Medico Research Chronicles

ISSN No. 2394-3971

Original Research Article

EVALUATION OF ANTIMICROBIAL EFFECTIVENESS OF OPHTHALMIC DROPS SOLD IN NIGERIA PHARMACY STORES AND MARKET PLACES

[1]Oluwayemisi A. Olorode (PhD, MLS)* [2]Ogba M. Ofonime (PhD, MLS) and [1]Azere E. Orowo (BPharm)

[1]Department of Pharmaceutical Microbiology and Biotechnology, Niger Delta University, Wilberforce Island, Bayelsa State.

[2]Department of Medical Laboratory Science, University of Calabar, Nigeria.

Submitted on: January 2017
Accepted on: February 2017
For Correspondence
Email ID:
yemisiolorode@yahoo.co.uk

Abstract

Eye drops are sterile liquids for instillation into the conjunctiva sac worldwide; they are formulated and packaged in order to maintain their sterility throughout the period of use. This study was conducted to evaluate the pharmaceutical quality and antimicrobial effectiveness ophthalmic drops offered for sale in Nigeria. A total of thirty-three (33) sterile eye drops of 11 different brands (3 of each) were examined; these include Gentamicin, Chloramphenicol, Ciprofloxacin, Betaxolol, Betamethasone, Artificial tears, Hypromellose, Diclofenac, and Timolol were purchased in pharmacy stores and the other two which are natural drops were purchased from the market and bus vendor (Oster and Quick action). A standardized (using 0.5 Mac Farland turbidity standard) clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were added (according to British and United State Pharmacopoeia) to newly opened eye drops and stored at room temperature away from light. At 1hour, 3days, 7days, 14days, 21days and 28days samples were collected, plated and the number of viable microorganisms was counted after 48hrs incubation at 37^{o}C. Seven 7 (Gentamicin, Betamethasone, Chloramphenicol, Hypromellose, Artificial tears, Ciprofloxacin and Betaxolol)out of the test eye drops had significant antimicrobial activity with their anti-infective having the highest rapidity in bactericidal activity; Timolol and Diclofenac showed a reduced and poor antimicrobial activity. The findings had shown 77.8 of the test eye drops passed the antimicrobial effectiveness test while 22.2% failed. Quick action and Oster were found to contain heavy microbial growth. In conclusion, the ophthalmic drops offered for sale in Nigeria from approved medicine stores are of acceptable standard.

Keywords: Ophthalmic drops, Antimicrobial effectiveness, *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*

Introduction

Eye drops (ophthalmic drops) are a popular dosage formulation meant for instillation to the conjunctival sac. They have been used successfully for the treatment of various eye infections and are either prescribed by an ophthalmologist or sold over the counter. The use of eye drops is widespread in all regions of the world especially during the harmattan or dry season like in Nigeria, where dust particles, as well as other irritants, are easily blown into the eye. They contain medicaments dissolved or suspended in aqueous or oily vehicles. Depending on the condition being treated, they may contain steroids, antihistamines, sympathomimetics, beta receptor blockers, parasympathomimetics, parasympatholytics, prostaglandins, non-steroidal anti-inflammatory drugs (NSAID's), antibiotics, antifungal or topical anesthetics. Eye drops do not sometimes have medications in them and are only lubricating and tear replacing solutions. Since eye drops are formulations meant to be instilled into the conjunctiva sac of the eyes, there is a need for them to be sterile (totally free from microbial contaminants). For eye drop products, the requirement for sterility should be maintained throughout the period of their use. This is because while in use microbial contamination may lead to product degradation or result in ocular infections (Samadi *et al.*, 2013).

They are convenient and easily administered without causing irritation to the eye. A major disadvantage of eye drops is its imprecise dosage as there is the danger of instilling more than or less than the required dose. Another disadvantage of eye drops is the rather high possibility of contamination with micro-organism as well as foreign matters. Contamination with microorganisms is frequently seen in multiple dosage eye drop formulations as repeated use can lead to contamination. These eye drops are mostly packaged in plastic containers with droppers and patients are advised to avoid touching the tip with their fingers and the eyes (conjunctiva sac) to which they are being instilled thus to prevent contamination of the product with micro-organisms present in the fingers and conjunctiva sac. The use of contaminated eye drops may lead to a range of eye diseases, some easily treated while others may lead to blurred vision or permanent loss of sight. These reasons, therefore, demand the use of antimicrobial preservatives in the formulation of eye drops at a concentration that will impede microbial growth without causing irritation to the ocular tissues of the patient/user. A preservative is a substance that is added to products such as foods, pharmaceuticals, paints, biological samples, wood, beverages, etc. to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product while physical preservation may entail refrigeration and drying (Msgati and Titus,2012).The preservatives used in ophthalmic drops (eye drops) include; phenylmercuric nitrate or acetate, benzalkonium chloride, chlorhexidine acetate, thiomersal, para hydroxybenzoate, EDTA, chlorobutanol, benzyl alcohol, phenyl ethyl alcohol at in use concentrations. These preservatives are added to multidose eye-drops to inhibit microbial contamination and may also act synergistically with other preservatives or with other components of the formula to enhance the total effects for microbial control. Their inclusion should be at a concentration that is effective but non-toxic

to humans. The single most dominant factor characteristic of all ophthalmic products is a specification on sterility not only after preparation but throughout the period of use. This is a legal requirement that dates back to 1955 (Marchese *et al.*,2001) and is still in force today. Despite this requirement, extensive researches have not been done on the quality and antimicrobial effectiveness of commercially available eye drops purchased and used by patients. This study helps to evaluate the effectiveness of antimicrobial preservatives used in eye drops sold in Bayelsa and Rivers states, Nigeria.

Materials and Method

Study setting

The study was carried out in Bayelsa state, Nigeria and most of the eye drops were purchased in her capital city, Yenagoa. Bayelsa is a state in southern Nigeria in the core Niger Delta region, between Delta state and Rivers state. The four main languages spoken are Izon, Nembe, Epie-Atissa and Ogbia. Like the rest of Nigeria, English is the official language. Bayelsa has a riverine and estuarine setting and a lot of her communities are almost completely surrounded by water hence making these communities inaccessible by road. Two of the eye drops used was purchased in Rivers state, Nigeria. Her capital city is Port-Harcourt and the state is bounded on the south by the Atlantic Ocean, to the North by Imo, Abia and Anambra states, to the east by Akwa Ibom state and to the west by Bayelsa and Delta states.

Eye drops used

A total of 33 eye drops of 11 different brands (3 from each brand) were used in this study. Nine (9) brands of the eye drops were purchased from registered pharmacy premises in Yenagoa, Bayelsa state, Nigeria. These include preparations of Gentamicin sulfate, Chloramphenicol, Timolol, Betaxolol hydrochloride, Hypromellose, Diclofenac sodium, Artificial tears, and Betamethasone eye drops from different manufacturers. Two (2) of the brands of eye drops (3 from each brand) were purchased from medicine vendors one from a marketplace and the other from a bus vendor in Port Harcourt, Rivers state. The eye drops were; Quick action® natural eye drop and Oster ® eye drop. Both of them are locally produced and they contain natural ingredients. The prices of the study eye drop ranged from #200 to #1300 (Nigerian money). Each container label was noted for the following: Contents, Manufacturer, Manufacturing and Expiry dates, preservatives used and batch number.

Materials used

Sterile McCartney bottles, syringes (2ml and5ml), sterile pipette, cotton wool, Petri dishes, wire loop, autoclave, dryer, incubator, foil paper, water bath, beakers, colony counter, sterile hockey stick, analytical balance.

Media used

Nutrient agar, Nutrient broth, freshly prepared 0.5Mcfarland standards and thioglycollate medium (serving as neutralizer) were used.

Preparation of inoculum for the challenge test

Reference strains of microorganisms used were from medical samples culture collections maintained in pharmaceutical microbiology laboratory, faculty of pharmacy, Niger Delta University and these include inoculums of *Pseudomonas aeruginosa, Escherichia coli,* and

Staphylococcus aureus. The inoculums of the study organisms were taken from nutrient agar slope culture and subculture in nutrient broth and incubated at 37^{o}C overnight. The inoculum was then streaked on nutrient agar plates to get discrete colonies. The identity of each microorganism was reassessed using colony morphology and biochemical tests. Three to five colonies were then picked using a sterile wire loop and transferred into sterile saline to obtain a microbial count of about $1*10^{8}$ colony forming a unit (CFU) per ml on comparison with the turbidity of 0.5Macfarland standard previously prepared.

Negative control test

The negative control test was carried out by plating out 0.1ml of each eye drop on nutrient agar of each eye drop before the inoculation of the microorganism to determine the initial microbial load. The plates were then incubated for 24hours after which the colony forming units were then enumerated.

Challenge test of the eye drops with the microorganisms

The eye drops were transferred aseptically into sterile McCartney bottles and capped. Challenging organisms of 0.15ml, 0.1ml, and 0.05ml (equivalent to 1% of the total volume of the 15ml,10ml, and 5ml eye drops) was inoculated into each eye drop so that for a brand of eye drop of 3 one contained *Pseudomonas aeruginosa,* the other *Staphylococcus aureus,* and the third *Escherichia coli.* The eye drops were then mixed thoroughly to obtain even distribution of the microorganism throughout the eye drop. The inoculated product was then maintained at 20−25°c (room temperature) away from light, throughout the test period. At 1hour, 3 days, 7 days, 14 days, 21 days and 28 days interval, 1ml of the sample were withdrawn and inoculated into 9ml thioglycollate medium to neutralize the preservative before plating out on nutrient agar in duplicates and incubated at 37°c for 48hours to determine the number of viable organisms on each plate using the colony counter and the mean value were computed from the number of colonies formed on

Results

Properties of the eye drops

Table 1.0 showed the properties of the study eye drops. Most of the products have their manufacturing date, expiry date, batch number and NAFDAC number clearly stated on the package. The exceptions to this were products J (the manufacturing date, expiry date, batch number and NAFDAC number was not indicated on the package), K (batch number and NAFDAC number not indicated) and D (NAFDAC number not indicated). The eye drops were all within their expiry date and the volumes ranged from 5-15ml. From the nine(9) brands of eye drops, six (6) (Products A, B, D,E,F, and G) contained benzalkonium chloride as preservative ranging from 0.01%-0.02% with product D having a combination of benzalkonium chloride (0.01%) and disodium edetate (0.05%) as a preservative. Product C contained thiomersal (0.02mg/ml) as preservative while Product I contained phenylmercuric nitrate (0.001%). On the other hand, Products H, J and K's specific preservative used was not stated.

Results

Table1: Shows Properties of the Different Brands of Eye drops

Product code	Content	Preservative	Country of manufacture	Mfd date	Expiry date	Batch number	Nafdac number	Volume (ml)	Duration after first opening
A	Gentamicin sulphate	Benzalkonium chloride (0.02%)	India	Jun-14	May-17	Yes	Yes	10ml	1 month
B	Betaxolol HCl	Benzalkonium chloride (0.1mg)	Belgium	May-13	Aug-16	Yes	Yes	5ml	1 month
C	Chloramphenicol	Thiomersal (0.02mg/ml)	India	Oct-13	Sep-16	Yes	Yes	10ml	28 days
D	Artificial tears	Benzalkonium chloride (0.01%), disodium edetate (0.05%)	Belgium	Jul-14	Jun-17	Yes	No	15ml	Nil
E	Ciprofloxacin	Benzalkonium chloride (0.01 %)	Nigeria	Jul-13	Jun-16	Yes	Yes	10ml	1 month
F	Hypromellose	Benzalkonium chloride (0.01%)	United kingdom	Jul-14	Jul-17	Yes	Yes	10ml	28 days
G	Timolol	Benzalkonium chloride (0.01%)	India	Sep-14	Aug-17	Yes	Yes	10ml	28 days
H	Betamethasone + neomycin	Nil	Nigeria	Nov-14	Oct-17	Yes	Yes	10ml	28 days
I	Diclofenac sodium	Phenylmecuric nitrate (0.001%)	India	Feb-14	Jan-17	Yes	Yes	10ml	1 month
J	Oster natural eye drop	Nil	Nigeria	Nil	Nil	no	No	10ml	2 months
K	Quick action natural eye drop	Nil	Nigeria	Feb-15	Feb-18	No	No	10ml	1 month

Table2 shows the result of the negative control test of the eye drops when cultured on nutrient agar and incubated at 37◦c for 24 hours. All the eye drops were found to be sterile with the exception of Oster and Quick action eye drops.

Table 2: Negative control test of the eye drops

Product code	**Eye drop brand**	**Number Examined**	**Colony forming unit/ml**
A	Gentamicin	1	0
		2	0
		3	0
B	Betaxolol	1	0
		2	0
		3	0
C	Chloramphenicol	1	0
		2	0
		3	0
D	Artificial tears	1	0
		2	0
		3	0
E	Ciprofloxacin	1	0
		2	0
		3	0
F	Hypromellose	1	0
		2	0
		3	0
G	Timolol	1	0
		2	0
		3	0
H	Betamethasone	1	0
		2	0
		3	0
I	Diclofenac	1	0
		2	0
		3	0
J	Oster eye drop	1	$>10^5$
		2	$>10^5$
		3	$>10^5$
K	Quick action	1	$>10^5$
		2	$>10^5$
		3	$>10^5$

TABLE 3: Negative Control Test of the Eye drops

Product code	Eye drop brand	Number examined	Colony forming unit/ml
A	Gentamicin	1	0
		2	0
		3	0
B	Betaxolol	1	0
		2	0
		3	0
C	Chloramphenicol	1	0
		2	0
		3	0
D	Artificial tears	1	0
		2	0
		3	0
E	Ciprofloxacin	1	0
		2	0
		3	0
F	Hypromellose	1	0
		2	0
		3	0
G	Timolol	1	0
		2	0
		3	0
H	Betamethasone	1	0
		2	0
		3	0
I	Diclofenac	1	0
		2	0
		3	0
J	Oster eye drop	1	$>10^5$
		2	$>10^5$
		3	$>10^5$
K	Quick action	1	$>10^5$
		2	$>10^5$
		3	$>10^5$

Figures 1.0 to 6.0 depict bar charts of the challenge tests of ophthalmic drops to the test organisms. Figure 1.0 shows the bar chart of *Escherichia coli* against the study brands of eye drops, Gentamycin, Chloramphenicol, Ciprofloxacin, Timolol, Betaxolol, Betamethasone, Hypromellose, Artificial tears, Quick action, Oster. Figure 2.0 showed the bar chart of *Staphylococcus aureus* against the study eye drops listed above, while figure 3.0 depicted the graph of *Pseudomonas aeruginosa* against the study eye drops. Figures 4.0 to 6.0 expressed the graph of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* challenge test against the Quick action and Oster respectively.

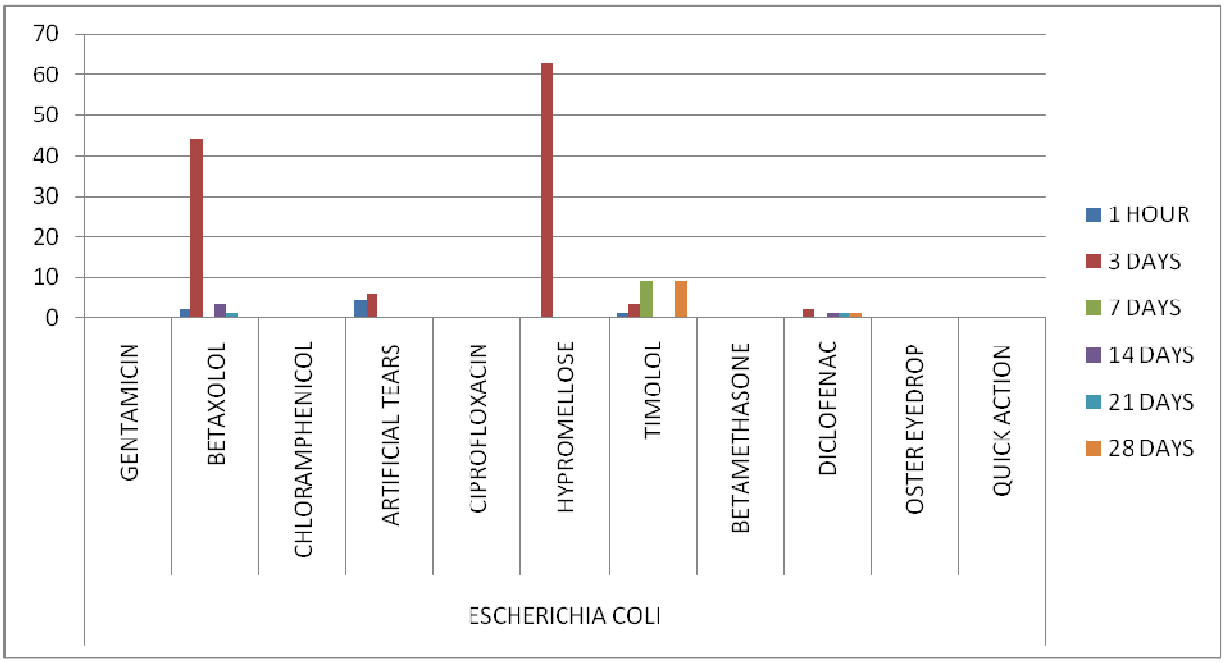

Figure 1: Showing Challenge test of Ophthalmic drops to *Escherichia coli*

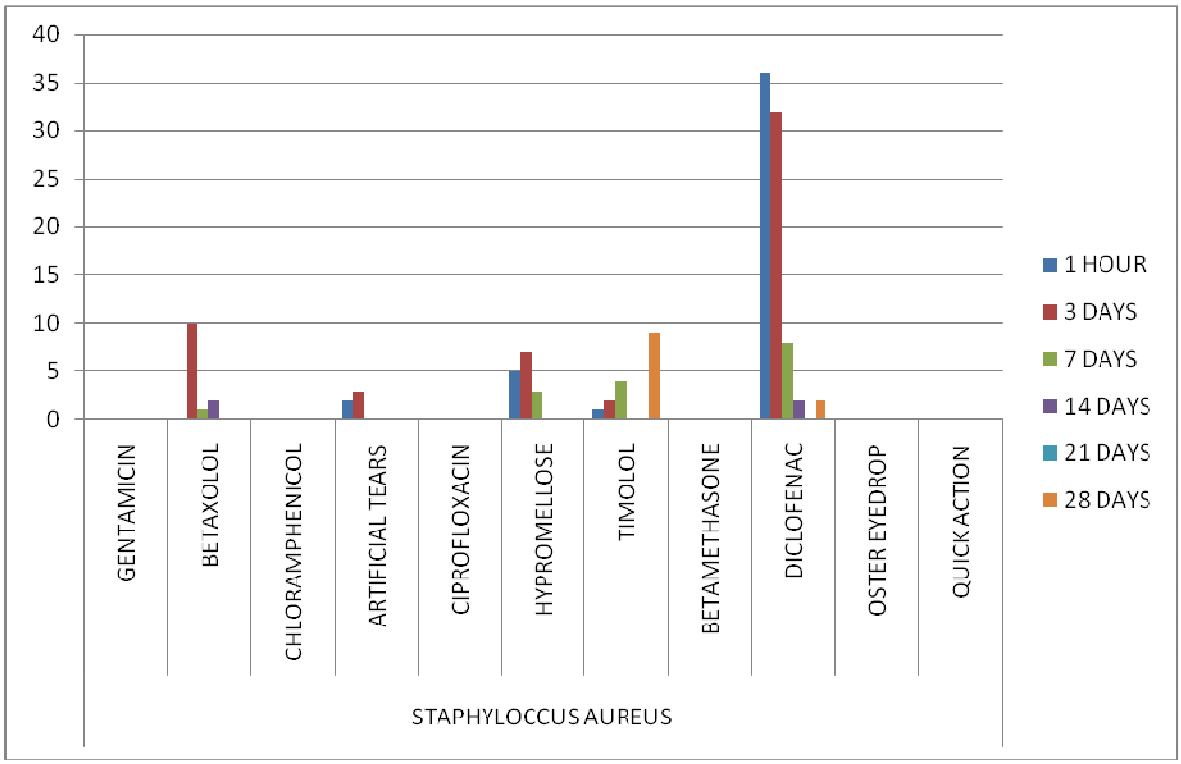

Figure 2: showing challenge test of the ophthalmic drops to *Staphylococcus aureus*

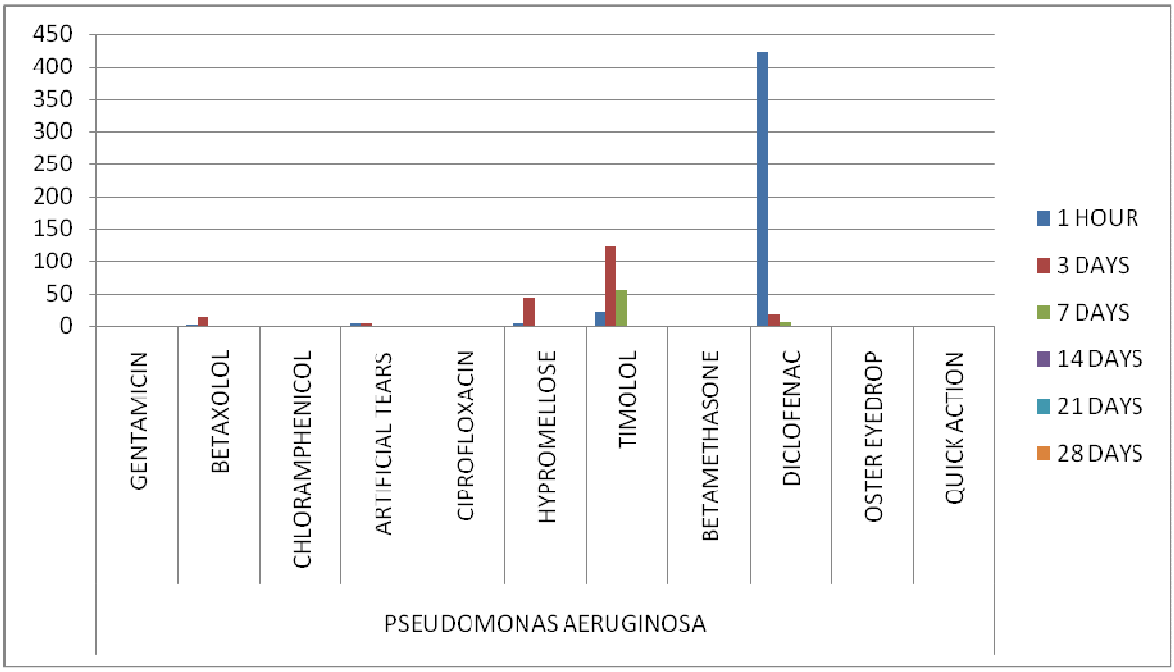

Figure 3: showing challenge test of the ophthalmic drops to *Pseudomonas aeruginosa*

Note: From the bar charts (1, 2 and 3), a growth batch pattern is observed at 1 hour, 3 days, 7 days, 21 days and 28 days when the eye drops are inoculated with the test organisms. It should be noted however that the supposed no growth value as seen in the charts for Oster® and Quick action® eye drops is due to the high number of colony forming units observed relative to the other eye drops, hence the need for a separate bar chart to show the microbial growth pattern for these eye drops.

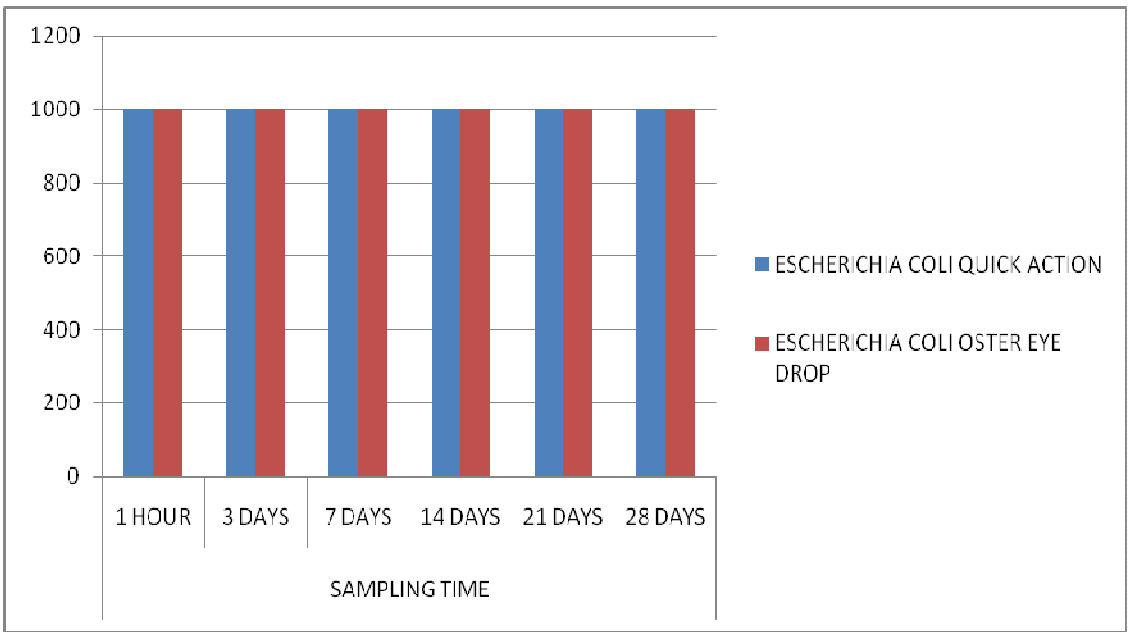

Figure 4: Showing challenge test of Quick action and Oster eye drops to *Escherichia coli*

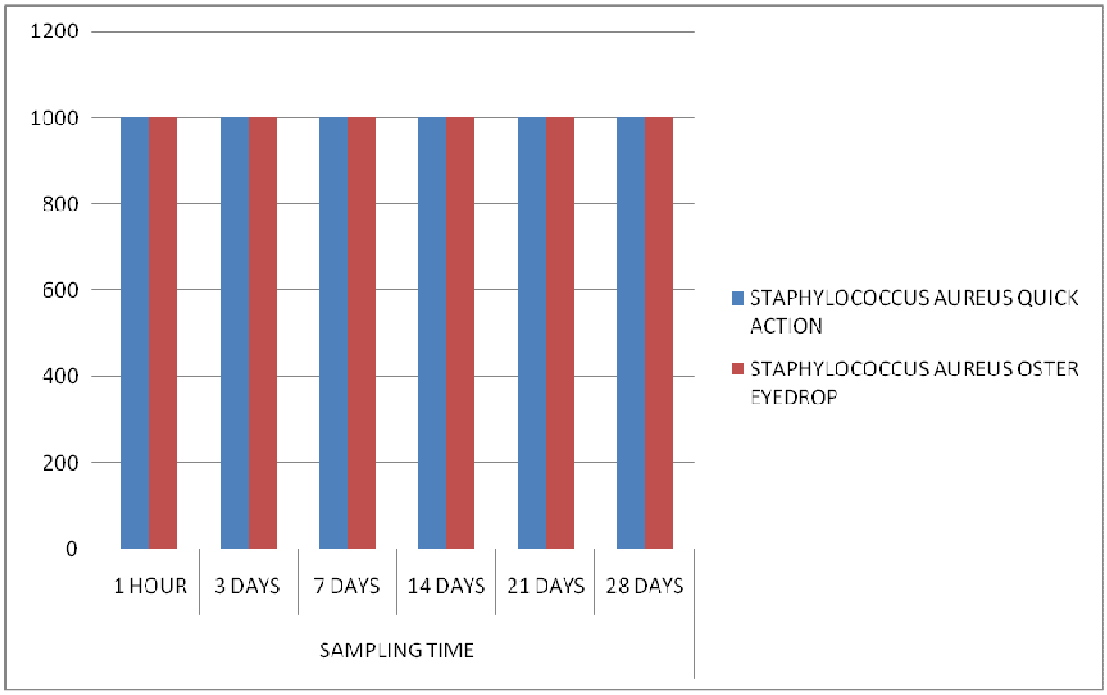

Figure 5: showing challenge test of Quick action and Oster eye drops to *Staphylococcus aureus*

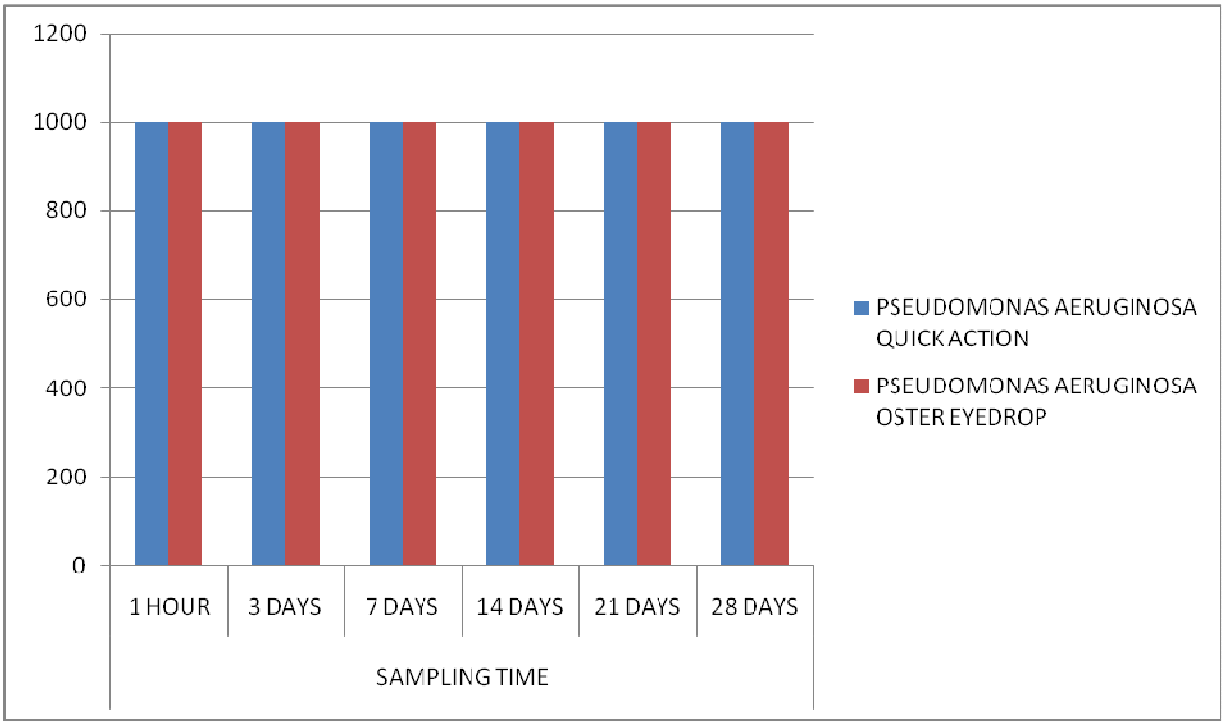

Figure 6.0: Showing challenge test of Quick action and Oster eye drops to *Pseudomonas aeruginosa*

Discussion

The antimicrobial preservative efficacy of the eye drops challenged with *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* is shown in Table 3 and the negative control test is shown in Table 2. The negative control test of the eye drops without inoculation of the microorganism showed that the eye drops were sterile with the exception of the natural eye drops (Quick action ® and Oster ® eye drops) which showed a high level of microbial contamination.

The eye drop products used for evaluation in this study were purchased in Yenagoa, Bayelsa state and Port- Harcourt, Rivers state. It should be noted that the same eye drop brands are available and on sale throughout the country.

From the antimicrobial preservative challenge test carried out, it was observed that the anti-infective eye drops (Gentamicin, Chloramphenicol and Ciprofloxacin eye drops) exhibited rapid bactericidal activity showing no growth at 1 hour, 3days, 7days, 14 days, 21 days and 28 days this is consistent with a study carried out by Akinkunmi (2013). These results are not unexpected since gentamicin, chloramphenicol and ciprofloxacin are antimicrobial agents with broad spectrum activities against a wide range of bacteria including *S. aureus, Pseudomonas sp, Proteus* and many coliform bacilli (Rosenthal *et al.,* 2006). This produces a synergistic effect with the preservative against any invading microorganism (Akinkunmi, 2013). The Betamethasone eye drop also exhibited this property although the preservative used in its formulation was not stated on the label. This eye drop, however, is a combination formulation containing Betamethasone (0.1%^{w}/v) and Neomycin (0.5% ^{w}/v). Neomycin is an aminoglycoside and has excellent activity against Gram-negative and good activity against Gram-positive bacteria. This broad spectrum of activity of Neomycin may be responsible for its activity on *E. coli, S. aureus,* and *Ps. aeruginosa* observed during the challenge test. Artificial tears challenged with *E. coli* and *S. aureus* showed no growth from the 7th day and no growth at the 14th day challenged with *Ps. aeruginosa* and no recovery of the microorganism by the 28th day which is in compliance with the No recovery (N.R) term of British Pharmacopoeia (BP) 'A' criteria which requires no recovery of viable cells after the 28 days. Hypromellose eye drop also showed no growth from the 14th day with no recovery of viable cells on the 28th day when challenged with the test organisms. Betaxolol eye drop challenged with *Escherichia coli* showed no growth at the 7th day to the 28th day while challenged with *Staphylococcus aureus* and *Pseudomonas aeruginosa* there was no growth from the 14th day onwards.

Diclofenac eye drop challenged with the micro-organism showed microbial presence even after a 14th day. The active ingredient (Diclofenac) does not possess any antimicrobial activity and the preservative employed in its formulation (phenylmercuric nitrate 0.001%) may not possess adequate antimicrobial activity against the selected bacteria used in this study. It should also be noted that the recommended concentration of phenylmercuric nitrate as preservative system in eye drops is 0.002% according to the British pharmacopeia (2005) and United states pharmacopeia (2012) hence the concentration of the preservative used in this eye drop formulation falls below the recommended standard and may be responsible for its poor activity against the study organisms. Timolol eye drop had *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* growth except on 14 days, but re-growth of bacteria cells on 28 days. This pattern previously documented for *Pseudomonas aeruginosa* by Akers and Taylor (1990 and T I P 2015), occurs for a variety of reasons; of these, the loss of preservative stability/activity, mutational or

physiological adaptive changes of the microbial cells and the selection of more resistant survivors have to be considered. In *Pseudomonas aeruginosa* and other *Pseudomonas spp* the genetic ability to synthesize an alginate based biofilm playing an important role in cell adhesion and in the protection against the inhibitory effects of antibacterial agents is extensively described (Williams, 2011; Nicole, 2014). Natural eye drops (Oster and Quick action) only showed a reflection of their negative control test. There was heavy microbial growth from 1 hour to the 28th day when challenged with the test organisms.

Ophthalmic products are required to be packaged in such a way that they will retard contamination. This is because multi-dose containers may be opened and closed and used many times by the consumer. During administration, the pipette attached to the cap of the bottle comes completely out of the container and this exposes the contents of the bottle. Previous studies on preserved eye drops have concluded that pathogenic Gram-negative bacteria are more likely to grow in the bottle reservoir than Gram-positive organisms which are mainly commensals in the environment (Sunita, 2013; Schein *et al.*, 1992; Geyer *et al.*, 1995). All the study eye drops have their tips attached to the bottle. This is of a great advantage of the old types of eye drops container that comprised a dropper separately packaged from the main container. These old container types have been reported to encourage contamination during use leading to serious ocular infections (Stevens and Matheson, 1992). Spillage of the contents can also increase the chance of contamination. Poor technique in administering the drops is a further risk factor for contamination especially if patients are self-administering in an outpatient setting. Elderly patients with provision and co-ordination may inadvertently touch their eyes or skin with the pipette dropper and on insertion of the dropper back into the container after use, may again contaminate the container (Rahman *et al*., 2006). Previous studies on preserved eye drops by Schein *et al* (1992) have found high contamination rates in beta blockers, steroid drops, and ocular lubricants and concluded that acetylcysteine, hypromellose and prednisolone drops are prone to contamination even in the presence of preservatives (David and Malik 2009).

The requirement on the efficacy of antimicrobial preservation in the USP for bacteria requires not less than 1 log reduction from the initial count after 7 days and not less than 3 log reductions from the initial count after 14 days and no increase from the 14 days count after 28 days (USP 29, 2006). The results obtained from this study showed that the Gentamicin, Chloramphenicol, Ciprofloxacin, Artificial tears, Betamethasone+neomycin, Hypromellose and Betaxolol eye drops had significant activity against the test organisms due to the preservative system employed in their formulation and intrinsic antimicrobial activity of their active ingredient. The preservative systems employed in Timolol and Diclofenac eye drops did not possess adequate antimicrobial activity against the test organisms.

Conclusion

In conclusion, seven (7) out of the 9 study brands of eye drops (Gentamicin, Chloramphenicol, Ciprofloxacin, Betamethasone, Artificial tears, Hypromellose and Betaxolol eye drops) were of appropriate microbial quality since they were shown to have complied with official requirements with respect to sterility and demonstrated ability to effectively kill microbes as required. Timolol and Diclofenac had weak preservatives. Quick action and Oyster did not meet the sterility standard. Since 77.8% of the test eye drops showed significant antimicrobial effectiveness, while 22.2% showed poor antimicrobial effectiveness profile, it can be concluded that eye drops offered for sale

and use in Nigerian pharmacy stores are of acceptable microbial quality and possessed good antimicrobial profile.

Recommendations

For quality to be maintained throughout the use of the products, it is recommended that patients should adhere to standard guidelines of using eye drops. Each eye drop product should not be used by more than one person, the tip of the bottle should not come in contact with the hands or eye or other objects and the eye drops should be stored as recommended to avoid contamination and assurance of sterility of the eye drop product throughout its period of use. The regulatory agency in Nigeria, National Agency for Food and Drug Administration and Control (NAFDAC), should checkmate the illicit manufacture and sale of natural eye drop products in the country as those encountered in the course of this study (Quick action ® and Oster ® eye drops). The dates of manufacture, expiry is also important and should be specified, this would give an idea about the timeframe, the wholesomeness of the product can be assured and the general public should purchase NAFDAC registered products.

References

- Akers, M. J. and Taylor, C. J. 1990. Official methods of preservative evaluation and testing. In: Guide to Microbiological control in Pharmaceuticals e.d Denyer, S and Baird, R. pp 292- 312 Chichester: Ellis Horwood
- Akinkunmi E. O. 2013. An Evaluation of the Pharmaceutical Quality and Antimicrobial Effectiveness of some frequently used eye drop products available for sale in Nigeria. *Ann Trop Med Public health*; Vol. 6: pages 221-226
- British Pharmacopeia Codex (2005). Eye drops and preservatives used in eyedrops. 11th edition .London; The pharmaceutical press. Pages 346-347
- David, P.F and Malik Y.K. (2009). Preservatives in topical ophthalmic medications; historical and clinical perspectives. *Expert Rev Opthalmol* 2009; 4(1) 59-64
- Greyer, O. Bottom, E J. Podos, S. M. 1995. Microbial contamination of medications used to treat glaucoma. *Br J Ophthalmol* Vol. 7 pages 337 – 379
- Marchese, A. Bozzolasco M. Gualco, L. Schito G. C. and Debbia, E. A. 2001. Evaluation of Spontaneous Contamination of Ocular Medications. Chemotherapy. Vol 47 pages 304- 308 Msagati, A and Titus A.M. (2012).The chemistry of food additives and preservatives. Retrieved from http:/www.eblib.com
- Nicole Vu, Kevin Nguyen, and Thomas CK (2014). The essentials of United states pharmacopeia chapter 51 antimicrobial effectiveness testing and its application in pharmaceutical compounding. *International journal of pharmaceutical compounding* Vol. 18. No 2;123-130
- Rahman, M. O. Tejwani, D. Wilson, J. A. Butcher, I. and Ramaesh, K. 2006. Microbial Contamination of Preservative Free Eye drops in Multiple Application Containers. Br. J Ophthalmol 2 Vol. 90 no 2 pages 138- 141
- Rosenthal, R. A. Buck, S. I. Henry, C. L. and Schlich, B. A. 2006. Evaluation of the Preserving Efficacy of Lubricant eye drop with a novel preservative system. J. Ocul Pharmacol Ther Vol. 22 pages 440-448
- Samadi, N. Tarighi, P. Fazeli, M. R. and Mehrgan H. 2009. Evaluation of Antimicrobial Effectiveness of Ophthalmic drops according to the Pharmacopeia Tests Criteria DARU Vol. 17 pages no 1, 13 – 18
- Schein, O. D. Hibberd, P. L. Starck T. 1992. Microbial Contamination of In

uses Ocular Medications. Arch ophthalmol, pages 11082 – 11085

- Steven, I. D. and Matheson, M. M. 1992. Survey of the Contamination of Eye Drops of Hospital in Patients and Recommendations for the Changing of Current Practice in Eye Drop Dispensing. Br J Opthalmol Vol. 76, pages 36- 38
- Sunita R. and Andrew I. (2013). Glaucoma medications and their side effects. www.glaucoma.org
- The International Pharmacopeia TIP. (2015). Ophthalmic products. 5th edition published in accordance with World Health Assembly Resolution. WHA 3.10, WHO Handbook of resolutions and decisions. Vol. 1 page 127.
- United States Pharmacopeia. 2012. National Formulary 31. Rockville MD, US Pharmacopeia convention inc pages 54 – 55
- United States Pharmacopeia. 2006. Antimicrobial Effectiveness Testing. Rockville MD: The United State Pharmacopeia convention INC; USP (29). Pages 2499 – 2500
- Williams B. Potter (2011). Steroids ; use with caution and with confidence. Pennsylvania college of optometry. Retrieved from: www.reviewofoptometry.com