Microbiological safety of commonly available eye ointments and their *in-vitro* anti-bacterial potency

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Current study attempted to check out the microbiological quality of some common sterile ointment commonly implemented for the treatment of eye infections in Bangladesh. Seventeen (17) different eye ointment (T-Mycin, Aprocin, Bactin, Optimox and Cloram, Hypomer gel, Sonexa, Polytracin, Cero, Aristobet, Lotepred, Herpigel, Gentob, Xoviral, Zirgan, Xovir, Tomycin, Tobirax, AFm-plus and Parafresh) were microbiologically examined through common and traditional cultural methods. Total viable bacterial and fungal contamination was found up to $10^6$ and $10^5$ cfu/ml respectively. Among the 17 samples T-Mycin, Aprocin, Sonexa, Polytracin and Tomycin were free from fungal contamination. All the samples significantly exceeded United States Pharmacopeia (USP) or British Pharmacopeia (BP) limit ($<10^2$ cfu/ml) in case of Total viable bacteria and fungus contamination. While the coliforms (*Escherichia coli* and *Klebsiella* spp.) were absent in all samples, the prevalence of *Staphylococcus* spp. was 100% in all samples up to $10^3$ while the *Bacillus* spp. was found up to $10^2$ cfu/ml. *Pseudomonas* spp. was cultivated in T-Mycin, Aprocin Hypomer gel, Sonexa, Polytracin, Cero, Aristobet and Lotepred up to 10³ cfu/ml. All the 5 drugs showed their antibacterial potency with satisfactory range of zone diameter against *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp. and *Bacillus* spp. In case of all 17 ointments the average zone diameter range was noticed within 13mm-22mm. The highest zone diameter (22mm) was recorded against *E. coli* produced by Aprocin and minimum zone diameter (13mm) was recorded against *Bacillus* spp. produced by Bactin, Optimox and Cloram. Gentamicin and Streptomycin were used as positive control against the tested bacteria.

Introduction

The extensive existence of microbial agent in the pharmaceutical products may hinder to prove its antimicrobial potency¹⁻³. Due to several inconsistency in the good manufacturing practice (GMP), poor quality of raw materials and manufacturing water, lack of microbiological monitoring of the equipments and unhygienic environment, packaging defect and inappropriate storage condition are the major causes of microbial spoilage in the pharmaceutical products⁴⁻⁷. Some common microflora like *Clostridium tetani*, *Pseudomonas aeruginosa*, fungi and viruses may generate the spoilage of the final products²,⁸⁻¹³.

According to the USP and BP guidelines the presence of contaminating total viable bacteria exceeding the acceptable limit of $<102$ cfu/g especially in sterile drugs such as eye drops and ointment brings a major threat for consumer¹⁴⁻²⁴. One of the studies reflected the presence of microbial contamination in the finished products as a result of the market objection¹²,¹⁹,²⁵,²⁶. As described in early study, several diseases have been noticed in Bangladeshi community due to the microbiological spoilage in different pharmaceutical drugs²¹,²⁷,²⁸⁻³². Huge bacterial and fungal contamination was observed in sterile liquid drugs those were commonly used to treat eye and ear infection³³. Furthermore, the increasing rate of drug resistant bacteria as well as the reduction of drug potency may recommend the necessity to sort out the antibacterial traits of the pharmaceutical drug³⁴⁻³⁸. However, the quality control and quality assurance department should take necessary action to eradicate the growth of objectionable microbes and execute the proper microbiological monitoring system following by GMP and HACCP guidelines. Considering all the things, the present study (1) assessed the bacterial and fungal load of the topical products commonly used to eradicate eye related complications along with their antibacterial activity.

Materials and Methods

Sample Collection, Processing, and Microbiological Analysis

Seventeen (17) different eye ointment samples (T-Mycin, Aprocin, Bactin, Optimox, Cloram, Hypomer gel, Sonexa, Polytracin, Cero, Aristobet, Lotepred, Herpigel, Gentob, Xoviral, Zirgan, Xovir, Tomycin, Tobirax, AFm-plus and Parafresh) labeled with manufacturing and expiry dates were collected from different retailer drug stores located within the city of Dhaka and were subjected to microbiological examinations; i.e., the total viable bacteria and fungi were quantified and the presence of specific pathogens was detected as well³⁹,⁴⁰. Briefly, 10 ml of

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samples were homogenized with 90 ml of buffer peptone water (BPW) and serial dilutions were prepared up to 10-4. An aliquot of 0.1 ml of each suspension from the 10-2 was spread onto Nutrient agar (NA) to enumerate the total bacteria (TVB) and on Sabouraud dextrose agar (SDA) plate for the estimation of fungal load. The NA and SDA plates were incubated at 37°C for 24 hours and at 25°C for 24 to 48 hours, respectively.

Detection of Specific Pathogenic Bacteria

An aliquot of 0.1 ml from the 10-2 dilution of each sample was spread onto Mac Conkey agar, Mannitol salt agar (MSA), Pseudomonas agar, and Mannitol egg yolk polymyxin (MYP) agar base media for the enumeration of Escherichia coli, Klebsiella spp. Staphylococcus spp., Pseudomonas spp., and Bacillus spp. consecutively. All the plates were incubated at 37°C for 24 hours except MFC agar which was incubated at 45°C for 18-24 hours. Confirmative identification of the specific pathogens was accomplished through the biochemical tests.

Determination of antibacterial activity of eye ointment against the laboratory stock culture

To examine the drug efficacy of T-Mycin, Aprocin, Bactin, Optimox, Cloram, Hypomer gel, Sonexa, Polytacin, Cero, Aristobet, Lotepred, Herpigel, Gentob, Xoviral, Zirgan, Xovir, Tomycin, Tobirax, AFm-plus and Parafresh against different tested bacteria isolated from the different sources: this study was introduced agar well diffusion methods on Muller Hington Agar. According to the suggested method by Clinical and Standarded Laboratory Institute; a loopful culture of the tested bacteria (Escherichia coli, Klebsiella spp., Pseudomonas spp., Staphylococcus spp. and Bacillus spp.) was inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England) and the bacterial lawn was prepared onto the surface of the MHA media. Then wells (8 mm) were made on the inoculated MHA media and 100/4L antibiotic solution was added in to the wells along with a positive control antibiotic disc (Gentamicin, 10 μg) and a negative control (normal saline). After incubation at 37°C for 24 hours the presence of clear zone around the sample solution (if any) was analytical for the existence of the antibacterial activity of the samples tested.

Results & Discussions

In Bangladesh Pharmaceutical sectors has huge contribution to earn foreign revenue. One of our previous research group discussed the effective in-process microbiological quality control may reduce the proliferation of microbial agent during manufacturing, packaging, distribution and storage as well as examine the quality measurement of both raw materials and finished product.

Prevalence of Microorganisms in Eye Ointments

In this study all the eye ointment revealed a huge aerobic bacterial and fungal prevalence up to 10^6 and 10^5 cfu/ml respectively (Table 1). The total viable bacterial and fungal load was found to be exceeded the USP limit (<10^2 cfu/g) in all the samples. Among the 17 samples only 5 samples were found free from fungal contamination. In case of T-Mycin, the propagation of Pseudomonas spp., Staphylococcus spp. and Bacillus spp. were quantified 2.3×10^2, 2.8×10^3 and 2.3×10^2 cfu/ml consecutively. Pseudomonas spp., Staphylococcus spp. and Bacillus spp. were found up to 10^3cfu/ml for Aprocin while only Staphylococcus spp was found up to 10^2 cfu/ml in Bactin, Optimox and Cloram. In Hypomer gel, Sonexa, Polytacin, Cero, Aristobet and Lotepred the load of Pseudomonas spp., Staphylococcus spp. and Bacillus spp. were observed up tp 10^4 cfu/ml. Staphylococcus spp. and Bacillus spp were found in Herpigel, Gentob, Xoviral up to 10^3 cfu/ml. Only Staphylococcus spp. was cultivated in Zirgan, Xovir, Tomycin, Tobirax, AFm-plus and Parafresh up to 10^3 cfu/ml. E. coli and Klebsiella spp. were totally absent in all the samples (Table 1). However, the bio-burden was assessed out of the acceptable limits recommended by USP or BP. Thus 80% of the samples studied were found to be microbiologically uncontrolled in case total viable and fungus contamination while the coliform was absent in every case. Among the specific pathogenic bacteria, the presence of Staphylococcus spp. was prominent (Table 1).

Detection of antibacterial activity of EyeOintments

The potency of 17 eye ointment against different tested bacteria (Escherichia coli, Klebsiella spp., Pseudomonas spp., Staphylococcus spp and Bacillus spp) was determined by observing the zone diameter through agar well diffusion method. Most of drugs showed their antibacterial potency with satisfactory range of zone diameter against the tested bacteria. In case of T-Mycin the zone was recorded 20mm, 17mm 18mm, 15mm and 13mm against E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp and Bacillus spp consecutively. Aprocin showed 22mm, 18mm, 15mm, 17mm and 15mm of zone diameter for E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp and Bacillus spp consecutively. Bactin showed 18mm, 15mm, 15mm, 17mm, 13mm of zone diameter for E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp and Bacillus spp consecutively. Optimox showed 18mm, 17mm, 18mm, 16 mm and 13mm of zone diameter against E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp and Bacillus spp consecutively. Polytracin showed 17mm, 17mm, 18mm and 17mm against E. coli and Klebsiella spp consecutively. Hypomer gel showed its potency 20mm for E. coli and Klebsiella spp. (Table 2). Sonexa exhibited zone diameter 20mm and 19mm for E. coli and Klebsiella spp. while Polytacin showed 17mm, 17mm, 18mm and 17mm against E. coli, Pseudomonas spp., Staphylococcus spp., Klebsiella spp and Bacillus spp. Cero showed antibacterial potency 18mm, 17mm, 17mm and 15mm against E. coli, Pseudomonas spp., Klebsiella spp and Bacillus spp. 18mm, 17mm, 17mm and 15mm zone were produced by
### Table 1: Prevalence of pathogenic microorganisms in eye ointment

| Name of Samples | TVB         | Fungi | E. coli | Pseudomonas spp. | Staphylococcus aureus spp. | Klebsiella spp. | Bacillus spp. |
|-----------------|-------------|-------|---------|------------------|----------------------------|-----------------|--------------|
| T-Mycin (n=5)   | 2.0×10^6    | 0     | 0       | 2.3×10^6         | 2.8×10^3                  | 0               | 2.3×10^2     |
| Aprocin (n=5)   | 4.0×10^5    | 0     | 0       | 1.9×10^3         | 4.3×10^2                  | 0               | 1.9×10^2     |
| Bactin (n=5)    | 7.5×10^6    | 7.5×10^5 | 0     | 7.0×10^2         | 0                          | 0               |              |
| Optimox (n=5)   | 2.5×10^5    | 2.5×10^4 | 0     | 2.9×10^2         | 0                          | 0               |              |
| Cloram (n=5)    | 4.5×10^5    | 4.5×10^4 | 0     | 4.7×10^2         | 0                          | 0               |              |
| Hypomer gel     | 5.3×10^3    | 2.8×10^4 | 0     | 1.8×10^4         | 2.8×10^3                  | 0               | 1.1×10^3     |
| Sonexa          | 1.0×10^3    | 0     | 0       | 4.5×10^3         | 4.3×10^3                  | 0               | 4.7×10^4     |
| Polyclacin      | 1.6×10^4    | 0     | 0       | 3.7×10^4         | 7.0×10^5                  | 0               | 5.7×10^3     |
| Cero            | 3.3×10^2    | 1.1×10^3 | 0     | 2.0×10^3         | 2.9×10^3                  | 0               | 2.0×10^3     |
| Aristobet       | 2.2×10^3    | 4.7×10^4 | 0     | 4.5×10^3         | 4.7×10^3                  | 0               | 4.4×10^3     |
| Lotepred        | 1.0×10^3    | 5.7×10^3 | 0     | 6.7×10^4         | 3.5×10^3                  | 0               | 6.0×10^3     |
| Herpigel        | 2.5×10^4    | 2.0×10^3 | 0     | 0               | 2.8×10^3                  | 0               | 2.7×10^3     |
| Gentob          | 4.5×10^5    | 4.4×10^3 | 0     | 0               | 1.5×10^3                  | 0               | 1.1×10^3     |
| Xoviral         | 3.5×10^5    | 6.0×10^3 | 0     | 0               | 4.0×10^3                  | 0               | 4.7×10^4     |
| Zirgan          | 2.0×10^4    | 2.7×10^3 | 0     | 0               | 2.8×10^3                  | 0               | 0            |
| Xovir           | 1.5×10^5    | 2.0×10^4 | 0     | 0               | 2.8×10^3                  | 0               | 0            |
| Tomycin         | 4.5×10^4    | 0     | 0       | 0               | 4.3×10^3                  | 0               | 0            |
| Tobirax         | 2.0×10^5    | 2.5×10^4 | 0     | 0               | 7.0×10^3                  | 0               | 0            |
| AFm-plus        | 2.8×10^4    | 1.7×10^3 | 0     | 0               | 2.9×10^3                  | 0               | 0            |
| Parafresh       | 4.0×10^5    | 3.7×10^4 | 0     | 0               | 4.7×10^3                  | 0               | 0            |

All the experiments were performed in triplicates and the results were reproducible.

### Table 2: Detection of antibacterial activity of eye ointment against laboratory strain.

| Name of Samples | E. coli | Pseudomonas spp. | Staphylococcus aureus | Klebsiella spp. | Bacillus spp. |
|-----------------|---------|------------------|-----------------------|----------------|--------------|
| T-Mycin (n=5)   | 20      | 17               | 18                    | 15             | 13           |
| Aprocin (n=5)   | 22      | 18               | 15                    | 17             | 15           |
| Bactin (n=5)    | 18      | 15               | 15                    | 17             | 13           |
| Optimox (n=5)   | 18      | 17               | 18                    | 16             | 13           |
| Cloram (n=5)    | 20      | 17               | 18                    | 15             | 13           |
| Hypomer gel     | 20      | 0                | 0                     | 20             | 0            |
| Sonexa          | 20      | 0                | 0                     | 19             | 0            |
| Polyclacin      | 17      | 17               | 0                     | 18             | 17           |
| Cero            | 18      | 17               | 0                     | 17             | 15           |
| Aristobet       | 18      | 18               | 0                     | 17             | 15           |
| Lotepred        | 20      | 17               | 0                     | 16             | 18           |
| Herpigel        | 18      | 15               | 0                     | 17             | 18           |
| Gentob          | 15      | 0                | 0                     | 16             | 17           |
| Xoviral         | 17      | 0                | 17                    | 17             | 17           |
| Zirgan,         | 17      | 0                | 17                    | 18             | 15           |
| Xovir           | 15      | 0                | 18                    | 19             | 14           |
| Tomycin         | 15      | 15               | 18                    | 18             | 14           |
| Tobirax         | 18      | 17               | 17                    | 18             | 17           |
| AFm-plus        | 18      | 17               | 20                    | 18             | 16           |
| Parafresh       | 17      | 18               | 20                    | 17             | 15           |

Positive control

### USP Limit:
- Total viable bacteria <10^2 cfu/g
- Total fungal load <10^1 cfu/g.

All the experiments were performed in triplicates and the results were reproducible.
Aristobet against *E. coli*, *Pseudomonas* spp., *Klebsiella* spp and *Bacillus* spp. Lotepred showed zone diameter 20mm, 17mm, 16mm and 18mm for *E. coli*, *Pseudomonas* spp., *Klebsiella* spp and *Bacillus* spp. Herpigel showed zone diameter 18mm, 15mm, 17mm and 18mm for *E. coli*, *Pseudomonas* spp., *Klebsiella* spp and *Bacillus* spp. Gentob showed antibacterial potency 15mm, 16mm and 17mm for *E. coli*, *Klebsiella* spp and *Bacillus* spp. The zone diameter 17mm was produced by Xoviral against *Pseudomonas* spp., 18mm, 19mm, 14mm zone were produced by Xovir for *E. coli*, *Staphylococcus* spp., *Klebsiella* spp., *Bacillus* spp. Tomycin showed 15mm, 15mm, 18mm, 18mm and 14mm for *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp and *Bacillus* spp. AFm-plus exhibited 18mm, 17mm, 20mm, 18mm and 16mm for *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp and *Bacillus* spp. Parafresh showed antibacterial potency 17mm, 18mm, 20mm, 17mm and 15mm for *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp and *Bacillus* spp. (table 1).

**Conclusion**

To ensure the consumers health safety as well as increase the product quality of different pharmaceuticals it has no alternative to eradicate the growth of undesirable microbial agent. Day by day disease medication is going to more difficult due to the propagation of spoilage micro-flora more than the marginal limit. During the production period every Good hygiene practices, proper handling, clean environment are necessary for avoiding microbial contamination and maintenance of drug quality. To ensure the patient safety as well as to maintain the public health harmony, a customary microbiological examination of sterile drugs is suggested, especially in the developing countries, where the ease of microbial contamination is usual.

**Competing interests**

The authors declare that they have no competing interests.

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