Pharmaceutical Evaluation and Microbiological Properties of Three Brands of Tobramycin Eye Drops Marketed in Retail Pharmacies of Albayda, Libya.

Samia Alsawi Majeed

Lecturer of pharmaceutics

Department of Pharmaceutics, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya.

The 25th Conference of The Scientific Association of Colleges of Pharmacy in The Arab World

The 1st International Conference of The Faculty of Pharmacy at LIMU

1.INTRODUCTION

- Tobramycin is a water-soluble aminoglycoside antibiotic produced by *Streptomyces tenebrarius*, which is formulated in different pharmaceutical dosage forms, including ophthalmic solutions, suspensions, ointments, inhalation solutions, and intravenous administration

- Tobramycin is prescribed for the treatment of a number of infections caused by bacteria, particularly Gram-negative bacteria and it appears to be effective against various *Pseudomonas species.*

- Tobramycin provides a treatment for a wide range of ocular pathogens, it has established a high incidence of bacterial eradication in patients with bacterial conjunctivitis (94.3–98.5%)

- Tobramycin attaches to the 16s rRNA of the bacterial 30s ribosomal subunit, thereby preventing the initiation of translation.

- Specifically, it binds to the A-site, causing mistranslation and misreading of the codons, resulting in the delivery of incorrect aminoacyl units. Consequently, these misfolded proteins accumulate and disrupt the cell membrane and cellular processes; tobramycin is classified as a bactericidal agent

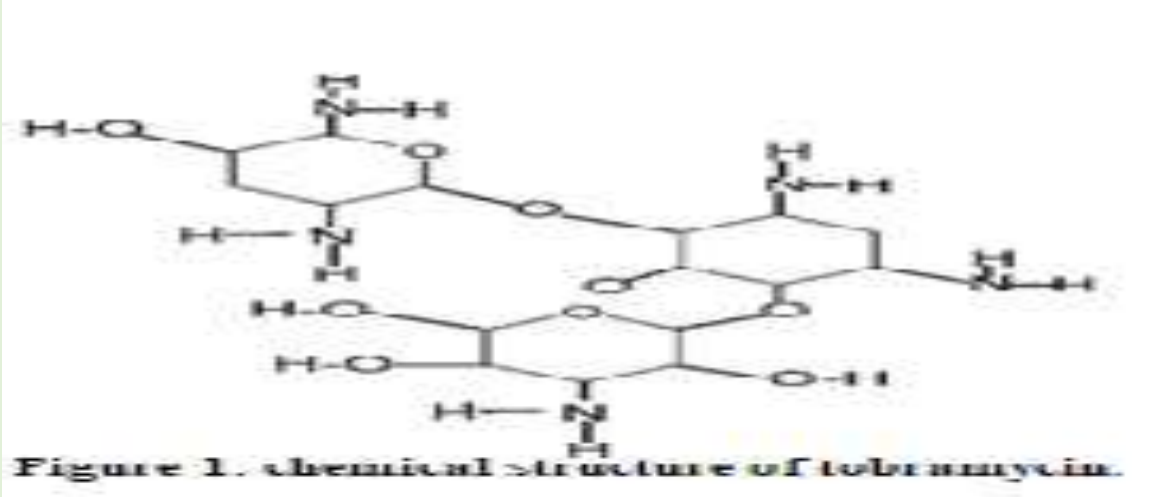

figure 1*: chemical structure of tobramycin*

- A sterile tobramycin ophthalmic solution (eye drops) with a tobramycin concentration of 0.3% is commercially available.

- Tobramycin eye drops are commonly used, widely abused, and easily obtainable as an over-the-counter medication for the treatment of most eye infections in Libya.

- Because of the importance of tobramycin eye drops in the treatment of ocular infection, there is a need to apply quality evaluation of these eye products to avoid poor-quality eye products that can be a source of hazard to the health of patients and prevent the spread of unqualified eye drops in the Libyan drug market.

AIM OF STUDY

this study aimed to evaluate the quality of three brands of tobramycin eye drops sold in retail pharmacies in Al-Bayda, Libya by visual inspection, organoleptic examination, particulate matter, refractive index, pH, sterility, antibacterial activity, and antimicrobial effectiveness of the preservative.

2 MATERIALS AND METHODS

2.1 Materials used:

1. Culture medium, Chemicals and Reagents:

The culture medium included; Fluid Thioglycollate Medium (FTM), nutrient agar (NA), Sabouraud Dextrose Agar (SDA), Nutrient broth (NB), and Mueller Hinton Agar (MHA), which were obtained from Hi-Media Laboratories Pvt. Ltd. (India). Culture media, and chemicals were purchased from reputable suppliers to ensure purity and quality.

2. Eye drops used:

Three brands of tobramycin eye drops Tobrex (Switzerland), Tobracol (Tunisia), and Tobrin (Egypt) were collected from the local Al-Bayda market, Libya, and are described in **Table 1 & Figure 2**.

table 1. *Description of eye drops used in the study*

Name Of eye drop	Production Company	Active Ingredient	Batch Number	Expiry Date
Tobrex (A)	Novartis pharma AG, Switzerland	Tobramycin 0.3%	VN460C	1/ 2025
Tobracol (B)	Unimed laboratories, Tunisia	Tobramycin 0.3%	19	6/ 2025
Tobrin (C)	E.I.P.I.CO. Egypt	Tobramycin 0.3%	2300088	1/ 2025

3. Microorganisms used:

The organism's strains used Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli*, and fungi *Candida albicans* strains were obtained from the culture collections maintained in the Department of Biomedical Science, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya.

4. Media used:

Fluid Thioglycollate medium (FTM), Nutrient agar (NA), Nutrient broth (NB), Mueller Hinton Agar (MHA), freshly prepared 0.5 McFarland standards and Sabouraud Dextrose Agar (SDA) were used in this study.

2.2 Methods :

1. Visual inspection of packaging:

The primary and secondary packages of eye drops were examined carefully for proper packaging, sealing, labeling, brands name, name of the active ingredient, name of the company, drug strength, dosage form, batch number, expiration date, storage information, safety features, and leaflets.

2.Organoleptic examination:

The eye drops were visually inspected for particulate matter, color, and clarity against a black-and-white background with adequate illumination. Black particles were detected using a white background, while white particles were identified against a black background, as shown in **Figure 2**.

3. Assessment of the pH of eye drops:

The pH values of the three eye drop brands were measured using a calibrated pH meter (pH Sevenmulti, Mettler Toledo, Germany). Five milliliters of each eye drop brand was poured into a clean and dry beaker, and the sensitive probe of the pH meter was dipped inside it and allowed to stabilize before tacking the pH value.

4. Refractive index measurement:

The refractive indices of three brands of eye drops were measured using a refractometer (RX-5000 ATAGO, Kyoto, Japan).

5. Preparation of bacteria suspensions:

1. Broth cultures of test organisms were distributed on nutrient agar slopes and incubated for 24 h at 37°C.

2. Following incubation, the bacterial growth was collected and washed with normal saline to produce a suspension with a concentration of 10^8-10^9 CFU/ml.

3. The suspension was stored at 4°C and the average number of viable organisms per mL was determined using the surface viable count technique (Miles et al., 1938).

4. The stock suspension was serially diluted by combining appropriate volumes with a sterile 0.9% saline solution.

5. A micropipette was used to dispense 0.02-milliliter aliquots of the diluted suspension onto nutrient agar plates, which were then left to dry.

6. Following a 24-hour incubation period at 37°C, the colonies that formed on each agar surface were counted. The average colony count was multiplied by 50, and the dilution factor was used to calculate the viable count of the stock suspension.

7. This result was expressed as colony-forming units (CFU) per ml of suspension.

6. Sterility testing of the eye drops:

1. Using the direct inoculation method under aseptic conditions in a laminar airflow cabinet, tobramycin eye drops (approximately 0.5 mL) from various brands were aseptically transferred into 20 mL of different media (fluid thioglycollate medium (FTM) for anaerobic bacteria and nutrient agar (NA) for aerobic bacteria) with a sterile pipette and incubated at 35-37°C for 24-48 h.
2. Approximately 0.5 mL of the same tobramycin eye drop (0.5 mL) was transferred into Sabouraud dextrose agar (SDA) and incubated at 20-25°C for 72 h to obtain colony-forming units of fungi.
3. For the positive controls, we used 20 mL of fluid thioglycollate media in a sterile universal bottle and aseptically inoculated it with *S. aureus* (0.1 mL) (standardized using McFarland) to serve as a control for anaerobic bacteria. Similarly, we inoculated 20 mL of nutrient agar with 0.1 mL of *E. coli* to serve as a control for the aerobic bacteria , and we used 20 mL Sabouraud dextrose agar and inoculated it with 0.1 mL of *C. albicans* as a control for fungi. All experiments were conducted in triplicates (Kusuma et al., 2020).

7. Evaluation of preservative effectiveness of the eye drops:

The preservative effectiveness test was performed by challenging the tobramycin eye drops with E. coli, S. aureus, and C. albicans.

1. For each challenged microorganism, 5 ml of each tobramycin eye drop was taken in sterile tubes (10 ml).
2. Each sample tube was inoculated with a standardized inoculum to achieve a final microorganism concentration between 1×10^5 and 1×10^7 CFU/ml.
3. All the inoculated tubes were incubated at 25-37oC for 28 days and viable counts were periodically determined by the pour-plate method at 0, 7, 14, 21, and 28 days after inoculation.

8. Antibacterial activity of the eye drops:

The agar well diffusion method, described by Kavanagh (1972), was used with minor modifications to evaluate the antibacterial activity of the eye drops. In this method, Petri dishes with inoculated Mueller-Hinton agar were divided into four wells using a sterile cork borer, and the wells were filled with 0.1 ml of the prepared eye drops solutions. The plates were incubated at 37 °C for 18 h, and the growth inhibition zones were measured in millimeters (mm).

9. Statistical analysis:

The experiments were performed in triplicates to ensure statistical reliability. The mean ± standard deviation values obtained from three replicates were analyzed using the IBM Statistical Package for Social Sciences (SPSS-26) Statistics software (version 26, Statistical Package for Corp., Armonk, NY, USA.

3.RESULTS AND DISCUSSION

1. visual inspection of packaging

table 2. *results of visual inspection of brands of tobramycin eye drop*

Inspection category and specific question	Brand A	Brand B	Brand C
A. Container and closure, is the container is properly sealed?	yes	yes	yes
B. Label, does the label on package match the label on container ?	yes	yes	yes
1.Brand name, is it clear on container and package ?	yes	yes	yes
2. Scientific name, is it clear and correct on container and package ?	yes	yes	yes
3. Manufacturer's name and logo, is it present and correct on container and package ?	yes	yes	yes

4.Drug strength , is it clear on container and package ?	yes	Yes but it is clarified in leaflet	yes
5. Dosage form, is it clear on container	yes	Yes but in French language	yes
6. Batch NO, or lot NO, is it imprinted on label of container and package ?	yes	yes	yes
7. Date of manufacture and expiry date, are they imprinted clearly on the container and package?	yes	Expiry date only	yes
8. Storage information, are the storage conditions indicated on container and package?	yes	Written in French language	yes
9. Safety features a. Is data matrix code available in outer package? b. Is antitampering device available on the package?	Yes yes	NO NO	Yes NO
C. Leaflet or package insert, does the information in the leaflet match with that on the container?	yes	yes	yes

2 .Organoleptic examination of eye drops:

Figure 2*: Packaging and containers with tobramycin eye drops: (A): Tobrex, (B): Tobracol, and (C): Tobrin, and Clarity of eye drops.*

All brands were found very clear, colorless, without particulate matter against white and black backgrounds, the clarity, and absence of particulate matter in the eye drops indicated an efficient membrane filtration process in the manufacturing which excludes any visible particulate matter

3. pH evaluation of eye drops:

Table 3*: Physicochemical properties of the tobramycin eye drops:*

No	Brand of tobramycin eye drops	pH value	Colour and Clarity
1.	Brand A	6.88	Colorless and clear
2.	Brand B	6.91	Colorless and clear
3.	Brand C	6.90	Colorless and clear

Results of pH of the brands of tobramycin eye drops were 6.88, 6.91, and 6.90 for brand A, B, and C respectively, they were within the acceptable pH range for eye formulations and compatible with physiological pH of tear fluid (7.4).

4. Refractive index measurement of eye drops

Table 4*: Refractive index measurement of brands of the tobramycin eye drops:*

No.	Brand of tobramycin eye drops	Refractive index (RI) value
1.	Brand A	1.335
2.	Brand B	1.334
3.	Brand C	1.334

The refractive index of three brands of eye drop was 1.335, 1.334, and 1.334 for brand A, B, and C respectively. The reduced visual acuity after instillation is a limitation of eye drop which correlates with its viscosity and refractive index . To reduce the visual disturbance after application the eye drop should be transparent and ideally has a refractive index identical to that of the tear fluid (1.336–1.338).

5. Sterility testing of eye drops:

Table 5*: Assessment of the microbiological quality of various freshly opened samples of tobramycin 0.3% eye drops:*

No.	Brand of tobramycin eye drops	Number Examined	Fluid thioglycollate medium	Nutrient agar	Sabouraud dextrose broth
1.	Brand A	R_1	No	No	No
		R_2	No	No	No
		R_3	No	No	No
2.	Brand B	R_1	No	No	No
		R_2	No	No	No
		R_3	No	No	No
3.	Brand C	R_1	No	No	No
		R_2	No	No	No
		R_3	No	No	No

Key: NG: No changes in color and the absence of organism growth in the medium.

Sterility assessment revealed no observable microbial growth in any of the tobramycin eye drop brands, indicating that they were free from bacterial and fungal contamination. This result confirms that the products are sterile and can be safely administered to the patients.

Figure 3: *sterility test of three brands of tobramycin eye drops*

6. Evaluation of preservative effectiveness of the eye drops:

table 6*: Preservative effectiveness of the eye drops challenged with microorganisms used:*

No.	Microorganisms used	Eye drops used	Sampling time/Microbial count (CFU/ml)					
			0 day	1th day	7th day	14th day	21th day	28th day
1.	E. coli	Brand A	4×10^6	2×10^4	4×10^2	0	0	0
		Brand B	4×10^6	3×10^4	5×10^2	0	0	0
		Brand C	4×10^6	3×10^4	5×10^2	0	0	0
2.	S. aureus	Brand A	5×10^6	4×10^3	6×10^2	0	0	0
		Brand B	5×10^6	4×10^3	5×10^2	0	0	0
		Brand C	5×10^6	4×10^3	5×10^2	0	0	0
3.	C. albicans	Brand A	2×10^5	-	1×10^3	1×10^1	0	0
		Brand B	2×10^5	-	1×10^3	2×10^1	0	0
		Brand C	2×10^5	-	1×10^3	2×10^1	0	0

Antimicrobial effectiveness testing indicated zero colony-forming units (CFU) of bacterial strains on days 14, 21, and 28. For fungal strains, the CFUs gradually declined over 14 day, becoming completely absent on days 21 and 28.

7. Antibacterial activity of the eye drops:

Table 7: *In-vitro* antibacterial activity of the eye drops:

No.	Microorganisms used	Eye drops used	Inhibition Zones (mm)±SD
1.	E. coli	Brand A	22±0.02
		Brand B	23±0.05
		Brand C	20±0.01
2.	Ps. aeruginosa	Brand A	25±0.03
		Brand B	26±0.08
		Brand C	23±0.04
3.	S. aureus	Brand A	25±0.02
		Brand B	25±0.09
		Brand C	25±0.05

This study's findings demonstrated that tobramycin eye drop brands exhibited considerable efficacy against the tested microorganisms. This effectiveness was attributed to two factors: the antimicrobial properties inherent in the active ingredient and the preservative system used in the eye drops' formulation

Figure 4: *Antibacterial activity of three brands of tobramycin eye drops*

4.CONCLUSION

Our study evaluated three brands of tobramycin eye drops in the Libyan market, finding that all brands were properly sealed and labeled per FIP guidelines.The eye drops were clear, colorless, and free of particulates, with pH values of 6.88, 6.91, and 6.90 for brands A, B, and C, respectively, matching the physiological pH of the tear fluid. The refractive indices were 1.335, 1.334, and 1.334, respectively, aligned with those of tear fluid. However, the microbial quality of tobramycin eye drop brands was found to be satisfactory, as they met official sterility standards and demonstrated effective antimicrobial properties as required.Based on these findings, eye drops available for purchase and application in Pharmacies of Albayda, Libya. demonstrated satisfactory pharmaceutical quality and effective antimicrobial properties.

RECOMMENDATION

Further research should adhere to recommended usage guidelines to ensure the quality of eye drop products. Each container should be reserved for use by a single person, and care must be taken to prevent the bottle tip from touching the hands, eyes, or other surfaces. Additionally, proper storage instructions are crucial to avoid contamination and to maintain sterility throughout use.

Thank you for your attention

It is appreciated…