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Microbial Quality Assessment of some Manufactured and Manually Packaged Eye and Nasal Drops

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Abstract

This study aimed to determine the magnitude and pattern of microbial contamination of eye and nasal drops. Method: sixty samples collected from different pharmacies in Khartoum State, Sudan. The samples were divided into three groups; group (A) "eye drops" containing chloramphenicol, group (B) manufactured "nasal drops" containing normal saline and group (C) manually packaged in pharmacies, drops containing normal saline for both eye and nose. Results: Microbial identification methods were performed, bacteria and fungi were isolated (B = 36) isolates and (C = 51) isolates respectively, their percentages were Staphylococcus aureus (25% and 19.608%), Bacillus sp (0% and 3. 922%), Escherichia coli (2.8% and 3. 922%), Proteus sp (11.11% and 9.803%), Citrobacter sp (52.8% and 5.882%), Klebsiella pneumonia (8.3% and 0%) and Pseudomonas aeruginosa (0% and 3.922%) % . The most common isolated fungi were Saccharomyces serivicae (3.33% and 5.490%), Aspergillus nigar (2.8% and 9.803%) %, Aspergillus flavus (5.55% and 5.882%) %, Penicillium sp (5.55% and 3.922%), Rhizubus sp (2.8% and 3.922%), Candida albicans (0% and 1.960%) and Actinomycetes (0% and 1.960%) Group A that contain chloramphenicol was (0%) isolate. Lipopolysaccharides been detected. Conclusion: Group A containing chloramphenicol is free from microorganism while group B and C are contaminated and do not meet the microbiological quality standards.

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Introduction

Different forms pharmaceutical products such as eye drops, and nasal sprays, which use as a medication for nasal cavity. Its uses locally for conditions such as allergic and nasal congestion. Assimilation of substances can be extremely quickly and directly through the nose. A pharmaceutical drug, many of these exist as a nasal sprays for systemic administration. (Rizzo, *et al*, 2007). The contamination of nasal saline-dispensing solution used as a spray with microorganisms that are part of the normal skin and nasal flora and gram-negative organisms of potential enteric origin. These data support the use of nasal saline-dispensing solution as drops, rather than as spray. They also illustrate the risk for cross infection if more than one

patient uses nasal solution. (Itzhak, 2002). Eye drops; liquid drops applied eye surface, in small amounts from single drop to a few drops. Eye drops usually contain saline to match eye salinity. Drops containing only saline and sometimes a lubricant to treat eye dryness and irritation "itching and redness. Eye drops may contain one or more medications to treat eye diseases; eye drops bottles contain preservatives to inhibit contamination after its opening. It is recommend to disposing of bottles not more than three months after opening**.** The dropper tip is more contamination than residual contents, and coagulase-negative *Staphylococcus species*, which are common commensal flora of the ocular conjunctiva and skin, were the most frequently identified organisms (Shee, *et al*, 2021**).**

Differing forms pharmaceutical products are susceptible to microbial contamination (Aulton, 2002). Presence of microorganisms in this products may affect consumers, when these contaminated preparations used it will be a hazardous to users health. There will be drug-borne infections to human (Coker, 2005). Microbial flora in preparations which are not sterile influenced by the nature of the ingredients "whether natural or synthetic", quality of the vehicle and the attitude and care of personnel involved in their handling (Parker, 2000). Presence of microorganisms whether pathogenic or non-pathogenic in these products Keep consumers to lose Their faith in that manufacturers, stability changes of products due to contaminants activities may cause considerable financial defeat of company (Mugoyela and Mwambete, 2010). Plants of Pharmaceutical are mainly divide into two categories: Sterile; the final products are such as eye drops, and non-sterile; the final product contain microorganisms within defined limit such as tablets and capsules. Final product require special construction limits, particle size of air, numbers of microorganisms in air and water. Environment of pharmaceutical plants must controlled with specific limits depending on the product it produces, (Magdy, 2014)**.** Non-sterile products, which contain a high degree of water content, may contaminant with microorganisms. These microorganisms cause product spoilage and loss of its therapeutic properties, if microorganisms are pathogenic, A serious infections can be arise (Kamil and Lupuliasa, 2011); (Denyer and Baird, 1990). Ophthalmic solutions found contaminated with pathogenic bacteria, cause serious ocular infections such as endophthalmitis and keratitis (Asegedech, *et al,* 2017). Microorganisms include *Aspergillus sp* and *Penicillium sp*,Study carried by (Obuekwe and Eichie, 2006)**,** involved structured selection of representative eye and nose drops, found that a majority of microbial contaminations in non-sterile products are; *Aspergillus sp*, *Bacillus sp*, *Candida albican*s and *Klebsiella spp*. Pharmaceuticals contamination with microorganisms can bring a changes in their physical characteristics, including breaking of emulsions, thinning of creams, fermentation of syrups, appearance of turbidity or deposit, and changes in odor and color, (Gad, *et al.* 2011). Microbial flora found in non-sterile preparations, influenced by the nature of the ingredients. Quality of vehicle, care and attitude of personnel; involved in their handling require a stringent limits for microorganisms, drugs used for an immune compromised patients, a smaller numbers of opportunistic pathogens become infectious agents, either by severe underlying disease or by using of immunosuppressive drugs (Mugoyela and Mwambete 2010). Contaminated eye drops or other ophthalmic solutions; potential cause the infection of ocular. They can be associated with keratitis and corneal ulcers, carry the risk of transmitting opportunistic microorganisms, as well as pathogenic, such as *Pseudomonas aeruginosa* and *Serratia marcescens*. Contamination rate of ophthalmic solutions varies widely, bacterial contamination of eye drops may alter the pH of the solution, for that reduce the efficiency of the drug (Nentwich, *et al.* 2007), (Raghad , *et al.* 2011). Isolated bacteria from eye drops, most of it identified belonged to the commensal normal flora. Contaminants were *Staphylococcus aureus*, *Micrococcus*, *Neisseria catarrhalis*, Gram-negative rods, *Candida albicans, and Staphylococcus epidermidis* responsible for ocular morbidity and blindness. In

tropical countries. Keratitis is the most frequently encountered fungal infections, although the orbit, lids, lacrimal apparatus, sclera, conjunctiva and intra-ocular structures may also be involved, microbes may infect the cornea, orbit and other ocular structures. Microbial keratitis and endophthalmitis are two well-known eye diseases, this caused by the implanting of microorganisms into cornea or other parts of the eye from the environment, or by the user. Causative agents include; *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Proteus species, Klebsiella species, Candida albicans, Nocardia species, Fusarium species, Aspergillus species*, and dematiaceous fungi. Lipopolysaccharide an endotoxin that constitutes the outer leaflet of outer membrane of most gram-negative bacteria. Lipopolysaccharide is comprised of hydrophilic polysaccharide and hydrophobic component known as lipid A which is responsible for the major bioactivity of endotoxin (wang and Quinn, 2010). High rate of contamination of eye drops in this setup. Gram-negative organisms more often contaminate the tip of eye drop rather than the drop. Eye drop contaminants were sensitive for chloramphenicol, gentamicin, norfloxacin, and ciprofloxacin. Multiple drug resistance was seen among Klebsiella spp and Pseudomonas spp (Lemlem, *e al*, 2019)

Materials and Methods:

This study aimed to detect the microbial contamination in eye and nose drops, sixty samples collected and examined, collected randomly from different pharmacies in Khartoum State, Sudan, sealed properly and packaged. Samples had been divided into three group ‘‘A, B and C’’ each group contain 20 samples, group A contain ‘chloramphenicol eye’ drops, group B ‘manufactured normal saline’ nose drops and group C ‘normal saline prepared manually’ in pharmacies used in both eye and nose. Collected samples cultured on solid media Nutrient agar, Blood agar, CLED, MacConkey agar, Mannitol Salt agar, Eosin Methylene Blue agar and DNase, media streaking using by sterile loop. Semi solid media “Kligler Iron Agar” cultured by stabbing sterile straight wire loop. Broth media “Peptone water, MR – VP and Citrate” media cultured by using sterile loop. Results of cultural characteristics, primary and secondary tests for bacterial identification were obtained and recorded; culture on mycological media; Sabouraud Dextrose agar and potato dextrose agar, cultures media were incubated at 37°C for 24 to 48 hrs, SDA incubated aerobically at 25°C - 27°C for a week. After incubation, microscopic examination was perforemed to all specimens, by using loop a part of culture taken from the colonies and examined under microscope, bacterial and fungal colonies. Fungal isolates were identified based on colony morphology on SDA and PDA and Candida, microscopic morphology on slide cultures, and differential tests (Zarei , *et al*, 2000) and (Zarei, 2003), Bacterial isolates identified by using standard microbial identification techniques (Larsen, 2000).

Detection of Lipopolysaccharide:

Bacterial Gram negative cells (*Pseudomonas aeruginosa, Klebsiella pneumonia, Citrobacter and E.coli.*) were grown to late log phase, one ml of suspension was centrifuged for a minute at 13200 rpm**,** resuspended in 100 µL of lysis buffer and incubated at 100°C for 10 min , 60 µg of proteinase K was added , incubated at 60°C for 1 h added 20 µL of sample buffer, it was been boiled for another 5 min then loaded 2.5 µL of the LPS preparation onto a standard SDS - acrylamide mingled containing 15% acrylamide Electrophorese in 1X Laemmli running buffer at 20 mA until the bromophenol blue had migrated 10 cm. The gel was placed in 200 mL fixation buffer overnightand the fixation buffer by 200 mL oxidation buffer, shaked at 40 rpm for 5 minutes then washed three times in 500–1000 mL ddH_2O on a shaker at 40 rpm for 15 minutes staining was done by mixture containing 500ml methanol ,500 distilled water and 100 glacial acetic acid then washed by distilled water (Campbell *et al*. 2003).

Results:

Contamination detected for 60 samples, some species of bacteria and fungi identified isolated microorganisms in both

Groups (B and C). Group A that contain chloramphenicol gave (0%) isolates. Isolated bacteria and fungi (B:36) isolates and (C:51) isolates respectively, their percentages were *Staphylococcus aureus* (25% and 19.608%), *Bacillus* sp (0% and 3. 922%), *Escherichia coli* (2.8% and 3. 922%), *Proteus sp* (11.11% and 9.803%), *Citrobacter sp* (52.8% and 5.882%), *Klebsiella pneumonia* (8.3% and 0%) and *Pseudomonas aeruginosa* (0% and 3.922%) % . The most common isolated fungi were *Saccharomyces serivicae* (3.33% and 5.490%), *Aspergillus nigar* (2.8% and 9.803%) %, *Aspergillus flavus* (5.55% and 5.882%) %, *Penicillium sp* (5.55% and 3.922%), *Rhizubus sp* (2.8% and 3.922%), *Candida albicans* (0% and 1.960%) *and Actinomycetes* (0% and 1.960%). Lipopolysaccharides been detected. Frequencies and percentages of isolated microorganisms obtained, as explained in tables (1), LPS detected, as shown in figure (1)

Table (1): Frequency and percentages of isolated bacteria and fungi in both B and C Groups

Name of Microorganism	Group B	Group C
Staphylococcus aureus	9 (25 %)	10 (19.608 %)
Bacillus sp	0 (0 %)	2 (3. 922 %)
Escherichia.coli	1 (2.8 %)	2 (3. 922 %)
Proteus sp	4 (11.11 %)	5 (9. 803 %)
Citrobacter sp	1 (2.8 %)	3 (5. 882 %)
Klebsiella pneumonia	3 (8.3 %)	0 (0 %)
Pseudomonas aureginosa	0 (0 %)	2 (3. 922 %)
Saccharomyces serivicae	12 (33.3 %)	13 2 (5.490%)
Aspergillus nigar	1 (2.8 %)	5 (9. 803 %)
Aspergillus flavus	2 (5.55 %)	3 (5. 882 %)
Penicillium sp	2 (5.55 %)	2 (3. 922 %)
Rhizubus sp	1(2.8 %)	2 (3. 922 %)
Candida alibicas	0 (0 %)	1 (1. 960 %)
Actinomycetes	0 (0 %)	1 (1. 960 %)
Total	**36 (100 %)**	**51 (100 %)**

Figure (1): Lipopolysaccharide band (g) - ve bacteria showed LPSappearances from left to right; *Pseudomonas aeruginosa, Klebsiella pneumonia, Citrobacter sp and E.coli.*

Discussion:

The isolation of microorganisms or contamination in group A (eye drops) were not detected due to presence of chloramphenicol, while microorganisms were isolated in both Groups B; manufactured "nasal drops" and group C manually packaged in pharmacies at different percentages These results of bacteria and fungi were found by (Kamil and Lupuliasa, 2011), (Denyer and Baird, 1990), (Nentwich, et al. 2007) and (Raghad, et al. 2011), isolation of *Staphylococcus aureus* (Asegedech, et al, 2017), and Gram negative Rods, *Candida albicans*, out result with them in isolation of *Micrococcus*, *Neisseria catarrhalis*, *Staphylococcus* epidermidis Also agreed with (Mehrgan et a.. 2006), who found *Bacillus* sp ,and *Staphylococcus* sp, and (wang and Quinn, 2010) who found that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* sp, *Klebsiella* species, *Aspergillus* species, *Candida albicans*, They disagreed in isolation of *Streptococcus pyogenes*, *Nocardia* species, *Fusarium* species. Diana Carrasco, et al 2020 found that; the most frequently isolated bacterial genus was *Bacillus*, followed by *Escherichia coli*, *Klebsiella* and *Enterobacter*, *Salmonella* and *Staphylococcus aureus* not found in any product, but potentially pathogenic microorganisms such as *Pseudomonas* were isolated in 40.0% of the eye drops. *Enterobacter* and *Escherichia coli* showed resistance to multiple compounds and *Pseudomonas* was not resistant to any antibiotic. Lipopolysaccharides been detected and that was due to presence of gram-negative bacteria in contaminated samples.

Conclusion:

This study concluded that eye and nasal drops manufactured in Sudan were highly contaminated with several types of bacteria and fungi, for the reasons these drops were not meet any standard limitation, and are completely unsuitable for human use, This indicated that there were not good manufacturing or practices, for that manufacturing standard must be improved in the future.

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