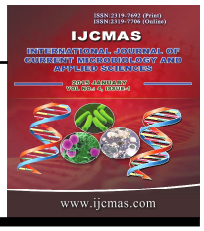
Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 716-724

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 1 (2015) pp. 716-724**
http://www.ijcmas.com



Original Research Article

Isolation and characterization of microorganisms associated with second hand female undergarments and children wear sold in Makurdi Metropolis

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Keywords

Second hand clothes, Pants, Bra, Children wear, Microorganisms

A B S T R A C T

The aim of the study was to assess the microbial contaminant of second hand clothes sold in Makurdi metropolis Second hand clothes are widely used but harbour microbial contaminants.. 60 samples of different second hand clothes (pants, bra, children wear) were sampled in four different market locations in Makurdi (Wurukum, Wadata, Modern and North Bank markets) and analyzed to study the microbial contaminant. The isolates were *Bacillus substilis (100%), Candida albican (29.9%), Candida tropicalis (65.5%), Penicillium* specie (1.8%), *Rhodotorula rubra(1.8%)* and *Trichophyton rubra(1.8%)* .Study shows that Wadata and North Bank Markets second hand clothes have more microbial load than Wurukum and Modern Markets and isolates from the second hand clothes are pathogenic especially to women and children. It recommended users should avoid use of these clothing items or use strong detergent to wash before wearing.

Introduction

Second hand clothes (SHC) are clothing items that have already been used by one person prior to the present user (Didymus, 2012). They include used panties, socks, shirts, skirts and household linens such as pillowcases, towels, beddings, curtains, table clothes e.t.c. (Didymus, 2012) These clothes are imported mostly from the West and Asia (Adow, 2012; Oyekanne, 2012).United state America (U.S.A) is said to the world largest exporter of second hand clothes follow by Germany, the United Kingdom (U.K) and Netherland, Sub-Sahara African, South East Asia and Eastern Europe are the largest importers of second hand clothing (Wallander, 2011).

Second hand clothing items, due to antecedents and widespread used pose a major health risks to the public. The recognition of this fact prompted the Rwanda Bureau Standard to ban second hand undergarment in Rwanda (Kamana, 2013) certain disease that may be transmitted via contact such as *candidiasis,* hepatitis A, B and C as well as skin infection such as scabies, ringworm could be transmitted through these popular clothing items. (Sharifzada, 2011; NAN, 2012). Also Bloomfield documented the ability of various types of clothing items to retain bacterial, fungi and viruses for varying

lengths of time (Bloomfield *et al*., 2011).

It is quite obvious that second hand clothing have an inherent ability to retain and transmit microorganisms from the first to the final user. And treating them with detergents and antiseptic has been shown to be an effective means of considerable disinfecting them, the effectiveness of the disinfection process is however dependent in a few variables (Muthaini *et al*., 2010).

In view of the widespread use of second hand clothing items in Nigeria, and the ability of clothing to retain microorganism, therefore Research is relevant to the isolation and characterization of microbial contaminant associated with second hand clothes and to justify that user of used clothing, especially undergarment should be public concern to all in view of likelihood of getting communicable disease.

The main objectives of this study was to isolate and characterize microorganism that maybe found on "second hand female under wears and children wear" sold in different market in Makurdi metropolis. And also to compare the microbial load of different types of second hand clothing items in Makurdi metropolis determining which one pose the greatest risk to the public

Materials and Methods

Area of Study

The Makurdi town area of Benue State lies within the middle belt zone of Nigeria, its bearing is 300km south East of Abuja, the federal capital territory and 887km north East of Lagos. The area lies within the hot humid zone with little seasonal temperature variation throughout the year Makurdi meteropolis.

Sample collection

Study was conducted on five samples of second hand clothes collected from three (3) categories: pants, bras, children wears from four markets. 60 samples of second hand clothes (panties, bras, children wears) were bought in groups in four (4) different markets (Wurukum, Wadata, Modern, North bank Markets). Each sample was collected in isolation in sterile polythene bags to avoid cross contamination. The entire area of sample was swabbed using the sterile cotton swab immersed in normal saline, the swab was then used for culture.

Media Preparation

All media were prepared according to the producers instructions.

Inoculation/culturing

Each of the swabbed stick was then used/inoculate in blood agar incubated at 37^0c for 24 hours for blood agar and 23^0c room temperature for 3 days for fungi culture and colonies growth were counted macroscopically

Identification of Bacteria: The identification of bacteria was based on their morphological and biochemical characteristics using procedure described by Cheesbrough, 2000

Morphological Identification: The colonies observed shows grey, opaque irregular colonies, non-Haemolytic and mucoid in appearance on blood.

Gram Reaction: Microorganisms were gram stained,Air dried and viewed under microscope under x10 objectives.

Coagulase Test: On a clean slide a drop of normal slide was placed. A small portion of suspected colony emulsified. A drop of human plasma was added and rocked. Developments of agglutination indicate coagulase positive and absence of agglutination indicate coagulase negative.

Motility Test:A small portion of suspected colony was introduced into peptone water and incubated for 1 hour at 37^0c. A drop of it was placed on a clean slide and observed under microscope under x40 objectives presence of motile cell indicate motility positive.

Oxidase Test:A piece of filter was placed in a clean Petri dish. Made few drops of oxidase reagent (tetramethyl-p-phenyldiamine) on a filtered paper such that it soaked completely. A small portion of the suspected colony was brought using the edge of a slide and observed for the presence of blue colour. Absence of blue colour indicates oxidase negative.

Catalase Test:A drop of hydrogen peroxide solution was placed on clean slide few suspected colonies growth was remove and brought in contact with the hydrogen peroxide solution (H_2O_2). Emergence of bubbles shows a positive result and absence of bubbles indicated a negative result.

Identification of Fungal: Growth on the sabouraud dextrose agar plates were observed for colour, pigment production, size of growth opaquecity. Germ tube test was done to differentiate budding yeast and lactophenol cotton blue test used in identification of mold.

Germ Tube Test: Few colonies of suspected yeast growth were introduced into human serum and incubated for 30 minutes. The preparation was observed under the microscope for budding yeast. Presence of bud shows *candida albican*. Absence of bud is other species of *candida*.

Lactophenol (Cotton blue test): On a clean slide a drop of methanol was placed and a portion of fungi growth was cut with the aid of surgical blade and tested in the methanol. A drop of lactophenol cotton blue added. A cover slip was placed on it gently to which it observed under microscope with x40 objectives the picture seen was compared with an identification chart (atlas). (Cheesbrough, 2000).

Statistical Analysis

Data was analyzed using the descriptive statistic SPss (version 20).

Result and Discussion

Three (3) of second hand clothes (pants, bras and children wears) from four different markets were examined and all pants, bras, children wears sampled in this study were contaminated with the following contaminant for bacteria they were all colonized by one type of bacteria, *Bacillus substilis*. Fungal includes *Candida albican, Candida tropicalis, Penicillium spp, Rhodotorula rubra, Trichophyton rubrum*.

Table 1 shows the morphological characteristics, shape of cell of bacterial isolate from samples

Table 2 shows the biochemical characteristics of bacterial isolate from samples.

Table 3 shows the number of colonies isolated from bacterial in their respective locations.

From the fourth analyses it shows the frequency of isolated bacterial on pathogenic organism found in the samples shown in table 4.

Table 5 shows the Macroscopic and Microscopic characteristics of fungal isolates.

Table 6 shows the number of colonies isolated from fungal in their respective locations.

From the seventh analyses it shows the frequency of isolated fungal found in samples shown in table 7

Table 8 shows the Counts of fungal pathogenic organisms and their cfu/ml count.
Table 9 shows microorganisms from various locations
Table 10: frequency and percentages of fungi from various markets

This study has shown that second hand pants, bras, and children wears are colonized with one type of bacterial, *Bacillus substilis*. Survival of microbes in formites is influenced by intrinsic factors which include formite properties or microbial characteristics and extrinsic factors including environmental temperature, humidity e.t.c (Muthaini *et al.,* 2010) the survival of other possible bacteria such as diptheriods, staphylococci e.t.c which are normal flora found to be isolated from clothes as reported by (Bloomfield *et al*., 2012) depend on these factors. Thus for only bacillus subtilis to be the only bacteria isolate it have the ability to withstand environmental temperature and for others could not survive possibly due heat as the second hand clothes sorted are packaged into bails, time of duration, the time taken to reach destination is another factor that influences the survival of microorganism on clothing, certain bacteria can only survive for certain period of time in days, weeks or months as reported by Neely and Maley (2012). Study shown that bacteria found in Wadata, Wurukum, Northbank and Modern markets are of equal percentage(25.0%). *Bacillus substilis* has a unique cell structure "endospore" with ability to remain dormant for years and resistant to damaging conditions such as heat, desiccation, toxic chemicals and ultraviolet irradiation (Nester *et al.,* 2004). It is rare for this *Bacillus substilis* to colonised on the human body, this is because it does not produce significant quantities of extracellular enzymes or possess virulent factors that will predispose it to cause infection (Environmental Protection Agency, 2012). Thus in order for infection with *Bacillus substilis* to occur, there must be high number of microbial load or the individual immune system is weaken (Environmental Protection Agency Report, 2012). Though *Bacillus substilis* has been found in human body, mostly in the skin or in the internal tract (Kirk, 2009). Study has demonstrated that *Bacillus substilis* to cause food poisoning and for one to control infection, involve washing hands after handling soiled bed linen or clothes. Some of fungi isolated from these clothing including *Candida tropicalis, Candida albican, Trichophyton rubrum, Rhodotorula rubra, Penicillium spp*.

These data indicate fungi survive for long period in clothes, panties had all the fungi isolates while in bras and children wears *Candida albican* and *Candida tropicalis* was found to only persist in all bras and children wear samples(9). The result shows *Candida tropicalis* to have the highest number of occurrence (65.5%) *Candida albican* (29.1%), *Penicillium spp* (1.8%), *Trichophyton rubrum* (1.8%) and *Rhodotorula rubra* (1.8%).These fungi isolated are associated with fungal infections(Table 7).

Yeast isolated includes *Candida tropicalis, Candida albican, Rhodotorula rubra* while

mold are *Penicillin spp, Trichophyton rubrum. Candida tropicalis* and *Candida albican* are closely related species. Candida ordinarily cause candidiasis depend on site of infection in both immunocompromised and immunocompetent patients, it prevalence and drug resistance has been of a great concern (Kothavade*et al.*,2014). In Indian *Candida tropicalis* is the most common cause of nosocomial candidaemia. *Candida albican* is common inhabitant of the skin, mouth, gastrointestinal tract, vagina, ordinary it causes no ill effects except among infant, pregnant women, diabetes. Yeast proliferates inside baby's nappy causing nappy rash, produce redness in vagina (Bakker, 2013). This fungal transmission can occur through direct contact with infected formites (Kothavade *et al.*, 2014). Onchomycosis a fungi infection of the nail can be due to *Candida albican* (DermNET, 2014). *Rhodotorula rubra* has been recognized as emerging yeast pathogens in human and have been found to be isolated from curtains (Wirth *et al.*, 2012) cause menignitis, disseminated endocandidiasis and systemic mycosis.

Penicillium spp a common mold can cause asthma hypersensitivity, pneumonitis. It has being isolated from cases of people with pneumonia, urinary tract infection and can be found on fabric (legal information and resource online, 2013). *Trichophyton rubrum* like *Candida albican* cause onychomycosis being a dermatophytes it found to grow on skin, hair, nails. This can cause fungi infection dependent on the site (DermNET, 2014). Isolates of fungi shows that Wadata and Northbank have the highest percentage (27.3%) followed by Modern market(23.0%),Wurukum market(21.8%). For these microbes to remain viable on the surfaces long enough to come in contact with a host, they only need to be present in small number to infect the next host (Mutaini *et al*., 2010).

From this study, it is concluded that Wadata and Northbank have the highest percentage in fungal and all the markets have the same percentages in bacteria isolates. But the existence of microorganism on second hand clothes could be independent of the location.Wadata and North bank Market's second hand clothes pose a greater risk to the public and microorganism isolated from second hand clothes are pathogenic to human especially among women and children

Table.1 Morphological characteristics, shape of cell of bacterial isolate from samples

Morphology	Gram reaction	Shape of cell	Arrangement	Organism
Cream colonies on blood agar	+ve	Rod	Irregular	*Bacillus substilis*

Table.2 Biochemical characteristics of bacterial isolate of samples

Catalase test	Coagulase test	Motility test	Oxidase test	Probable Organism
+	-	+	-	*Bacillus substilis*

Keys= +ve = positive; -ve=negative.

Table.3 The number of colonies isolated from bacterial in their respective locations

Location	Colonies	Cfu/ml
Wurukum market	61	61.6 X 10^5
Wadata market	37	37.3 X 10^5
North bank market	33	33.3 X 10^5
Modern market	19	19.0 X 10^5
Total	**150**	**151.2 x 10^5**

Table.4 The frequency of isolated bacterial found in the samples

Location	Colonies	Cfu/ml
Bacillus substilis	60	100.0%
Total	60	100.0

Table.5 Macroscopic/microscopic characteristics of fungal isolates

Colony characteristic	Microscopic appearance	Fungal isolate
Greenish, whitish colonies with fine extension	Septate hyphae condiophore in multilike chain like a paint brush	*Penicillium specie*
Velvety surface growth yellow in appearance	Septate hyphae presence of micro conidia	*Trichophyton rubrum*
White translucent colonies	With no budding	*Candida tropicalls*
White translucent colonies	With bud	*Candida albican*
Salmon red smooth colonies	No clamydiosphore No Arthrospores	*Rhodotorula rubra*

Table.6 The number of colonies isolated from fungal in their respective locations

Location	Colonies	Cfu/ml
Wurukum market	10	10.7 X 10^5
Wadata market	17	17.0 X 10^5
North bank market	7	7.3 X 10^5
Modern market	7	7.6 X 10^5
Total	41	42.6x10^5

Table.7 The frequency of fungal isolated found in samples

Organisms	Frequency	Percentage (%)
Candida tropicalis	36	65.5%
Candida albican	16	29.1%
Penicillium spp	1	1.8%
Trichophyton rubrum	1	1.8%
Rhodotorula rubrum	1	1.8%
Total	55	100.0

Table.8 The Counts of Fungal Pathogenic Organisms And Their cfu/ml Count

Organisms	Frequency	Cfu/ml
Candida tropicalis	36	36.0×10^5
Candida albican	16	16.0×10^5
Penicillium spp	1	1.0×10^5
Trichophyton rubrum	1	1.0×10^5
Rhodotorula rubrum	1	1.0×10^5
Total	**55**	**55.0×10^5**

Table.9 Microorganisms from samples locations

Samples	Numbers	Wurukum market	Wadata market	Northbank Market	Modern Market
Panties	20	*Bacillus subtilis, Candida tropicalis*	*B. substilis, C. tropicalis, Penicillium spp, Trichophyton rubrum*	*B. substilis, Rhodotorula rubra, Candida albican, Candida tropicalis*	*Candiada tropicalis*
Bras	20	*Bacillus substilis, candida albican*	*B. substilis, C. albican, C.tropicalis*	*B.substilis, C.tropicalis, C.albican*	*C. tropicalis*
Childre n wear	20	*B.substilis,C.tropicalis, C.tropicalis*	*B.substilis, C.tropicalis, C.albican*	*B.substilis, C.andida albican, C, tropicalis*	*C. tropicalis*

Table.10 Frequency and percentages of fungi from various markets

Location	Number of Organism	Percentage
Wurukum	12	21.8%
Wadata	15	27.3%
North bank	15	27.3%
Modern	13	21.8
Total	**55**	**100.0%**

Recommendation

The following control measures are recommended

- Total avoidance of second hand undergarment
- Avoidance of children wears especially for infant since their prone to infections but in the case where there is partial avoidance due to the standard of living of consumers, consumers should wash second hand clothes with strong detergents.

Acknowledgement

The Authors are grateful to Kuvempu University, Jnana Sahyadri, and Shankaraghatta for providing facilities to carry out the research work.

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