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Microbial Flora in Ukay-ukay Clothing from Flea Markets in Valencia City, Bukidnon

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ABSTRACT

Microflora from selected *ukay-ukay* clothing in Valencia City, Bukidnon were isolated and identified, and the effectiveness of commonly used antimicrobial agents against the microbial isolates was assessed through a standard antimicrobial disc diffusion assay. The effectiveness of conventional hand washing and sun drying in eradicating the microbes from *ukay-ukay* clothing was also assessed. Nine bacteria namely; *Micrococcus luteus, Staphylococcus* sp.*, Enterobacter agglomerans, E. aerogenes, E. hafniae, Citrobacter freundii, Salmonella arizonae, Serratia* sp.*, Edwardsiella* sp.*,* and seven fungi namely; *Aspergillus fumigatus, A. niger, A. nidulans, Penicillium glabrum, Rhizopus nigricans, Fusarium* sp.*, Monilia* sp. were recovered and identified. One fungal species remained unidentified. Antimicrobial susceptibility tests showed that majority of the bacteria were susceptible to doxocycline, tetracycline, and norfloxacin. However, majority of the isolates were resistant to penicillin. Tioconazole was the most effective among the antifungal agents tested. Majority of the fungal isolates were resistant to sulfur+ZnO+salicylic acid, clotrimazole, benzoic acid+salicylic acid, ketocona-zole+clobetasol propionate and terbinafine hydrochloride. Reduction in colony counts after hand washing and sun drying reached 92.40% and 96.86% for bacteria and fungi, respectively. This study shows that *ukay-ukay* clothing harbor microbial pathogens with varying resistance/ susceptibility to popular antimicrobial agents.

Keywords: *Ukay-ukay, bacteria, fungi, antimicrobial agents*

INTRODUCTION

The use of *ukay-ukay* clothing is becoming a trend nowadays. Aside from being cheap, *ukay-ukay* materials are good sources for fashion and style, corporate and career dressing, as well as for sports and leisure wear. Hence, *ukay-ukay* trade had expanded and patronized by all segments of society. However, there is an increasing health concern on the use of *ukay-ukay* clothing. Previous studies demonstrated that nosocomial infections could be caused by using contaminated hospital linens and medical staffs' uniforms (Fijan & Turk, 2012; Wiener-Well et al., 2011; Calaghan, 1998). Moreover, microbial transfer via clothing and household linens can also be a possible cause of an infection outbreak. Clothing and linen, therefore, are identified as a reservoir of microflora. Skin infections, such as athlete's foot, ringworm, jock itch, impetigo, cellulitis, erysipelas, and candidiasis, are fungal or bacterial in origin. Bacteria, such as Staphylococcus aureus, methycillin-resistant Staphylococcus aureus (MRSA), S. saprophyticus, S. epidermis, Acinetobacter spp., Pseudomonas spp.,and Serratia spp., and fungi such as *Trichophyton* rubrum, *Candida* spp., *Aspergillus* spp. *Fusarium* spp. *Paecilomyces* spp. are among the most common causes of skin infections (DermNet NZ, 2014; MedlinePlus, 2014). It was also established that bacterial and fungal infections are synergistic. Fungal infections are good creators of crack, break or wounds of the skin, giving entry points for bacteria. This makes skin infections more serious and needs immediate medical attention.

In the past years, individuals who acquired bacterial and fungal infections did not seek medical assistance but resorted to self-medication because of easy access to over-the-counter antimicrobial agents. Also, some patients who were in the prescribed treatment regimen with antibiotics did not finish the treatment period. This indiscriminate use of antimicrobial drugs has been discouraged because it can result in drug resistance.

Antimicrobial agents include antibiotics, antiviral, anti-fungal, and anti-parasitic medications that are helpful in preventing, controlling, and treating diseases (Bayarski, 2013). Antibiotics are natural substances produced by or derived from micro-organisms that slow down or inhibit the growth of bacteria or directly kill them (Bayarski, 2013; Levy, 1998). However, with the increasing number of multi-drug resistant bacteria, we are losing effective new antibiotics. Obviously, we are now facing antibiotic resistance crises.

Antibiotics are among the most commonly prescribed drug in human medicine because these are effective in treating disorders caused by bacterial infections. However, up to 50% of all the antibiotics prescribed are not really needed or are

not optimally effective (Roberts et al., 2009; Levy, 1998) and may cause unwanted side effects, such as diarrhea, nausea, stomach pain, vomiting, and even fungal infections of the mouth, digestive tract, and vagina (Bayarski, 2013; Roberts et al., 2009). Further, some individuals may develop allergic reactions to antibiotics, particularly penicillin.

Although antibiotics are vital in medicine, prolonged use, misuse, and indiscriminate use can encourage the growth of resistant strains that can produce hard-to-treat disorders (Khachatourians, 1998; Levy, 1998). Antibiotic resistance can be worsened when antibiotics are used to treat disorders in which they have no efficacy and when these are used as prophylaxis rather than treatment (Bayarski, 2013). Resistance to antibiotics is a serious concern because resistant bacteria do not respond to the treatment and may continue to cause infection (Balan et al., 2013; Bayarski, 2013; Roberts et al., 2009; Schito, 2006).

Another side effect of antibiotic therapy is increased growth of fungal microbiota (Nover et al., 2004) but literature shows that anti-fungal susceptibility testing is less developed and less utilized than antibacterial testing (Rex et al., 2001). Probably, this is because of the uncertainty surrounding the efficacy of anti-fungal agents. There is also uncertainty as to the optimal period of treatment, appropriate dosage of drug, and frequency of application (Crawford and Hollis, 2007). Just like in bacteria, anti-fungal agents are also losing their efficacy because of the spread of resistant strains. Therefore, use of antimicrobial agents should be limited only to situations where these are needed, and there should be a selection of the right antimicrobial agent to use.

This study endeavored to isolate and identify microbes present in selected *ukay-ukay* samples and assess their sensitivity/susceptibility to commonly used antimicrobial agents. This study also sought to determine if microbial flora from *ukay-ukay* clothing can be eradicated by conventional hand washing and sun drying.

METHODOLOGY

Ukay-ukay retailer outlets in Valencia City, Bukidnon, Philippines were visited. Based on the site observation and interview with owners/vendors, three sampling sites were chosen, and the sampling was done twice during the one-year study period.

Supplies, *ukay-ukay* samples, and anti-fungal drugs were purchased from Valencia City, Bukidnon. The samples were categorized into three groups namely a) shorts and long pants, b) shirts and blouses, c) and beddings.

Nutrient agar (NA) and Potato dextrose agar (PDA) were used to culture bacteria and molds/yeasts, respectively. Media were prepared following the manufacturer's instructions. Sterilization was set at 121°C for 15 minutes using a pressure-cooker-type sterilizer. The prepared media were used immediately or stored in the refrigerator until use.

To isolate bacteria, 10 cm^2 of sample's surface was swabbed using sterile cotton swabs which were directly swabbed to NA plates. The NA plates were incubated at 35°C for 24 hours then checked for growth. The number of bacterial colonies was counted, and each colony was examined for color and growth. Total microbial counts in colony forming units (CFU) were recorded for each plate. Representative colonies were streaked several times to NA plate to obtain a pure culture. Pure cultures were grown in NA slant and stored in the refrigerator as stock cultures. For identification, pure cultures were subjected to Gram-staining and standard biochemical tests namely; Indole, Methyl Red-Voges Proskauer and Citrate utilization tests (IMViC), catalase test, coagulase test, and mannitol fermentation test following standard procedures (Acharya, 2013; Baron, 1996). These tests were carried out at the Animal Disease Diagnostic Laboratory of Central Mindanao University.

To isolate fungi, the same method for bacterial isolation was followed except that PDA plates were used. The incubation was also extended to 48-72 hours at 35oC to allow the fungal spores to germinate. After incubation, the plates were checked, and the colonies were counted. The characteristic growth of the colonies was noted. Each distinct colony was grown in PDA slants for stock culturing. Total fungal counts (in colony forming units/CFU) were recorded for each plate. The isolated fungi were submitted to the Plant Disease Diagnostic Laboratory of CMU for identification. The isolates were described and identified based on macroscopic characteristics such as colonial form, surface color and texture, and microscopic features such as fruiting bodies and spores.

The pure cultures of bacteria were submitted to the Animal Disease Diagnostic Laboratory of Central Mindanao University for antimicrobial disc susceptibility test. For bacteria, Kirby-Bauer Disk-Diffusion assay was done on Mueller-Hinton agar plates following the standard procedures of the Clinical and Laboratory Institute guidelines (2005). The antibiotic discs used were penicillin (6 µg/10 IU), erythromycin (15 µg), tetra-cycline (30 µg), doxycycline (30 µg), and norfloxacin (10 µg). The same methods were used for fungi. The antifungal drugs used were tioconazole, sulfur+ZnO+salicylic acid, clotrimazole, benzoic acid+salicylic acid, ketoconazole+clobetasol propionate and terbinafine hydrochloride. The assay was done on PDA plates and was conducted at the Microbiology Laboratory of Biology

Department, Central Mindanao University.

For both bacteria and fungi, the diameter of the zones of inhibition after 24-h incubation period was measured using a caliper. Results were interpreted based on the Clinical and Laboratory Standards Institute guidelines (CLSI, 2005).

To determine if the microbial load can be reduced by conventional hand washing, the samples were washed using a laundry detergent then sun-dried. After this, routine isolation and culturing procedures were done. Colony counts of the pre-washed and post-washed samples were compared and percent reduction in microbial counts computed.

RESULTS AND DISCUSSION

Microflora Isolated from the Ukay-ukay Samples

Swabbing of *ukay-ukay* samples had recovered microflora that includes bacteria and fungi. Table 1 and 2 revealed that there were nine bacterial species identified under six genera. Of these, two are Gram-positive, and seven are Gram-negative.

Ukay-ukay garments are made from different types of cloth: cotton, polyester, nylon, or combination of these. Previous studies demonstrated the survival of bacteria and fungi in these types of cloth. Survivability depends on species/strains and type of cloth. Neeley and Maley (2000) reported the survival of 22 Gram-positive bacteria on cotton clothing, towels, cotton/polyester scrub suits, laboratory coats and privacy drapes. Enterobacter species survived from less than 1 hour to 10-50 days depending on inoculum size on cloth. According to Bloomfield et al. (2011), Gram-positive species such as S. aureus and some fungal species can survive extended periods of time (days to months) on fabrics. Gram-negative species are less resistant to drying than Gram-positive; however, the survival of some Gram-negative species such as Serratia marcescens and Pseudomonas aeruginosa is still sufficient for transfer to hands or skin and other body surfaces. Scott and Bloomfield (1990) also studied the survival of Salmonella spp. After cleaning cloth fabric. Results showed that growth of Salmonella spp. was reduced to 20 CFU/25 sq cm of cloth at 4 hours. However, re-growth of residual survivors occurred within 24 hours.

The presence of nine species of bacteria in this study is suggestive of the ability of bacteria to survive in cloth for prolong periods. This also suggests that people who use *ukay-ukay* products can contract these bacteria and can compromise their health since the identified bacteria are pathogenic.

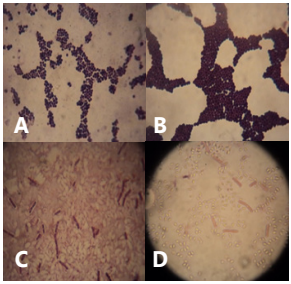

Figure 1. Representative Bacteria Isolated from the Samples (A. *Micrococcus luteus*; B. *Staphylococcus* sp.; C. *Salmonella arizonae*; D. *Citrobacter freundii*). 1600X

Table 1
Bacteria Isolated from Ukay-ukay Clothing and their General Characteristics

Species	Sample Category Present			Characteristics
	1	2	3	
Citrobacter freundii	✓	✓		Gram-negative rod; common in soil, water, sewage, food and intestinal tracts of animals and humans; opportunistic pathogens responsible for nosocomial infections of the respiratory and urinary tracts.
Edwardsiella sp.	✓			Gram-negative rod; occasionally opportunistic pathogens of humans; causes gastroenteritis and wound infections.
Enterobacter aerogenes	✓	✓	✓	Gram-negative rod; nosocomial and pathogenic bacterium; causes opportunistic infections including most types of infections.
Enterobacter agglomerans	✓	✓	✓	Gram-negative rod; opportunistic pathogen in the immunocompromised host causing wound, blood, and urinary tract infections.
Enterobacter hafniae			✓	Gram-negative rod; can cause opportunistic infections in immunocompromised host; urinary and respiratory tracts are common sites of infection.
Micrococcus luteus	✓	✓	✓	Gram-positive spherical; part of the normal flora of mammalian skin; colonizes mouth, mucosae, oropharynx and upper respiratory tract.
Salmonella arizonae			✓	Gram-negative rod; often pathogenic; can cause severe enteritis and septicemia.
Serratia spp.	✓		✓	Gram-negative rod; causes nosocomial infections of the bloodstream, lower respiratory tract, urinary tract, surgical wounds, skin and soft tissues in adult patients.
Staphylococcus sp.	✓	✓	✓	Gram-positive spherical; common cause of skin infections (boils), respiratory disease, and food poisoning; can survive from hours to weeks, or even months on dry environmental surfaces depending on the strain.

Legend: 1- shorts, long pants; 2- shirts, blouses; 3- beddings; ✓- present

Table 2.
Gram Stain Reaction and Biochemical Characteristics of Isolated Bacteria from Ukay-Ukay Clothing

Bacteria	Gram-stain reaction	Biochemical characteristics						
		Indole	Methyl Red	Voges-Proskauer	Citrate utilization	Catalase	Oxidase	Mannitol fermentation
Citrobacter freundii	–	–	+	–	+	+	–	–
Edwardsiella sp.	–	+	+	–	–	+	–	–
Enterobacter aerogenes	–	–	–	+	+	+	–	+
Enterobacter agglomerans	–	–	–	+	+	+	–	ND
Enterobacter hafniae	–	–	–	+	–	+	–	+
Micrococcus luteus	+	–	–	–	–	+	+	–
Salmonella arizonae	–	–	+	–	+	+	–	+
Serratia spp.	–	–	–	+	+	+	–	+
Staphylococcus sp.	+	–	+	+	+	+	–	+

Legend: (+): Positive; (–): Negative; (ND): Not determined

Figure 2 and Table 3 show fungi isolated from ukay-ukay samples which include three species of Aspergillus (A. fumigatus, A. niger, A. nidulans), Penicillium glabrum, Rhizopus nigricans, one unidentified spe-cies of Fusarium, two unidentified species of Monilia (Candida) and one unidentified species. The absence of fruiting bodies and reproductive structures made it difficult to identify these fungal species.

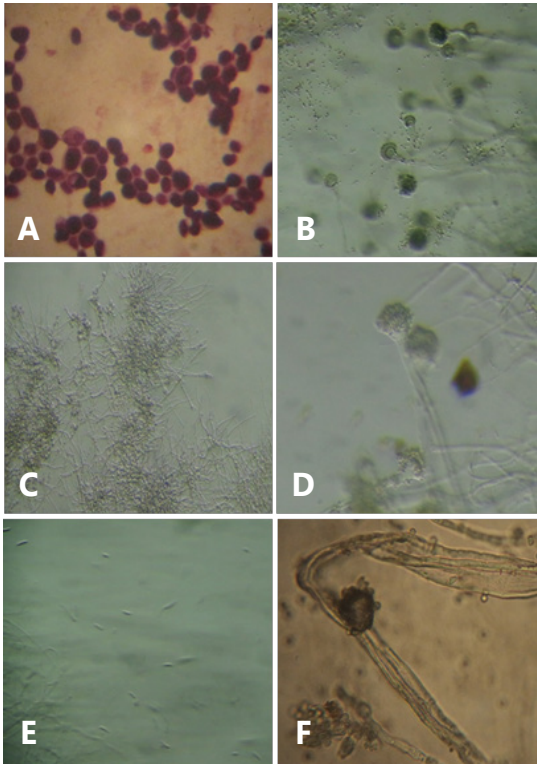

Figure 2. Representative Fungi. (A) *Monilia* sp., 1600X (B) A. fumigatus, 100X (C) P. *gla-brum*, 100X (D) *A. nidulans* 100X (E) *Fusarium* sp., 100X (F) *Rhizopus nigricans*, 400X

Table 3
Fungi from the Samples Collected during the Two Sampling Periods

Species	Sample Category Present			Characteristics
	1	2	3	
Aspergillus fumigatus	✓	✓	✓	Widespread in nature; produces conidiophores with green conidia that readily become airborne; the most common Aspergillus species that can cause diseases such as chronic pulmonary infections and allergic bronchopulmonary aspergillosis in immunocompromised patients.
Aspergillus nidulans	✓	✓	✓	Septate hyphae with a woolly colony texture and white mycelia; colonies usually appear green due to pigmentation of spores; known to cause invasive infections and osteomyelitis to patients suffering from chronic granulomatous disease.
Aspergillus niger	✓	✓	✓	Colonies dark-brown to black; conidial heads large; common cause of otomycosis (fungal ear infections) and in rare cases aspergillosis.
Fusarium sp.			✓	Colonies usually fast growing; pale or brightly colored; can cause fusarial infections in the nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis); can also cause opportunistic infections in immunocompromised humans.
Monilia spp.	✓	✓	✓	Monilia may refer to yeast Candida albicans; Monilia (yeast infection) is a causal agent of opportunistic oral and genital infections and candidal onychomycosis (infection of the nail plate)
Penicillium glabrum		✓		Ubiquitous soil fungi preferring cool and moderate climate; known to cause diseases in plants such as strawberries.
Rhizopus nigricans			✓	Commonly known as bread mold; the spores contain allergenic proteins that can cause respiratory and nasal problems such as chronic cough, dyspnea, allergenic rhinitis and chronic phlegm.
Unidentified sp.	✓	✓	✓	Colonies are white to milky white in color, wrinkled and dry. Usually large with approximately 20 to 30 mm in diameter.

Legend: 1- shorts, long pants; 2- shirts, blouses; 3- beddings; ✓- present

The presence of fungi on ukay-ukay clothing is not surprising. Neely and Orloff (2001) examined the survivability of some fungi including Candida spp., Aspergillus spp., Fusarium spp., Mucor spp., and Paecilomyces spp. on different hospital fabrics. Survival of fungal species depends on the species and type of cloth material. Most fungi can survive at least one day, but many survive for weeks. It was also reported that Aspergillus and Mucor survived around 26 days. Moreover, there is a tendency for fungi to have longer viability on synthetic materials (polyester) than on fabrics with natural fiber content such as cotton, terry, and blends (Neely and Orloff, 2001).

The majority of the isolated fungi are known to cause skin infections. In immunocompromised persons, these isolates can cause even more severe health problems. For instance, Aspergillus spp. can cause chronic pulmonary problems (CDC, 2014). Fusarium sp. can cause opportunistic infections in immunocompromised individuals (Howard, 2003). R. nigricans spores have 31 distinct allergens (Sridhara et al., 1990) which were reported to produce respiratory and nasal symptoms such as chronic cough, dyspnea, chest tightness, chronic phlegm, snuffle, and allergic rhinitis (Zhang et al., 2005). The result of this study can serve as a warning to the general public as to the presence of fungi in ukay-ukay clothing and the potential health risks they can cause.

Microbial Load of Ukay-ukay Samples

Figure 3 reveals the average bacterial colony counts during the two sampling periods. Beddings had the highest bacterial colony count in a given sample. The thickness and the material of the beddings make them good reservoir of microflora. Neeley and Maley (2000) reported that Gram-negative bacteria including Enterobacter spp. can survive 2-50 days in 100% cotton, 100% cotton terry, 60/40% polyester blend, and 100% polyester blend. Sampled beddings were made mostly of cotton/polyester blend materials. Seven bacterial species were isolated from the beddings namely; Enterobacter agglomerans, E. aerogenes, E. hafniae, Micrococcus luteus, Salmonella arizonae, Serratia sp., and Staphylococcus sp. Moreover, M. luteus, Entero-bacter spp., S. arizonae, and Staphylococcus sp. were observed in the other categories. Their presence in soil, dust, water, air and being part of the normal flora of the skin as reported by Chuku and Nwankiti (2013) may have contributed to the observation of this study.

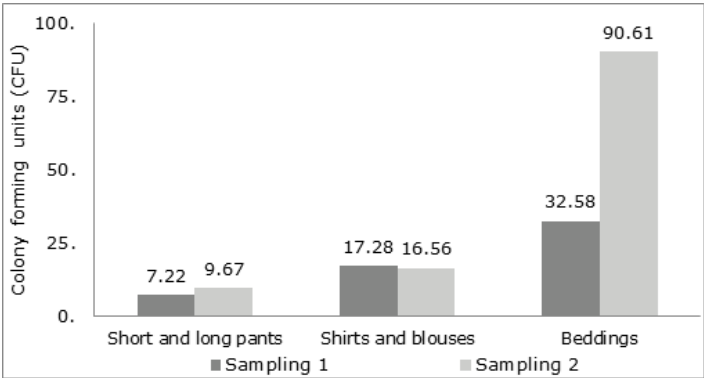

Figure 3. Average Bacterial Colony Counts on the Three Sample Categories during the Two Sampling Periods.

M. luteus is resistant to reduced water potential and have the ability to tolerate drying and high salt concentration (Madigan and Martinko, 2005). It can also survive in oligotrophic environments for extended periods of time (Greenbalt et al., 2004). Hence, it is not surprising that M. luteus were found in all sample categories.

For fungi, shorts and long pants had the highest colony count for the first sampling (36.29) while beddings showed the highest count for the second sampling (51.56). It should be noted that there are eight (8) fungal species isolated from the sampled beddings.

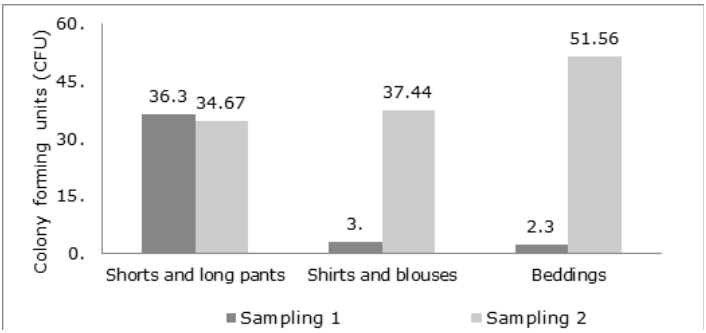

Figure 4. Average Fungal Colony Counts on the Three Sample Categories in Two Sampling Periods

The difference in fungal colony counts in two sampling periods suggests that the presence of fungi in ukay-ukay clothing is affected by season. More fungal colonies were observed in the samples collected during the second sampling (February 2014) compared to the samples from the first sampling (September 2013). February was more humid because of isolated rain showers that might contribute to the germination of fungal spores (Hassouni et al., 2007). Fungal spores are airborne and can easily contaminate other surfaces including clothing.

Figure 5 shows the microbial load per sample category. Beddings showed the highest bacterial colony count (61.59) while shorts and long pants had the highest fungal colony counts (35.48). The high recovery of bacterial colonies may be attributed to the type of cloth material since some bedding are thick, highly absorbent and tend to retain moisture. A similar result was reported by Oller and Mitchell (2009), they recovered more Staphylococcus colonies from high absorbency towels.

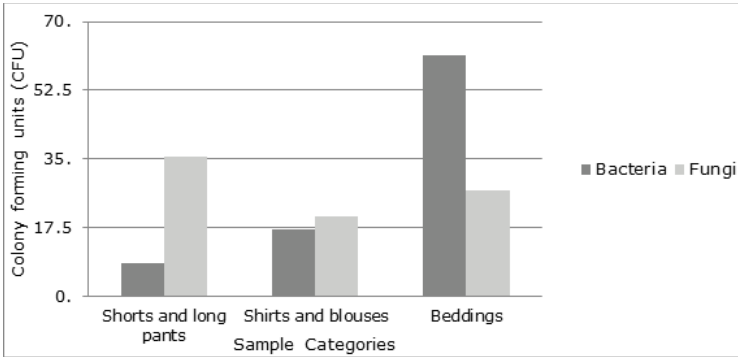

Figure 5. Average Colony Counts of Bacteria and Fungi Collected during Two Sampling Periods

Bacterial and fungal species are widely distributed among sample categories. This means that ukay-ukay, regardless of the type of garment and fabric, can harbor microflora of medical importance.

Antimicrobial Disc Susceptibility Test

Bacteria and fungi isolated from the sampled ukay-ukay clothing were subjected to antimicrobial disc susceptibility testing, and the response of the isolates to commonly used antimicrobial agents was determined by measuring the zone of inhibition (ZOI). ZOI is indicative of the inhibitory effect of the antimicrobial agents as well as a measure of the solubility of the agents (Kennell and Cunningham, 2014). Therefore, the presence of a clear area around the antimicrobial discs signifies not only the bacteriostatic effect of the compound but also the solubility of the drug.

Table 6 indicates the results of the antibiotic susceptibility testing as reflected by the ZOI (in mm). Except for Edwardsiella sp. with intermediate susceptibility (12 mm), the bacterial isolates [Citrobacter freundii (9 mm), Enterobacter agglomerans (8 mm), Micrococcus luteus (9 mm), Salmonella arizonae (11 mm), and Serratia sp. (0 mm.)] were resistant to penicillin. For erythromycin, both M. luteus (5 mm) and Serratia sp. (13 mm.) were resistant while the other bacteria [C. freundii (16 mm), E. agglomerans (16 mm), Edwardsiella sp. (19 mm), and S. arizonae (17 mm)] had an intermediate susceptibility. For tetracycline, both M. luteus (12 mm) and Serratia sp. (13 mm) had an intermediate response. The other bacterial isolates [C. freundii (18 mm), E. agglomerans (23 mm), Edwardsiella sp. (25 mm), S. arizonae (24 mm)] were susceptible tetracycline. All the bacterial samples [C. freundii (24 mm), E.

agglomerans (25 mm), Edwardsiella sp. (26 mm), M. luteus (24 mm), S. arizonae (24 mm), and Serratia sp. (17 mm.)] were susceptible to doxocycline. C. freundii (6 mm) was resistant for norfloxacin and the rest [E. agglomerans (23 mm), Edwardsiella sp. (25 mm), M. luteus (17 mm), S. arizonae (25 mm), and Serratia sp. (19 mm.)] were susceptible to norfloxacin.

Table 6
Results of Antibacterial Disc-Diffusion Assay. Zones of Inhibition were Measured in Millimeter

Bacteria	Penicilin	Erythromycin	Tetracycline	Doxycycline	Norfloxacin	Average Resistance
Citrobacter freundii	9	16	18	24	6	14.6
Edwardsiella sp.	12	19	25	26	25	21.4
Enterobacter aerogenes	n.t.	n.t.	n.t.	n.t.	n.t.	
Enterobacter agglomerans	8	16	23	25	23	19.0
Enterobacter hafniae	n.t.	n.t.	n.t.	n.t.	n.t.	
Micrococcus luteus	9	5	12	24	17	13.4
Salmonella arizonae	11	17	24	24	25	20.2
Serratia spp.	0	13	13	17	19	12.4
Staphylococcus sp.	n.t.	n.t.	n.t.	n.t.	n.t.	
Average Effectiveness	8.17	14.33	19.17	23.33	19.17	

Legend: n.t. – not tested

Figure 6 reveals that of the five (5) antibiotics tested, doxycycline had the highest average ZOI which reached 23.33 mm in diameter. Hence, it is most effective in inhibiting the growth and proliferation of the bacteria tested. Penicillin has low average effectiveness (8.17 mm). This finding coincides with the observation of Schito (2006) who reported that S. aureus is resistant to penicillin. Roberts et al. (2009) also mentioned that there is widespread resistance to penicillin among gram-negative bacteria. Contrary to this result, Daza et al. (2001) specified that Proteus mirabilis had a high susceptibility to penicillin and that over 92% of Enterococcus faecalis were sensitive to penicillin. The observed ineffectiveness of penicillin in this study can be attributed to antibiotic resistance acquired by bacteria due to the indiscriminate use of this antibiotic (Kawo & Musa, 2013). Penicillin was a popular choice for the treatment of bacterial infections before it was categorized by the Department of Health and Bureau of Food and Drugs as prescription medicines. Uncontrolled and indiscriminate use of this antibiotic had probably resulted in the development of resistance mechanism against the antibiotic by the bacteria (Balan et al., 2013; Schito, 2006; Levy, 1998). Once resistance is acquired, bacteria will no longer respond to antibiotics and may continue to cause infection (Bayarski, 2013).

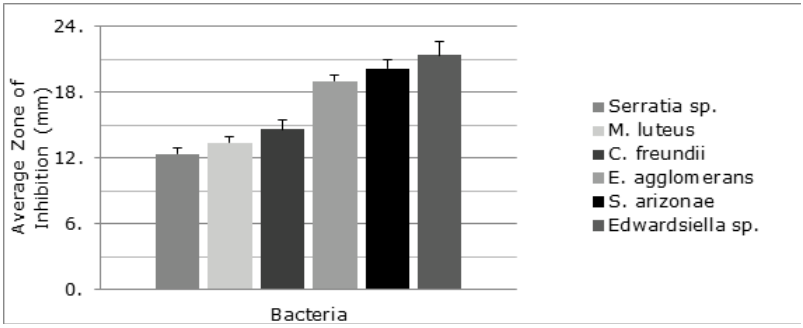

Figure 6. Average Effectiveness of the Antibiotics against all Isolates (bars indicate the standard deviation of four replicates)

Bacteria that showed sensitivity/susceptibility to the applied antibiotics can be successfully eliminated; those that are slightly insensitive (intermediate susceptibility) can be controlled by using more of the drug; whereas the resistant bacteria require other therapies (Levy, 1998). Among the bacterial isolates, Serratia sp. had the greatest resistance with an average ZOI of only 12.4 mm (Figure 7).

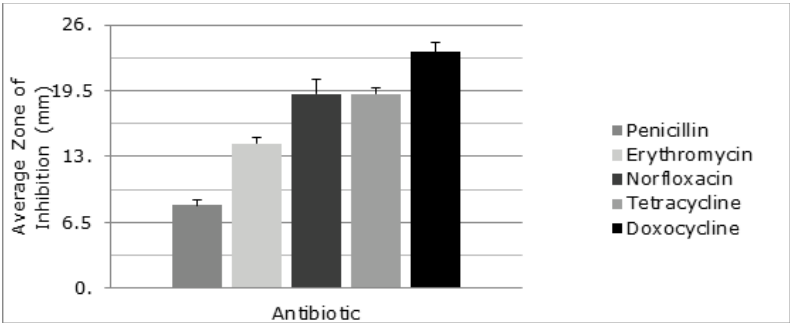

Figure 7. Average Resistance of the Isolates to Antibiotics (bars indicate the standard deviation of four replicates)

The fungi isolated from the ukay-ukay samples were subjected to antifungal susceptibility test as well. Table 7 presents ZOI (in mm) record. All fungal isolates can be considered resistant to sulfur+ZnO+salicylic acid because majority of fungal isolates (Aspergillus fumigatus, A.nidulans, Fusarium sp., Penicillium glabrum and Rhizopus nigricans) displayed no inhibitory zone (0 mm). A. niger, Monilia spp., and the unidentified species had relatively small zones of inhibition (8mm, 3mm, and 0.5mm, respectively). Tioconazole is considered effective antifungal agent because all the isolates were susceptible to the drug with zone of inhibition as follows: Aspergillus fumigatus (24 mm), A.nidulans (26 mm), A. niger (24.5 mm), Fusarium sp. (25 mm), Monilia spp. (27 mm), P. glabrum (24 mm), R. nigricans (27 mm), and the unidentified species (24 mm). For clotrimazole, except A. niger which was slightly susceptible (13.5 mm inhibitory zone), the rest [Aspergillus fumigatus (2.7 mm), A.nidulans (4 mm), Fusarium sp. (3 mm), Monilia spp. (9 mm), P. glabrum (8 mm), R. nigricans (7 mm), and the unidentified species (2.8 mm)] were resistant. Benzoic acid+salicylic acid showed no efficacy because of the fungi [Aspergillus fumigatus (1.7 mm), A.nidulans (3 mm), A. niger (12 mm), Fusarium sp. (0 mm), Mo-nilia spp. (10 mm), P. glabrum (2 mm), R. nigricans (5 mm), and the unidentified species (0 mm) were all resistant to it. The same observation applies to ketoconazole+clobetasol propionate. The fungal isolates [Asper-gillus fumigatus (3.3 mm), A.nidulans (10 mm), A. niger (10.5 mm), Fusarium sp. (0 mm), Monilia spp. (13.5 mm), P. glabrum (8 mm), R. nigricans (8 mm), and the unidentified species (3.3 mm)] were resistant to this drug. Most of the fungi [Aspergillus fumigatus (5 mm), A.nidulans (13 mm), Fusarium sp. (6 mm), Monilia spp. (12.5 mm), P. glabrum (6 mm), R. nigricans (8 mm), and the unidentified species (1.5 mm) were resistant to terbinafine hydrochloride. A. niger (14 mm) has minimal activity.

Table 7
Results of Anti-Fungal Disc Diffusion Assay (The zones of inhibition is measured in millimeter)

Bacteria	Katialis (Sulfur, zinc oxide, resorcnol, salicylic acid)	Trosyd (Tioconazole)	Canesten Thrush Cream (Clotrima-zole)	Whitfield Ointment (Benzoic acid, salicylic acid)	BL Cream (Ketoconazole, clobetasol propionate)	Lamisil (Terbinafine hydrochlride)	Lamisil (Terbinaf-ine hydrochloride)
Aspergillus fumigatus	0	24	2.7	1.7	3.3	5	6.12
Aspergillus nidulans	0	26	4	3	10	13	9.33
Aspergillus niger	8	24.5	13.5	12	10.5	14	13.75
Fusarium sp.	0	25	3	0	0	6	5.67
Monilia spp.	3	27	9	10	13.5	12.5	12.50
Penicillium glabrum	0	24	8	2	8	6	8.00
Rhizopus nigricans	0	27	7	5	8	8	9.17
Unidentified spp.	0.5	24	2.8	0	3.3	1.5	5.35
Average Effectiveness	1.44	25.19	6.25	4.21	7.08	8.25	

Legend: n.t. – not tested

Figure 8 shows that among the drugs tested, tioconazole is the only antifungal agent that was effective against the fungal isolates. It has an average effectiveness of 25.19 mm zone diameter. Probably, the other drugs require a longer duration of application to be effective. As reported by Crawford and Hollis (2007), clotrimazole can significantly reduce treatment failure (64%) when administered for four weeks compared to one-week administration. When clotrimazole was compared to ketoconazole, the effect did not show a significant difference. This finding is almost the same as the result of this study. Another factor that may be considered for the observed low effectiveness of the drugs is the development

of resistance that may be caused by under dosage or overuse of a certain drug or failure to complete treatment courses (Schito, 2006). Figure 9 indicates that as observed, the unidentified species and Fusarium sp. had the greatest resistance of 5.35 and 5.67 mm, respectively

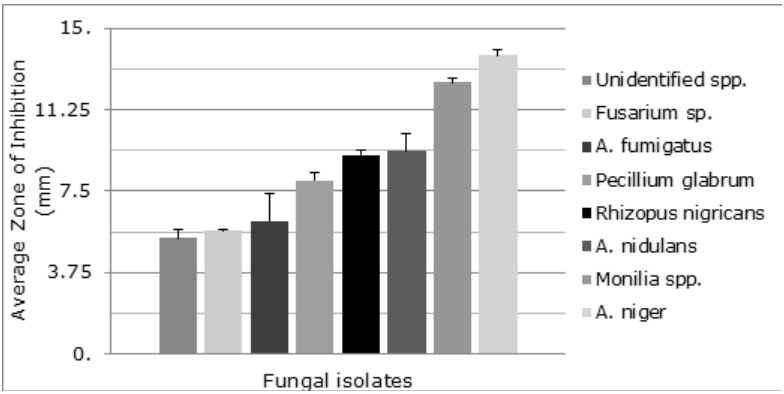

Figure 8. Average Effectiveness of the Anti-fungal Agents against all Isolates (bars indicate standard deviation of four replicates)

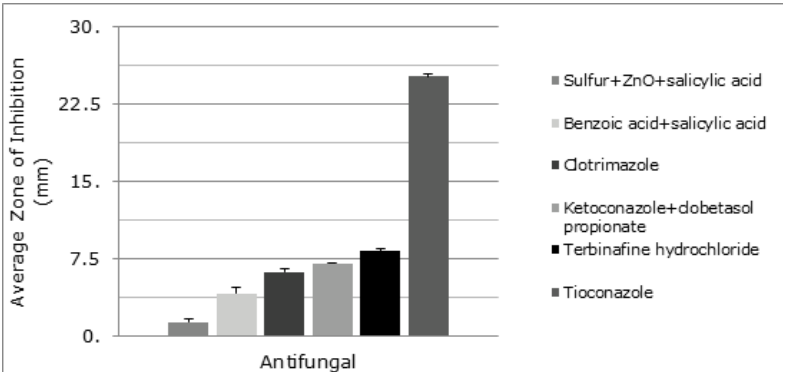

Figure 9. Average resistance of the isolates to anti-fungal agents (bars indicate the standard deviation of four replicates)

Hand Washing and Sun Drying

Isolation of micro-flora from hand washed and sun dried samples revealed a high reduction in colony counts. Table 8 presents that for bacteria, an average of

92.40% reduction was noted. This means that washing as a common and traditional mode of decontamination reduces microbial numbers in clothes and linens. However, ukay-ukay microbes were not 100% eliminated by hand washing. The remaining microbes probably could be resistant to this way of decontamination or simple hand washing, and sun drying may not be sufficient to eradicate the microbes in clothes totally.

Table 8.
Percent Reduction in Total Bacterial Colony Counts per Sample Category

Sample Category	Average Colony Counts		% Reduction
	Pre-laundry	Pre-laundry	
1	16.88	1.92	88.62
2	33.82	3.08	90.89
3	123.19	2.81	97.70
		Average	92.40

The previous study of Walter and Schillinger (1975) showed that Staphylococcus aureus survived a 10-minute laundering at 54°C followed by drying. The same study was also reported that S. aureus was eliminated when washed at 60°C. In this study, washing was done using tap water with an approximate temperature of 13°C. The tendency of survival of some bacteria such as S. aureus is highly possible and sufficient quantities may remain in washed clothes for host colonization.

Table 9 presents that there was a high reduction in fungal colony count in all sample categories (96.86%) following post-laundry. Although washing seems effective in removing fungi, washing process can potentially contaminate other clothing, other surfaces, or the workers because fungal spores are easily getting airborne. It is a well-established knowledge that fungal infections can be acquired by handling contaminated laundry and this can lead to infection outbreak (Sha et al., 1988). The effectiveness of washing and sun drying in eliminating fungi in ukay-ukay clothing should be confirmed by further studies involving large numbers of samples and other parameters.

Table 9.
Percent Reduction in Total Fungal Colony Counts per Sample Category

Sample Category	Average Colony Counts		% Reduction
	Pre-laundry	Pre-laundry	
1	70.96	0.82	98.84
2	40.44	2.5	93.81
3	53.81	0.11	97.93
		Average	96.86

CONCLUSION

This study shows that ukay-ukay clothing harbor pathogenic bacteria and fungi with varying resistance/susceptibility to popular antimicrobial agents. Although these pathogens can be eradicated by washing and sun-drying, recurrence of resistant ones is highly possible. This study also demonstrates that ukay-ukay materials could serve as a vehicle for transmission of pathogenic microbes.

RECOMMENDATION

It is recommended that a similar study with huge sample size should be conducted to establish the prevalence of microbes in ukay-ukay. Microbial identification based on DNA sequences is also recommended. Survival of ukay-ukay micro-flora to desiccation, hot water, and chemical treatments are also worthy of study.

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