Screening of Various Pharmaceuticals, Cosmetics (Non-Medicated & Medicated) and Packaged Food Items for Selected Bacterial and Fungal Strains Collected from Different Areas of Khyber Pakhtunkhwa, Pakistan

Saqib Jahan[1], Lateef Ahmad[1*], Abad Khan[1], Waqar Ahmad Kaleem[1], Muhammad Sohail Anwar[1], Abdul Mateen[1], Muhammad Faheem[1], Zia Ur Rahman[2]

[1]Department of Pharmacy, University of Swabi, Khyber Pakhtunkhwa, Pakistan

[2] Department of Health and Biological Sciences, Abasyn University Peshawar, Pakistan

Corresponding author Email: lateef.ahamad@uoswabi.edu.pk

Abstract

The research study aims to investigate and analyze bacterial and fungal strains isolated from non-medicated cosmetics, medicated cosmetics, pharmaceuticals, and food products. Samples from various brands of these products were collected from District Peshawar, District Swabi, District Mardan and District Bannu and subjected to microbiological examination using standard methods. The isolation process involved inoculating the samples onto specific culture media such as nutrient agar, broth, MSA (Mannitol Salt Agar), MacConkey agar, and SDA (Sabouraud Dextrose Agar). The plates were then observed after an incubation period of 3 days, with yeast and molds cultured at 25°C and bacteria at 32.5°C. Morphological characterization of the colonies was done by examining their appearance, while microscopic characterization involved Gram staining and biochemical tests. The study revealed the presence of different bacterial strains, including Bacillus cereus, Pseudomonas spp., Staphylococcus aureus, Staphylococcus epidermidis, and Actinomycetes, as well as fungal strains. These findings highlight the need for strict safety regulations in the manufacturing and handling of these products. Contamination resulting from improper practices can have severe consequences on human health and the overall quality of these products. Moreover, the study suggests the potential of these isolated strains for further exploration. By understanding their characteristics and capabilities, they could be utilized to improve both human health and environmental aspects. This implies that future research should focus on harnessing the beneficial properties of these isolates. In conclusion, the research study emphasizes the importance of ensuring the safety and quality of non-medicated cosmetics, medicated cosmetics, pharmaceuticals, and food products. The identification and characterization of bacterial and fungal strains provide valuable insights for developing and implementing stricter regulations in the industry. Additionally, further

investigations can explore the potential applications of these isolates to enhance health and environmental conditions.

Keywords: Bacterial, fungal, non-medicated cosmetics, medicated cosmetics, pharmaceuticals, and Packaged food products

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INTERODUCTION

The number of microorganisms present in any pharmaceutical or cosmetic product can have a significant impact on its quality during the manufacturing process or during normal use. Microflora introduced from one or more sources, such as unprocessed materials, may be present in the final product [1], the processing equipment, the environment, the water used for the manufacture, the use of important additives like preservatives and the manufacturing personnel [2]. The microbial instability of pharmaceuticals, packaged food items and cosmetic products is a critical performance metric. The instability of microorganisms affects both sterile and nonsterile dosage forms. In pharmaceutical, packaged foods and cosmetic products, a variety of preservatives and combinations of preservatives are used to combat microbial instability. In some instances, it has been reported that microorganisms have developed resistance to these preservatives. [3]. In the second half of the 20th century, the International Pharmaceutical Federation (FIP) established a special committee entrusted with drafting regulations governing drug production in response to reports of infections caused by microbial contamination of drugs. [4]. There are numerous varieties of (pathogenic or non-pathogenic) foodborne microflora that adhere to food contents and surfaces for extended periods of time [5]. These microorganisms serve an essential role in food degradation, toxification, and consumer pathogenicity. This impacts the purity and safety of food consumed by consumers [6]. Among these microorganisms, the enteropathogenic entrobacteriaceae family, i.e. Escherichia coli, Salmonella, and Shigella, are the most dangerous to human health [5, 7]. Foodborne diseases are caused by the ingestion of microbial pathogens, compounds, or biotoxins produced by microorganisms. Cosmetics have become an integral part of daily life and are extensively employed for beauty, sun protection, and the removal of extraneous material [8]. Risk of skin infection to consumers can occur as a result of use of skin products such as powder and cream, eye products like mascara and eyeliner [9], and hairdressing [10]. Some pathogenic microorganisms including Staphylococcus aureus and Pseudomonas aeruginosa are detected in beauty products [11]. In hairdressing and beauty, S. aureus and Staphylococcus epidermidis were the most important bacteria that cause human maladies such as skin infection, boils, bullous impetigo, hair follicles, and scalded-skin syndrome [12]. Bacillus, Staphylococcus, Pseudomonas, Enterobacter, Escherichia coli, and

Klebsiella were the most common bacteria isolated from cosmetic products, according to another study [13]. Skincare products, hair preparations, and facial make-up were responsible for the majority of allergic contact dermatitis in some regions of the Middle East [14]. Herpes causes blisters on the lips and around the mouth from shared make-up tools. Lipsticks and powder brushes that touch these parts of the face can then spread the allergic contact dermatitis infection to other people [15]. The most common bacteria such as P.aeruginosa and Pseudomonas putida presented on eye makeup include mascara and eyeliner. P. aeruginosa can cause irritation, conjunctivitis, pink eye, redness, and watery discharge, which could lead to irreversible blindness [16]. Makeup brushes also have the potential to act as suitable homes for bacteria to thrive. Although the microbial standards of cosmetics have been progressively improved by strict law; their contamination has been frequently reported and even in some cases, has generated serious problems for consumers [17]. Unfortunately, cosmetic contamination awareness and health risks are very poor among the users of all age groups. There is no established law, guidelines, and best practices for many public make-up testers. The main objective of this study was to assess the bacterial and fungal contamination from different Non-medicated cosmetics, medicated cosmetics, pharmaceutical and Packaged food products in different part of Khyber Pakhtunkhwa, Pakistan.

MATERAILS AND METHOD

Samples Processing

To isolate different bacterial species and fungal species different samples of cosmetics, packaged juices/food items and pharmacy products. Samples were inoculated on different media i.e. Nutrient agar and Broth, MSA, MacConkey agar media and SDA media. The dishes were incubated for 24 h at 37°C while SDA plates for fungi identification were incubated at 28°C and observed daily for 7 days. All plates were observed for the growth of microbial colonies after incubation to identify colonial morphology. Gram stains were carried out to determine if bacteria were Gram-positive bacteria, Gram-negative bacteria, and yeast.

Microscopy/Gram Staining

Gram staining Bacterial culture of 18 to 24 hours old, distilled water, Immersion oil, lens cleaner and lens paper, microscope, bibulous paper, Bunsen burner, inoculating loop and clean glass slides were needed to carry out the procedure. The prepared heat fixed smear was gently flooded with crystal violet dye and remain on stand for 1 minute and then washed it using wash bottle of distilled water followed by gently flooded with gram iodine. The smear was allowed for 1 minute on the stand followed by washing with distilled water. The appearance of the smear on the slides was purple circle. For the decolourization of the smear, 95% ethyl alcohol was drop over and over again for 5 to 10

seconds until almost all alcohol becomes clear. For the counter straining of the smear safranin was used and then the smear was kept the stand for 45 seconds and washed with distilled water. The slide was dried. In the 40x and 100x lens of the light microscope the smear was studied.

Biochemical tests

Biochemical tests fall under phenotypic methods for microbial isolation. Protocols for standard biochemical tests were followed and used to identify the bacteria and fungi in respect of the genus and the species level [18]. For bacteria, we have used the following tests: catalase, coagulase, oxidase, Triple sugar iron, H_2S Test, urease test, indole test, and citrate biochemical tests were used to identify. Various Candida species can be detected by a germ test tube and sugar fermentation tests including glucose, maltose, and sucrose, which cause a color change as an indicator.

RESULTS AND DISCUSSION

Biochemical Tests

Various biochemical tests were performed to identify bacterial isolates which included catalase, coagulase, oxidase, Triple sugar iron, H_2S Test, urease test, indole test, and citrate biochemical tests.

Indole test

This test aimed at the differentiation of gram negative bacteria based on the production of hydrolysis of tryptophan. Indole-positive bacteria produced pink to red color rings on the top surface of the media as shown in (Table 1) while no color change was observed for indole-negative bacteria. One bacterium gave indole positive tests while the rest of them gave negative tests.

Simmons Citrate Test

Citrate-positive bacteria changed the color of the media from green to bright blue while citrate negative bacteria did not change the media color. Four isolates were found to utilize citrate as an energy source while the rest of the isolates were unable to do so as shown in Table 1.

Triple sugar iron

The isolated species were cultured in test tubes containing Triple sugar iron followed by incubation at 37 °C for 2-12 hours. When incubated for mentioned period then observed for a yellow and red, color of Butt and Slope, production of H_2S and Gas also in the medium. Four isolates were found to Alk positive while the one of the isolates were acidic in Table 1.

Urease Test

The bacterial isolates were cultured in test tubes containing Christensen urea broth followed by incubation at 37 ˚C for 12 h. when incubated for required time observe for a pink colour in the medium. All the isolates show urease negative as mentioned in Table 1.

Catalase Test

In the catalase test H_2O_2 is used to detect catalase enzyme in bacteria. A bacterial colony was taken and spread on a microscopic slide followed by the mixing of some drops of H_2O_2. Bubble formation was observed due to the presence of catalase enzyme in test organism. All the isolates show catalase postive as mentioned in Table 1.

Oxidase Test

A few drops of reagent were poured on the sterile paper and mixed with colony of test bacterial isolates; smear was formed with help of sterile stick. Deep purple colour is produced when test is positive.Three isolates were show oxidase positive while two isolates show negative as shown in Table 1.

Table 1. Detail Description of Microscopy and Biochemical Test

S. No.	Gram Reaction	Coagulase	Oxidase	H_2S Test	Indole Test	Citrate Test	Urease Test	Catalase Test	TSI Test	Identified Organisms
1.	+ve Rods	- ve	+ ve	-ve	- ve	+ve	- ve	+ve	Alk	Bacillus cerus
2.	-ve Rods	- ve	+ ve	- ve	- ve	+ve	- ve	+ve	Alk	Pseudomonas spp
3.	+ve Cocci	+ve	- ve	- ve	- ve	+ ve	- ve	+ve	Acidic	Staphylococcus aureus
4.	+ve Cocci	-ve	- ve	- ve	- ve	- ve	- ve	+ve	Alk	Staphylococcus epidermitis
5.	+ve Rods	+ ve	+ve	- ve	+ve	+ ve	-ve	+ve	Alk	Actinomycetes

In this study, we revealed the diversity of bacteria and fungi isolated from different Non-medicated cosmetic products, Pharmaceutical products, packaged food products and medicated cosmetic products of various brands. The tested products were taken from famous brands. The names of brands are not mentioned in the study due to commercialization issues.

Isolation of Microbes from Pharmaceutical products

Microbial contamination of pharmaceutical products is a matter of a great importance to the industry and it can become a major cause of both product and economic losses. Results shown in (Table 2) investigated that pharmaceutical products shown contaminated with bacteria (in varying degrees including Gram negative bacteria such (PHM2) have Pseudomonas spp and Gram Positive bacteria such as PHM4, PHM5 and PHM10 were contaminated by Bacillus cerus while other tested products Shown negative results. Singh et al. investigated the fungal contamination of the raw materials of some therapeutic allopathic drugs. They reported the genus Aspergillus as the dominant microorganisms detected in the raw materials [19]. Some of them may produce highly toxic materials, such as aflatoxin B. The microbial contamination of the raw materials of seven kinds of Jamu Gendong. They concluded that, in most of the cases, these plants are heavily contaminated with microorganisms due to improper handling of the raw materials and the inadequate process of production, which severely affect the quality of the final products [20].

Table .2 Show Microbial contaminations of pharmaceutical products

S.No	Naming Code of the Product	Available marketed Dosage form/State	Bacteria/Fungi
1	PHM-1	Suspension	Negative
2	PHM-2	Suspension	Pseudomonas spp
3	PHM-3	Syrup	Negative
4	PHM-4	Syrup	Bacillus cerus
5	PHM-5	Syrup	Bacillus cerus
6	PHM-6	Syrup	Negative
7	PHM-7	Syrup	Negative
8	PHM-8	Syrup	Negative
9	PHM-9	Suspension	Negative
10	PHM-10	Syrup	Bacillus cerus
11	PHM-11	Syrup	Negative
12	PHM-12	Suspension	Negative
13	PHM-13	Suspension	Negative
14	PHM-14	Syrup	Negative
15	PHM-15	Syrup	Negative
16	PHM-16	Suspension	Negative

17	PHM-17	Suspension	Negative
18	PHM-18	Syrup	Negative
19	PHM-19	Syrup	Negative
20	PHM-20	Cream	Negative
21	PHM-21	Parenteral	Negative
22	PHM-22	Cream	Negative
23	PHM-23	Cream	Negative
24	PHM-24	Parenteral	Negative
25	PHM-25	Ointment	Negative

Microbes in Non- Medicated Cosmetics Products

Cosmetics also act as an important vehicle for the transmission of pathogens to humans due to which concern about their use and safety is rising with time. Microorganisms can grow on almost every substances existing in nature and often able to attack or even decompose them. Cosmetic ingredients are rich in nutrients that provide organic substrates in the form of sugar, starch , protein , amino acids , organic acids , alcohols , lipids and etc. for microbial growth. The products may be contaminated when applying by users and especially it may be observed that unwanted microorganisms may contaminate the products at varying rates. In (Table 3) represented that cosmetic Non-Medicated products COS-N13 and COS-N16 were contaminated with Staphylococcus epidermitis and COS-N14 have Staphylococcus aureus while others all cosmetic Non-Medicated products shown negative results. The presence of bacterial contamination in lip glosses and lipsticks that were used by 16 dentists. They revealed contamination in which P. putida, Bacillus spp., Staphylococcus salivarius, Enterococcus faecalis, and S. aureus were effective [21]. A study conducted between 2005 and 2008, they reported that the highest strain isolated from 24 different used cosmetic products was P. aeruginosa (42%). In a study conducted in Iran, it was reported that the highest rate of contamination in 24 used moisturizing creams and hand and face creams belonged to gram-positive bacilli (54%). The second-most common bacterium was revealed to be S. aureus (38%) [21, 22].

Table .3. Microorganism Contamination in Non- Medicated Cosmetics Products

S. No	Naming code of the Product	Available marketed Dosage form/State	Bacteria/Fungi
1	COS-N1	Shampoo	Negative
2	COS-N2	Antibacterial Hand wash	Negative
3	COS-N3	Anti-bacterial Hand wash	Negative

4	COS-N4	Face Mask	Negative
5	COS-N5	Sunblock	Negative
6	COS-N6	Moisturizing Lotion	Negative
7	COS-N7	Cream	Negative
8	COS-N8	Facial Scrub	Negative
9	COS-N9	Face Wash	Negative
10	COS-N10	Cream	Negative
11	COS-N11	Foundation Cream	Negative
12	COS-N12	Serum	Negative
13	COS-N13	Facewash	Staphylococcus epidermitis
14	COS-N14	Hair Cream	Staphylococcus aureus
15	COS-N15	Body Scrub	Negative
16	COS-N16	Cream	Staphylococcus epidermitis
17	COS-N17	Whitening Cream	Negative
18	COS-N18	Jelly	Negative
19	COS-N19	Face Mask	Negative
20	COS-N20	Foam	Negative
21	COS-N21	Skin Care Cream	Negative
22	COS-N22	Hand Wash	Negative
23	COS-N23	Face Wash	Negative
24	COS-N24	Brightening Face Wash	Negative
25	COS-N25	Beauty Cream	Negative

Isolation of Microbes in Food Products

There are a number of methods that can be used to monitor the microbiological safety and quality of foods. According to another literature review [23] each year an estimated 5.5-6.5 million cases of food poisoning are reported in the USA. It is interesting to see the ubiquity of microbes in a home environment and in food samples that we consider safe and devoid of microbes. In this study twenty five food samples were investigated for isolation of bacteria and for determination of HPC. In [Table 4] shows that the food items were contaminated with different types of bacteria and fungi. In food items FJ-13 and FJ-14 were contaminated. Staphylococcus aureus was the most abundant bacteria in the food items while in FJ-5, FJ-6, FJ-10 and FJ-14 were observed for the fungal growth. Others all Food products shown negative results. Hence results were in agreement with other studies, which found that among food samples the highest contamination was found in raw food, followed by cooked food and juices [24]. Another study [24] found that a high incidence of cross-contamination

in 25 domestic kitchens by potential pathogens (Salmonella spp., Campylobacter spp., E. coli and S. aureus) was also detected during the preparation of a chicken lunch.

Table 4. The Microflora in Food products

S. No	Naming code of the Product	Available marketed Dosage form/State	Bacteria/Fungi
1	FJ-1	Fruit Drink	Fungus
2	FJ-2	Fruit Drink	Fungus
3	FJ-3	Cocktail fruit drink	Negative
4	FJ-4	Fruit Drink	Negative
5	FJ-5	Fruit Juice	Fungus growth
6	FJ-6	Grape Nectar	Fungus growth
7	FJ-7	Carbonated Drink	Negative
8	FJ-8	Stimulant Drink/Energy Drink	Negative
9	FJ-9	Sauce	Negative
10	FJ-10	Fruit Juice	Fungus growth
11	FJ-11	Low caloric Drink	Negative
12	FJ-12	Fruit nectar	Negative
13	FJ-13	Coconut Milk	Staphylococcus aureus
14	FJ-14	Fresh Juice	Fungus growth and S. aureus growth
15	FJ-15	Fruit Drink	Negative
16	FJ-16	Ketchup	Negative
17	FJ-17	Sauce	Negative
18	FJ-18	Stimulant Drink	Negative
19	FJ-19	Apple fruit drink	Negative
20	FJ-20	Mango Nectar	Negative
21	FJ-21	Flavored Tea	Negative
22	FJ-22	Fruit Drink	Negative
23	FJ-23	Fruit Drink	Negative
24	FJ-24	Mango Nectar Juice	Negative
25	FJ-25	Apple fruit Drink	Negative

Isolation of Microbes from Medicated Cosmetics Products

They harbor a wide variety of microbes due to contamination from various sources which cause mild to severe allergic reactions leading to complicated life-threatening infections [25]. In this section the microbiological contamination of different medicated cosmetic products was evaluated. In Table 5 represented that MC-10 products contaminated with Actinomycetes while the medicated cosmetics MC-19 was contaminated by Staphylococcus aureus. Other tested products Shown negative results. Similarly, in other study have reported the presence of Staphylococcus in unpreserved cosmetic tools after use [26]. The fungal contaminants of cosmetics consisted largely of Aspergillus fumigatus , Pencillium and Microsporium spp., [27]

Table 5. The bacterial and fungal isolates from Medicated Cosmetics Products

S. No	Naming Code of the Product	Available marketed Dosage form/State	Bacteria/ Fungi
1	MC-1	Shampoo	Negative
2	MC-2	Shampoo	Negative
3	MC-3	Sunblock Cream	Negative
4	MC-4	Cream	Negative
5	MC-5	Antidandruff Shampoo	Negative
6	MC-6	Cream	Negative
7	MC-7	Lotion	Negative
8	MC-8	Cream	Negative
9	MC-9	Cream	negative
10	MC-10	Shampoo	Actinomycetes
11	MC-11	Cream	Negative
12	MC-12	Cream	Negative
13	MC-13	Moisturizer cream	Negative
14	MC-14	Body wash	Negative
15	MC-15	Cream	Negative
16	MC-16	Face wash	Negative
17	MC-17	Conditioner& Shampoo	Negative
18	MC-18	Face wash	Negative
19	MC-19	Cream	S aureus
20	MC-20	Facial Scrub	Negative
21	MC-21	Cream	Negative

22	MC-22	Cream	Negative
23	MC-23	Cream	Negative
24	MC-24	Cream	Negative
25	MC-25	Cream	Negative

CONCULSION

Bacillus cerus, Pseudomonas spp., Staphylococcus aureus, Staphylococcus epidermitis, and Actinomycetes have been isolated from all of these products, in addition to a fungal strain. Some of these microorganisms are pathogenic and can induce a variety of infectious diseases. From these bacteria, however, beneficial bacterial components have been isolated that have the potential to provide maximal benefits to us and the environment. All of these businesses should develop a monitoring system for evaluating product quality and ensuring product safety. For product manufacturing, a sanitary environment is required. Personnel should adhere to hygienic standards. The officer in charge of quality assurance must verify and designate the raw materials used to make a product. The closed system should be favored for product manufacturing. Before releasing the produced products to the market, quality control management must approve them. In addition to adhering to the safety protocols during the use of a product, consumers must also observe these measures. Before purchasing products, they should verify the manufacturing and expiration dates and rely on high-quality brands rather than low-priced, low-quality products. Bacteria and fungi should not be present in these products because, at a certain point, they pose a grave threat to human health. However, the pathogenicity of some strains is still unknown, and there is an urgent need to investigate and verify the metabolic profile of these bacteria and fungi so that they can be utilized for therapeutic purposes.

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