ISOLATION OF MICROBIAL CONTAMINATION FOUND IN COUNTERFEIT EYE COSMETIC PRODUCTS

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ABSTRACT

Eye cosmetics such as mascaras, eye shadows and eye liners are liable to microbial contamination prior to poor hygienic conditions either through preparation or distribution. This research aimed to isolate the microorganisms present in counterfeit eye cosmetic products. The isolations of microbial contamination from cosmetic products (n=5) were based on samples collected using convenience sampling method, then diluted with Tween80 and peptone water prior to isolation on growth media. Quantitative analysis was conducted using serial dilution method for total viable count and differential count whilst, qualitative analysis was done by identifying the isolate(s) against Gram staining. Antibiotic sensitivity testing was also conducted. Counterfeit eye cosmetic products of Brand A showed higher bacteriological count (95 CFU/g) compared to Brand B (no growth). Fungal and moulds count of counterfeit products of Brand A (11 CFU/g) was higher than Brand B (1 CFU/g). Microscopic observations showed higher numbers of gram negative bacilli compared to gram positive cocci. Antibiotic sensitivity testing showed high sensitivity towards Gentamicin and Penicillin V with zone of inhibition of 1.4 and 1.5 inches respectively. The higher microbial contamination in counterfeit eye cosmetic products compared to genuine eye cosmetic products suggest that the counterfeit products may have harmful effects on consumers with sensitive skin.

INTRODUCTION

Cosmetic products are principally considered health care items since they are largely in the non-sterile pharmaceutical categories and the products may undergo microbial contamination[1], suggesting lack of process control in pharmaceutical environments, especially involving water systems and raw materials[2,3]. According to Seigert[4], cosmetic products are quite likely to consist of various substrates such as water, lipids, polysaccharides, alcohol, proteins, amino acids, glycosides, peptides and vitamins for the survival and to facilitate the development of a large variety of pathogenic bacteria and fungi. Research done by Noor et al.[5] revealed the presence of an enormous number of bacteria, actinomycetes and fungi within commonly used cosmetics.

Keywords: Counterfeit, Eye, Cosmetics, Microbial, Contamination

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Counterfeiting is defined as creating a similar or lookalike product and labeling it as original and publicized as a true original[6]. Counterfeit products are manufactured legally but are out-of-specifications as a result of inadequate manufacturing or poor storage conditions[7,8]. Generally, consumers are unaware of the serious risks arise from microbiological contamination associated with counterfeit products[9]. Therefore, the main focus of this study is to observe loads of microbiological contamination presence in counterfeit cosmetic products.

MATERIALS & METHOD

Sample selection

The approach of this study was based on the techniques adapted from Tamalli et al.[10]. The samples were selected using convenience sampling methods obtained from different local night markets, supermarkets, pharmacy/personal care stores and cosmetic stores located in Shah Alam, Selangor.

Sample selection was done based on popularity of the brands of locally available eye cosmetic products. A total of four (4) eye cosmetic products of two (2) different brand and talcum were chosen for the study. Each brand requires both genuine and counterfeit eye cosmetic products and one sample represent a control. The samples were obtained from different local night markets, supermarkets, pharmacy/personal care stores and cosmetic stores located in Shah Alam, Selangor and selected using convenience sampling method. Broken seals or unsealed eye cosmetic products such as used cosmetics or testers were excluded in this research. Other exclusion criteria of this research include all eye cosmetic products which have exceeded the expiration dates.

The samples were differentiated into 4 groups according to the brands. Group 1 is classified to genuine eye shadow of Brand A, Group 2 is classified to counterfeit eye shadow of Brand A, Group 3 is classified to genuine eye shadow of Brand B and Group 4 is classified to counterfeit eye shadow of Brand B. Talcum is a basic ingredient of dry, pressed-powdered eye shadows thus, a clinically proven talcum was served as a control for this research.

Isolation of Microorganisms

Sampled products of each eye cosmetics were prepared by dispersing 1g of sample into 8ml of 0.1% peptone water and 1ml of Tween80. A three-fold dilution was made and incubated for 2 hours at 37°C.

Total Microbial Count & Differential Count

Although three-fold dilution was made, only the first aliquot was inoculated on culture media using spread plate method. All culture plates were incubated for 24-48 hours except for Sabouraud Dextrose agar which needs to

be incubated for 48-72 hours at 37°C followed by colony count. Results of total microbial viable count and differential count were expressed as colony forming unit per gram (CFU/g).

RESULTS

Total Microbial Viable Count & Differential Count

The results from isolation of microorganisms showed high number of bacterial count which is 95 CFU/g on Nutrient agar on counterfeit eye cosmetic products compared to genuine eye cosmetic products. Whilst, on Sabouraud Dextrose agar showed high fungal count which is 23 CFU/g on genuine eye cosmetic product compared to counterfeit cosmetic products.

Differential count was done to determine whether certain bacteria present were able to utilize specific nutrients on Blood agar and MacConkey agar. Group 2 showed the highest number of differential count (137 CFU/g) on Blood agar compared to Group 4 (3 CFU/g) on Mannitol Salt agar (Fig 1).

Colony Morphology and Microscopic Observation

Figure 2 shows the total microscopic observation and characterization of bacteria colonies isolated from each group which were done using Gram staining. There were more Gram negative bacteria isolated.

Antimicrobial Sensitivity Testing

Table 1 shows the antibiotic sensitivity testing done on isolates 1 to 7 using Gentamicin, Penicillin V and Optochin whilst, isolates 8 to 10 were done using Nystatin. The antibiotic agents were used against microorganisms to determine susceptibility of microbial species.

Isolates 1 to 7 showed sensitivity towards Gentamicin and Penicillin V especially Isolate 1 with highest sensitivity test measured by zone of inhibition of 1.4 inches and 1.5 inches respectively for Gentamicin and Penicillin V. Optochin showed minimal inhibition and most isolates were resistant towards the antibiotic except Isolate 1 However, for fungi, only Isolate 9 showed sensitivity towards Nystatin with minimal zone of inhibition measured at 0.5 inches which may reflect Isolate 9 is *Candida* spp.

DISCUSSION

Total Microbial Viable Count & Differential Count

High total microbial viable count and differential count do not portray Group 2 is dangerous to consumers since Group 4 only grew on Sabouraud Dextrose agar and Mannitol Salt agar. Mannitol Salt agar is used to specifically to identify *Staphylococcus aureus* and Sabouraud Dextrose agar is for fungi. Hence, low total microbial viable count. and differential count of Group 4 may pose higher risk of the growth of pathogenic bacteria.

Compared to research done by Tamalli et al.[10] on microbial contamination on three different brands of eye shadow, total bacteriological count was less than 1000 CFG/g ranging from 1.0×10^3 to 9.0×10^2 CFU/g and fungal count was 10 CFU/g ranging 1.0 x 10 to 3.0 x 10 CFU/g. The samples were also contaminated with *Staphylococcus epidermidis, Bacillus subtilis, Alternaria* spp. and *Penicillium* spp. The result showed big differences of total microbial viable count as it may reflect the modification of spread plate method to obtain desired results.

Colony Morphology and Microscopic Observation

According to Ochei and Kolhatkar[11], antimicrobial therapy aims to treat infections with antibiotics to which microorganisms are sensitive and which are selective only for the infecting organism harming the host. Besides, antimicrobial drugs differ according to the type and range of bacteria. Broad spectrum antibiotics such as Penicillin V and Gentamicin are effective against both Gram positive bacteria and Gram negative bacteria however, broad spectrum antibiotics may have adverse effects on the normal flora whilst, narrow spectrum antibiotics such as Optochin are effective against only Gram positive bacteria. It is recommended to use narrow spectrum antibiotics against known pathogen to reduce adverse effect on the normal flora.

Penicillin V is a derivative of Penicillin which is broader antibacterial spectrum and can be administered orally. Depending on radicals attached to aminopenicillanic acid, the penicillin can either have highest activity against Gram positive bacteria, spirochetes and some other or relatively resistance to ß-lactamase with low activity against Gram positive bacteria and inactivity against Gram negative bacteria or high activity against both Gram positive and Gram negative bacteria or relatively stable to gastric acid and appropriate for oral administration[12]. This antibiotic is usually used against Gram positive bacteria such as *Staphylococcus* spp. and *Streptococcus* spp. ß-lactamase producing bacteria such as *Staphylococcus aureus, Haemophilus influenza* and *Neisseria gonorrhoeae* are resistant towards Penicillin.

Gentamicin is bactericidal in concentrations of 0.5 to 5 µg/ml for most Gram positive and Gram negative bacteria which includes *Proteus* spp., *Serratia* spp. and *Pseudomonas* spp. however, Gentamicin is ineffective against bacteroids and *Streptococcus* spp. Gentamicin also useful in treating serious infections caused by Gram negative bacteria resistant to other antimicrobial agents[12].

According to Kaijalainen[13], Optochin is usually used and often the only identification test for *Streptococcus pneumoniae* but is more reliable in differentiation of *Streptococcus pneumoniae* and other *Streptococci*. However, in recent years, pneumococcal cells had lost their sensitivity towards Optochin and producing optochin-resistant strain such *Enterococcus faecalis* and the origin of optochin-resistant variants is still unclear. Referring to Cowan and Steels's Manual for the Identification of Medical Bacteria, in appropriate concentration of Optochin on culture media, a small zone

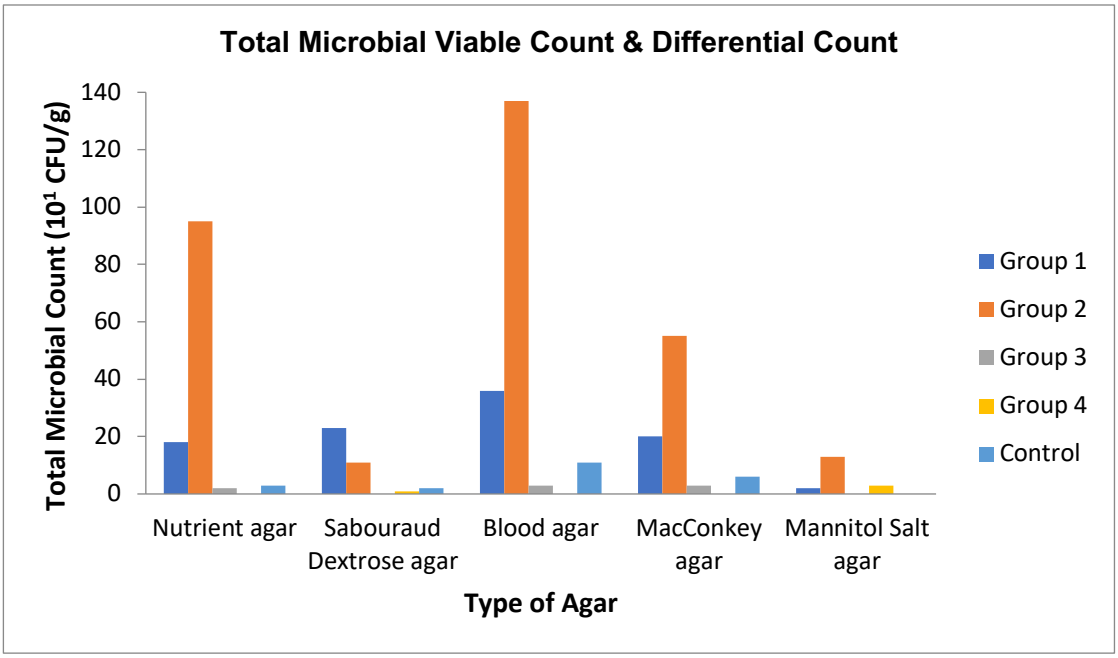

Figure 1: Total Microbial Viable Count and Differential Count of microorganisms of culture media.

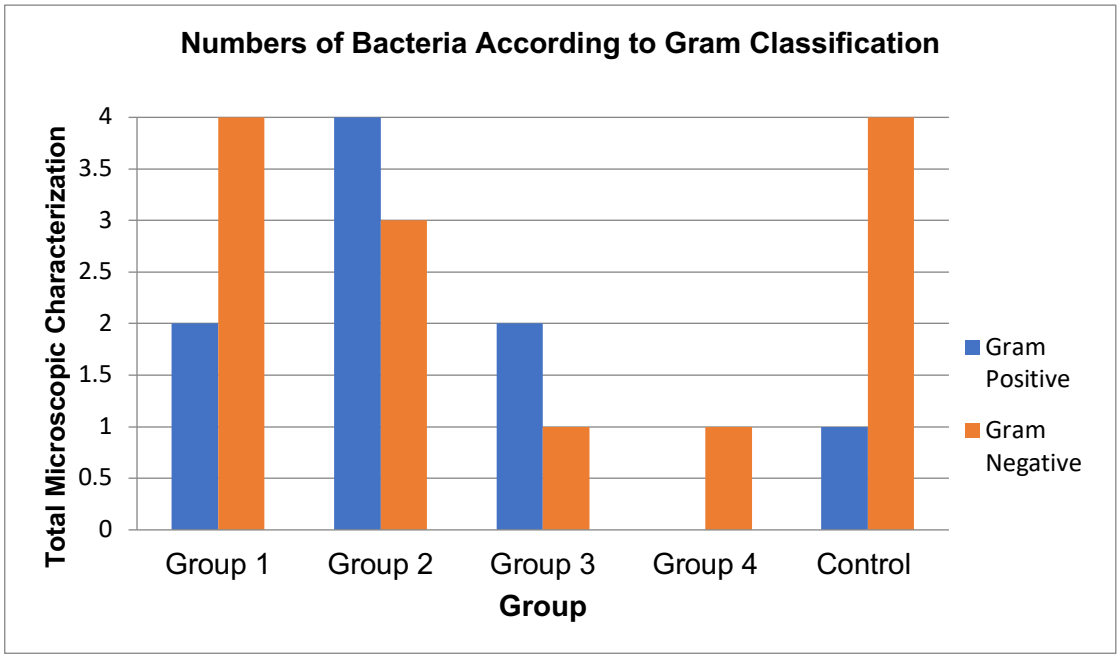

Figure 2: Numbers of Gram positive and Gram negative bacteria observed in each group. These are reported according to Medical Laboratory Science: Theory and Practice (2010).

of inhibition ranging 1-2 mm may occur with few viridans of *Streptococci* but *Streptococcus pneumoniae* showed higher zone of inhibition which extend more than 5 mm. Lastly, Nystatin is an active antifungal against most pathogenic *Candida spp.*, including *Candida albicans,* commonly used to treat candidal infections on the mouth and vagina. It is also used in suppressing subclinical esophageal candidiasis and gastrointestinal overgrowth of candida[12]. This antibiotic works by binding to fungal cell membrane which allows leakage of cellular contents[11]. However, if administered parentally, can causes toxicity.

CONCLUSION

The presence of microbial contamination is higher in counterfeit eye cosmetic products compared to genuine eye cosmetic products suggest that the counterfeit products may be harmful to consumers with sensitive skin and this contamination are possibly due to errors in manufacturing of selected brand and deteriorate storage conditions; indicating that it is necessary to comply with Good Manufacturing Practices (GMP) during production.

Table 1: Antibiotic Sensitivity Testing done on isolates against various antibiotics to measure levels of inhibitory activity of microorganisms.

Isolate	Zone of Inhibition			
	Gentamicin	Penicillin V	Optochin	Nystatin
1	1.4961	1.5748	0.5906	
2	1.0236	0.9843	Resistance	
3	0.9843	0.6299	Resistance	
4		No growth		Not applicable
5	0.8268	0.4724	Resistance	
6	0.8268	Resistance	Resistance	
7	1.2598	1.0629	Resistance	
8	Not applicable			Resistance

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