Page 340 to 344

Volume 2 • Issue 2 • 2018

Scientia Ricerca

Research Article

Clinical Biotechnology and Microbiology

ISSN: 2575-4750

Microbiological Quality and Preservative Efficacy of Some Eye Drops Sold in Uyo- Nigeria

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Received: January 19, 2018; **Published:** May 05, 2018

Abstract

Medications to be instilled directly into the eye are expected to be sterile and so throughout the period of use. They must, also, be properly preserved and packaged. Some eye drops offered for sale and used for the treatment of infections in Uyo-Nigeria were therefore, evaluated for their microbiological quality and preservative efficacy. Four different brands of eye drop samples commonly found in pharmacy shops in Uyo-Nigeria containing mainly gentamycin and chloramphenicol were opened and tested for bacterial contamination after exposure to the atmosphere for seven days using standard microbiological methods. This was done to assess the possibility of contamination due to handling during use. The anti- infectives were also challenged with a range of organisms to ascertain their microbiological quality. Results obtained indicated no viable microorganisms were detected in the products within the seven days of exposure confirming a high microbiological quality and preservative efficacy. Results also showed no further multiplication of organisms when they were used to challenge the products confirming antimicrobial effectiveness as well as good microbiological quality. Generally, the multi dose eye drops offered for sale in Uyo, Akwa Ibom state, Nigeria and tested are of acceptable microbial quality and high preservative efficacy.

Key words: *Medications; Microbial quality; Preservative efficacy; Organisms; Multi dose*

Volume 2 Issue 2 May 2018

Introduction

Eye drops (Ophthalmic products) are sterile preparations which contain active pharmaceutical ingredients that are intended for application to the eye. Depending on the condition being treated, eye drops may contain steroids, antibiotics, antifungals or anti-inflammatory drugs. Generally, they may be available over the counter in many pharmacies or they may be obtained from hospitals on prescription. They are sometimes supplied as multi- dose preparations and it is expected that the active agents contained therein should remain effective throughout the entire period of use (Liang., *et al*. 2006). Probably, the most important attribute of any ophthalmic product besides its quality is its ability to maintain the sterile nature of the product. Sterility in sterile products like eye drops is an absolute requirement because the presence of a single surviving microbial cell is sufficient to render the product non- sterile (Schein., *et al*. 1992).

Microorganisms form an integral part of our environment and they have so much potential for contaminating medicines during process manufacture and use (Lamikaran., *et al*. 2002). Ophthalmic preparations are expected to meet the appropriate microbiological quality criteria stipulated by standard reference books. When contamination is detected in preparations which are required to be sterile, there may be consequences which may include deterioration, product degradation (Samadi., *et al*. 2009, Rahman., *et al*. 2006) or even further contamination e.g. when used in the treatment of eye infections. Typically, it has been associated with keratitis and corneal ulcers as well as being responsible for transmitting some opportunistic as well as pathogenic organisms (Nentwich., *et al*. 2006).

The protection of multiple dose products like eye drops against microbial contamination is therefore best achieved by the addition of a suitable preservative. A preservative is an agent natural or artificial that helps to maintain the original state or quality of a product thereby preventing either degradation or extraneous contamination. They are mandatorily added to multi- dose medications like eye drops to suppress contaminants and prevent microbial contamination and biodegradation (Tasli and Cosa, 2001). It is noted however that those products intended for use during eye surgeries do not contain preservatives because they can be irritating to the tissues in the eye and are therefore, mostly packaged in single dose containers (Amrite., *et al*. 2006).

Due to the persistent problem of microbial resistance and therapeutic failures resulting from the use of various multi dose eye drops in the treatment of various eye infections, this work has become expedient. It is intended to evaluate the microbiological quality of some eye drops sold in Uyo-Nigeria as well as asses their preservative efficacy or their ability to protect themselves from possible contamination during the period of use.

Materials and Methods

Procurement of Eye drops

The eye drops used in this work were obtained from registered Pharmacy outlets in Uyo, Akwa Ibom state, Nigeria. Each container label was noted for the preservative used, batch number, manufacturing and expiry dates as well as National agency for food and drugs (NAFDAC- Nigeria) registration number.

Physical and Organoleptic Examination.

The various eye drop solutions were examined visually for acceptable appearance, clarity and presence of particles.

Evaluation of Efficacy of Preservative.

The ability of the eye drops to withstand contamination when exposed to air for a specific period of time was investigated by exposing the samples of eye drops to the atmosphere. This was done by opening the eye drop solution and placing them on the microbiology laboratory shelves over a period of seven days. After seven days, 1mL was serially diluted to achieve different concentrations ranging from 101 to 109. Aliquots of appropriate dilutions were aseptically plated out on sterile nutrient agar and Sabouraud dextrose agar plates and then incubated at 37°C for 24 hours for bacteria and 25°C for 72 hours for fungi respectively. Thereafter, growth was looked out for with the intention of determining the number of colony forming units in the product. Equal aliquots were also introduced into sterile bottles of thioglycolate broth to monitor the growth of any obligate anaerobes that may find their way into the product. The tubes were incubated for seven days after which growth was looked out for.

Evaluation of Antimicrobial Effectiveness/Microbiological Quality.

1mL of each eye drop sample was serially diluted in ten folds to achieve different concentrations ranging from 10^1 to 10^9. 1 mL of appropriate dilutions was aseptically transferred into labeled petri dishes containing adequate amounts of nutrient agar and Sabouraud dextrose agar respectively. The plates were gently swirled to mix and allowed to solidify. They were then incubated for 24 hours at 37°C for bacteria and 72 hours at 25°C for fungi. After incubation, the plates were carefully observed for growth with the intention of determining the colony forming units in the original sample of the eye drop. Equal aliquots were also introduced into sterile bottles of Thioglycolate broth and treated as in the previous experiment.

Results

Physical Examinations

All the eye drops tested had their manufacture date, expiry date and batch numbers clearly stated on their packs. They were all within their expiry dates and carried instructions that the contents should be used up within one month of opening. The volume of the content ranges from 5 to 10 mL and all eye drop samples used had National Agency for Food and Drug Administration and Control (NAFDAC) Nigeria seal of approval and number. The label disclosures also indicated the preservatives included in the eye drops.

Evaluation of Efficacy of Preservatives

Results obtained showed that no growth was observed on both the Nutrient and Saboraud agar plates after the products were exposed to the atmosphere for seven days. No surface growth, deposit or cloudiness was observed in all the media throughout the period of incubation (Tables 1 and Table 2).

Media	Dilutions															
	Sample A								Sample B							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-2}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Nutrient agar (After 24 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabouraud dextrose agar (After 72 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thioglycolate broth. (after 7 days)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1: *Microbial growth on different media after exposure of products A and B in the laboratory for seven days.*

Key: - = No growth

\+ = Growth

All results are average of triplicate plate or tube readings

Media	Dilutions															
	Sample C								Sample D							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-2}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Nutrient agar (After 24 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabouraud dextrose agar (After 72 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thioglycollate broth (After 7days)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: *Microbial growth on different media after exposure of products C and D in the laboratory for seven days.*

Key: - = No growth

\+ = Growth

All results are average of triplicate plate or tube readings

Evaluation of Antimicrobial Effectiveness/Microbiological Quality

Results obtained showed that none of the products tested showed any sign of growth after they were directly checked for the presence of any organisms. They were confirmed to be effective antimicrobial agents and therefore, of good microbiological quality (Tables 3 and 4).

Media	Dilutions															
	Sample A								Sample B							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-2}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Nutrient agar (After 24 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabouraud dextrose agar (After 72 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thioglycollate broth. (after 7days)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: *Antimicrobial effectiveness/Microbiological quality of products A and B.*

Key: - = No growth

\+ = Growth

All results are average of triplicate plate or tube readings

Media	Dilutions															
	Sample C								Sample D							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-2}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Nutrient agar (After 24 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabouraud dextrose agar (After 72 hrs.)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thioglycollate broth. (after 7days)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: *Antimicrobial effectiveness/Microbiological quality of products C and D.*

Key: - = No growth

\+ = Growth

All results are average of triplicate plate or tube readings

Discussion

Every ophthalmic product must be manufactured under conditions validated to render it sterile in its final container for the shelf life of the product (Yasukawa., *et al*. 2006). It is also known that all preparations for administration into the eye should be sterile at the point of administration. Except in some peculiar situations like during surgery, appropriate preservatives should also be added to ensure that the product remains sterile through the period of use. This is even more important because multi dose containers which are commonly used as packaging materials are opened and closed many times while the product is in use. Other information like the manufacture and expiry dates which assures the consumer of the wholesomeness of the product should also be supplied.

All the eye drops used in this work were appropriately packaged and labeled. They had their National Agency for Food Drug Administration and Control (NAFDAC) numbers, batch numbers and expiry dates properly stated. This is an indication that the products must have undergone appropriate regulatory screening before they were released into the market. The label disclosures showed that samples A, B and C had their preservatives stated while that used for sample D was not. This is an unacceptable practice which may have been as a result of carelessness on the part of the manufacturers.

Results obtained from the study showed that all the eye drops tested contained suitable preservatives since they were able to withstand microbial contamination when exposed to the laboratory environment (air) over a period of seven days (Tables 1 and Table 2).

Product D which did not have its preservative indicated on its label was also able to withstand contamination confirming that the non-disclosure may have been as a result of carelessness on the part of the manufacturers. The Thioglycolate tubes also showed no signs of growth after seven days of incubation confirming that no obligate microbes which found their way into the product survived.

The results of the microbiological quality test, was as observed in the preservative efficacy test confirming that all the products were of good microbiological quality. In conclusion, the tested eye drops sold in Uyo, Akwa Ibom State Nigeria, were found to be of good quality since they met the requirement of sterility and ability to preserve the products from the effects of contaminating organisms as required by reference books.

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