## Original Research Article

# EFFICACY OF NEWLY EMERGING DETERGENTS, LAUNDRY BLEACH, AND TOILET SOAP AGAINST BACTERIA ISOLATED FROM FAIRLY USED CLOTHES, MALE /FEMALE UNDERWEAR

#### **Abstract**

The aim of this study is to determine the potency and investigate the efficacy of these detergents, toilet soap, and laundry bleach against bacteria on fairly used clothes and Female Underwear. The current study included sample collection from used clothing and female underwear and compared the efficacy of detergents, toilet soap and laundry bleach (Jik) used to reduce the microbial load in the used cloth. The isolated organism was isolated from the used cloth and female underwear, identified, and microbiologically characterized using the conventional method of microbiology assay. i.e, biochemical test, a compilation of cultural colonial and characteristics. Test Samples were washed using four (4) types of detergents and five (5) types of toilet soap and laundry bleach. The result of the colony-forming unit (CFU) before and after washing with detergent and toilet soap and laundry bleach were recorded, washing with detergents and toilet soap. the results showed that detergents are more effective in reducing bacterial load than toilet soap in all test samples. the addition of laundry bleach (jik) also shows the effectiveness in reducing bacterial load in fairly used clothes and Female Underwear The antibiotics susceptibility test was carried determined using disc diffusion and agar well diffusion methods respectively. Eight (8) bacteria were identified include Aerobacter aerogenes (3), Bacillus polymyxa (5), Staphylococcus aureus (2), Veillonella parvula (5), Escherichia freundii (1), Escherichia coli (3), Enterobacter aerogenes (2) and Aerobacter cloacae (1). Bacillus polymyxa, and Veillonella parvula were the most common organism isolated and followed by Escherichia coli from fairly used clothes and Female Underwear. In this study, some of the Gram-positive isolates were sensitive to commonly used antibiotics like Streptomycin, Tarivid, Reflacine Ciprofloxacin, Augmentin, Gentamycin, Ceporex, Nalidixic acid, Septrin, and Amplicin. Gram-negative bacteria isolated were sensitive to Ciprofloxacin and all the isolated organisms were resistant to Ceporex. Gram-positive bacteria were sensitive to Streptomycin and were resistant to Rifampicin. To determine the growth dynamics and killing kinetics of the isolated organisms, an Ultraviolet spectrophotometer. The addition of antibiotics to the isolated organisms at the 48th-hour speed up the death rate of the isolates from fairly used clothe washed with different detergent Laundry Bleach and toilet soaps. It can be deduced that this study validates the efficacy of newly emerging detergents, Laundry bleach, and toilet soap against bacteria isolated from fairly used clothes. It is also recommended to wash fairly used clothes using different methods of soaking the fairly used clothes and female underwear in the Laundry Bleach for some days before finally washing in detergents and ironing before use. This method will drastically reduce and remove bacterial load in the fairly used clothes and female underwear.

Keywords; Efficacy, Newly Emerging Detergents, Toilet Soap, Bacteria, Fairly Used Clothes,

Female Under-wear.

#### INTRODUCTION

Fairly used clothes in Nigeria are known as "Okrika" and are clothing materials like shirts, trousers, towels, socks, panties, pillowcases, curtains, and bed sheets imported from the United States of America, United Kingdom, Asia, Germany, and the Netherlands after been used for a while by the initial buyer (1). They are packed in bundles known as bales during importation. Nigerians' desire for foreign goods (including wears) increases daily because of our economic situation and poverty under-tunes. These clothes were imported commonly from Asia and Europe, the largest exporter of second fairly used clothes were the united states of America (U.S.A) followed by Germany, the United Kingdom (U.K), and the Netherland while the largest importers of fairly used clothes are Sub-Sahara African, South East Asia and Eastern Europe. (1).

Though the wears were cheaper, usually the driving force is the quality of the products which are adjudged better than the new ones, an erroneous belief. These were purchased without giving consideration to any attending health and risk implications. Clothes have the potential, just as any other hand contact site, to be a component in the chain of infection transmission during normal daily activities (2).

However, Some of the risks associated with fairly used clothing consist of; Tinea, Impetigo, Scabies, Body lice, Pubic lice, Head lice are possible diseases that could be transported through fairly used clothes and their tendency to spread skin diseases.

Overtime man has been fighting for several centuries with the ever-persistent bacterial infections. The threat of bacterial infection was threatening with high mortality rates. This type of predicament which was faced by patients and also put the credibility of doctors to the task made the scientists of that time find ways to solve this problem (3).

At the turn of the century with the ever-growing number of disinfectants claiming to possess antibacterial properties, it has been left to the consumer to decide for oneself which is best. In the light of disinfectant used for cloth, availability, efficacy, and potency is our watchword. Local entrepreneurs haven't been left behind by the "clean bus" and there are quite a number of products produced each with its own testimony of how effective they are against bacteria. Through this myriad of confusion, the consumer is the one to suffer most of our laundry but does not disinfect. (4).

Increased life expectancy since the first half of the 20th century can be attributed to improved hygiene status resulting in decreased infectious disease incidence. Simple personal hygiene including soap utilization was the silent success of public health in the pre-disinfectant era. (5).

In our day-to-day interactions with microorganisms, we employ a variety of ways to reduce otherwise their numbers so as to avoid contamination with the microbes leading to eventual infection and illness. One major way of achieving these goals is the use of various types of disinfectants and detergents which are being sold in the market. Various types of disinfectants being sold have various activities ranging from –cidal to –static activities. (6)

A detergent is a surfactant or a mixture of surfactants with cleansing properties when in dilute solutions Disinfectants are specifically designed to kill bacteria, viruses, and mildews that are found on clothes and even kitchen and bathroom fixtures, and flourish on counters and drain gates in the sink (7). Using disinfectants is important in households because they prevent the spread of bacteria.(6).

Detergent is used to refer to synthetic cleaning compounds distinguishable from soap even though soap is also a detergent in its true sense. There are a large variety of detergents used in Nigeria, their potency should be questioned but the most commonly found are alkylbenzene sulfonates, a family of compounds that are similar to soap but are more soluble in hard water because the polar

sulfonate (of detergent) is less likely than the polar carboxylate (soap) to bind to calcium and other ions found in hard water. (8).

In domestic contexts, the term detergent by itself refers specifically to laundry detergent or dish detergent, as opposed to hand soap or other types of cleaning agents. Detergents, like soaps, work because they are amphiphilic; partly hydrophilic (polar), and partly hydrophobic (non-polar). Their dual nature facilitates the mixture of hydrophobic compounds (like oil and grease) with water. Because air is not hydrophilic, detergents are also foaming agents to varying degrees.(9). Toilet Soap on the other hand is a salt of a fatty acid used in a variety of cleansing and lubricating products. Soap when dissolved in water, possesses the ability to remove dirt from surfaces such as the human skin, textiles, and other solid. In a domestic setting, soaps are surfactants usually used for washing, bathing, and other types of housekeeping. In industrial settings, soaps are used as thickeners, components of some lubricants, and precursors to catalysts. (10).

Early studies on bacterial contamination on fairly used clothes were conducted in investigating the bacterial load of hand towels and dishcloth towels. Such clothes were heavily contaminated with bacteria and suspected as one of the main vectors for spreading and dissemi0nation of the bacteria (Speirs *et al.*, 2001). Bacterial infections are among the important infectious diseases, hence, over 50 years of extensive research have been launched for achieving new antimicrobial medicines isolated from different sources (11), Triclosan has been shown to inhibit the growth of both Grampositive and Gram-negative bacteria situ, with varying effectiveness across bacterial species. For, example, triclosan is relatively ineffective at inhibiting the growth of Gram-negative bacteria (12).

Although the antibacterial activity of triclosan involves some non-specific killing mechanisms, research finding suggests that the bacteriostatic action occurs by inhibiting a specific bacterial target, known as the enoyl-acyl carrier protein reductase. Triclosan shares this bacterial biosynthetic fatty acid pathway target with the antibiotic isoniazid. These findings have led researchers to explore whether triclosan may influence the emergence of resistance to antibiotics (13,14),(15).

Several investigators have reviewed studies examining the mechanisms of antiseptic resistance (13; 14; 16), there are few systematic reviews that have attempted to summarize the potential risks associated with triclosan in the context of the purported effectiveness of this antibacterial ingredient used in hygiene products in the community setting (16). The efficacy of soap containing triclosan

generally refers to the additional level of effectiveness beyond the ability of plain soap to simply remove transient organisms via surfactants and the mechanical action of the wash procedure.

The level of effectiveness may be measured at the microbiological level or at the population level, as added protection against bacterial contamination or the occurrence of common infectious illnesses. Risks, on the other hand, include the potential for bacteria to become unsusceptible to triclosan, for the emergence of cross-resistance to antibiotics, and for the ingredients to become toxic to the environment and to humans (16)

#### MATERIALS AND METHOD

#### DETERGENTS, LAUNDRY BLEACH AND TOILET SOAP USED

Toilet soap used (4): - Test sample 1(Premier cool), Test sample 2 (Eva), Test sample 3(Lux), Test sample 4(Joy, Detergents used(4): -Test sample 1(Waw), Test sample 2(Canoe), Test sample 3(Good mama), Test sample 4(So easy). (Laundry Bleach (1) Jik.. All detergents, Laundry Bleach (Jik), and toilet soap used for this research were purchased from a Market in Akungba Akoko, Ondo State, Nigeria.

#### **SAMPLECOLLECTION**

Two categories of fairly used cloth samples, Towel, and Singlet were procured in May 2021 from Akungba Akoko market, located in Ondo-State, Nigeria. The two categories of fairly used clothes, male and female underwear were labeled accordingly. The samples were then placed in sterile polythene bags and brought to the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko for analysis. Standard methods were used in the isolation and identification of the microorganisms.;

### ISOLATION OF MICROORGANISMS FROM SAMPLES

To obtain microorganisms from fairly used clothes, male and female underwear (Before and after washing); the test tube was partly full of sterilized water. Dipped bacteria-laden swabs into the water, then transferred some of the bacteria collected into the water. To ensure maximum representation of the microorganisms in the samples during culturing and to obtain good results, a test run is were determined with the concentrated extract (After washing) and the raw sample

(Before washing). Inoculate the dish by pouring 0.5m/ls of the water into the dish and pouring already sterilized and cooled nutrient agar on it, then incubated 37 for 24hrs.(17)

#### **CULTURING AND SERIAL DILUTION**

#### Serial Dilution Process

This was done to determine the population of the organisms by reducing the count in the series of dilutions. An aliquot of 1ml from the sample was transferred into 9ml of prepared sterile distilled water in the first test tube with a sterile syringe and mixed, from the first dilution, 1ml was then transferred into the second test tube and repeated until the last test tube was inoculated, and was incubated for 24hrs for the organism to grow. (18).

Culturing of isolates from fairly used clothes, male and female underwear. Pour plate methods were used to inoculate the test sample. The technique is carried out for each of the sample extracts by inoculating 0.5µl onto the dry agar plate and already prepared cooled sterile 20ml 0f nutrient agar was poured on it and are then incubated at room temperature and results were obtained after 24 hours. This is followed by measuring the zone of inhibition so as to identify the most effective detergent, laundry breach, and toilet soap that is found to have antimicrobial properties. (18)..

# BACTERIOLOGICAL ANALYSIS OF FAIRLY USED CLOTHES, MALE AND FEMALE UNDERWEAR.

Using the pour plate method of inoculation,0.5ml of the five-fold dilution of 10<sup>5</sup> and 10<sup>3</sup> of fairly used clothes, male and female underwear waters which has already been washed with toilet soap, laundry bleach, and detergents was aliquoted into sterile Petri dishes. The nutrient medium was prepared by dissolving 6.16grams of Agar into 220 liters of distilled water in a sterile conical flask, corked with cotton wool and aluminum foil, and then homogenized to dissolve. It was sterilized in an autoclave at a temperature of 121 for 15minutes, after the sterilization the medium was allowed to cool but not solidify, 20ml amount of the medium was then poured into different sterile Petri dishes containing 0.5ml of the inoculum and allowed to set, then the plates were incubated for 24hrs. After 24hrs, the cultural characteristics on the plates were studied and recorded; resultant colonies were sub-cultured on fresh nutrient agar and then incubated for 24hrs. Pure isolates were

# IDENTIFICATION OF ISOLATES FROM FAIRLY USED CLOTHES, MALE AND FEMALE UNDERWEAR

#### Macroscopic Examination and Biochemical Characteristics of Isolates

Cultural and macroscopic examinations were done to identify the pure isolate. identification of the isolates was based on the cellular morphology characteristics. In addition, various biochemical tests which include: Catalase, Indole, Motility, Gram staining, Fermentation of sugars (Sucrose, Lactose, Dextrose), Coagulate, Urease, Hydrogen sulphide, Gas production, and oxidase tests were done for conventional identification of the isolates. (18)...

#### Microscopic Examination and Gram Staining Techniques of Isolates

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell constituents. The Gram stain procedure distinguishes between Gram-positive and Gram-negative groups by coloring these cells red or violet. The working solution of reagents used for the Gram staining technique was prepared according to the manufacturer's instructions. Staining was carried out by emulsifying approximately one isolated 18- 24hours old colony in a drop of water placed at the center of a clean grease-free slide until a thin smear was made. The smear was air heat-fixed by passing the slide through a Bunsen burner flame and then air-dried. The heat-fixed smear was flooded with a basic aniline dye (crystal violet) for 60 seconds. This was flooded with Lugol's iodine and allowed to remain for 60 seconds. This was then rinsed off with running tap water. The smear was decolorized with 70% ethanol which was immediately washed out to avoid total decolorization. The smear was counter-stained with Safranin for 60 seconds, washed off with running tap water, and blot-dried. The slide was then examined under an oil immersion objective microscope. Organisms that retained the purple colour of crystal violet- iodine complex (CV-1 complex) were recorded as Gram-positive, while those that appeared pink were Gram-negative. The slide was then air-dried and the bottom was dried. The slide was then observed under a compound microscope using oil emulsion lens magnification of X100. (19).at a

# BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES FROM FAIRLY USED CLOTHES, MALE AND FEMALE UNDERWEAR

#### Oxidase Test

The nutrient broth was prepared and autoclaved at 121 for 15minutes, it was then allowed to cool. A sterile inoculating loop was used to pick a distinct colony from the organism for about 18-24hrs old and was inoculated inside the prepared nutrient broth in the test tube which was incubated for 24hrs, after incubation, then the colour changed was observed which was purple within few second when the kovac'soxidase reagent is added which indicates oxidase-positive, while no colour changes indicated a negative result. (20).

#### **Motility Test**

Test tubes of motility, indole, urease (MIU) broth was prepared and labelled. The inoculating loop was flamed and colony of the 18-24h old culture sample was picked and stabbed 2/3 of the way down to the bottom of the test tube and then incubated at room temperature for 24hrs. A well dispersed growth which could be cloudy or diffuse away from the line of inoculation indicated a positive result while growth in the distinct zone along the stab indicated a negative result. (21).

#### **Indole Test**

Motility, indole, and urease broth were prepared, test tubes containing 5mls of media were autoclaved at 121 for 15minutes, it was then allowed to cool. The test tube was inoculated with the 18-24h old test organism was aligned and 0.5ml of kovac's reagent was dropped into them and shaken gently and allowed to stand for 20minutes, the appearance of red ring coloration of the surface layer of the media was confirmed the result to be indole positive while no red colouration indicated indole negative. (21).

#### Urease Test

Motility, indole, and urease broth were prepared,10ml 0f the broth was dispensed into clean test tubes and autoclaved at 121 for 15minutes, it was then allowed to cool. A sterile inoculating loop was used to pick a colony of the organism and was introduced into a urea agar slant and was incubated at 37 for 24hrs. After incubation, colour changes observed were bright pink from the initial straw colour which indicated a positive result and no colour changes indicated a negative result. (21).

Sugar Fermentation Test (Dextrose, Lactose, and Sucrose) and Hydrogen Sulfide, Gas Production. The triple sugar iron agar was dispensed 5ml into test tubes and then sterilized. The slant of the test tubes was made, each labeled test tube was inoculated with the isolates of 18-24hrs old by making a stab in the solid part and streaking the slanted part of the agar. The test tubes were incubated for 18-24hrs at 37. After incubation, an alkaline or acid (red slant/yellow butt) reaction indicates dextrose fermentation only, a yellow butt reaction indicates the fermentation of dextrose, lactose, and sucrose, a red slant reaction indicates the absence of carbohydrates fermentation results, blackening of the medium occurs in the presence of hydrogen sulfide, bubbles or cracks in the agar indicates the production of gas. (18).

# ANTIBIOTICS SENSITIVITY TESTING OF ISOLATES FROM FAIRLY USED CLOTHES, MALE AND FEMALE UNDERWEAR

The antibiotics sensitivity test was performed in other to determine the sensitivity or resistance of the bacteria isolates to some selected antibiotics of certain concentrations. Kirby-Bauer National Committee for Clinical and Laboratory Standard (CLSI, 2014) modified disc diffusion technique was used to determine the antibiotics sensitivity of the isolates. Using a sterile inoculating loop, a colony of the organisms was taken and inoculated into a Nutrient agar broth in the test tubes and inoculated for 24hrs. A sterile swab stick was dipped into the test tubes to pick the organisms and the swab stick was rotated severally and pressed firmly on the side of the wall to remove excess inoculums and used to streak the entire dried surface of the already prepared, sterilized, and solidified nutrient agar in the Petri dishes, the steaking was repeated two (2) times by rotating the plate approximately each time to ensure that every part of the plate was inoculated. The plates were allowed to stay for 15minutes so as to allow any excess surface moisture to be absorbed before applying the drug impregnated discs. Pre-determined commercial (Oxoid), antibiotics disc of both gram-positive and gram-negative was fixed on the media using sterile forceps to ensure complete contact with agar. The plates were inverted and incubated overnight at 37 for 24hrs. After 24hrs of incubation, the plates were examined, the susceptibility of each isolate to each antibiotic was indicated by a clear zone of growth of inhibition around each disc. The diameter of the zone of inhibition was measured using a calibrated ruler from the underside of the plate and recorded in millimeters. The results were interpreted according to the Clinical laboratory standard institute (22).

The following antibiotics used were; For gram positive (+ve)Streptomycin (S,30mcg), Norfloxacin (NB, 10mcg), Chloramphenicol (CH, 30mcg), Ciprofloxacin (CPX, 10mcg), Erythromycin (E, 30mcg), Levofloxacin (LEV, 20mcg), Gentamycin (CN, 10mcg), Ampiclox (APX, 20mcg), Rifampicin (RD, 20mcg), Amoxil (AMX, 20mcg) For gram negative (-ve) Tarivid (OFX, 10mcg), Reflacine (PEP,10mcg), Ciprofloxacin (CPX, 10mcg), Augmentin(AU, 30mcg,) Gentamycin (CN, 10mcg), Streptomycin (S, 30mcg), Ceporex (CEP, 10mcg), Nalidixic acid (NA,30mcg), Septrin (SXT, 30mcg), Amplicin (PN, 30mcg). Each disc was pressed down to ensure complete contact with agar surface and incubated at 37for 24hrs. The zones of inhibition were measures using calibrated ruler in millimeters. (18).

# GROWTH DYNAMIC / DEATH RATE (KILLING KINECTICS ) OF ISOLATES USING ULTRA VIOLET SPECTROPHOTOMETER

Growth dynamic refers to the rate at which cells of microorganisms grow at a given time. The test was done to determine the rate of growth of the isolates as well as their killing time in due time. The colony was picked from the stocked culture slant and inoculated into a nutrient broth which was incubated for 37for 24hrs. A loopful of organisms was picked from the broth culture into nutrient broth in two sets which are set A and C respectively. The ultraviolet spectrophotometer was set at 480 wavelengths, warmed up for 15minutes and then the control was first read, the first reading was taken at zero hours and it continues after every 12hours for 8times, at the 5th reading which is the 48th hour of set B, ciprofloxacin was added to determine up the rate of the kill. (23)

#### **RESULTS**

The result obtained from this research work, the efficacy of newly emerging detergents, laundry bleach, and toilet soap against bacteria isolated from fairly used clothes, male and female underwear were collated and represented in the table and graphical forms.

- TABLE .1; Shows cultural characteristics (Macroscopic examination) of isolated bacteria from fairly used clothes, male and female underwear washing with detergents, laundry bleach and toilet soaps. Cultural characteristics include the size, shape, colour, texture, opacity and edges. It was observed that some isolated bacteria were big, small, and medium in size, irregular and regular in shape, rough and smooth in texture, transparent, translucent and opaque in opacity, rough or smooth edges and the bacteria also exhibit different colour; whitish, orange, yellowish and creamy in colour.
- 1. Samples washed with Toilet soap (Test sample 1(Premier cool), Detergent (Test sample 1(Waw) with the addition of laundry bleach (Test sample 1(Jik), Test sample 4(Joy) with laundry bleach (Test sample 1(Jik), Test sample 1(Jik), Test sample 2(Canoe) with laundry bleach (Test sample 1(Jik), Test sample 2(Canoe) and Test sample 2 (Eva).has a large-sized isolates.
- 2. Samples washed with Test sample 2 (Eva), Test sample 2(Canoe), Test sample 3(Lux) with laundry bleach(Test sample 1(Jik), Test sample 2(Canoe), with laundry bleach(Test sample 1(Jik), Test sample 3(Good mama), with laundry bleach(Test sample 1(Jik), Test sample 4(Joy), Test sample 1(Premier cool) with laundry bleach(Test sample 1(Jik), Test sample 1(Waw), Test sample 1(Premier cool), has small-sized isolates.

TABLE 2; Shows the morphological characteristics (Macroscopic examination) of isolated bacteria from fairly used clothes, male and female underwear. In this table it was observed that the isolated bacteria has different morphological characteristics; transparent, translucent and opaque opacity, rough with creamy, whitish, milky, and yellowish in colour.

TABLE 3; Shows the result of Gram staining of the isolates from isolated bacteria from fairly used clothes, male and female underwear. It was observed from this table, all isolate after washing with different detergent and toilet soap, were the result obtained.

Test sample 3 (Lux– $1\times10^5$ B), Test sample 3(Lux)+ laundry bleach(Test sample 1(Jik- $1\times10^3$ ), Test sample 1(Premier cool)+ laundry bleach(Test sample 1(Jik - $4\times10^5$ B), Test sample 1(Waw) + laundry bleach(Test sample 1(Jik - $2\times10^5$ ), Test sample 2(Canoe- $1\times10^3$ ), Test sample 4(Joy)+ laundry bleach(Test sample 1(Jik  $8\times10^5$ ), Test sample 3(Good mama),+ laundry bleach(Test sample 1(Jik- $5\times10^3$ ), Test

sample 3(Good mama  $-2\times10^5$ ), Test sample 1(Waw)+ laundry bleach(Test sample 1(Jik- $6\times10^3$ A), Test sample 1(Waw- $3 10^5$ ), Test sample 1(Waw)+ laundry bleach(Test sample 1(Jik- $6\times10^5$ B), Test sample 4(So easy)+ laundry bleach(Test sample 1(Jik  $-7\times10^3$ ), Test sample 2 (Eva)+ laundry bleach(Test sample 1(Jik- $3\times10^3$ ) were negative to Gram staining, and Test sample 1(Premier cool- $4\times10^5$ ), Test sample 1(Premier cool+ laundry bleach(Test sample 1(Jik- $4\times10^5$ ), Test sample 2 (Eva- $3\times10^3$ ), Test sample 3(Lux- $1\times10^3$ A), Test sample 4(So easy- $4\times10^3$ ), Test sample 1(Waw- $3\times10^3$ ), were all Gram-positive

Microscopic examination shows that all isolate obtained after washing with; Test sample 3(Lux- $1\ 10^5$ B), Test sample 3(Lux)+ laundry bleach(Test sample 1(Jik- $1\times10^3$ ), Test sample 1(Waw),+ laundry bleach(Test sample 1(Jik- $6\times10^5$ B), Test sample 1(Premier cool- $4\times10^5$ ), Test sample 1(Premier cool) + laundry bleach(Test sample 1(Jik- $4\times10^5$ B) were Tiny rods shape,

Test sample 1(Premier cool)+ laundry bleach(Test sample 1(Jik  $-4 \times 10^3$ A), Test sample 4(Joy)+ laundry bleach(Test sample 1(Jik  $-8 \times 10^5$ ), Test sample 3(Good mama)+ laundry bleach(Test sample 1(Jik  $-5 \times 10^3$ ), Test sample 2 (Eva- $3 \times 10^3$ ), Test sample 3(Lux- $1 \times 10^3$ A), Test sample 4(So easy- $4 \times 10^3$ ) has Long rods shape.

Test sample 3(Good mama  $-2\times10^5$ ), Test sample 1(Waw)+ laundry bleach (Test sample 1(Jik- $6\times10^3$ A) has Short rods shape.

Test sample 1(Premier cool)+ laundry bleach(Test sample 1(Jik- $4\times10^5$ B), Test sample 2(Canoe -  $1\times10^3$ ), Test sample 1(Waw - $3\times10^5$ B), Test sample 4(So easy)+ laundry bleach(Test sample 1(Jik- $7\times10^3$ ), Test sample 2 (Eva)+ laundry bleach(Test sample 1(Jik- $3\times10^3$ ), Test sample 1(Waw- $3\times10^3$ A) were all cocci in shape.

### Key - NEWLY EMERGING DETERGENTS, LAUNDRY BLEACH AND TOILET SOAP

- 1. Toilet soap used (4): Test sample 1(Premier cool), Test sample 2 (Eva) Test sample 3(Lux), Test sample 4(Joy),
- 2. Detergents used(4): Test sample 1(Waw), Test sample 2(Canoe), Test sample 3(Good mama), Test sample 4(So easy)
- 3. Laundry Bleach (1) Test sample (1) Jik...



TABLE 1; Cultural Characteristics (Macroscopic Examination) of Isolated Bacteria from Fairly Used Clothes, Male /Female Underwear against Newly Emerging Detergents, Laundry Bleach and Toilet Soap

NEWLY EMERGING	DILUTION	SIZE	SHAPE	COLOUR	TEXTURE	OPACITY	EDGES
DETERGENTS,	FACTOR						
LAUNDRY BLEACH							
AND TOILET SOAP							
Premier cool+jik	10 <sup>5</sup>	Big	Irregular	Creamish	Rough	Translucent	Rough
Premier cool+jik	$10^{3}$	Small	Regular	Creamish	Smooth	Opaque	Rough
Lux	$10^{3}$	Big	Regular	Yellowish	Smooth	Transparent	Smooth
Lux	10 <sup>5</sup>	Medium	Irregular	Milky	Rough	Opaque	Rough
Waw	$10^{5}$	Small	Regular	Whitish	Smooth	Opaque	Smooth
Waw+jik	$10^{3}$	Small	Regular	Yellowish	Smooth	Translucent	Smooth
Waw+jik	$10^{5}$	Big	Regular	Milky	Smooth	Translucent	Smooth
Premier cool	10 <sup>5</sup>	Small	Regular	Creamish	Smooth	Opaque	Smooth
Premier cool	$10^{3}$	Small	Regular	Brownish	Rough	Opaque	Smooth
Waw	$10^3$	Medium	Irregular	Golden yellow	Smooth	Translucent	Rough
Joy	$10^{5}$	Small	Irregular	Creamish	Rough	Transparent	Rough
Joy+jik	10 <sup>5</sup>	Big	Irregular	Creamish	Rough	Opaque	Rough
Eva+jik	10 <sup>3</sup>	Medium	Regular	Yellowish	Smooth	Translucent	Smooth
Easy	10 <sup>3</sup>	Medium	Regular	Creamish	Smooth	Transparent	Smooth
Good mama	10 <sup>5</sup>	Medium	Regular	Whitish	Smooth	Transparent	Smooth
Easy+jik	$10^3$	Medium	Regular	Milky	Smooth	Transparent	Smooth

Good mama+jik	$10^3$	Small	Regular	Yellowish	Rough	Translucent	Smooth
Lux+jik	10 <sup>5</sup>	Small	Regular	Whitish	Smooth	Opaque	Smooth
Canoe+jik	$10^{3}$	Small	regular	Whitish	smooth	Opaque	Smooth
Canoe +jik	10 <sup>5</sup>	Big	Irregular	Creamish	Rough	Transparent	Smooth
Lux+jik	$10^3$	Small	Regular	Whitish	Smooth	Opaque	Smooth
Canoe	10 <sup>5</sup>	Big	Irregular	Creamish	Smooth	Translucent	Smooth
Canoe	10 <sup>3</sup>	Small	Regular	Whitish	Smooth	Opaque	Smooth
Eva	10 <sup>5</sup>	Big	Irregular	Creamish	Smooth	Translucent	Smooth
Eva	10 <sup>3</sup>	Small	Irregular	Whitish	Smooth	Transparent	Smooth

### Key – NEWLY EMERGING DETERGENTS, LAUNDRY BLEACH AND TOILET SOAP

- 1. Toilet soap used (4): Test sample 1(Premier cool), Test sample 2 (Eva) Test sample 3(Lux), Test sample 4(Joy),
- 2.Detergents used(4): Test sample 1(Waw), Test sample 2(Canoe), Test sample 3(Good mama), Test sample 4(So easy)
- 3.Laundry Bleach (1) Test sample (1) Jik..

TABLE 2; Morphological Characteristics (Macroscopic Examination) of Isolated Bacteria from Fairly Used Clothes, Male /Female Underwear against Newly Emerging Detergents, Laundry Bleach and Toilet Soap

NEWLY EMERGING	DILUTION	SUB-CULTURE
DETERGENTS,LAUNDRY	FACTOR	
BLEACH AND TOILET		
SOAP		
Premier cool+jik	10 <sup>5</sup>	Motile, translucent with creamy colour
Premier cool+jik	$10^3$	Swamy, opaque with creamy colour
Lux	$10^{3}$	Non-motile, transparent with yellowish colour
Lux	$10^{5}$	Swamy organism, opaque with creamy colour
Waw	$10^{5}$	Motile, opaque with whitish colour
Waw+jik	$10^{3}$	Motile, translucent with golden yellow colour
Waw+jik	10 <sup>5</sup>	Motile, translucent with milky colour
Premier cool	10 <sup>5</sup>	Non-motile, opaque with creamy colour
Premier cool	$10^3$	Swamy, opaque with brownish colour
Waw	$10^3$	Motile, translucent with golden yellow
Joy	10 <sup>5</sup>	Swamy, translucent with creamy colour
Joy+jik	10 <sup>5</sup>	Motile, opaque with creamy colour
Lux+jik	$10^5$	Non-motile, opaque with whitish colour
Canoe+jik	$10^3$	Swamy, opaque with creamy colour
Canoe+jik	10 <sup>5</sup>	Swamy, transparent with creamy colour
Lux+jik	$10^{3}$	Motile, opaque with whitish colour
Canoe	10 <sup>5</sup>	Non-motile, translucent with creamy colour
Canoe	10 <sup>3</sup>	Motile, opaque with whitish colour
Eva	10 <sup>5</sup>	Non-motile, translucent with creamy colour
Eva	10 <sup>3</sup>	Motile, transparent with whitish colour
Eva+jik	$10^{3}$	Motile, translucent with yellowish colour
Easy	$10^{3}$	Motile, transparent with creamy colour
Good mama	10 <sup>5</sup>	Motile, transparent with whitish colour

Easy+jik	$10^{3}$	Motile, transparent with milky colour
Good mama+jik	$10^{3}$	Motile, translucent with brownish colour

TABLE 3: Gram Staining for identification of Isolated Bacteria From Fairly Used Clothes, Male/Female Underwear (Towel, Singlet and Panties) against Newly Emerging Detergents, Laundry Bleach and Toilet Soap-why bolden? Is this your style?

NEWLY EMERGING DETERGENTS,	GRAM STAINING	MICROSCOPY
LAUNDRY BLEACH AND TOILET SOAP		EXAMINATION
Lux 1×10 <sup>5</sup>	Short rods	-ve
Lux 1×10 <sup>3</sup>	Long irregular rods	+ve
Eva 3×10 <sup>3</sup>	Long irregular rod	+ve
Premier cool 4×10 <sup>5</sup>	Tiny irregular rod	+ve
Canoe 1×10 <sup>3</sup>	Irregular cocci	-ve
Good mama 2×10 <sup>5</sup>	Short rods	-ve
Waw 3×10 <sup>5</sup> B	Cluster cocci	-ve
Waw 3×10 <sup>3</sup> A	Cluster cocci	+ve
Easy 4×10 <sup>3</sup>	Long irregular rods	-ve
Lux+jik 1×10 <sup>3</sup>	Tiny irregular rods	-ve
Eva +jik 3×10 <sup>3</sup>	Cluster cocci	-ve
Premier cool +jik 4×10 <sup>5</sup> B	Cluster cocci	-ve
Premier cool +jik 4×10 <sup>3</sup> A	Long irregular rods	-ve
Good mama +jik 5×10 <sup>3</sup>	Long irregular rods	-ve
Waw+jik 6×10 <sup>5</sup> B	Tiny irregular rods	-ve
Waw+jik 6×10 <sup>3</sup> A	Short rods	-ve
Easy+jik 7×10 <sup>3</sup>	Cocci	-ve
Joy+jik 8×10 <sup>5</sup>	Long rods	-ve

KEY: +(POSITIVE) - (NEGATIVE)

TABLE 4; Shows the biochemical tests carried out on the isolates. The tests include; the Motility test, Urease test, Indole test, Triple sugar fermentation (Sucrose, Lactose, Dextrose), Gas production, and Hydrogen sulphide test (H<sub>2</sub>S). It was observed in this table that some

isolates are positive and negative to motility test, it was observed that the isolates from different samples of newly emerging detergents, laundry bleach, and toilet soap, were the following observation, Test sample 3(Lux-1×10<sup>5</sup>B), Test sample 3(Lux -1×10<sup>3</sup>A), Test sample 1(Premier sample  $1(\text{Jik-4}\times10^5)$ , Test sample (1) laundry bleach(Test easy) + laundry bleach(Test sample  $1(\text{Jik-}7\times10^3, \text{Test sample }4(\text{So easy }-4\times10^3), \text{Test sample}$ 1(Waw -3×10<sup>3</sup>A), Test sample 1(Waw- 3×10<sup>5</sup>B), Test sample 1(Premier cool + laundry bleach sample  $1(\text{Jik-}4\times10^3)$ , Test sample 1(Premier cool) $-4 \times 10^{5}$ ), Test (Eva)+ laundry bleach(Test sample 1(Jik- 3×10<sup>3</sup>), Test sample 2 (Eva)- 3×10<sup>5</sup>), Test sample  $1(\text{Waw} + \text{laundry bleach}(\text{Test sample } 1(\text{Jik} - 6 \times 10^3), \text{ Test sample } 3(\text{Good mama } -2 \times 10^5). \text{ Test}$ sample 3(Good mama + laundry bleach (Test sample 1(Jik- 5×10<sup>3</sup>), Test sample 2(Canoe - $1\times10^3$ ), Test sample  $4(Joy + laundry bleach(Test sample <math>1(Jik - 8\times10^5)$  were Urease positive while, Test sample  $3(\text{Lux} + \text{laundry bleach}(\text{Test sample } 1(\overline{\text{Jik}} - 1 \times 10^{3}))$  were Urease negative. It  $3(\text{Lux- }1\times10^{5}\text{B}), \text{ Test}$ was also observed that Test sample easy + laundry bleach(Test sample 1(Jik -7×10<sup>3</sup>), Test sample 1(Waw- 3×10<sup>3</sup>A), Test sample  $1(\text{Waw -}3\times10^5\text{B})$ , Test sample  $3(\text{Lux} + \text{laundry bleach}(\text{Test sample }1(\text{Jik - }1\times10^3), \text{Test sample }1(\text{Jik - }1\times10^3))$ 1(Premier cool + laundry bleach(Test sample  $1(Jik - 4 \times 10^3)$ ), Test sample 1(Premier cool -4×10<sup>5</sup>), Test sample 2 (Eva -3×10<sup>5</sup>), Test sample 2 (Eva +jik 3×10<sup>3</sup>, Waw+jik 6×10<sup>5</sup>B, Waw+jik  $6\times10^3$ AGood mama  $2\times10^5$ , Canoe  $1\times10^3$ , Joy + laundry bleach(Test sample 1(Jik- $8\times10^5$ ) were indole positive test while, Test sample 3(Good mama + laundry bleach(Test sample 1(Jik-5×10<sup>3</sup>), Test sample 4(So easy-4×10<sup>3</sup>, Test sample 1(Premier cool -4×10<sup>5</sup>, Test sample 3(Lux- $1\times10^3$ A) were indole negative test. It was also observed that Test sample 3(Lux -1×10<sup>5</sup>B), Test sample 1(Premier cool), -4×10<sup>5</sup>), Test sample 3(Good mama -2×10<sup>5</sup>, Test sample 1(Waw -3×10<sup>3</sup> A), Test sample 2 (Eva + laundry bleach(Test sample 1(Jik-3×10<sup>3</sup>), Test sample 1(Premier cool + laundry bleach(Test sample 1(Jik-4×10<sup>3</sup>A), Test sample 1(Premier cool + laundry bleach (Test sample 1(Jik -4×10<sup>5</sup>B, Test sample 3(Good mama + laundry bleach(Test sample 1(Jik- $1(\text{Waw} + \text{laundry bleach}(\text{Test} \text{ sample } 1(\text{Jik} - 6 \times 10^3 \text{A}), \text{ Test}$  $5\times10^3$ , Test sample 1(Waw + laundry bleach(Test sample 1(Jik - 6×10<sup>5</sup>B), Test sample 4(So easy + laundry bleach (Test sample  $1(\text{Jik} - 7 \times 10^3)$ were positive for oxidase test while Test sample 4(Joy + laundry bleach(Test sample 1(Jik - 8×10<sup>5</sup>), Test sample 3(Lux + laundry bleach(Test sample 1(Jik -1×10<sup>3</sup>). Test sample 4(So easy -4×10<sup>3</sup>), Test sample 1(Waw -3×10<sup>5</sup>B), Test sample 2(Canoe  $-1 \times 10^3$ , Test sample 2 (Eva  $-3 \times 10^3$  and Test sample 3(Lux  $-1 \times 10^3$ A were negative for oxidase test....This can be put in a table

Table 5; Sugar fermentation for identification of isolated bacteria from Fairly Used Clothes, Male/ Female Underwear (Towel, Singlet, and Panties) against Newly Emerging Detergents, Laundry Bleach, and Toilet Soap. All the isolates from the sample were positive for the triple sugar fermentation test except Test sample  $3(Lux + laundry bleach (Test sample <math>1(Jik - 1 \times 10^3)$  is negative for the triple sugar fermentation test. All isolates were positive for Hydrogen sulphide production test (H<sub>2</sub>S). Also, Test sample 4(Joy + laundry bleach (Test sample 1(Jik- $8\times10^5$ ), Test sample 1(Waw-3×10<sup>3</sup>A), Test sample 4(So easy -4×10<sup>3</sup>), Test sample 4 (So easy + laundry bleach (Test sample 1(Jik- 7×10<sup>3</sup>), Test sample 3(Lux -1×10<sup>3</sup>) were positive for gas production tests while the rest isolate was negative for gas production. All the isolates are positive for glucose test except Test sample 1(Waw + laundry bleach (Test sample 1(Jik- 6×10<sup>5</sup>) were negative for the glucose test, all the isolates were also positive for mannitol test. It was also observed that Test sample 3(Lux -1×10<sup>5</sup>B), Test sample 1(Premier cool), -4×10<sup>5</sup>), Good mama 2×10<sup>5</sup>, Waw 3×10<sup>3</sup> A, Eva+jik 3×10<sup>3</sup>, Premier cool+jik 4×10<sup>3</sup> A, Premier cool+jik 4×10<sup>5</sup> B, Test sample 3(Good mama + laundry bleach(Test sample 1(Jik- 5×10³, Test sample 1(Waw + laundry bleach(Test sample 1(Jik -6×10<sup>3</sup>A), Test sample 1(Waw + laundry bleach(Test sample 1(Jik -6×10<sup>5</sup>B), Test sample 4(So easy + laundry bleach(Test sample 1(Jik - 7×10<sup>3</sup>), Test sample 3(Lux -1×10<sup>3</sup>) were positive for gas production tests while the rest isolate was negative for gas production. All the isolates are positive for the glucose test except Test sample 1(Waw + laundry bleach (Test sample 1(Jik- 6×10<sup>5</sup>) were negative for the glucose test, all the isolates were also positive for mannitol test.

TABLE 6; Probable organism isolated from fairly used clothes, male/female underwear (towel, singlet, and panties) against Newly emerging detergents, laundry bleach, and toilet soap. The isolated organisms include; *Aerobacter aerogenes* (3), *Bacillus polymyxa* (5), *Staphylococcus aureus* (2), *Veillonella parvula* (5), *Escherichia freundll* (1), *Escherichia coli* (3), *Enterobacter aerogenes* (2), *Aerobacter cloacae* (1).

TABLE 4 Biochemical Examinations for identification of Isolated Bacteria from Fairly Used Clothes, Male/ Female Underwear (Towel, Singlet and Panties) against Newly Emerging Detergents, Laundry Bleach and Toilet Soap.

Newly Emerging	Motility	Urease	Indole	Catalase	Oxidase	coagulate	$H_2S$	Gas production
Detergents, Laundry								
Bleach And Toilet Soap								
Lux 1×10 <sup>5</sup> B	+	+	+	+	+	+	+	-
Lux 1×10 <sup>3</sup> A	+	+	-	+	-	+	+	+
Fancy 2×10 <sup>3</sup>	-	-	-	+	+	+	+	-
Eva 3×10 <sup>3</sup>	+	+	+	-	-	+	+	-
Premier cool 4×10 <sup>5</sup>	+	+	+	7	+	+	+	-
Canoe 1×10 <sup>3</sup>	+	+	+	+	-	+	+	-
Good mama 2×10 <sup>5</sup>	+	+	+	+	+	+	+	-
Waw 3×10 <sup>3</sup> A	-	+	+	+	+	+	+	+
Waw 3×10 <sup>5</sup> B	-	+	+	+	-	+	+	-
So Easy 4×10 <sup>3</sup>	+	+	-	+	-	+	+	+
Lux+jik 1×10 <sup>3</sup>	-	-	+	+	-	+	+	-
Fancy+jik 2×10 <sup>5</sup>	+	-	-	-	-	-	+	-
Eva+jik 3×10 <sup>3</sup>	+	+	+	+	+	-	+	-
Premier cool+jik 4×10 <sup>5</sup> B	+	+	-	+	+	-	+	-
Premier cool+jik 4×10 <sup>3</sup> A	-	+	+	-	+	-	+	-
Good mama+jik 5×10 <sup>3</sup>		+	-	+	+	-	+	-

Waw+jik 6×10 <sup>5</sup> B	-	+	+	+	+	+	+	-
Waw+jik 6×10 <sup>3</sup> A	-	+	+	+	+	+	+	-
So Easy 7×10 <sup>3</sup>	+	+	+	+	+	+	+	+
Joy+jik 8×10 <sup>5</sup>	-	+	+	+	1	-	+	+

TABLE 5; Sugar fermentation for identification of Isolated Bacteria From Fairly Used Clothes, Male/ Female Underwear (Towel, Singlet and Panties) against Newly Emerging Detergents, Laundry Bleach and Toilet Soap.

Newly Emerging Detergents, Laundry	Lactose	Sucrose	Dextrose	Glucose	Mannitol
Bleach And Toilet Soap					
Lux 1×10 <sup>5</sup> B	+	+	+	+	+
$Lux 1 \times 10^{3} A$	+	+	+	+	+
Fancy 2×10 <sup>3</sup>	+	+	+	+	+
Eva 3×10 <sup>3</sup>	+	+	+	+	+
Premier cool 4×10 <sup>5</sup>	+	+	+	+	+
Canoe 1×10 <sup>3</sup>	+	+	+	+	+
Good mama 2×10 <sup>5</sup>	+	+	+	+	+
Waw 3×10 <sup>3</sup> A	+	+	+	+	+
Waw 3×10 <sup>5</sup> B	+	+	+	+	+
So Easy $4 \times 10^3$	+	+	+	+	+
Lux+jik 1×10 <sup>3</sup>	-	-	-	+	+

Fancy+jik 2×10 <sup>5</sup>	+	+	+	+	+
Eva+jik 3×10 <sup>3</sup>	+	+	+	+	+
Premier cool+jik 4×10 <sup>5</sup> B	+	+	+	+	+
Premier cool+jik 4×10 <sup>3</sup> A	+	+	+	+	+
Good mama+jik 5×10 <sup>3</sup>	+	+	+	+	+
Waw+jik 6×10 <sup>5</sup> B	+	+	+	-	+
Waw+jik 6×10 <sup>3</sup> A	+	+	+	+	+
So Easy 7×10 <sup>3</sup>	+	+	+	+	+
Joy+jik 8×10 <sup>5</sup>	+	+	+	+	+

TABLE 6; Probable organism Isolated From Fairly Used Clothes, Male/ Female Underwear (Towel, Singlet and Panties) against Newly Emerging Detergents, Laundry Bleach and Toilet Soap.

Newly Emerging Detergents, Laundry Bleach And Toilet Soap	Organisms identified
Test sample 3(Lux-1×10 <sup>5</sup> B)	Aerobacter aerogenes
Test sample $3(\text{Lux} - 1 \times 10^3 \text{A})$	Bacillus polymyxa
Test sample 1(Premier cool -4×10 <sup>5</sup> )	Bacillus polymyxa
Test sample 2(Canoe $-1 \times 10^3$ )	Veillonella parvula
Test sample 3(Good mama -2×10 <sup>5</sup> )	<u>Citrobacter</u> freundii
Test sample $1(\text{Waw} - 3 \times 10^3 \text{A})$	Staphylococcus aureus
Test sample 1(Waw - 3×10 <sup>5</sup> B)	Veillonella parvula

Test sample $4(So \text{ easy } -4 \times 10^3)$	Bacillus polymyxa
Test sample $3(\text{Lux} + \text{laundry bleach}(\text{Test sample } 1(\text{Jik } 1 \times 10^3)$	Escherichia coli
Test sample 2 (Eva + laundry bleach(Test sample 1(Jik -3×10 <sup>3</sup> )	Veillonella parvula
Test sample 1(Premier cool + laundry bleach(Test sample 1(Jik - 4×10 <sup>5</sup> B)	Aerobacter aerogenes
Test sample 1(Premier cool + laundry bleach(Test sample 1(Jik - 4×10 <sup>3</sup> A)	Veillonella parvula
Test sample 3(Good mama + laundry bleach(Test sample 1(Jik - 5×10 <sup>3</sup> )	Aerobacter cloacae
Test sample 1(Waw + laundry bleach(Test sample 1(Jik - 6×10 <sup>5</sup> B)	Escherichia coli
Test sample 1(Waw + laundry bleach(Test sample 1(Jik - 6×10 <sup>3</sup> A)	Escherichia coli
Test sample $4(\text{So easy -}7\times10^3)$	Veillonella parvula
Test sample 4(Joy + laundry bleach(Test sample 1(Jik - 8×10 <sup>5</sup> )	Aerobacter aerogenes

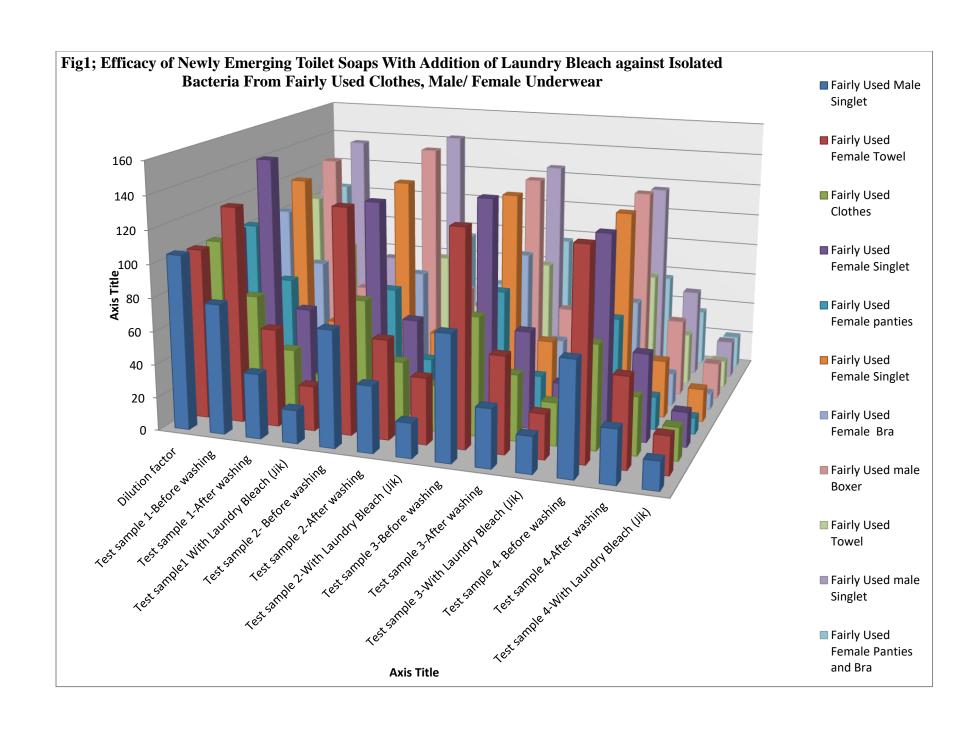
### Key – NEWLY EMERGING DETERGENTS, LAUNDRY BLEACH AND TOILET SOAP

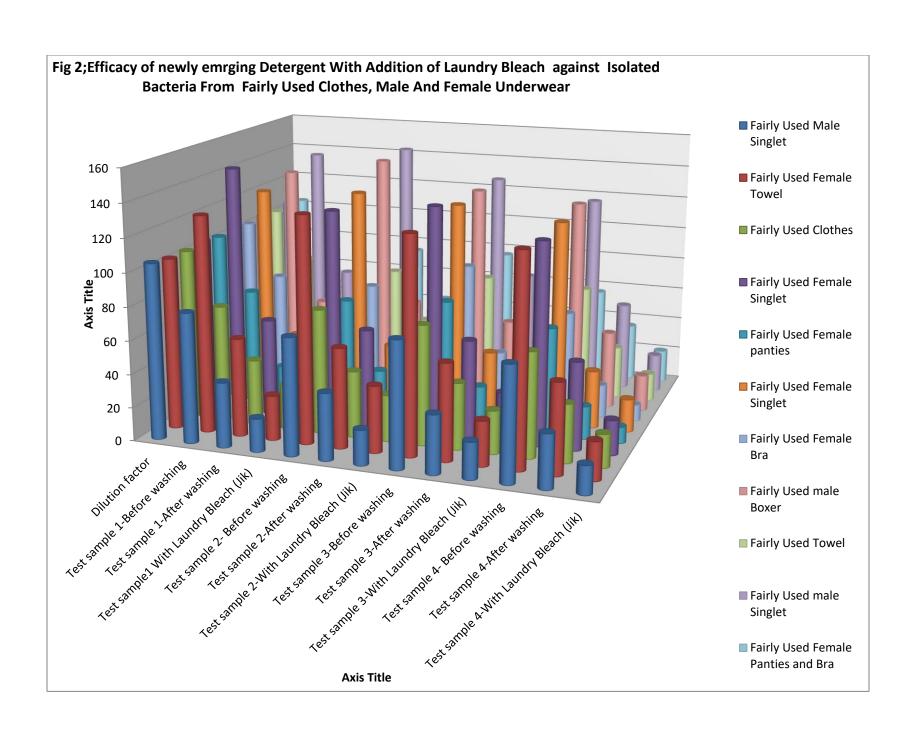
- 1. Toilet soap used (4): Test sample 1(Premier cool), Test sample 2 (Eva) Test sample 3(Lux), Test sample 4(Joy),
- 2.Detergents used(4): Test sample 1(Waw), Test sample 2(Canoe), Test sample 3(Good mama), Test sample 4(So easy)
- 3.Laundry Bleach (1) Test sample (1) Jik..

This section of this research work shows the efficacy of newly emerging toilet soaps with the addition of laundry bleach against isolated bacteria from fairly used clothes, male and female underwear

Fig 1; The result shows the efficacy of using toilet soap with the addition of laundry bleach (Jik) against the isolated bacteria from fairly used clothes, male and female underwear, this shows the effectiveness of toilet soap with the addition of laundry bleach against the isolated bacteria from fairly used clothes, male and female underwear on colony plate count. The results show that toilet soap with the addition of laundry bleach was most effective against the isolated bacteria from fairly used clothes, male and female underwear. From this study Test sample 4(Joy) toilet soap and addition of laundry bleach (jik) show the lowest colony counts of bacteria on a plate, before washing (10<sup>5</sup>) 68 numbers of colony counts on a plate, (10<sup>3</sup>) 128 numbers of colony counts, After washing with Test sample 4(Joy) toilet soap with the addition of laundry bleach (jik) (10<sup>5</sup>) 19 number of colony counts, (10<sup>3</sup>) 30 numbers of colony counts, After washing with laundry bleach (jik) (10<sup>5</sup>) 5 numbers of colony counts, (10<sup>3</sup>) 19 numbers of colony cunts, which implies it is the most effective toilet soap among the toilet soap against bacteria on fairly used clothes in ascending order.

Fig 2; The results show the efficacy of using detergents and laundry bleach (Jik) against the isolated bacteria from fairly used clothes, male/female underwear, the result shows how effective the detergent is against the isolated bacteria from fairly used clothes, male and female underwear. The result shows which detergent is most effective against the bacteria against the isolated bacteria from fairly used clothes, male and female underwear, From this study Test sample 4 (So Easy) detergent shows the lowest colony counts of bacteria on a plate, before washing (10<sup>5</sup>) 68 numbers of the count, (10<sup>3</sup>) 128 number of counts. After washing with Test sample 4 (So Easy) detergent (10<sup>5</sup>) 20 number of counts, (10<sup>3</sup>) 33 number of counts, with laundry bleach (jik) (10<sup>5</sup>) 10 number of counts, (10<sup>3</sup>) 22 number of counts, which implies Test sample 4 (So Easy) detergent is the most effective detergent on bacteria isolated from the sample among the detergents used for the study. Fig 2 is arranged accordingly showing the efficacy of detergent against bacteria isolated on fairly used cloth in ascending order.





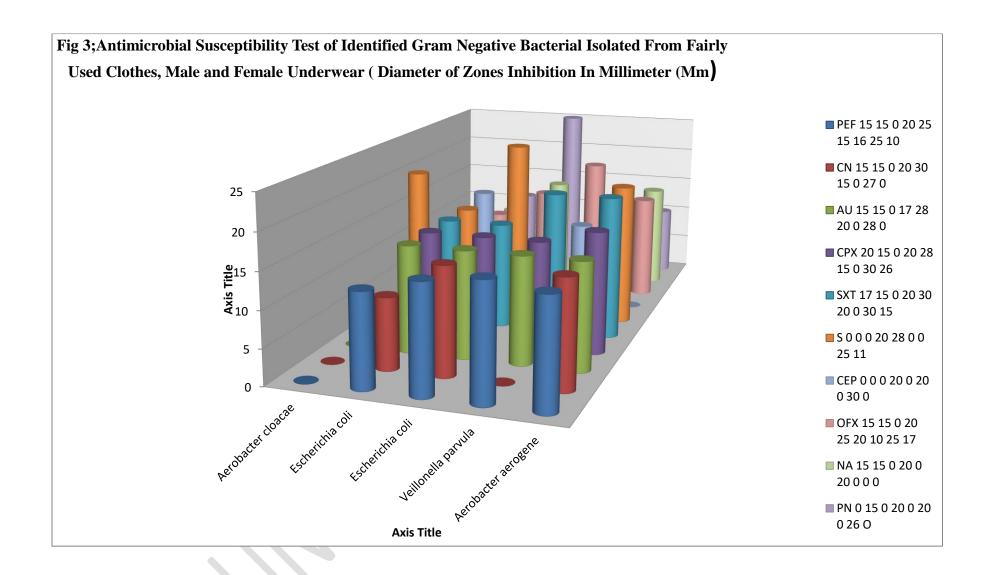
- Fig 3; Shows the antibiotics susceptibility test of the identified Gram-negative organisms isolated from fairly used clothes, male and female underwear. It was observed that *Escherichia coli* and *Aerobacter aerogenes* has the highest zone of inhibition to Reflacine (25mm) while *Citrobacter freundi and Aerobacter cloacae* has no zone of inhibition to Reflacine (0mm). *Escherichia coli* has the highest zone of inhibition to Gentamycin (30mm), *Escherichia coli and Aerobacter aerogenes* has the highest zone of inhibition to Augmentin (28mm), *Aerobacter aerogenes* has the highest zone of inhabitation to Septrin (30mm), *Escherichia coli* and *Aerobacter aerogenes* has the highest zone of inhibition to Streptomycin (28mm), *Aerobacter aerogenes* has the highest zone of inhibition to Ceporex (30mm), *Escherichia coli* and *Aerobacter aerogenes* has the highest zone of inhibition to Tarivid (25mm), *Veillonella parvula* and *Enterobacter aerogenes* has the highest zone of inhibition to Nalidixic acid (20mm), *Aerobacter aerogenes* has the highest zone of inhibition to Amplicin (26mm).
- Fig 4; Percentage zones of inhibition of antibiotics against *Aerobacter aerogene* isolated from fairly used clothes, male and female underwear. It was observed that Nalidixic acid (NA) 13%, Tarivid(OFX)13%, Septrin(SXT)15%, Ciprofloxacin(CPX)18%, Augmentin(AU)13%, Gentamycin(CN)14%, and Reflacine (PEF)14%
- Fig 5; Percentage zones of inhibition of antibiotics against *Veillonella parvula* isolated from fairly used clothes, male and female underwear. It was observed that Nalidixic acid (NA) 13%, Tarivid(OFX)13%, Septrin (SXT) 13%, Ciprofloxacin(CPX) 12%, Augmentin(AU)12%, Gentamycin (CN)12%, Reflacine (PEF)12% and Amplicin (PN)13%
- Fig 6; Percentage zones of inhibition of antibiotics against *Enterobacter aerogenes* isolated from fairly used clothes, male and female underwear. It was observed that Nalidixic acid (NA) 12%, Tarivid(OFX)12%, Ceporex (CEP) 12%, Septrin (SXT) 12%, Ciprofloxacin (CPX) 9%, Augmentin (AU)13%, Gentamycin (CN)9%, Reflacine(PEF)9% and Amplicin (PN)12%
- Fig 7; Percentage zones of inhibition of antibiotics against *Escherichia coli* isolated from fairly used clothes, male and female underwear. It was observed that Tarivid(OFX)13%, Streptomycin(S)14%, Ciprofloxacin (CPX) 14%, Augmentin (AU)14%, Gentamycin (CN)16%,, Reflacine(PEF)13% and Amplicin (PN)13%

Fig 8; Shows the antibiotics susceptibility test of the identified Gram-positive organisms isolated from fairly used clothes, male and female underwear. It was observed that *Bacillus polymyxa* has the highest zone of inhibition to Ampiclox (20mm), *Bacillus polymyxa* has the highest zone of inhibition to Rifampicin (20mm), *Aerobacter aerogenes* has the highest zone of inhibition to Amoxil (23mm), *Bacillus polymyxa* and *Veillonella parvula* has the highest zone of inhibition to Streptomycin (20mm), *Aerobacter aerogenes* has the highest zone of inhibition to Norfloxacin (22mm), *Aerobacter aerogenes* has the highest zone of inhibition to Chloramphenicol (20mm), *Staphylococcus aureus* has the highest zone of inhibition to Ciprofloxacin (22mm), *Bacillus polymyxa* has the highest zone of inhibition of Erythromycin (20mm), *Bacillus polymyxa* has the highest zone of inhibition to Levofloxacin (20mm), *Bacillus polymyxa* and *Aerobacter aerogenes* has the highest inhibition of Gentamycin (20mm).

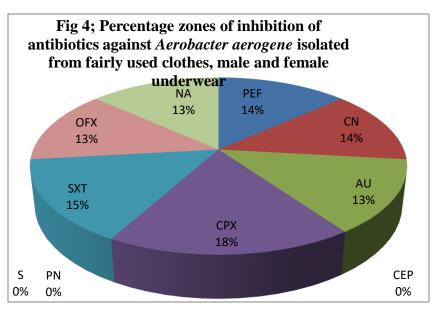
Fig 9; Percentage zones of inhibition of antibiotics against *Bacillus polymyxa* isolated from fairly used clothes, male and female underwear. It was observed that Gentamycin (CN)13%, Levofloxacin (LEV)9%, Ciprofloxacin (CPX)9%, Chloramphenicol (CH)10%, Norfloxacin (NB)14%, Streptomycin(S)11%, Amoxil.(AMX) 15%, Ampiclox (APX) 10%

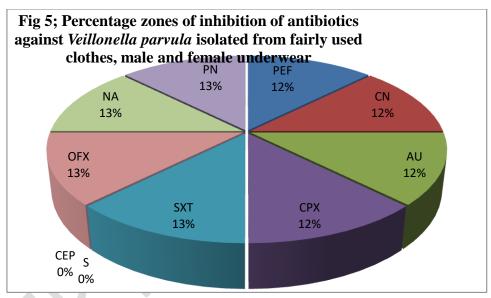
Fig 10; Percentage zones of inhibition of antibiotics against *Staphylococcus aureus* isolated from fairly used clothes, male and female underwear. It was observed that Gentamycin (CN)19%, Levofloxacin (LEV)20%, Erythromycin(E)20%, Ciprofloxacin (CPX) 24%, Streptomycin (S) 20%.

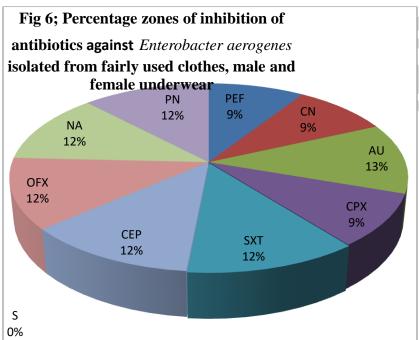
Fig 11; Percentage zones of inhibition of antibiotics against *Veillonella parvula* isolated from fairly used clothes, male and female underwear. It was observed that Gentamycin (CN)16%, Erythromycin(E)9%, Ciprofloxacin(CPX)16%, Chloramphenicol (CH)14%, Norfloxacin (NB) 8%, Streptomycin(S)17%, Amoxil (AMX) 12%, Ampiclox (APX) 8%

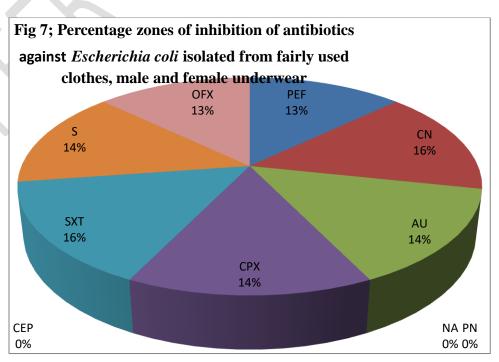


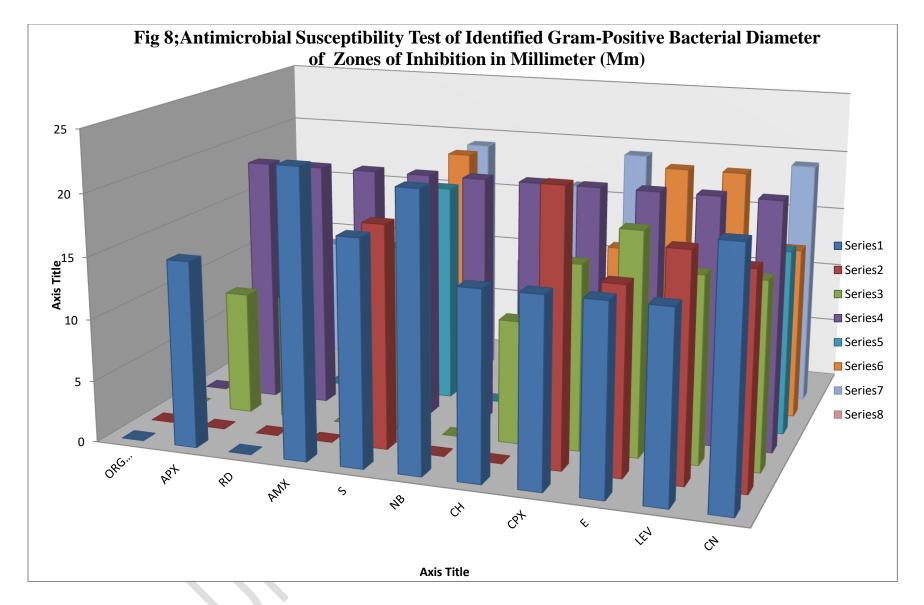
KEY: S- Streptomycin, OFX- Tarivid, PEP- Reflacine CPX- Ciprofloxacin, AU- Augmentin, CN- Gentamycin, CEP- Ceporex, NA- Nalidixic acid, SXT- Septrin, PN- Amplicin





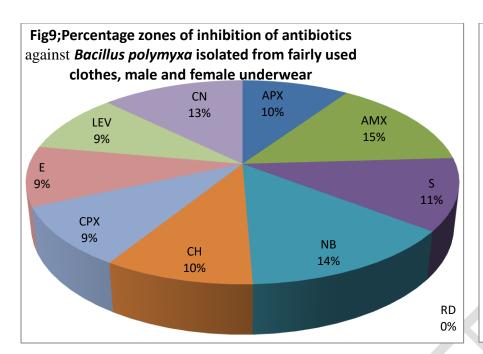


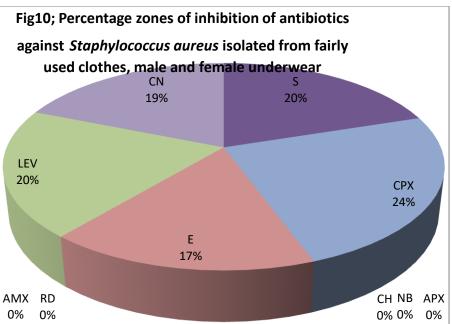




KEY: S- Streptomycin, NB- Norfloxacin, CH- Chloramphenicol, CPX- Ciprofloxacin,

E- Erythromycin, LEV- Levofloxacin, CN- Gentamycin, APX- Ampiclox, RD- Rifampicin, AMX- Amoxil.





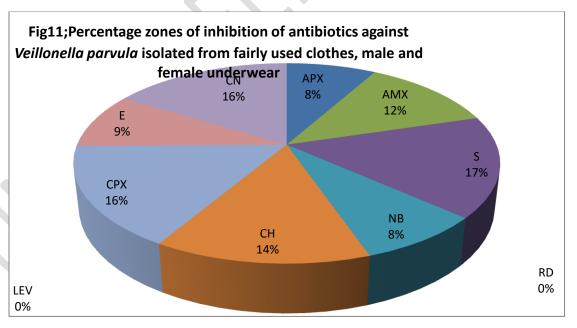


Fig 11; Shows the growth dynamics of bacteria isolated from fairly used clothes, male and female underwear using Ultraviolet spectrophotometer with the wavelength 480λ. It was observed that at 0hr *Citrobacter freundll* has the highest growth rate of 0.9003λ and *Aerobacter cloacae* have the lowest growth rate of 0.070λ. At 84<sup>th</sup> hour *Aerobacter cloacae* has the highest death rate of 0.296λ and *Escherichia coli* has the lowest death rate of 0.095λ.

Fig 12; Shows the growth dynamics and killing kinetics of bacteria isolated from fairly used clothes, male and female underwear, and the addition of Ciprofloxacin antibiotics at the  $48^{th}$  hour using an Ultraviolet spectrophotometer. In this table, it was observed that at 0hr *Aerobacter aerogenes* have the highest growth rate of  $0.142\lambda$ , and *Veillonella parvula* has the lowest growth rate of  $0.059\lambda$ . At the  $48^{th}$  hour *Escherichia coli* has the highest death rate of  $0.138\lambda$  and *Bacillus polymyxa* has the lowest death rate of  $0.079\lambda$ 

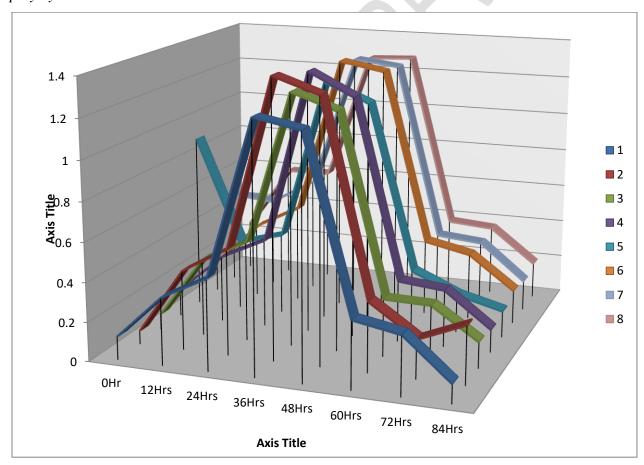


Fig 12. Growth Dynamics of Bacteria Isolated From Fairly Used Clothes, Male and Female Underwear Using Ultraviolet Spectrophotometer With The Wavelength 480λ. (With Control: 0.000)

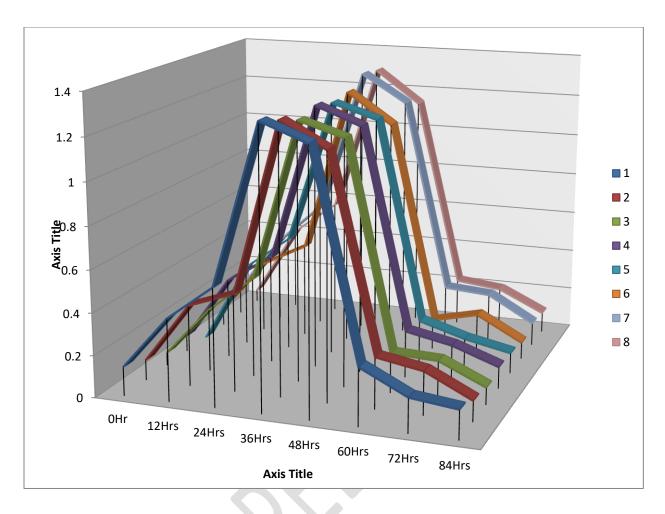


Fig 13; Growth Dynamics and Killing Kinetics of Bacteria Isolated From Fairly Used Clothes, Male/Female Underwear and Addition of Ciprofloxacin Antibiotics At 48<sup>th</sup> Hour Using Ultraviolet Spectrophotometer. (With Control: 0.000)

### 4.0 DISCUSSION

The aim of this study is to determine the potency and investigate the efficacy of these detergents, toilet soap, and laundry bleach against bacteria on fairly used clothes and Female Underwear. The different samples of fairly used cloth examined in this study showed varying degrees of microbial load with bacteria. The level of patronage given to these fairly used clothes by all in the society makes it a matter of public health importance. Though there are no data to show the link between the wearing of fairly used clothes and any infectious disease or disease outbreaks, the organisms isolated have been incriminated in one disease condition or the order. Towel, Female singlet, female bra, and panties, which is one of the commonly purchased fairly used

clothes, has the highest bacteria count from this study. This brings to surface facts, the need to know the source of these fairly used clothes. (12). (24) demonstrated the colonization of Towel and curtains in a Japanese hospital with pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, and *Vancomycin-resistant Enterococci (VRE)*. If these Towels get to the markets as fairly used clothes, the lives of buyers/users become endangered and increased related health risks. The varied efficacy of the different detergents and toilet soap used for different fairly used cloth types recorded in this study provides a guide as to which detergent and toilet soap may be used with a particular fairly used cloth/wear.

From this research work, towel, adult wears, male boxer and female panties and bra, Test sample 3(Good mama) detergent performed better than other detergents, and J Test sample 4(Joy) and Test sample (1) Laundry Bleach (Jik) performed better than other soap the reason for this might be in the components of the detergents and toilet soap, the texture of the cloth materials and the primary level of microbial load. Towels with heavier textures are better rewashed with Laundry Bleach (Jik). However, there was a general reduction in the microbial load with the use of the detergents and toilet soap, this agrees with the studies (25).

Fairly used wears from this research work. *Staphylococcus aureus* was the most commonly isolated bacterium from the examined fairly used cloth samples. This organism has also been isolated from previous studies by (25) (26) and (27). *Staphylococcus aureus* has been incriminated largely in various infections with multiple drug-resistant strains, skin infections such as boils and furuncules (28). This organism is also associated with pneumonia, bacteremia, and endocarditis (29; (30); *Staphylococcus aureus* isolation from these cloth samples no doubt is of public health concern.

Bacillus sp was the principal bacterium isolated by (1) and was also isolated from this study. Bacillus sp is not known as a human pathogen and is another health risk. Its isolation might be related to the fact that Bacillus sp generally can withstand harsh environmental conditions like heat, desiccation, and ultraviolet irradiation because of its ability to form endospores by which it can remain dormant for years. (31;1), especially if the cloth is been transported from one region to the other, a very good example is the importation of this fairly used cloth from the United kingdom and the United States of America. Bacillus polymyxa, was implicated during this research work (23). Other isolated organisms from the fairly used

cloth and male and female underwear includes <u>Citrobacter</u> freundii, Veillonella parvula Enterobacter aerogenes, and Aerobacter aerogene

Veillonella parvula is an anaerobic gram-negative coccus that is part of the normal human flora. It has rarely been identified as a pathogen in humans, and the most frequently reported infection caused by Veillonella parvula is osteomyelitis. a case of bacteremia unrelated to a central venous catheter and without an underlying source of infection (32). They are usually recovered as part of polymicrobial infection and they are often regarded as a contaminant. However, they have been isolated in pure culture from various sterile body sites such as sinuses, lungs, liver, central nervous system, heart, and bone. The presence of this organism depends on the previous users of the fairly used cloth. But it is worthy of note, to find Veillonella parvula in the fairly used cloth during this research work. (33).(34;(35) (28; 34).

Enterobacter aerogenes have been reported as important opportunistic and multiresistant bacterial pathogens for humans during the last three decades in hospital wards. These Gramnegative bacteria have been largely described during several outbreaks of hospital-acquired infections in Europe and particularly in France where there is an influx of this imported fairly used cloth. The dissemination of *Enterobacter* sp. is associated with the presence of redundant regulatory cascades that efficiently control the membrane permeability ensuring the bacterial expression detoxifying protection and the of enzymes involved in antibiotic degradation/inactivation. **Emphasis** should be taken to cognizant that Enterobacter aerogenes species are able to acquire numerous genetic mobile elements that strongly contribute to antibiotic resistance. Moreover, this particular fitness helps them to colonize several environments and hosts and rapidly and efficiently adapt their metabolism and physiology to external conditions and environmental stresses(36), to corroborate the finding of this research work, the isolated organism may travel from coast to coast i.e. horizontal transmission of mobile elements containing antibiotic resistance genes,

<u>Citrobacter</u> freundii is another isolated organism, <u>Citrobacter</u> is gram-negative enterobacteria classified as a genus in the family Enterobacteriaceae. <u>Citrobacter</u> species are often found in the stools of children, where they are generally regarded as normal components of the intestinal microflora. <u>Citrobacter</u> freundii may cause urinary tract infections, and are found

in wounds, respiratory, meningitis, and sepsis. They can cause <u>healthcare-associated infections</u>, especially in pediatric and <u>immuno-compromised patients</u> *Citrobacter* species have been reported previously in association with enteritis in humans, but thus far little effort has been made to clarify the nature of this association. It has been well established that Escherichia coli and some non-*E. coli* enterobacteria can cause diarrhea by producing either heat-stable (ST) or heat-labile (LT) enterotoxins (37).

Hence, isolation of these different organisms like *Staphylococcus aureus* from fairly used cloth samples calls for concern from this study which has been documented in serious wound infections, many strains of which are multidrug-resistant (38), *Staphylococcus aureus* was isolated in 17.1% of the cloth samples, this is in agreement with the work of (12). *Enterobacter aerogenes* is associated with nosocomial infection, eye and skin infection, vagina infections especially among diabetics, pregnant women, and immunocompromised patients (39). They are equally isolated from this study. Bacteria stay long on fabrics hence they are constantly present on fairly used cloth materials (40). The counts of microbial load on used fairly used clothes might be due to factors like customers' hand touching to feel texture and quality, environmental pollution, sewing, and packaging system or seller's carrier status. It is therefore advisable to display new wear in covered or glass-cased showrooms as against the open display commonly encountered to attract buyers common in Nigeria (41). (42),

It was observed that Ciprofloxacin(CPX) has 18% percentage zones of inhibition of antibiotics against Aerobacter aerogene, Nalidixic acid (NA) and Tarivid(OFX) has 13% percentage zones of inhibition of antibiotics against Veillonella parvula, Augmentin (AU) has 13% percentage zones of inhibition of antibiotics against Enterobacter aerogenes, Augmentin (AU) has 14% percentage zones of inhibition of antibiotics against Escherichia coli, Amoxil 15% (AMX) percentage zones of inhibition of antibiotics against Bacillus polymyxa, Ciprofloxacin (CPX) has 24% percentage zones of inhibition of antibiotics against Staphylococcus aureus and Streptomycin(S) has 17% percentage zones of inhibition of antibiotics against Veillonella parvula. The practice of formulating the toilet soap and detergent with specialized antibiotics should be upheld, this will give us leverage on the action of toilet soap /detergent on microbes isolated from the fairly used cloth and male /female underwear. The antibiotics will increase the killing action of the microbe thereby decontaminating the fairly used

clothes to reduce the disease risk factors. One such example was demonstrated in the killing kinetics during the course of the research work, Ciprofloxacin, these antibiotics accelerate the death rate of the isolates before reading was taken by the Ultraviolet spectrophotometer. if antibiotics are added to the toilet and detergent then risen with the laundry bleach, the microbe will be eradicated permanently (18,23).

The emergence of new toilet soap, detergent laundry bleach will help to eradicate the so-called emerging normal flora organism if correctly formulated and effectively used Exposure of strains of bacteria, to lethal doses of hypo chloric acid (Laundry bleach, toilet soap and detergent) causes a decrease in ATP production. Chlorine dioxide present in the detergent and soap acts on the permeability of the external membrane of the bacteria e.g. E. coli, through a primary lethal phenomenon that consists in a substantial leakage of K+ ion; such leakage does not occur for macromolecules. Sub-lethal doses inhibit cellular respiration due to a nonspecific oxidizing effect (43). All fairly used cloth should be washed with toilet soap and a mixture of detergent and a high concentration of laundry (Jik) bleach before use, this will drastically reduce the microbial load on the cloth and prevent major health hazards, that may emanate from this fairly used cloth.

#### CONCLUSION

Potential pathogens were isolated from the fairly used clothes with Towel, Singlet, bra, and panties have been implicated as the ones with the highest microbial load. These isolated microbes were incriminated in life-threatening diseases. Though the use of detergents, toilet soap, and laundry bleach in washing and re-washing the fairly used cloths reduces the microbial load drastically if not totally eradicated. fairly used clothes consumption is becoming a big industry in the third world like Nigeria because they are relatively cheaper and a little more durable quality better than the new cloth, it is very important and imperative, to educate the populace, about the danger and health implications of fairly used cloth, if not properly washed and rewashed before use and different soap and detergent, To remove the intending danger of fairly used cloth.

#### RECOMMENDATION

From the result obtain from the Efficacy of newly emerging detergents and toilet soap against bacterial isolated from fairly used clothes and underwear, I recommend that detergent or toilet soap can be used to wash any fairly used clothes purchased before use to reduce the microbial load present on the cloth and to prevent contacting of infectious disease, I also recommend that (Good mama)detergent and toilet soap with a high concentration of laundry bleach is best for washing of fairly used cloth.

#### **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### References

- 1. Agbulu, C.O., Gberikon, G.M., and Ajine, B.O. (2015) *Isolation and characterization of microorganisms associated with second hand female undergarments and children wear sold in Makurdi Metropolis*. (Int. J. Curr. Microbiol. App. Sci. 4(1): 716-724 [2]).
- 2. Malnick, S., Bardenstein, R., Huszar, M., Gabbay, J and Borkow, G. (2008). *Pyjamas and sheets as a potential source of nosocomial pathogens*. (Journal of Hospital Infection; 70:89-92).
- 3. Scott, T. A., Batey, S. F. D., Wiencek, P., Wiencek, G., Alt, S., Francklyn, C. S. (2019). Immunity-Guided Identification of Threonyl-tRNA Synthetase as the Molecular Target of Obafluorin, a β-Lactone Antibiotic. *ACS Chem. Biol.* 14 (12), 2663–2671. doi:10. 1021/acschembio.9b00590

- 4. Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., and Sayler, G. (2006) Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Appl Environ Microbiol* 72: 4214–4224.
- 5. Greene, V.W. (2001). Personal Hygiene and Life expectancy improvements since 1850; Historicand Epidemiological association. (American journal of infection control 29:203-206).
- 6. Matiru, V. (2009). Mapping of organizations engaged in climate change adaptation in Kenya: Final draft. Unpublished report prepared for the Worldwide Fund for Natur
- 7. Frumkin, H. (2001) Beyond toxicity human: health and the natural environment. *American Journal of PreventativeMedicine*20-234–240.
- 8. Hussain, S., Khan, F., Hussain, H. A., and Nie, L. (2016). Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. *Front. Plant Sci.* 7:116. doi: 10.3389/fpls.2016.00116
- 9. Olajubu, F. A; V.T. Folorunsho, O. Olajede(2017). *The Microbial Diversity of Fairly Used Wears Sold in Lagos Market, Nigeria*. 2017. Department of Microbiology, Adekunle Ajasin University, Akungba- Akoko, Nigeria. 12(2): 63-68
- 10. Aiello AE Larson EL and Sedlak R.( 2008) *Hidden heroes of the health revolution sanitation* and personal hygiene. American Journal of Infection Control 36:128-51
- 11. Pilonetto M, Rosa EA, Brofman PR, Baggio D, Calvário F, Schelp C, Nascimento A and Messias-Reason I. (2004) Hospital gowns as a vehicle for bacterial dissemination in an intensive care unit. Brazilian Journal of Infectious Diseases. 8: 206-10.
- 12. Shiomori T, Miyamoto H, Makishima K, Yoshida M, Fujiyoshi T, Udaka T, Inaba T and Hiraki N. (2002) Evaluation of bed making-related airborne and surface methicillin resistant *Staphylococcus aureus* contamination. Journal of Hospital Infection; 50:30-5.
- 13. Aiello AE and Larson EL. (2002) Causal inference: the case for hygiene and health. American Journal of Infection Control 2002; 30:503-11.
- 14. Levy, S. B. (2001). Antibiotic resistance: consequences of inaction. *Clinical Infectious Diseases*, 5124–9
- 15. Olajubu, F. A; V.T. Folorunsho, O. Olajede. *The Microbial Diversity of Fairly Used Wears Sold in Lagos Market, Nigeria.* 2017. Department of Microbiology, Adekunle Ajasin University, Akungba- Akoko, Nigeria. 12(2): 63-68

- 16. Russell, A. D., Tattawasart, U., Maillard, J.-Y. & Furr, J. R. (1998). Possible link between bacterial resistance and use of antibiotics and biocides. *Antimicrobial Agents and Chemotherapy* 42, 2151
- 17. Osuntokun OT, Ibukun AF, Yusuf-Babatunde AM, Abiodun S(2019). Pre/post-plasmid profile analysis, killing- kinetics and secondary metabolites screening of *Adenopus breviflorus* (Benth) fruit extract against multiple drug resistant isolates using *Staphylococcus aureus* (MDRSA) as a case study. Journal of Advanced Research in Biotechnology. J Adv Res Biotech. 2019;4(1):1-17.
- 18. Osuntokun, O. T., Thonda, O. A., Akele, E. O., Adedokun, L. O., Adedayo, S. A., & Bello, O. A.(2021). Pathogenic Bacteria Found on Surfaces of Canned Drinks and Wines Being Sold In Retail Shops in Ondo state, Nigeria, Health Implications, Food Safety and Quality Assessment. *South Asian Journal of Parasitology*, *5*(4), 68-94.
- 19. Fawole MO, Oso BA. ISSN 1597-6343 Published by Faculty of Science; 2007.
- 20.Cheesbrough, M. (2006) (District Laboratory Practice in Tropical Countries Cambridge University Press. Pp434)
- 21.Tankeshwar, A. (2013). Citrate utilization test: principle procedure, expected results and positive organisms. Microbe, 20-21
- 22.Clinical and Laboratory Standards Institute (CLSI) (2016): Performance standards for antimicrobial susceptibility testing; Twenty-sixth informational supplement. CLSI document M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute.
- 23.Osuntokun OT, Azuh VO, Adejoro BF, Akele EO.(2021) Antimicrobial Spectrum, Growth/Killing Kinetics, Conventional/Molecular Assay of Characterizing Non-Leguminous Endophytic Bacteria and Fungi from *Helianthus annuus*, *Carica papaya* and *Lycoperesicum solanum*. J Biomed Res Environ Sci. 2021 Oct 30; 2(10): 1018-1034.
- 24. Trillis, F., Eckstein, EC, Budavich, R, Pultz M J and Donskey CJ.(2008). *Contamination of hospital curtains with health care associated pathogens.Infection Control and Hospital* Epidemiology 2008;29: 1074-6).
- 25. Muthaini, Y.M., Matiru V.N. and Bii, C. (2010). Potential second hand clothes and the effectiveness of disinfection methods. Kenyan Journal of Microbiology. 1: 1-19

- 26. Sattler, C. and Armando, C. (2004). Coagulase Positive Staphylococcal Infections (Staphylococcus aureus), In: Ralph D. Feign (Editor), Textbook of Paediatric infectious diseases, 5th Edition, Saunders publishers, pp. 1099-1123.
- 27.Gaspard P, Eschbach E, Gunther D, Gayet S, Bertrand X and Talon D. (2009) *Methicillin resistant Staphylococcus aureus contamination of healthcare workers' uniforms in long-term care facilities*. (Journal of Hospital Infection.71:170–5).
- 28. Khadri, H. and Alzohairy, M.( 2010). Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative staphylococci in a tertiary care hospital in India. Int. J. Med. Med. Sci. 2(4): 116-120
- 29. Tekalign, K. and Bacha, K. (2013). Prevalence and antibiotic susceptibility pattern of methicillin resistant Staphylococcus aureus among primary school children and pioneers in Jimma town, (South West Ethiopia. Annals of Clinical Microbiology and Antimicrobials. 12: 11) 30. Fowler, V.G., Helen M.H.S., Boucher, W., Corey, R. G. and Abrutyn, E.( 2006) Daptomycin versus standard therapy for bacteremia and Endocarditis caused by Staphylococcus aureus. (The new England Journal of Medicine. 355 (7): 1812-17)
- 31. Nester, E.W., Anderson, G.D., Roberts E.C., Pearsall, N.N and Nester, M.T 2004. (*A Human Perspective Microbiology*, 4th Edition pp 535-645)
- 32.Barnhart RA, Weitekamp MR, Aber RC. Osteomyelitis caused by Veillonella. Am J Med. (1983),(5):902–904
- 33. Chaturvedi VN, Methwani A, Chaturvedi P, Narang P. Bacterial flora in chronic tonsillitis. Indian Pediatr. (1989) (1):52–56.
- 34.Koksal F., Yasar, H. and Samasti, M. (2009). Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. (Microbiological Research 164:404-410)
- 35. Huang, S.Y., Tang, R.N. and Chen, S.Y. (2003) Coagulase-negative staphylococcal bacteremia in critically ill children: risk factors and antimicrobial susceptibility. (J Microbiol Immunol Infect. 36: 51-55)
- 36.Hammerum AM, Hansen F, Nielsen HL, Jakobsen L, Stegger M, Andersen PS, Jensen P, Nielsen TK, Hansen LH, Hasman H, Fuglsang-Damgaard D. Hammerum AM, et al. J Antimicrob Chemother. (2016)(11):3117-3124. doi: 10.1093/jac/dkw289. Epub 2016 Aug 1. J Antimicrob Chemother. 2016. PMID: 27494919

- 37.Back, E., R. Mollby, B. KaUser, G. Stintzing, T. Wadstrom, and D. Habte.(1980). Enterotoxigenic E. coli and other gram-negative bacteria of infantile diarrhea: surface antigens, hemagglutinins, colonization factor antigen and loss of enterotoxigenicity. J. Infect. Dis. 142:318-327. 3.
- 38. Nkang, A.O. Okonko, I.O., Mejeha, O.K, Adewale O.G, Udeze, A.O, Fowotade, A., Fajobi, E. A., Adedeji, A.o and Babalola, E.T. (2009). (Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. J. Microbiol. Antimicrobial. 1(2): 19-26
- 39. Kothavade R.J., Kura M.M., and Panthaki, M.H. (2014). *Candida tropicalis, its prevalence, pathogenicity and increasing resistance to fluconazole*.(Journal of Medical Microbiology. 5(2): 35-39)
- 40. Muthaini, Y.M., Matiru V.N. and Bii, C.(2010). *Potential second-hand clothes and the effectiveness of disinfection methods* (. Kenyan Journal of Microbiology. 1: 1-19)
- 41. Ertek, M., Yazgi, H. and Erol, S. 2002 Demonstration of in vitro antagonism between fusidic acid and quinolones. (J Int. Med. Res. 30:525-8)
- 42. Pankaj K Tyagi and ShrutiTyagi (2013) Bacterial contamination in kitchens of rural and urban areas in Meerut District of Utter Pradesh (India), African journal of Microbiology Research.
- 43. Bettelheim KA, Evangelidis H, Pearce JL, Sowers E, Strockbine NA. Bettelheim KA, Clin Microbiol. (1993) (3):760-1. doi: 10.1128/jcm.31.3.760-761.1993. J Clin Microbiol. 1993. PMID: 7681442 Free PMC article.