



Antibacterial susceptibility pattern of Bacteria isolated from second hand Clothes sold in Potiskum Market, Yobe State, Nigeria.

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Abstract: The study investigated the types of bacteria present in second hand clothing's and the effect of antibacterial agents on the bacteria load. Six samples of second-hand clothes were purchased from Potiskum market: Bed spread, Baby cloth, Adult male shirt, Adult skirt, Panties, Brassiere. Plate Count Methods was used to determine the bacteria colony. Baby cloth has the highest bacteria count followed by bed spread, the lowest being Brassiere. Gram staining tests and biochemical test was used to identify the isolates. Five bacteria were identified, which include: *Corynebacterium* spp., *Bacillus* spp., *Micrococcus* spp., *Aeromonas* spp. and *Protens* spp. The clothes samples were subjected to sensitivity tests, three antibacterial agents were used for the susceptibility test on the bacteria. Hypo is most effective followed by Izal and lastly Detol. At 3.125% to 6.25% concentration, hypo can conveniently clear all the bacteria in the fabric samples, except micrococcus which are insensitive to any of the antibacterial agent. Consumers should approach second hand clothing's with caution since this study has demonstrated that clothes have high bacteria counts, it is not safe to wear without proper washing.

Keywords: Second hand clothing's, bacteria, biochemical test, sensitivity tests, antibacterial agent.

Introduction

One of the major functions of clothes is the protection of human body from vagaries of weathers. (Callaham and Paoleti., 1999). . Used or second hand clothes are clothing items that have been used by one or more persons before for the current user, it include all categories of clothing materials ranging from adult to children clothes, interior decoration and bed covers.

Second-hand or used clothing's are getting more popular nowadays, not only because it is cheap, affordable and different brand names, but most consumers believed it is more quality than new ones. This may be as a result of price hike of the new clothes brought about by high rate of inflation in Nigeria. The largest used clothing exporter in the world is the United States (US) followed by Germany, the UK and the Netherlands, while the largest used clothing importer is Sub-Saharan Africa, Southeast Asia and Eastern Europe. (Agbulu et.al., 2015). Second hand clothing makes up a small part of global trade in textiles and clothing, for some countries like Nigeria, the trade supports hundreds of thousands of livelihoods which include jobs in trading, distributing, repairing, restyling, and washing. Though it has been stated that second hand clothing import is one of the factors that is responsible for the closure of Nigerian textile industries, data has not been generated to make exact comparisons with employment generated by domestic production to the one generated by the second hand value chain. The latter may be much as it covers wider scope, the trade booms in every nooks and crannies of the country and it involves many people irrespective of age, sex, academic qualifications and by extension did not require any technical experience. Unlike conventional textile industries which are concentrated in three major cities of Kano, Lagos and Kaduna, is age restricted, sex preference and requires academic and technical experience. As wide spread as the trade seems to portray, because of the packaging, transportation and above all, no one knows the history of previous users so there is a possibility for contracting a very large infectious disease from bacteria, fungi etc. on the clothing.

The use of used clothing causes disease that starts from direct contact with the skin or is transmitted by human hands and then carries the infection through the mouth, nose and eyes. Contamination of bacteria and mold can cause health problems.

Based on this, the research is to isolate different types bacteria from the samples of second hand clothing's purchased from Potiskum Market, Yobe State and the effect of three types antibacterial agents purchased from the same market on the identified bacteria so as to make the clothes safe to wear and reduce risk of contracting diseases.

Materials and methods

Materials

Five samples of second hand clothing's were purchased from central market in Potiskum for the purposes of identifying the presence of bacteria. They were labeled as follows: Samples A. Bed spread, sample B. Baby cloth, sample C. Adult male shirt, sample D. Adult skirt, sample E. Panties, sample F. Brassiere.

Methods

Isolation of Bacteria

10g of the sample materials was swabbed in 90ml of sterile saline solution, 1ml was taken from the solution to make a serial dilution to 10³, and 0.1ml of the serial dilution was collected, and spread on the media plates and incubated for 18-24hours at 37⁰C. The bacteria on the plates were counted using colony counter.

Gram staining tests

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.

The slide of cell sample to be stained was prepared, the sample was heat fixed to the slide by carefully passing the slide with a drop or small piece of sample on it through a Bunsen burner three times.

The primary stain (crystal violet) was added to the sample/slide and was incubated for 60 seconds. The slide was later rinsed with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.

Gram's iodine was added for 60seconds- this is a mordant that fixes the crystal violet to the bacteria cell wall.

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The sample/slide was rinse with acetone for 3 seconds and later rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, if the alcohol remains on the sample for too long, it may also decolorize Gram positive cells.

The secondary stain, safranin, was added to the slide and was incubated for 1 minute. It was wash with a gentle stream of water for 5 seconds. The essence is that, if the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacteria are Gram negative, it will lose the primary stain and take the secondary stain, causing it to appear red when viewed under a microscope.

Microscope observation

Using oil immersion with x 100 power objective to examine the colours, shapes, sizes of the Bacteria.

Biochemical test

Indole test:

It screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used to distinguish among the family enterobacteriaceae.

All tubes of peptone broth was inoculated with the organism and incubate at 37°C for 48hrs and after incubation period a few drops of Kovacs reagent was added and was shaken gently and was allowed to stand for about 20 minutes. (This is to permit the reagent to rise to the top) A Red colour at the reagent layers indicates indole positive.

Methyl red test: it is used to detect the ability of an organism to produce and maintain acid end products from glucose fermentation. The tubes of Methyl Red-Voges Proskauer (MRVP) broth was inoculated with the organism and was incubated at 37°C for 48hrs. After the incubation, the methyl red test was carried out on each of the bacterium and 5 drops of methyl red indicator was added to each tube. A red colour gives a positive (acid) test while a yellow colour indicates negative (alkaline) test

Voges Proskauer test (VP): the test is used to detect acetoin in a bacterial broth culture.

The tubes of the MRVP broth were inoculated with the organism and were incubated at 37°C for 48hrs and VP test was carried out with the addition of 1ml of naphthol solution followed by 1ml of 40% potassium hydroxide solution, it was agitated and allow to stand for about 1hr and then observe a pink to red colour indicates the presence of acetyl methyl carbinol (VP + PECAH).

Simmon Citrate test: it is used for differentiating gram-negative on the basis of citrate utilization. The tubes of citrate agar were inoculated with the organism and were incubated at 37°C for 48hrs after the incubation period. A colour change to deep blue is a positive reaction while no colour change is negative reaction.

Coagulase test: it is an enzyme that clots blood plasma. The test is performed on gram-positive, catalase positive species to identify the coagulase positive staphylococcus aureus.

A colony of the test organism was emulsified separately in to two drops of normal saline, and one drop of citrated clot blood plasma was added and mixed together. A “clumping” indicated a positive reaction.

Catalase test: Facilitates the detection of the enzyme catalase in bacterial. It is essential for differentiating catalase positive Micrococcaceae from catalase negative streptococcaceae.

A colony was emulsified in a drop of 3 or 6% hydrogen peroxide was placed on a clean glass slide frothing or bubbling is indicative of a positive reaction.

Motility test: The motility test is used to determine whether an organism is motile or non motile, a motile organism are generally bacilli although a few motile cocci motile do exist.

Into motility medium a fine stabbing about one to two centimeter from the bottom was made with the test organism. It was incubated 37°C for 1 hr. A cloud medium along the line is negative while a cloudy medium spread through is positive.

Oxidise test: The test is used for the differentiation of Neisseria, Moraxella, Campylobacter and pasteurilla species (oxidase positive).

Two to three drops of oxidize reagent was put on to a filter paper and a colony was picked and emulsified on to the reagent. A blue purple indicated a positive reaction while no colour change indicated negative reaction.

Sensitivity test

The zone of inhibition is a circular area around the spot of the antibiotic in which the bacterial colonies do not grow. The zone of the inhibition can be used to measure the susceptibility of the bacterial towards the antibiotic.

The standardize inocula of both the bacterial and fungal isolate were streaked on stabilized Mueller Hinton plates respectively with the aid of sterile swab sticks. Four wells were punched on each inoculated agar plates with a sterile cork borer. The wells was properly labeled according to different concentrations of extracts prepared which were 100%, 75%, 50% and 25% respectively. Each well was filled up with approximately 0.2 ml of the extracts.

The inoculated plates with the extracts were allowed to stay on the bench for about one hour, this is to enable the extracts to diffuse on the Agar. The plates were then incubated at 37°C for 24 hours; (Plate of Mueller Hinton Agar) while the plate of potato dextrose agar was incubated at room temperature for about 3-5 days.

At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of the zones was measured using transparent ruler calibrator in millimeter and result was recorded.

Determination of Minimum inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) testing define test materials potency in terms of the concentration at which it will inhibit growth of (MIC) or completely kill (MBC) 1×10^6 challenge microorganism during an 18-to-20 hour period of incubated $35 \pm 2^\circ\text{C}$.

The minimum inhibitory concentration of the extract was determined using tube dilution method with the Mueller Hinton broth used as diluents. The lowest concentration of the extract showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The standardize organisms were inoculated into each tube containing the broth and extract. The inoculated tubes were then incubated at 37°C for 24hours.

At the end of the incubation period, the tubes were examined / observed for the presence or absence of growth using turbidity as criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The result was also recorded.

Determination of Minimum Bacteriocidal Concentration (MBC)

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extract.

A sterilized wire loop was dipped into the test tube(s) that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on sterile nutrient agar plate. The plates were incubated at 37°C for 18-24 hours. At the end of the incubation period, the plates were examined /observed for the presence or absence of growth.

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Results and Discussion

Isolation of the bacteria

Table 3.1 below shows the colony of isolated bacteria in the samples. Sample B has the highest no of bacteria colony, followed by sample A while the sample F has the lowest no of colony. The higher the no of colony, the more bacterial is present and the more chances that any consumer that wears it will be susceptible to disease. None of the second hand clothing's is safe to wear without proper washing.

Table: 3.1 Colony of isolated bacteria

Samples no	No of colony	DF	Vol/no (ml)	Cfu/g x10 ⁵
A	55	10 ³	0.1	5.5
B	68	10 ³	0.1	6.8
C	49	10 ³	0.1	4.9
D	47	10 ³	0.1	4.7
E	52	10 ³	0.1	5.2
F	40	10 ³	0.1	4.0

Gram staining tests

The results of the gram staining tests in table 3.2.1 below show that five of the six samples shows gram positive (fig.3.2.1) while one samples show gram negative with cocci as predominant bacterial when Mannitol salt agar was used as the media, while all the result of the six samples in Table 3.2.2 were gram negative and all cell morphology were short rod bacterial when using Macconkey agar as the media.

Table 3.2.1 Gram staining test using Mannitol salt agar

Code Name	Gram reaction	Cell mophology
A	positive	Cocci
B	positive	Cocci
C	Positive	Short rod

Table 3.3.1. Biochemical test using Mannitol salt agar as the media

Samples No	IND test	M/R test	V/P test	CIT test	H ₂ S test	MOT test	CAT test	COU test	GENERAL ORG
A	-	+	-	-	-	-	+	-	Corynebacterium spp
B	-	+	-	-	-	-	+	-	Corynebacterium spp
C	-	+	-	-	-	+	-	-	Bacillus spp
D	-	+	-	-	-	-	-	-	Micrococcus spp
E	-	+	-	-	-	+	-	-	Micrococcus spp
F	-	+	-	-	-	-	-	-	Micrococcus spp

Table 3.3.2 Biochemical test using Mac Conkey agar.

Samples No	IND test	M/R test	V/P test	CIT test	H ₂ S test	MOT test	CAT test	COU test	GENERAL ORG
A	+	+	-	-	-	+	-	-	Aeromonas spp
B	+	+	-	-	+	-	-	-	Proteus spp
C	+	+	-	+	-	+	-	-	Aeromonas spp
D	+	+	-	+	-	+	-	-	Aeromonas spp
E	+	+	-	+	+	-	-	-	Proteus spp
F	+	+	-	+	-	-	+	-	Aeromonas spp

Key: IND- Indole Test, M/R- Methyl Red Test, VP- Voges Proskauer test, CT- Citrate Test, H₂S- Hydrogen Sulphide Test, MT- Motility Test, CAT- Catalase Test, COU- Coagulase Test

D	Positive	Cocci
E	Positive	Cocci
F	Positive	Cocci

Table 3.2.2 Gram staining test using Macconkey agar

Code Name	Gram reaction	Cell mophology
A	Negative	Short rod
B	Negative	Short rod
C	Negative	Short rod
D	Negative	Short rod
E	Negative	Short rod
F	Negative	Short rod

Biochemical tests

These are the tests that are performed on different bacterial for their identification on the basis of their biochemical activities towards different biochemical compounds. Eight different types of tests were used for the identification and the results are as shown bellow in table 3.3.1 and 3.3.2 using two different media. In total, five different types of bacterial were identified on the fabric samples. These include: Corynebacterium spp, Bacillus spp, Micrococcus spp, Aeromonas spp and Proteus spp.

Determination of zone of inhibition

The zone of inhibition is a circular area around the spot of the antibiotic in which the bacterial colonies do not grow. The zone of the inhibition can be used to measure the susceptibility of the bacterial towards the antibiotic.

Table 3.4.1. Dettol as antimicrobial agent (All the measurement is in millimeters)

Bacterial types	100 %	75 %	50 %	25%
Bacillus Subtilis	0	0	0	0
Corynebacterium	0	0	0	0
Micrococcus	0	0	0	0
Aeromonas	18	16	14	12
Protens	18	16	14	12

Table 3.4.2. Hypo as antimicrobial agent (All the measurement is in millimeters)

Bacterial types	100 %	75 %	50 %	25%
Bacillus Subtilis	35	31	28	24
Corynebacterium	36	32	28	24
Micrococcus	0	0	0	0
Aeromonas	36	32	28	24
Protens	32	28	28	20

Table 3.4.3. Izal as antimicrobial agent. (All the measurement is in millimeters)

Bacterial types	100 %	75 %	50 %	25%
Bacillus Subtilis	28	26	24	20
Corynebacterium	0	0	0	0
Micrococcus	0	0	0	0
Aeromonas	26	24	22	20
Protens	28	24	22	18

Sensitivity tests

Determination of Minimum inhibitory Concentration (MIC)

Table 1.5.1. Dettol as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	-
Corynebacterium	-	-	-	-
Micrococcus	-	-	-	-
Aeromonas	-	-	±	±
Protens	-	-	-	±

Table 3.5.2. Hypo as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	±
Corynebacterium	-	-	-	±
Micrococcus	-	-	-	-
Aeromonas	-	-	-	±
Protens	-	-	-	±

Table 3.5.3. Izal as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	±
Corynebacterium	-	-	-	-
Micrococcus	-	-	-	-
Aeromonas	-	-	-	±

Determination of Minimum Bactericidal Concentration (MBC)

Table 3.6.1 Dettol as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	-
Corynebacterium	-	-	-	-
Micrococcus	-	-	-	-
Aeromonas	-	-	±	±
Protens	-	-	-	±

Table 3.6.2 Hypo as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	±
Corynebacterium	-	-	-	±
Micrococcus	-	-	-	-
Aeromonas	-	-	±	±
Proteus	-	-	-	±

Table 3.6.3 Izal as antimicrobial agent

Bacterial types	25 %	12.5%	6.25%	3.125%
Bacillus Subtilis	-	-	-	±
Corynebacterium	-	-	-	-
Micrococcus	-	-	-	-
Aeromonas	-	-	±	±
Proteus	-	-	-	±

Discussion

From the samples of second hand clothing's tested, the followings bacterial were identified and isolated; Corynebacterium spp, Bacillus spp, Micrococcus spp, Aeromonas spp and Protens spp. *Bacillus* sp was one of the bacteria isolated from this study, was found in sample C which is adult male shirt, its isolation might be related to the fact that *Bacillus* sp generally can withstand harsh environmental conditions like heat, desiccation, toxic chemicals and ultraviolet irradiation because of its ability to form endospores by which it can remain dormant for years. Some types of Bacillus bacteria are harmful to humans, plants, or other organisms, an example is *B. anthracis*, which causes anthrax in humans and domestic animals. (Bottone E. J., 2010) asserted that some bacillus species have been reported to be a cause of serious and potentially fatal non gastrointestinal tract infections.

Corynebacterium was found in samples A and B (Bed spread and baby cloth) it is a sexually transmitted organisms, it appears to utilize glycogen stored in the vagina epithelia cell, causing a malodorous vaginal discharge characterized by an abnormally high pH 5.5 and composed of epithelia cells and hords of bacilli. Corynebacterium Diphtheria is the most important species, with the most important human pathogenic significance strain of these species produces a strong exotoxine and are responsible for causing diphtheria. (Alina O., 2012). Diphtheria bacteria spread from person to person, usually through respiratory droplets, like from coughing or sneezing.

Micrococcus is another disease that was isolated in the samples, micrococcus was found in samples D, E, F (Skirt, Panties and Bra), (Ross, A.C et.al., 2008) said that micrococci and staphphlococci have been grouped together based upon similar cellular morphology and positive catalase activity. Monococci are normal residence of the skin and are usually considered pathogenic. *Microoccus luteus* can cause serious

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disease; they have been isolated from patients with underlying immune dysfunction who have an indwelling medical device e.g. micrococci have been reported to cause catheter-related infection in patient with leukemia. Micrococci have occasionally been reported as the cause of pneumonia, meningitis associated with ventricular shunts, septic arthritis, bacteremia, peritonitis, endophthalmitis, CR-BSI and endocarditis. (Eiff V. et.al., 1996)

Proteus are Gram-negative aerobic bacteria. It is present in samples E and B (Baby cloth and brassiere), they are named based on their ability to undergo morphological changes of colonies. With peritrichous flagella, *Proteus* spp. are motile. Some characteristics of a *Proteus* culture are swarming and an ammonia smell. The *Proteus* habitat is widely distributed in the environment. Chi yu Chen et.al 2012 concluded that *proteus mirabilis* is a common pathogen responsible for complicated urinary tract infection UTIs that sometimes causes bacteremia, occasionally in normal hosts but more often in those with indwelling catheters or anatomic or functional urinary tract abnormalities. *Proteus* are common among the gram-negative bloodstream isolates, with most secondary to UTI and often associated with urinary catheters. (Bennett J.E. 2020). *Aeromonas* is a genus of Gram-negative; they are found in samples A, C, D, and F. facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family enterobacteriaceae. It is an etiologic agent responsible for a variety of infections complications in immune-competent and immune-compromised person. Gastroenteritis is the major clinical illness associated with *Aeromonas* species, although causation and estimates of disease burden are controversial. (Qamar, F.N., 2020).

From the results of number and types of the bacteria in the second hand clothes, most of which are pathogenic, are therefore not safe to wear without proper washing. Previous studies carried out shows that ordinary detergents and have not successfully eradicate the bacteria in the fabrics as carried out by (Muthaini *et al.*, 2010). Three antibacterial agent: Hypo (Bleach), Dettol, and IZAL were purchased from central market in Potiskum, this serve as washing assistance that may kill the bacteria. Hypo in table 3.4.2 produces the highest zone of inhibition, that is the more sensitive the bacteria is to the hypo even at 25%, the result is better than Dettol and IZAL at 100% concentration. *Bacillus subtilis* and *Corynebacterium* are not sensitive to dettol and micrococci are not sensitive to any of the antimicrobial agents.

At 3.125% concentration, the entire antibacterial agents have stated inhibiting the bacteria except those that are insensitive to the agents like *Bacillus Subtilis*, *Corynebacterium* and micrococcus. However it takes 6.25% concentration for Dettol in table 3.5.1 to inhibit *Aeromonas* bacteria.

Conclusion

Diverse pathogenic bacteria were present in the second hand clothes with baby cloth, bed spread and panties have the highest bacterial counts. Five different types of bacteria were identified; *Corynebacterium* spp., *Bacillus* spp., *Micrococcus* spp., *Aeromonas* spp. and *Proteus* spp. In addition to washing with detergents, the use of antibacterial agent was able to clear these bacteria, hypo being most effective followed by IZAL and Dettol. It can conveniently conclude that at 6.125%, in washing bath, Hypo will clear all the bacteria except micrococcus. IZAL at 6.125% will clear three of the bacteria except *Micrococcus* and *Corynebacterium*, while dettol can only clear two of the bacterial except *Bacillus Subtilis*, *Corynebacterium* and *Micrococcus*. None of the antibacterial agent is able to clear micrococcus.

Recommendation

The use of second hand clothing's if it is a must, should be washed thoroughly with disinfectants and strong detergents and properly ironed as most of these bacteria cannot survive high temperatures of ironing so as to reduce the microbial load of the clothes thus preventing infections as no one knows the history of the former user health

The use hypo, which is the best among the antibacterial agents used, is mainly for white clothes, there is need to find another agent through research that can be used for multi coloured clothes

IZAL which also produces a fairly good result has a horrible odour, which most people do not like, more research should be carried out to produce antibacterial agent with good scent.

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