive for a longer period of time on used clothes. Secondhand clothing may have been contaminated with pathogens that survive even after washing.

Used clothes have been found in the transmission the infectection from the previous user or first consumer to the second buyer. Used clothing is not recommended for use since it does not know the history of past users, posing the risk of developing a serious infectious disease from bacteria on the garment. Purchasing secondhand clothing is thought to be one source of skin disease transmission among users, especially when worn without being washed(Briones et al., 2016). Even after washing with detergent, children's clothes must be thoroughly cleaned before use since it is infested with a bacterial colony.(Stephens & Gilbert, 2019).

A research found that germs such as Staphylococcus aureus, including MRSA and MSSA, were often colonised on secondhand towels, underwear, and socks, which is a major public health risk. MRSA detection rates were found to be greater on undergarment surfaces than on other clothing(Sciences, 2015). Staphylococci are widely distributed in the environment and may be cultivated from almost any source. The study demonstrated.(Baharuddin & Author, 2020) Staphylococci are resistant to drying and can live for years in dust and dirt. They can withstand high temperatures and have a strong resilience to drying, allowing them to survive for lengthy periods of time on fomites and garments. It causes skin infections like sties, and furunculous, as well as more serious diseases such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, as well as deeper infections such as osteomyelitis and endocarditis(Journal, 2000)

Undergarments from a prominent secondhand market were evaluated in this study. The goal of the research was to determine the quantity microorganism’s contamination on secondhand clothing in

order to determine the risk of pathogenic microorganism transmission. After multiple cleaning treatments, a systematic study of the survival of different microbial isolates on used clothing was conducted. The current study collected samples from several used clothes market places in Haripur, Pakistan and compared the efficiency of detergents used to kill the microbial contamination clothes. Adult clothing, baby clothing, underwear, and fabric toys were among the examples. To assess which textile samples had the greatest bacterial contamination and dominant pathogenic microorganisms linked with skin illness, bacteria were isolated and identified using CFU. Culture and biochemical methods, as well as antibiotic susceptibility tests and PCR, were used to identify the bacteria. Samples were washed with granulated detergent to test how efficient it is at eliminating bacterial contamination in such clothing. The most prevalent isolate bacteria in adult clothing were Staphylococcus aureus and Bacillus subtilus, whereas the most common isolate germs in infant clothes and fabric toys were Staphylococcus aureus and Bacillus subtilus. The highest concentrations of CFU were found in newborn apparel (4x106), fabric toys (2.2x104), and adult clothing (20x102). The findings demonstrated that following washing with common house hold detergents. The granulated detergent was more effective in removing germs from a plate. In Staphylococcus aureus, different antibiotics revealed varied patterns of susceptibility. Ampicillin, Cefepime, imipenem, Lincimycin, and cefixime were resistant to Staphylococcus aureus, while oxytetracycline, Gentamicine, ciprofloxacin, and cefoxitin were sensitive. Bacillus subtilis was resistant to erythromycin, streptomycin, Gentamicine, Amoxicillin, Septrin, Zinnacef, and Ampiclox, however Bacillus subtilis was susceptible to ciproflaxacine and Rocephin. According to the findings, babies' clothes are the most susceptible to bacterium and fungal contamination, followed by fabric toys, and finally adult garments.

## 2. Material and Methods

### 2.1. Samples Collection

For this research 60 samples were collected from flea (Landa) market of District Haripur. Samples included Adult second hand clothes, baby clothes, undergarments, bags, bed covers and fabric toys. 10 samples of each category were collected by a sterile cotton swab and then taken to the lab in sterile cotton swab tubes.

### 2.2. Isolation of bacteria

For initial isolation of bacterial strains, Nutrient Agar media was used and for differentiation of isolated strains as Gram positive and Gram negative bacteria, (Chakraborty & Karmahapatra, 2011) Mac Con Key Agar was used. Media preparation and culturing wad done according to the standard procedures under controlled conditions.

### 2.3. CFU/ml before and After Washing Samples with Detergent

Each sample was washed with granulated detergent to check the efficacy of detergent. CFU of each sample was checked before and after washing. CFU /ml (colony finding unit) Samples (10 mL) were pipette aseptically to sterile Erlenmeyer flask and diluted with 90 mL peptone water pH 7.2, followed by series of dilution ($10^{-2}$- $10^{-8}$ ). Three replicates of 0.1 ml of each dilution lawn at N.A and incubated at 37°C for 24 hours (Reyes-Toscano et al., 2020). The bacterial growth was checked by a digital colony counter. The number of colonies was calculated as CFU /ml using the calculation below:

CFU/ml= $\frac{\text{no of colonies} \times \text{dilution factor}}{\text{volume plated in ml}}$ (Bogomolny et al., 2013)

Colony finding digital counter was used just before and after washing of samples.

### 2.4. Identification of Bacteria

Positive growth of samples was identified by colony morphology (colony texture, color and growth pattern),(Fluit et al., 2001) Gram staining, biochemical tests including catalase, oxidase, coagulase, $H_2S$, motility test and methyl red, and further molecular identification by polymerase chain reaction.(Basil et al., 2014)

### 2.5. Antimicrobial susceptibility

To check the susceptibility of isolates 9 antibiotics were used for each isolate according to the standard method of disc diffusion which was recommended by CLSI (Clinical and Laboratory Standards Institute). Muller Hinton Agar was prepared by following standard protocols and then the freshly cultivated bacterial suspension was wiped all over the Petri plates containing MHA. Antibiotic discs were placed on the marked point and incubated overnight at 37℃.

### 2.6. Molecular identification of Staphylococcus aureus

For PCR, DNA of the isolated Staphylococcus aureus was extracted by boiling method followed by Gel electrophoresis. (Makgotlho et al., 2009)

## 3. Results

Total 60 different samples were taken from three different Flea markets of District Hapripur, Pakistan. The 60 samples included undergarments, baby toys, clothes, bed covers and shoes (10 samples of each category). The isolation of samples on General purpose Media (Nutrient Agar) and differential media (Mac Con Key Agay) indicated that the isolated bacterial strains were Staphylococcus aureus and Bcillus subtilis.

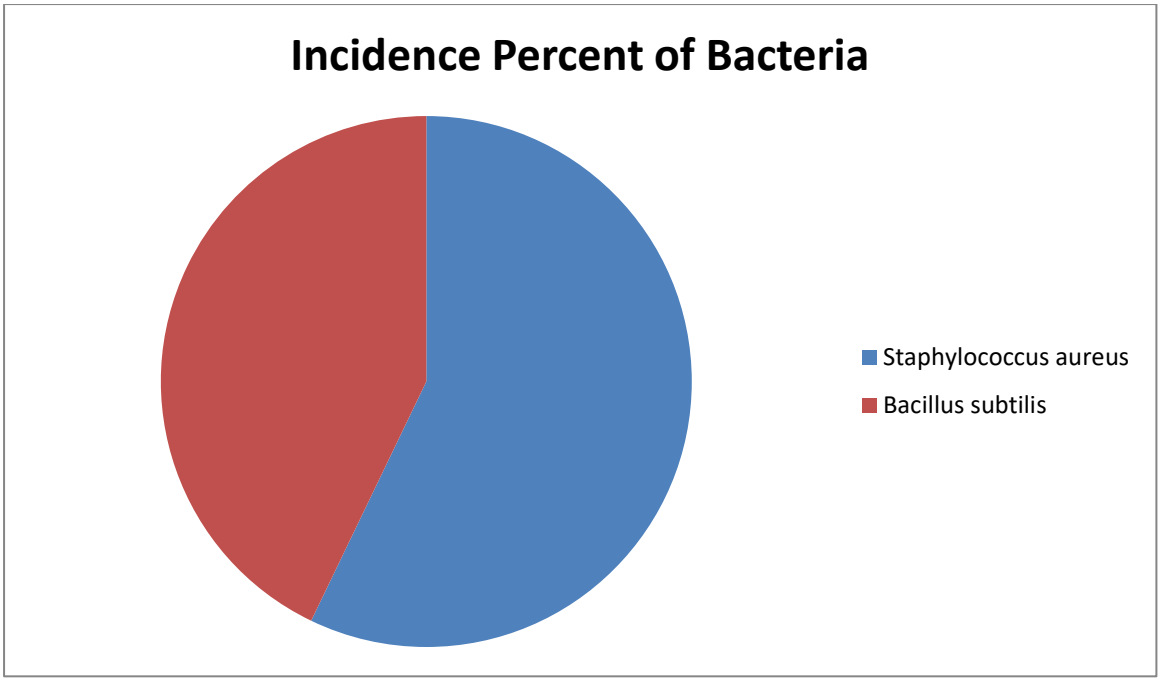
### 3.1. Morphological Identification

Colony morphology of Staphylococcus aureus and Bacillus subtilis on Nutrient Agar and was gold and creamy respectively, and on Manitol Salt Agar the conlonies appeared yellow in case of both strains.

### 3.2. Incidence Percent of Bacteria

Incidence percentage of Staphylococcus aureus was 80% and bacillus was 60%.

**Fig:1**




### 3.3. Effect of Washing with Detergent

Each of the sample was washed with granulated detergent (surf axel) and then CFU was checked. The result showed that the second hand fabric toys and undergarments were bearing the highest number of bacterial contaimination even after washing with detergents. The average CFU/ml of adult clothes before and after washing was $20x10^2$ and $1.6x10^1$ respectively. The average CFU/ml of undergarments before and after washing was $23x10^3$ and $1.6x10^1$ respectively. The average CFU/ml of baby clothes before and after washing was $20.3x10^1$ and $1.6x10^1$ respectively. CFU/ml of fabric Toys was $28x10^2$ and $1.6x10^1$ before and after wash respectively.

**Fig 2**

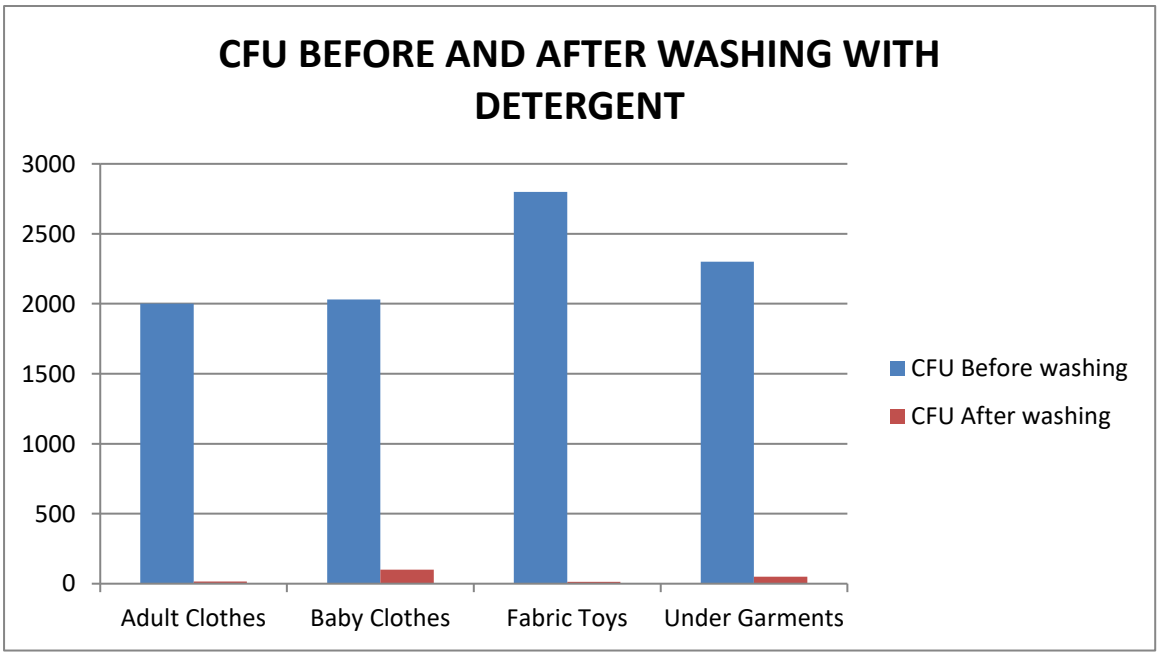


**Fig 2 shows the highest bacterial count in fabric toys and undergarments even after washing with granulated detergent. The CFU is baby clothes and adult clothes in comparatively low.**

### 3.4. Antimicrobial Susceptibility Pattern

In Staphylococcus aureus, different antibiotics revealed varied patterns of susceptibility. Ampicillin, Cefepime, imipenem, Lincimycin, and cefixime were resistant to Staphylococcus aureus, while oxytetracycline, Gentamicine, ciprofloxacin, and cefoxitin were sensitive. Bacillus subtilis was resistant to erythromycin, streptomycin, Gentamicine, Amoxicillin, Septrin, Zinnacef, and Ampiclox, however Bacillus subtilis was susceptible to ciproflaxacine and Rocephin.

**Fig 3**

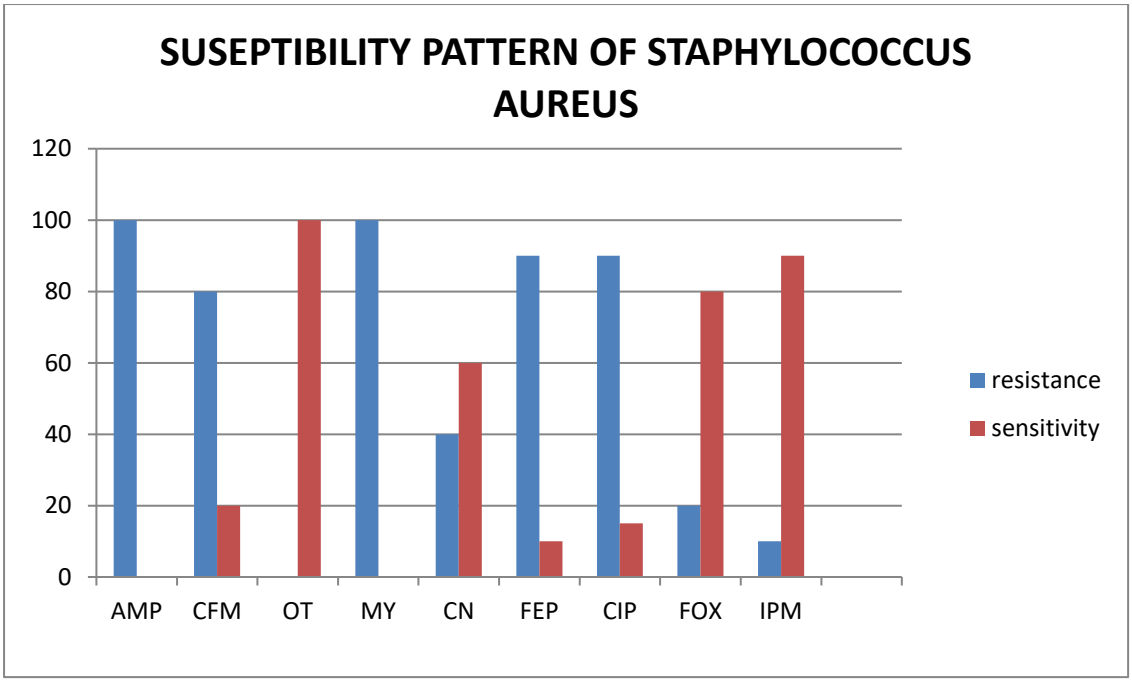
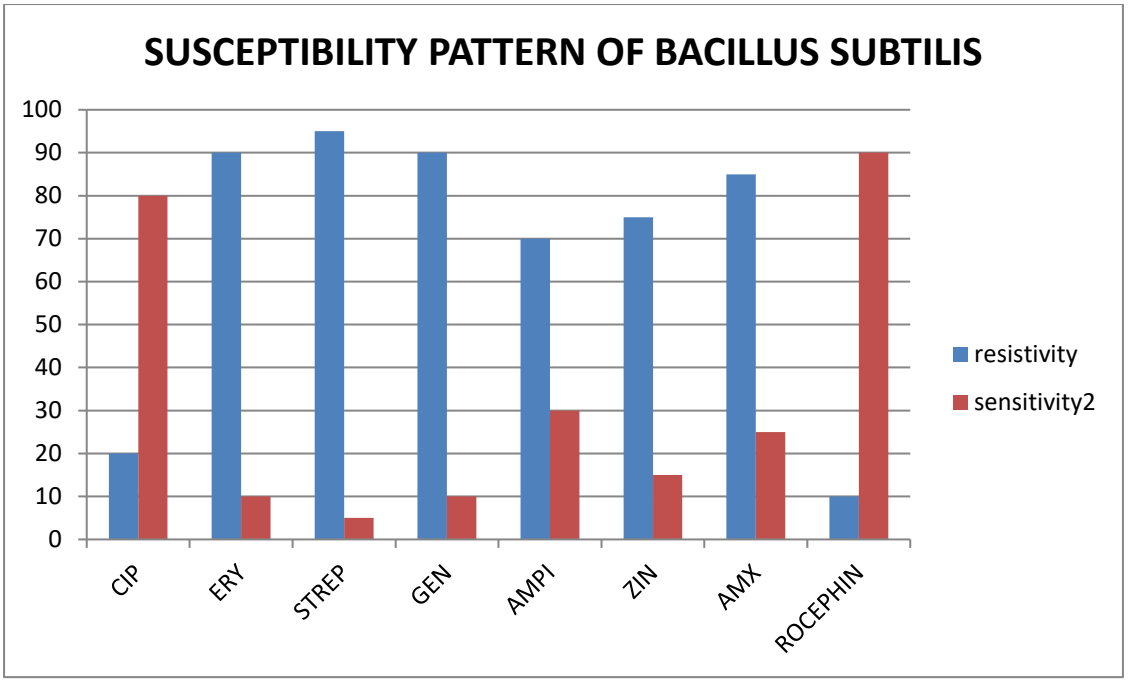



**Fig 3; S.aureus show highest resistance against Ampicillin and Lincimycin. S.aureus was sensitive towards Imipenem, Oxytetracycline, cefoxitin and Gentamicine but highest degree of sensitivity was towards Oxytetracycline.**

**Fig 4.**




**Fig;4, Bacillus subtilis show highest degree of resistance towards Streptomycin, Erythromycin, Gentamicine and Amoxicillin and highest sensitivity towards ciprofloxacin**

### 3.5. Biochemical Identification

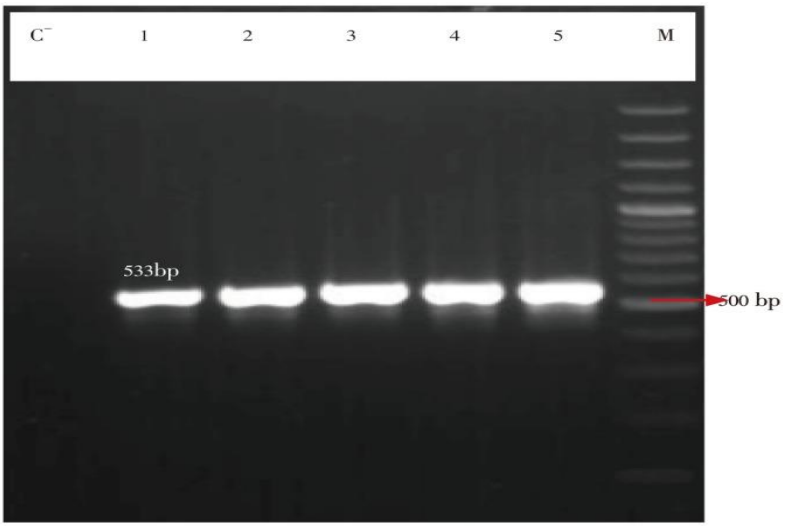
The biochemical tests were performed to identify the selected bacterial isolates.Catalase test was positive for both isolated bacterial strains (staphylococcus aureus and Bacillus subtilis). Oxidase test was performed for both isolated strains; the result was negative for staphylococcus aureus and positive for Bacillus subtilis. Motility Test was negative for Staphylococcus aureus and positive for Bacillus subtilis. Hydrogen Sulphide Test was positive for staphylococcus aureus and negative for Bacillus Subtilis. Methyl Red Test was performed for the confirmation of Bacillus subtilis. A distinct red color was observed in the tube due to the formation of stable acid. Coagulase test was also positive for Staphylococcus aureus.

**Table 1**

| Biochemical tests | Staphylococcus aureus | Bacillus subtilis |
|---|---|---|
| Methyl Red Test | – | – |
| Catalase Test | + | + |
| Oxidase Test | – | + |
| Coagulase Test | + | – |
| H2s Test | + | – |
| Motility Test | – | + |

### 3.6. Molecular Identification of Staphylococcus aureus

Staphylococcus aureus was further identified using a PCR technique that detected the mec A gene in all S. aureus strains. Eighteen of the Staphylococci isolates tested positive for the mec A gene. Figure 9.13 shows the PCR-amplified DNA products of this gene from five clinical isolates.




**Fig; 5, PCR amplification of mec A gene in five selected isolates of Staphylococcus. Lane C: mecA negative S.aureus; lane 1-5: PCR products of mec A gene; (533bp); M: 100 bp DNA size marke**r.

## 4. Discussion

Though the hard economic realities may make it difficult to prohibit the sale and use of used goods, careful handling and use must be practiced. The way they're handled at the source is critical; if they're not properly stored and bundied together, they could become more polluted. The majority of consumers are unaware of the dangers of wearing secondhand clothing without following necessary disinfection procedures, putting them at risk of illness. Because used clothing does not know the history of past users, there is a risk of developing a highly serious infectious disease from bacteria on the clothing, as most germs are extremely resistant and can survive hard environments for long periods of time on clothing. Some bacteria, particularly those that come with food, may not be removed by a routine wash.

The differences in microbe counts between the sample types, as well as the differences in CFU before and after washing could be attributed to a variety of factors, including fumigation carried out on clothes from the country of origin, or some of the clothes being hanged in the open in the used clothes market where the m icrobes can1be killed1by ultraviolet from the sun. All of the above findings from this study are quite noteworthy. Moreover, due to different disinfected sprays r chemical treatment while importing second hand stuff from one region to another might make the bacteria more resistant and tolerant. As in this study is is indicated that, most of the isolated bacterial strain showed high level of resistance towards different antibiotics. One of the reason might b that the region wise variation in the antimicrobial susceptibility of different bacteria, that could be a health concern to the new buyers. One of the isolated strain Staphylococcus aureus showed a variety of resistance and sensitivity towards different antibiotics. Staphylococci are common in the environment and can be cultured from almost any surface, including clothing. Because of the frequent personal touch with the previous

owner's skin and the fact that the undergarments come from people from various walks of life, it's logical to assume that they're colonised with potentially harmful bacteria and fungus, as the study proved. Staphylococci are common in the environment and can be discovered on clothing as well as environmental surfaces. The current Staphylococci sp. is thought to be the primary source of sporadic illnesses as well as outbreaks of various sizes. It causes boils, pneumonia, meningitis, and urinary tract infections on the surface, as well as deep-seated diseases like osteomyelitis. The ability of Staphylococcus aureus to develop antibiotic resistance is well-known. The ability of S. aureus to colonise normal humans asymptomatically is a fundamental biological characteristic. S. aureus is considered normal flora, and around 30% of individuals are asymptomatic nasal carriers1. S. aureus carriers are at a greater risk of infection and are thought to be a major source of S. aureus strain transfer among people. Also the isolated in our investigation, Bacillus sp. is not a known human pathogen, although it can poison food. Bacillus spp isolation's may be linked to its capacity to endure harsh environmental conditions such as heat, desiccation, poisonous chemicals, and ultraviolet irradiation due to its ability to generate endospores, which allow it to remain dormant for years. Detergent was found not to be very effective against bacterial contamination in the samples that is of great health concern. So, even after washing with detergents, children's clothes should be thoroughly cleansed before usage because they are colonised with multidrug resistant bacteria colonies.

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