

Original Article

Pharmaceutical Evaluation and Microbiological Properties of Three Brands of Tobramycin Eye Drops Marketed in Retail Pharmacies of Al-Bayda, Libya

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ABSTRACT

Background: Tobramycin (TBM) is a water-soluble aminoglycoside antibiotic in various formulations, including ophthalmic solutions. It has been demonstrated to be effective against numerous ocular pathogens.

Objectives: This study aimed to determine the pharmaceutical and microbiological efficiency of three brands of tobramycin eye drops sold in retail pharmacies in Al-Bayda, Libya. **Materials and Methods:** Three sterile eye drops from different tobramycin brands sourced from the Al-Bayda market in Libya were analyzed. The physical appearance of each brand was assessed for packaging and closure integrity. Pharmaceutical analyses (color and clarity evaluation, pH), sterility testing, antibacterial activity, and antimicrobial effectiveness were conducted by inoculating nutrient agar and Sabouraud dextrose medium with each eye drop brand. **Results:** The findings showed that all brand packaging adheres to proper sealing standards with intact container closures. The labels complied with FIP guidelines for prescribed medicines. All brands were transparent and particle-free when examined against white and black backgrounds, respectively. The pH values for brands A, B, and C were 6.88, 6.91, and 6.90, respectively, matching the tear fluid's physiological pH. The refractive indices were 1.335, 1.334, and 1.334 for brands A, B, and C, respectively, consistent with tear fluid. Sterility tests indicated no microbial growth, and the preservatives were effective against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. **Conclusion:** This preservative system confers resistance to microbial contamination during its utilization. Further clinical investigations are necessary to assess the safety and long-term effectiveness of these pharmaceutical agents.

Keywords: Tobramycin, Eye drops, Pharmaceutical properties, Antimicrobial efficacy,

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INTRODUCTION

Eye drops (ED) are topical dosage forms utilized to treat anterior ocular conditions[1,2]. They are preferred over alternative formulations owing to their convenience, non-invasiveness, localized effects, and reduced side effects [3]. Eye drops constitute more than 70 percent of all ophthalmic preparations[4,5]. These sterile multidose formulations emphasize the maintenance of sterility during usage and shelf life[6,7]. Eye drops must remain sterile during storage and contain preservatives to inhibit microbial growth. Packaging in multidose containers poses the microbial contamination risk[8,9]. Preservatives may change pH, potentially causing ocular irritation and degradation of active ingredients, resulting in decreased efficacy and an increased risk of infection[10,11]. Patients are advised to discontinue the use of multidose eye drops 28 days after opening[12]. Antibiotic eye drops contain antibacterial agents and preservatives such as benzalkonium chloride and phenyl mercuric nitrate to ensure adequate preservation [5,13].

Tobramycin, a water-soluble aminoglycoside antibiotic derived from *Streptomyces tenebrarius*, is available in various pharmaceutical formulations, including ophthalmic solutions, suspensions, ointments, inhalation solutions, and intravenous preparations [14,15]. It is used to treat multiple bacterial infections, especially those caused by Gram-negative bacteria [16,17] and is effective against various *Pseudomonas* spp. It is used in the treatment of infections of the lower respiratory tract, bones, skin, and urinary tract [18], as well as a wide range of ocular pathogens [19,20]. Tobramycin has demonstrated high bacterial eradication rates in bacterial conjunctivitis (94.3–98.5%) and blepharoconjunctivitis, with a bacterial reduction rate of 88.9% [21,22].

The chemical structure of tobramycin comprises a central 2-deoxystreptamine ring (B), ring (A) with a glycosidic bond at C6-O, and ring (C) with a glycosidic bond at C4-O. It contains an amino group

connected to a carbohydrate molecule via the glycosidic chain of aminocyclitol. The compound's five amino and five hydroxyl groups confer the basic and hydrophilic properties, respectively. The protonation of the amino groups, occurring between pH 5.67 and 9.29, establishes five protonation equilibria in either basic or acidic environments [23,24]. Tobramycin binds to the 16s rRNA of the bacterial 30s ribosomal subunit, thereby inhibiting translation initiation [25]. It associates with the A-site, resulting in codon misreading and the incorporation of incorrect aminoacyl units. This process leads to disruption of cell membranes and cellular functions owing to the presence of misfolded proteins. Tobramycin is a bactericidal agent [26].

Tobramycin, such as aminoglycosides, is not absorbed through the gastrointestinal tract and must be taken intravenously or intramuscularly for systemic use [27]. A sterile 0.3% tobramycin ophthalmic solution (eye drops) was commercially available. These eye drops are frequently used, often inappropriately, and are readily accessible over the counter to treat most ocular infections in Libya. Given their significance in the treatment of ocular infections, it is imperative to assess the quality of these products to prevent potential health risks to patients and the proliferation of substandard eye drops on the Libyan market. This study aimed to evaluate the quality of three tobramycin eye drop brands sold in Al-Bayda, Libya, through visual inspection, organoleptic examination, particulate matter analysis, refractive index, pH, sterility, antibacterial activity, and preservative effectiveness.

MATERIALS AND METHODS

Materials used

Culture medium, Chemicals, and Reagents

All the chemicals used in the experiments had a minimum purity of 99.0%. The culture media, Fluid Thioglycollate Medium (FTM), Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), Nutrient Broth (NB), and Mueller-Hinton Agar (MHA) were obtained from HiMedia Laboratories Pvt. Ltd. (India). Three ophthalmic solutions of high quality, Tobrex (Novartis Pharma AG, Switzerland),

Tobracol (Unimed Laboratories, Tunisia), and Tobrin (E. I. P. I. Co., Egypt), were used. Culture media, chemicals, and ophthalmic solutions were

acquired from reputable suppliers to ensure purity and quality.



Figure 1. Packaging and containers with tobramycin eye drops: (A): Brand (A), (B): Brand (B), and (C): Brand (C), and Clarity of eye drops.

Table 1. Description of eye drops used in the study:

Code of eye drops	Production Company	Active Ingredient	Batch Number	Expiry Date
Brand (A)	Novartis pharma AG, Switzerland	Tobramycin 0.3%	VN460C	1/ 2025
Brand (B)	Unimed laboratories, Tunisia	Tobramycin 0.3%	19	6/ 2025
Brand (C)	E.I.P.I.CO. Egypt	Tobramycin 0.3%	2300088	1/ 2025

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

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.

Microorganisms used

The microbial strains employed in this research, including the Gram-positive bacterium *Staphylococcus aureus*, the Gram-negative bacterium *Escherichia coli*, and the fungus *Candida albicans*, were sourced from culture collections housed in the Department of Biomedical Science at the Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya.

Visual inspection of packaging

Primary and secondary packaging of ophthalmic solutions was thoroughly performed to assess the adequacy of packaging, sealing, labeling, brand name, active ingredient nomenclature, manufacturer identification, drug concentration, dosage form, batch number, expiration date, storage instructions, safety features, and accompanying livlets.

Organoleptic examination

The eye drops were visually inspected for particulate matter, color, and clarity on black and white backgrounds under appropriate illumination conditions. Black particles were detected using a white background and white particles were identified using a black background.

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 transferred

into a clean and dry beaker, and prior to recording the pH value, the sensitive probe of the pH meter was submerged in the solution and given time to stabilize.

Refractive index measurement

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

Preparation of bacteria suspensions

Broth cultures were grown on nutrient agar slopes and incubated for 24 h at 37°C. Bacterial growth was harvested, washed with saline to create a suspension of 10^8 - 10^9 CFU/ml, and stored at 4°C. Viable counts were determined using the surface viable count technique ⁽²⁸⁾. The stock suspension was diluted with a sterile 0.9% saline solution. A micropipette was dispensed in 0.02 ml aliquots onto nutrient agar plates, which were dried and incubated for 24 h at 37°C. The colonies were counted and multiplied by 50. At each time point, new stock suspensions were prepared.

Sterility testing of the eye drops

Tobramycin eye drops (0.5 mL) were added to 20 mL of various media using a sterile pipette in a laminar airflow cabinet under sterile conditions. For anaerobic bacteria, Fluid Thioglycollate Medium (FTM) was used, whereas Nutrient Agar (NA) was employed for aerobic bacteria. These were then incubated at temperatures between 35-37°C for a period-24-48 hours. Additionally, eye drops (0.5 mL) were placed on Sabouraud Dextrose Agar (SDA) and incubated at 20-25°C for 72 h to cultivate fungal colony-forming units. Positive controls consisted of 20 mL of Fluid Thioglycollate Medium inoculated with 0.1 mL of *S. aureus* (standardized using McFarland) for anaerobic bacteria, 20 mL of Nutrient Agar inoculated with 0.1 mL of *E. coli* (standardized using McFarland's method) for aerobic bacteria, and 20 mL of Sabouraud Dextrose Agar inoculated with 0.1 mL of *C. albicans* (standardized using McFarland) for fungi. Each experiment was conducted in triplicate [29].

Evaluation of preservative effectiveness of the eye drops

An antimicrobial effectiveness test was used to assess the activity of preservatives in pharmaceutical products using microbiological

methods and adhering to the USP41 protocol. The results were evaluated against acceptance criteria from three pharmacopeias ⁽³⁰⁻³²⁾. Tobramycin eye drops were administered to *E. coli*, *S. aureus*, and *C. albicans*. For each microorganism, 5 ml of tobramycin eye drops were placed in sterile 10 ml tubes and inoculated to achieve a final concentration of 1×10^5 - 1×10^7 CFU/ml. The inoculated tubes were incubated at 25-37°C for 28 days, and viable counts were determined using the pour-plate method at 0, 1, 7, 14, and 28 days post-inoculation.

Antibacterial activity of the eye drops

The agar well diffusion technique, as outlined by Kavanagh ⁽³³⁾, was utilized with slight adjustments to evaluate the antibacterial properties of the eye drops. In this procedure, Petri dishes inoculated with Mueller-Hinton agar were divided into three wells using a sterile cork borer. Each well was then filled with 0.1 ml of the eye drop solutions prepared for the test. The plates were incubated at 37 °C for 18 h, after which the growth inhibition zones were measured in millimeters (mm).

Data analysis

All experiments were performed three times to ensure statistical accuracy. The average $\pm$ standard deviation from these three trials was evaluated using the IBM SPSS Statistics software (version 26, Corp., Armonk, NY, USA).

RESULTS

Properties and Visual inspection of eye drops

Eye-drop formulations are packaged in sealed droppers and containers to protect their contents from environmental factors. Packaging includes information regarding the production company, country of manufacture, active ingredients, batch number, manufacturing date, expiration date, and volume (mL), as presented in Table 1. The visual assessment of tobramycin ophthalmic solution brands included the examination of containers and closures, labels, brand names, scientific names, manufacturer names and logos, drug strength, dosage form, batch number or lot number, date of manufacture and expiration date, storage instructions, safety features, and accompanying leaflets or package inserts Table 2.

Table 2. Results of visual inspection of tobramycin eye drop brands.

Inspection category and specific question	Brand (A)	Brand (B)	Brand (C)
Container and closure: Is the container properly sealed?	Yes	Yes	Yes
Label: Does the package label match the label on the container?	Yes	Yes	Yes
Brand name: Is it clear in containers and packages?	Yes	Yes	Yes
Scientific name: Is it accurately labeled in containers and packages?	Yes	Yes	Yes
Manufacturer name and logo: Is it present and correct on the container and package?	Yes	Yes	Yes
Drug strength: Is it clear in containers and packages?	Yes	Yes But it is clarified in the leaflet	Yes
Dosage form: Is it clear on the container?	Yes	Yes But in the French language	Yes
Batch No. or lot No.: Is it imprinted on the label of the container and package?	Yes	Yes	Yes
Date of manufacture and expiry date: Are they imprinted clearly on containers and packages?	Yes	Expiry date only	Yes
Storage information: Are the storage conditions indicated in containers and packages?	Yes	Yes Written in the French language	Yes
Safety features: Is data matrix code available in the outer package? Is an anti-tampering device available in packages?	Yes Yes	No No	Yes No
Leaflet or package insert: Does the information in the leaflet match that in the container?	Yes	Yes	Yes

Organoleptic examination of eye drops

Using a board for visual inspection with white and black backgrounds and suitable lighting, eye drops were examined for the presence of particles, as well as for their color and clarity. Organoleptic examination and physical assessment of tobramycin

pH evaluation of eye drops

Table 3 presents the visual characteristics of the eye drops, including their clarity, pH, and color. The pH values of the tobramycin eye drops A, B, and C were 6.88, 6.91, and 6.90, respectively

Table 3. Physicochemical properties of the tobramycin eye drops

No	Brand of tobramycin eye drops	pH value	Color 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

Refractive index measurement of eye drops

The refractive indices of the three brands of eye drops were measured and are listed in Table 4.

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

Sterility testing of eye drops

Testing for sterility on different eye drop brands, using three separate nutrient media that supported

the growth of both aerobic and anaerobic bacteria, as well as fungi, showed no microbial growth [Table 5](#).

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

N o.	Brand of tobramycin eye drops	Number Examined	Fluid Thioglycollate Medium	Nutrient Agar	Sabouraud Dextrose Broth
1.	Brand A	R1	No	No	No
		R2	No	No	No
		R3	No	No	No
2.	Brand B	R1	No	No	No
		R2	No	No	No
		R3	No	No	No
3.	Brand C	R1	No	No	No
		R2	No	No	No
		R3	No	No	No

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

Evaluation of the preservative effectiveness of the eye drops

The efficacy of the preservative against microbial contamination of ophthalmic solutions was

evaluated using a preservative challenge test. Microbial survival was assessed at 0, 1, 7, 14, 21, and 28 d post-inoculation to determine the effectiveness of the preservative system [Table 6](#).

Table 6. Preservative effectiveness of the eye drops challenged with microorganisms used.

N o.	Microorganisms used	Eye drops used	Sampling time (day/s)/Microbial count (CFU/ml)					
			0	1 th	7 th	14 th	21 th	28 th
1.	<i>E. coli</i>	Brand A	4 X 10 ⁶	2 X 10 ⁴	4 X 10 ²	0	0	0
		Brand B	4 X 10 ⁶	3 X 10 ⁴	5 X 10 ²	0	0	0
		Brand C	4 X 10 ⁶	3 X 10 ⁴	5 X 10 ²	0	0	0
2.	<i>S. aureus</i>	Brand A	5 X 10 ⁶	4 X 10 ³	6 X 10 ²	0	0	0
		Brand B	5 X 10 ⁶	4 X 10 ³	5 X 10 ²	0	0	0
		Brand C	5 X 10 ⁶	4 X 10 ³	5 X 10 ²	0	0	0
3.	<i>C. albicans</i>	Brand A	2 X 10 ⁵	-	1 X 10 ³	1 X 10 ¹	0	0
		Brand B	2 X 10 ⁵	-	1 X 10 ³	2 X 10 ¹	0	0
		Brand C	2 X 10 ⁵	-	1 X 10 ³	2 X 10 ¹	0	0

Antibacterial activity of the eye drops

Table 7 displays the results, highlighting the antibacterial efficacy of eye drops against

pathogenic strains of *E. coli*, *P. aeruginosa*, and *S. aureus*.

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

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

DISCUSSION

Quality control assessments using pharmaceutical standards regulate ophthalmic solutions. This study examined the physicochemical properties and antimicrobial efficacy of three commercially available tobramycin ophthalmic formulations in Al-Bayda, Libya. The absorption of pharmaceutical agents from ophthalmic solutions depends on their physicochemical characteristics, such as molecular weight, osmolality, viscosity, pH, lipophilicity, concentration, and presence of excipients[34].

Upon examination of the various ophthalmic solution brands, each was placed in a multidose vessel with an application dropper. All packaging remained intact in properly sealed containers to ensure the preservation of ophthalmic solutions. Labels on both packaging and containers were consistent across brands, delineating brand, scientific, and manufacturer names. The containers indicated the strength, dosage form, batch number, and expiration dates; however, the manufacturing dates were specified only for brands A and C. Storage conditions were noted for all containers and packages; however, brand B was presented in French. A data matrix code was present on the outer packages of brands A and C, but absent for brand B; only brand A incorporated an anti-tampering device. The information in the package inserts corresponded to that in the containers for all three brands. According to the results, all brands adhered to the FIP guidelines for prescribed medicine labeling[35]. When examined against white and

black backgrounds, all brands appeared transparent, colorless, and free of particulate matter. The clarity and lack of visible particulates in ophthalmic solutions suggests an efficient membrane filtration process during manufacturing[3].⁽⁶⁾

A stable pH in eye drops is crucial for ocular compatibility and comfort. Previous studies have reported varying eye pH levels [2]. The pH of ophthalmic solutions affects drug solubility and stability, ideally matching the natural tear fluid pH of 7.4 for optimal comfort. However, challenges in drug stability and solubility may impede this process. The acceptable pH range for ophthalmic solutions is 6.5–7.8 to prevent discomfort, which can induce lacrimation and pain. Buffer systems, such as citrate or acetate, maintain pH during storage [37,38]. Incompatible pH levels in eye drops can irritate and increase lacrimation, reducing tobramycin bioavailability by 0.3%⁽³⁹⁾. An appropriate pH is essential to minimize the adverse effects and mitigate the risks of microbial contamination during preparation. Tobramycin eye drops exhibited pH values of 6.88, 6.91, and 6.90 for brands A, B, and C, respectively, within the acceptable range and compatible with tear fluid pH (7.4). Extreme pH can cause irritation and drug degradation[3]. Buffering ensures pH stability and influences drug lipophilicity, absorption, ocular tissue distribution, and bioavailability by affecting the ionization state of the drug, which is pH dependent [5]. The refractive indices of the three eye drop brands were 1.335 for brand A and 1.334 for

brands B and C. Reduced visual acuity after instillation is a limitation of eye drops and is linked to viscosity and the refractive index [5]. To reduce visual disturbance, after application, drops ought to be clear and have a refractive index of 1.336–1.338, which is comparable to tear fluid [40]. The refractive indices of the brands in this study were aligned with those of tear fluid. A higher refractive index, as observed with oily eye drops (1.44–1.50), can cause foreign body sensations and frequent blurred vision reports [41].

Microbial contamination with multidose eye drops due to inadvertent entry or patient misuse presents significant risks to public and ocular health. Preservatives provide only temporary protection against microbial growth, limiting the shelf life of open preservative-containing products to 28 d[42,43]. Sterility is essential for ophthalmic products such as ophthalmic solutions[144]. However, inadvertent contamination during use and domestic storage can be detrimental to patients, necessitating the use of preservatives. Antimicrobial preservatives in ophthalmic drops mitigate spoilage risks by eliminating low levels of contaminants introduced during manufacturing, storage, or use[45]. Preservative effectiveness testing involves inoculating a sample with a specific number of microbial colony-forming units and assessing the preservative efficacy over time by monitoring CFU counts[46]. This study investigated the growth of *E. coli*, *S. aureus*, and *C. albicans* in various eye drop solutions obtained from various pharmaceutical companies. Sterility assessments demonstrated the absence of microbial growth in any tobramycin eye drop brand, indicating that they were devoid of bacterial and fungal contamination. This confirms the sterility and safety of the patient. The preservative efficiency test, or microbial challenge test, evaluates the resistance of an eye product to microbial contamination, particularly multidose eye drops[9]. The microbial challenge test revealed significant growth of *E. coli*, *S. aureus*, and *C. albicans* on day zero. Antimicrobial effectiveness testing revealed the absence of colony-forming units (CFU) of the bacterial strains on days 14, 21, and 28. For fungal strains, the CFUs gradually decreased over 14 days, becoming absent on days 21 and 28.

The antibacterial efficacy of the ophthalmic solution against Gram-positive and Gram-negative bacteria was evaluated using the agar well diffusion method. The activity of the solution was assessed against *E. coli*, *Ps. aeruginosa*, and *S. aureus*, respectively. Brand (A) exhibited inhibition zones of 22 ± 0.02 , 25 ± 0.03 , and 25 ± 0.02 mm, respectively. Brand (B) demonstrated zones of 23 ± 0.05 , 26 ± 0.08 , and 25 ± 0.09 mm, while brand (C) displayed zones of 20 ± 0.01 , 23 ± 0.04 , and 25 ± 0.05 mm for the aforementioned bacteria in the specified order. The results of this study demonstrated that tobramycin eye-drop formulations exhibited significant efficacy against the tested microorganisms. This effectiveness has been attributed to two factors: the inherent antimicrobial properties of the active ingredient and the preservative system utilized in the formulation of ophthalmic preparations[47,48].

However, this in vitro model has limitations and cannot be directly applied to real-world patient scenarios. Additionally, the study did not investigate the long-term effects of tobramycin ophthalmic solutions or the effects of storage conditions on their physicochemical properties and antimicrobial efficacy. Therefore, clinical trials are recommended to determine whether the observed physicochemical variations and antimicrobial efficacy reflect the long-term efficacy and safety of preservative-free ophthalmic preparations.

CONCLUSION

In conclusion, this study examined the pharmaceutical and microbiological characteristics of three commercially available tobramycin eye drops from pharmacies in Al-Bayda, Libya. The results show that all packaging meets the sealing standards with intact closures. The solutions were clear and particulate-free, with pH values between 6.88 and 6.91, and refractive indices ranging from 1.334 to 1.335. Sterility tests did not reveal any signs of microbial growth. The antimicrobial efficacy of the preservatives against *E. coli*, *S. aureus*, and *C. albicans* was assessed, revealing no colony-forming units (CFU) on days 14, 21, and 28. Future studies should focus on the long-term stability of tobramycin eye drops and the effect of storage conditions on their pharmaceutical and antimicrobial properties. Furthermore, examining the prevalence of substandard tobramycin eye drops

in other regions of Libya and comparing the results with those obtained in this study could provide valuable insights into the regulations in the pharmaceutical market.

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Ethical Considerations

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Not applicable.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Author Contributions

Conceptualization, S.A.M. and A.S.K.; proposed and designed compounds, A.S.K. and S.A.M.; conducted experiments, S.A.M. and A.S.K.; wrote original draft preparation, A.S.K. and S.A.M.; wrote review and editing, S.A.M. and A.S.K.; this work has been reviewed and approved by all authors.

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