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Journal of Bioscience and Applied Research
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Evaluation of the sterility condition of commonly used eye drops present in the Egyptian pharmacies

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Abstract

Fourty samples of commonly used eye drops were collected from The Egyptian Pharmacies and divided into 2 groups: group A (with antimicrobial activity) and group B (without antimicrobial activity), the two groups cultured on blood agar medium and Sabouraud agar medium two times after opening these samples in open air, 9 samples showed microbial growth. The number of colonies counted and the organisms isolated and identified as (*Staphylococcus auereus*, *Micrococcus*, *Candida albicans*, and coagulase negative *Staphylococcus*). After that group (A) samples were taken and inoculated with saline containing *Candida albicans* and *Staphylococcus auereus* then they cultured on the same two media, after the period of incubation *Candida* show growth in all samples while *Staphylococcus auereus* show positive growth in four samples.

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Key words: Eye drops, Microbial contamination, Preservatives, Ophthalmic solutions, Antibiotics, Cultures and Risk of infection.

1. Introduction

Eye vision plays a very important part of our lives. It provides about 75% of all sensory information transmitted to the brain. Thus, any factor that might affect vision quality such as environmental, biological or traumatic issues must be given immediate attention.One of these factors is the microbial contamination as many of the viruses, bacteria and fungi that can invade the human body are also capable of attacking the surface or interior of the eye and cause many microbial diseases such as conjunctivitis or keratitis (Templeton et al., 1982). Also Teuchner et al (2015) found that the contaminants varied between pathogenic and normal commensal flora of the skin. Microbial contamination may be transmitted by contaminated eye drops and ophthalmic solutions .Lalitha et al (2014) reported postoperative endophathalamitis from contaminated anaesthetic eye drops also may be transmitted by direct contact eye injuries such as those caused by accidental encounters with a stray finger, thumb or instrument. Eye drops usually contain saline as a base ingredient. Depending on their intended use, they may also contain lubricating, tear-replacing (artificial tears) or anti-redness substances as well as medications.

Some Eye drop preparations are only lubricating and contain tear-replacing solutions; these may be a source of eye contamination (Tasli & Cosar, 2001).Contaminated eye drops and ophthalmic solutions are a potential cause of ocular infection (Geyer et al.,1995 and Rahman etal,2006) also responsible for corneal ulcers and carry the risk of transmitting opportunistic as well as pathogenic organisms e.g. Pseudomonas Aeruginosa (Tasli & Cosar, 2001).

Bacterial contamination of eye drop containers may alter the pH of the solution and therefore reduce the efficacy of the drug (Perry& Donnenfeld, 2003). In order to prevent contamination, most ophthalmic preparations contain antimicrobial substances, unless the solution by itself has an antimicrobial effect (Oldham & Andrews, 1996). The addition of antimicrobial substances (Preservatives) to eye drop containers should prevent or at least inhibit the growth of harmful microorganisms. In a recent study researchers from Tennent Institute of Ophthalmology, Gartnavel General Hospital, Glasgow, UK, found that preservative-free eye drops are at risk of contamination by potentially pathogenic microorganisms (Rahman etal,2006).These Preservatives must be non-toxic,

compatible with other ingredients and efficient during the entire duration of use of the eye drops. As preservatives interfere with the metabolism and inhibit the growth of micro-organisms, they may have similar effects on human cells, explaining potential cytotoxic effects and inflammatory cell responses (Ash, 2004 and Perry& Donnenfeld, 2003). The antimicrobial activity is important for the rate of infection resulting from contamination during the process of instillation. Contact with fingers or lids, ciliaries, conjunctiva and cornea are possible causes of contamination even if instilled by healthcare professionals.The present work studied the sterility condition of commonly used eye drops present in the Egyptian pharmacies .

2. Materials and Methods

A total of (40) commonly used eye drops were collected for microbial examination and divided into two groups:

Group A: Eye drops with Antimicrobial Activity Preparations containing: Antibiotic – Antiviral - Antifungal & Antiseptic Preparations

Group B: Eye drops without antimicrobial activity preparations for: allergy – inflammation – glaucoma – mydriatics – anaesthetics & liquifilm for dryness.

The microbial analysis was performed on the solution dropped from the dropper tip for each eye drop in the two groups, These eye drops were obtained and opened in open air for 24 h at the Microbiology laboratory of the Research Institute of Ophthalmology, after 3 days these vials were opened and one drop was directly inoculated on each of the media (blood agar and sabouraud agar plates) then it spread across the plates. The blood agar incubated at 37 oC for 48hrs and evaluated after 24 and 48h, The sabourand agar plates were incubated at 30oC for up to 10 days and evaluated for growth on days 1, 5 and 10, after that the vials were cap for another 4 days then they cultured by the same way on blood agar and sabouraud agar plates and incubated with the same precautions.

On the other hand we decided to evaluate the efficiency of group A samples (eye drops with anti microbial activity), pure cultures of *Staphylococcus aureus* and *Candida albicans* were obtained. *Staphylococcus aureus* subcultured on blood agar to obtain a pure culture and *Candida albicans* subcultured on sabouraud agar which is selective media for fungi after that a number of these colonies picked and dissolved carefully in 1 ml saline then the mixture incubated for 24h at 37oC, after that 10 μ of these mix inoculated to each vial in the group then the vials incubated at 37oC for 48h, after the incubation period these samples cultured on blood agar media and sabouraud agar media , the blood agar incubated at 37oC for 48h and evaluated after 24 and 48h, The sabourand agar plates were incubated at 30oC for up to 10 days and evaluated for growth on days 1, 5 and 10.

A significant growth was considered a growth on the main inoculation site or on two or more streaks on the plate, the colonies were counted and all organisms identified by microscopy after Gram staining and biochemical tests.

Screening for antimicrobial susceptibility for the isolated bacteria:

Disk diffusion method: In vitro screening of antimicrobial susceptibility for the isolated bacteria was carried out by the Kirby-Bauer disk diffusion method (Baure et al., 1966) according to clinical laboratory standards institute (USA), document M100-S18. The first and the second lines of antibiotics were arranged**.** The zones showing complete inhibition are measured with a ruler including the diameter of the disc. For each antibiotic tested bacteria were classified as susceptible, intermediate and resistant according to interpretative criteria recommended by the (CLSI) M100-S18.

Statistical methods:

The 95% and 99% confidence intervals of the incidence of contamination were found using the binomial distribution. The statistical significance of the incidence in contamination between antibiotic preparations (A) and non-antibiotic preparations (B) compared using Fishers exact test.

3. Results

There is no microbial growth in the cultures of step one (cultures after 3 days from opening samples), but there is 9 cultures from 40(22.5%) give a microbial growth in the cultures of step two (cultures after 7 days from opening samples) as shown in table (1). This represents an overall incidence of 22.5% with a 95% confidence interval of 10% to 35% and a 99% confidence interval 5.5% to 42.5%. Non of the 16 antibiotic eye drop bottles showed signs of contamination, but the overall incidence of contamination in the 24 non-antibiotic bottles was 37.5% and the difference between these two groups was statically significant ($p<0.01$).

Table (1) screening of eye drops

Samples	First screening (3days)	Second screening (7days)
Antibiotic eye drops	No contamination	No contamination
Non antibiotic eye drops	No contamination	9 samples give microbial growth: 1. Liquifilm tears 2. Trillerg 3. Lubrivisic 4. Relestat 5. Refresh tears 6. Nevanac 7. Voltaren 8. Timolol 9.Artelac

Identification of the contaminants:
The colonies were counted and all organisms identified by microscopy, Gram staining and biochemical tests. There are 9 samples give positive results and all these results are shown in table (2).

Screening for antimicrobial susceptibility
The antimicrobial susceptibility for the isolated bacteria according to interpretative criteria recommended by the (CLSI) M100-S18and all the readings are recorded in table (3)

5. Discussion
Eye vision plays a very important part of our lives. Thus, any factor that might affect vision quality such as environmental, biological or traumatic issues must be given immediate attention. One of these factors is the microbial contamination as many of viruses, bacteria and fungi are capable of attacking the eye and cause many microbial diseases such as conjunctivitis or keratitis (Templeton et al., 1982). Microbial contamination may be transmitted by contaminated eye drops and ophthalmic solutions. Lalitha et al (2014) reported postoperative endophathalamitis from contaminated anaesthetic eye drops, many eye drop preparations are only lubricating and contain tear-replacing solutions; these may be a source of eye contamination (Tasli & Cosar, 2001). In addition, contamination of eye drop containers may alter the pH of the solution and therefore reduce the efficacy of the drug (Perry& Donnenfeld, 2003).So there has been much interest in the question of the sterility of eye drops and their contamination during use, as not all eye drops were sterilized and the methods of sterilization were not always what they should be.
In our study, 40 of samples of eye drops varied between (preserved and preservative free), were carefully opened in the microbiology lab of the RESEARCH INSTITUTE OF OPHTHALMOLOGY for 24 hours and cultured in two steps (after 3 days then after 7 days from the date of opening samples), then the cultures were microbiologically examined. We noticed that there is no microbial growth in the cultures of step one, but there is 9 samples from 40 (22.5%) showed a microbial growth in the cultures of step two. The colonies were counted and all organisms identified by microscopy after Gram staining and classical biochemical tests.
Five different microorganisms were detected in the nine samples varied between pathogenic and normal flora of the skin (Micrococcus, Gram-Negative *Bacilli*, coagulase negative *Staphylococcus, Staphylococcus aureus, Candida albicans).* Similar results were reported in other published studies Rahman et al.(2006) found 7 pathogenic microorganisms with a small proportion of the microorganism (*coagulase negative Staphylococcus)* identified as anormal commensal flora when studying the contamination of un preserved eye drops.
In addition, Raghad et al. (2011) published similar results (coagulase negative *Staphylococci, Staphylococcus aureus, Micrococcus spp* and*Candida spp*) when studying the contamination of eye drops in Iraq. Also Teuchner et al. (2015) found *Staphylococcus aureus* in glaucoma eye drops used in the hospitals.
On the other hand Kim et al. (2009) reported that the contaminants in unpreserved eye drops weren't pathogenic and the identified bacteria were all (coagulase-negative *Staphylococcus*) normal commensal flora of the skin.
Normal flora can be defined as microorganisms that normally reside at a given site of the human body and under normal circumstances do not cause disease but if they penetrate the mucosa that they are living in, they can change the local environment and causing disease such as Staphylococcus epidermidis.The most common contaminant was *Staphylococcus aureus* as it was found in 5 samples from the 9 contaminated samples (5/9). Similar results were reported by Teuchner et al.(2015) who studied the contamination rate in glaucoma eye drops used in the hospital .In addition, Rahman et al.(2006) noted that the most common contaminant was *Staphylococcus aureus* when study the microbial contamination of preservative free eye drops. *Staphylococcus aureus* still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. In recent years, *S. aureus is* becoming famous because of its resistance to antibiotics, mainly methicillin, and in most cases to all related antibiotics (β-lactam antibiotics) (Klevens et al. 2007and Francois et al. 2008).Contamination of eye drops can lead to serious ocular infections especially when the ocular surface defenses are compromised with topical steroids. Application of contaminated eye drops may lead to potentially devastating consequences in patients with ocular surface disease and after intraocular surgery where there are wound leaks.Templeton et al. (1982) reported three cases post keratoplasty, in which Serratia marcescens keratitis developed as result of the contamination of eye drops with this organism.
Our results proved that eye drops may become contaminated during the usage period once they opened in the open air for many times and the longer the duration of use, the greater the chance that eye drops may become contaminated. This result was similarly reported by Rahman et al. (2006). Many factors were found to play an important role in increasing or decreasing the rate of contamination of the in use eye drops. In our study, the two most important factors were the active ingredients in the eye drop especially the antibiotic substances and the presence of preservatives such as Benzalkonium Chloride also Rahman etal. (2006) reported that preservative-free eye drops may be at a greater risk of contamination than preserved drops.
Our results showed that, contamination was common in non- antibiotic bottles while the antibiotic bottles showed negative cultures such as Orchazide(Ketotifen)and Ciloxan(Ciprofloxacinhydrochloride). Many factors may have contributed to such high rates of resistance in the

Table (2) contaminated eye drops in non- antibiotic bottles

Sample	Indication	Contamination	Preservatives
Liquifilm tears	Lubricant eye drops	• *Micrococcus*	Benzalkonium chloride
Trillerg	Anti-inflammatory and antiallergic eye drops	• *Gram-Negative Bacilli*	Benzalkonium chloride
Lubrivisic 0.1%	Lubricant eye drops	• *Micrococcus* • *coagulase negative staphylococcus*	Benzalkonium chloride
Relestat	Antiallergic eye drops	• *coagulase negative staphylococcus*	Benzalkonium chloride
Refresh tears	Irritation and dryness of eye	• *Staphylococcus aureus*	Benzalkonium chloride
Nevanac	Treatment of inflammation and pain after cataract surgery	• *Staphylococcus aureus*	Benzalkonium chloride
Voltaren	Anti-inflammatory eye drops (after surgery)	• *Staphylococcus aureus*	Pactericidal properties of active ingredient
Timolol	Treatment of elevated intraocular pressure	• *Micrococcus*	Benzalkonium chloride
Artelac	Lubricant eye drops	• *Staphylococcus aureus* • *Candida albicans*	Preservative free

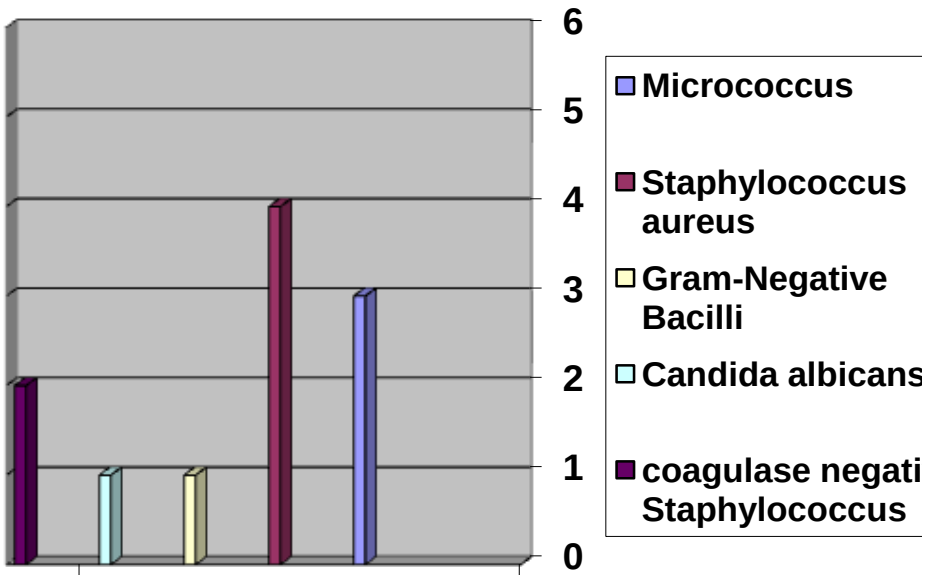

Figure (1) Isolated contaminants

The most common microorganism found in these samples is *Staphylococcus aureus*(4/9) followed by *Micrococcus* (3/9) and coagulase negative *staphylococcus(*2/9) ,finally *candida albicans*(1/9) and *Gram-Negative Bacilli* (1/9) as shown in fig (1)

Table (3) Culture and sensitivity results

Sample	Contamination	Sensitive	Inhibition zone (mm)	Moderate	Inhibition zone(mm)	Resistance	Inhibition zone(mm)
Liquifilm tears	*Micrococcus*	• Novobiocin 30 • Amikacin30 • Ciprofloxacin5 • Gatafloxacin5	24 21 23 25	• Polymyxin B300 • Fucidic acid10 • Sulphamethazole 25	15 16 17	• Gentamycin10 • Tetracycline30	9 8
Trillerg	*Gram-Negative Bacilli*	• Gentamycin 10 • Amikacin 30 • Gatafloxacin 5 • Tecoplanin 30	24 22 21 20	• Novobiocin30 • Sulphamethazole25 • Polymyxin B300	15 14 12	• Fucidic acid 10	7
Lubrivisic 0.1%	*Micrococcus*	• Ciprofloxacin5 • Gatafloxacin5 • Novobiocin30 • Gentamycin10	23 24 22 25	• Polymyxin B300 • Sulphamethazole25	14 18	• Amikacin 30 • Fucidic acid10 • Tetracycline30	7 10 6
	Coagulase negative staphylococcus	• Neomycin30 • Ciprofloxacin5 • Sulphamethazole25	23 25 26	• Gentamycin20 • Gatafloxacin 5 • Tetracycline30 • Novobiocin30	18 18 16 15	• Amikacin 30 • Fucidic acid10 • PolymyxinB300	6 10 9
Relestat	*Coagulase negative staphylococcus*	• Gentamycin 20 • Amikacin 30 • Ciprofloxacin 5 • Gatafloxacin5 • Tetracycline30	23 20 22 25 21	• Fucidic acid • Novobiocin30 • Sulphamethazole25	16 16 14	• PolymyxinB3	9
Artelac	*Staphylococcus aureus*	• Neomycin 30 • Ciprofloxacin5 • Ampicillin/sulbactam • Gentamycin 20	25 22 24 25	• Novobiocin 30 • Teicoplanin30 • Sulphamethazole25	18 14 15	• Polymyxin 300 • Tetracycline30	6 8
Refresh tears	*Staphylococcus aureus*	Gatafloxacin 5	26	• Neomycin 30 • Tetracycline 30 • Novobiocin 30 • Ciprofloxacin 5 • Gentamycin 10 • Polymyxin B300	19 16 17 15 18 14	• Teicoplanin30 • Fucidic acid 10 • Sulphamethazole25	9 8 8
Nevanec	*Staphylococcus aureus*	• Gatafloxacin 5 • Neomycin 30 • Ciprofloxacin 5	27 25 23	• Tetracycline 30 • Novobiocin 30 • Gentamycin 10 • Polymyxin B300	16 15 18 15	• Teicoplanin30 • Fucidic acid 10 • Sulphamethazole 25	8 10 7
Voltaren	*Staphylococcus aureus*	• Gatafloxacin 5 • Neomycin 30	27 23	• Teicoplanin30 • Ciprofloxacin 5 • Tetracycline 30 • Novobiocin 30 • Gentamycin 10 • Polymyxin B300	18 19 15 16 18 15	• Fucidic acid 10 • Sulphamethazole 25	11 8
Timolol	*Micrococcus*	• Ciprofloxacin5 • Gatafloxacin5 • Novobiocin30	23 26 22	• Polymyxin B300 • Sulphamethazole25 • Fucidic acid10 • Amikacin 30	14 18 15 17	• Gentamycin10 • Tetracycline30	8 6

antibiotic bottles such as high concentrations of the antibacterial substances or presence of preservatives which also act as antibacterial substances such as Benzalkonium chloride.

In this study, contamination occurred in both preserved and preservative free eye drops. A similar finding was reported in previous studies on preserved eye drops which found high contamination rates in steroid drops and ocular lubricant and the contamination rate varies widely from 0.07% to 35.8% even in the presence of preservatives (Wessels et al. 1999). Contamination of preserved eye drops was observed since 1970. Hugo & Wilson (1970) found 3 contaminated samples out of 204 examined and were preserved with Benzalkonium chloride. In addition Ford et al. (1985) found 46 contaminated samples from 184 examined were preserved with Benzalkonium Chloride. Till 2004 Mohammed et al. (2004) reported contamination rate of 34% in the preserved eye drops In this study in vitro screening for antimicrobial susceptibility for each contaminant was performed against the first and second line antibiotics by the Kirby-Baure disk diffusion method for the empirical treatment of such infections caused by these contaminants.

Obtained results revealed that, *Micrococcus* which found in sample (Liquifilm tears , Lubrivisic 0.1% and Timolol) from EPICO company was highly sensitive to Ciprofloxacin, Gatafloxacin and Novobiocin while highly resistant to Gentamycin,Amikacin,Fucidic acid and Tetracycline.

Gram negative *Bacilli* which found in sample (Trillerg) from Orchidia company was highly sensitive to Gentamycin,Amikacin,Gatafloxacin,Polymyxin and Tecoplanin while highly resistant to Fucidic acid .

Coagulase negative *staphylococcus* which found in sample (Lubrivisic 0.1%) from EPICO Company was highly sensitive to Neomycin while highly resistant to Amikacin , Fucidic acid, Polymyxin.

Coagulase negative *staphylococcus* which found in sample (Relestat) from Allergan Company was highly sensitive to Gentamycin, Amikacin, Ciprofloxacin, Gatafloxacin and Tetracycline while highly resistant to Polymyxin.

Staphylococcus aureus which found in sample (Artelac) from Mina Pharm Company was highly sensitive to Neomycin, Ciprofloxacin, Ampicillin/ sulbactam and Gentamycin while highly resistant to Polymyxin and Tetracycline.*Staphylococcus aureus* which found in sample (Refresh tears) from Allergen company was highly sensitive to Gatafloxacin while highly resistant to Teicoplanin, Fucidic acid and Sulphamethazole.

Staphylococcus aureus which found in sample (Nevanec) from Alcon was highly sensitive to Gatafloxacin, Neomycin and Ciprofloxacin while highly resistant to Teicoplanin, Fucidic acid and Sulphamethazole . *Staphylococcus aureus* which found in sample (Voltaren) from Novartis Company was highly sensitive to Gatafloxacin and Neomycin while highly resistant to Fucidic acid and Sulphamethazole.

On the other hand all the samples of group A (eye drops with antimicrobial activity) give no growth on the two media (blood agar and sabouraud agar) in the first and second analysis, so that we decided to test their inherent antimicrobial efficiency by intentionally inoculation with two different microorganisms *Staphylococcus aureus* and *Candida albicans* which were most common contaminants in eye drops. The results showed that, after the incubation period all the eye drop samples showed positive *Candida albicans* growth, this may be due to the absence or low concentrations of the antifungal substances in these samples. On the other hand, four eye drop samples showed positive *Staphylococcus aureus* growth 4/16 (25%), these positive cultures result from samples (Vigamox) from Alcon company,(Isoptofenicol 0.5%) from Ramida company ,(Oflox 0.3%) from Allergan and (Dexaflox) from Jamjoom pharma. These samples varied between preserved such as (Vigamox, Dexaflox, Isoptofenicol 0.5%) and un preserved such as (Oflox 0.3%). The contamination may be occurred as a result of the low concentration of antibiotics in these eye drops or these microorganisms were resistant to these antibiotics. On the other hand 12 samples give negative cultures.

Finally, non of the examined eye drops is expired and non of them was opened during storage period and all precautions were taken during handling samples and culturing process. This study underlines the importance of hygienic handling of eye drops and the risk of contamination raises the question of whether single-use medication might be preferred to reduce the risk of contamination especially for patients with compromised ocular surface because they can't attack the contamination caused by multi used eye drops although they contain preservatives or antibiotics.

Conclusion

Many eye drops are at risk of contamination with potentially pathogenic microorganisms during the usage period even if they contain preservative components. This may place some patients at increased risk of developing serious ocular infections especially the post operative patients. The prescription of these drops to patients with compromised ocular surface defense needs to be considered with caution and single use eye drop vials are recommended especially for post operative patients.

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