Topical Ocular Anesthetics Harbour Clinically Important Microbes

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Abstract

Purpose: The study was to determine clinically important microbial contaminants of topical ocular anesthetic medications used in eye centers in Ghana.

Methods: A cross-section of eye clinics was sampled for the topical ocular anesthetic agents. Standard laboratory procedures and protocols were observed in culturing the samples on different Agars. Microscopy and various biochemical tests were performed to identify microbial species. Antimicrobial susceptibility tests were also performed to ascertain the clinical importance of the isolated microbes.

Results: A total of 27 anesthetic agent were obtained (which consisted 15 Proparacaine and 12 Amethocaine), from which 87 bacteria were isolated which included *Bacilli spp.* 26(29.89%), *Coagulase Negative Staphylococci spp.* 17(19.54%), *Moraxella spp.* 17(19.54%), *Staphylococcus aureus* 8(9.19%), *Streptococcus spp.* 3(3.45%), *Klebsiella spp.* 3(3.45%), *Pseudomonas spp.* 1(1.15%), *Proteus spp.* 7(8.05%), *Escherichia coli.* 2(2.30%), and *Shigella spp.* 3(3.45%). There were 22 isolated fungal contaminants mainly *Penicillium spp.* 7(31.82%), *Cephalosporium spp.* 5(22.73%), *Aspergillus spp* 4(18.18%), *Cercospora spp.* 2(9.09%), and *Cladosporium spp.* 4(18.18%). The anesthetic agent with the most bacterial contamination was Proparacaine 44(50.57%) followed by Amethocaine 43(49.43%). Also, both agents were equally contaminated with fungus 11(50.0%) in each. Gentamicin was the only antibiotics that showed 100% activity against all the bacterial isolates. Fungal contaminants were more susceptible to Ketoconazole as compared to Fluconazole ($p \leq 0.05$).

Conclusion: Topical ocular anesthetic preparations used in clinical settings in Ghana are contaminated with clinically important microbes as the isolated bacteria were susceptible only to Gentamicin and fungi to Ketoconazole and Fluconazole.

Keywords: Anesthetics, Ocular infections, Amethocaine, Proparacaine

Introduction

Topical ocular anesthetics are usually pharmaceutical products that are capable of blocking the transmission of pain signals from the nerve endings of the eye to the brain.[1] They may be in the form of eye drop, gel or ointment and are used on the eyes before surgery, after injury, or before certain procedures such tonometry and foreign body removal.[2] Examples of local anesthetics include Alcaine (generic name: proparacaine), Altacaine, Ocu-Caine and Amethocaine (a derivative of Tetracaine).[3] Due to their importance

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in eye care, the possibilities of abuse by patients usually occurs.[4] Given the utility of these agents in eye care, contamination of their containers or solution itself could possibly pose a risk of infection to patients. The risk of ocular infections from contaminated anesthetic solution is deepened by the potency of a mere single instillation of anesthetic solutions to cause intercellular spacing, decrease in the microvilli and microplicae, and prominence of the cell nucleus.[5]

While precautions for use have been documented to ensure optimum performances,[6] improper handling of these preparations may compromise their potency and in some cases cause deleterious effects. The presumption that they are sterile at first opening[7] and the expiry date are easily perceived by both professional and non-professional users, contamination from microbial agents is usually understated. Again these contaminations are not noticeable to the naked eye and require microbial analysis which in most cases requires expertise. The issue of contaminated eye drops by microbes has been reported by several authors,[7-10] that notwithstanding, local ophthalmic anesthetics have been touted to possess supplemental antimicrobial function and could be resilient against microbial contamination.[7,11] It is in the light of this that a particular attention ought to be paid to in use local ophthalmic anesthetics to ascertain their sterility in the course of time. Commonly isolated bacteria from eye drops include coagulase-negative *Staphylococcus, Staphylococcus aureus, Pseudomonas, Bacillus, Proteus, Haemophilus, Enterobacter, Serratia, and Klebsiella* species.[7] Some of which results in serious infections of the eye especially among contact lens wearers, patients who have undergone recent ocular surgeries and worsening of preexisting ocular surface disease, dry eye, and lid deformity.[12-14] Bacterial keratitis in particular is known to be the leading cause of monocular blindness in the developing countries[15,16] with contaminated ophthalmic solutions indicated as a common risk factor.[12]

Methods

Sampling procedure

Data was collected across the ten regions of Ghana from 50 different eye clinics chosen from a list of 188 eye clinics provided by the Ghana Eye Secretariat. The criterion for the selection of the 50 clinics was informed by whether they have the complement of eye care staff (Ophthalmologists, Optometrists, Opticians and Ophthalmic nurses) or referral eye centers most of which perform procedures that require the use of a variety of ocular anesthetic agents. Selected clinics were not given prior notice of the research team's visit to collect samples. This was to forestall any changes and modifications to their usual practices regarding their use of these agents at their facilities. In each of the selected clinics, the anesthetics were collected based on their availability once access was granted to the researchers.

Sample collection procedure

Some 2ml each of the anesthetic was collected using sterile 2.5 ml syringe (one for each sample) fitted with a 20 gauge needle into a Sterile hermetic bottles (one for each sample) enveloped in a pre-sterilized plastic zip bags. The samples were transported under aseptic conditions to laboratory for microbiological assay. All samples were processed within 12 hours of collection.

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Culturing and identification of microbes

Serial dilution of each sample was done to the fifth dilution using peptone water. After which they were plated on nutrient agar using pour plating method and incubated for 18 to 24 hours at 37°C. Both qualitative and quantitative analyses were used to examine the growth pattern and characteristics on nutrient agar and the findings were documented. For each colony of bacteria obtained after isolating them from nutrient agar, they were inoculated in nutrient broth and incubated overnight under aerobic condition at 37˚C. Colonies of bacteria growth from nutrient broth were sub-cultured on MacConkey and Blood agar (all from Oxoid Limited, Thermo Fisher Scientific Inc., UK) using streaking method to obtained pure colonies by incubating overnight under aerobic condition at 37˚C. To avoid contamination of other samples, Loop flaming was always done per plate streaking. Morphological identification was made on the colonies. For pure colonies obtain from both Blood and MacConkey agars, Gram staining and microscopy was done to differentiate Gram positive bacteria from Gram negative bacteria. Further identification of Gram positive bacteria obtained was done using Catalase, Coagulase and Mannitol agars and gram negative bacteria were further identified using Oxidase, Indole, Urease, Citrate and Triple sugar agar (TSI). Identified bacteria were subjected to antibiotic sensitivity testing.

Sabouraud Dextrose Agar, SDA (Oxoid Limited, Thermo Fisher Scientific Inc., UK) was prepared in conical flask. The SDA was allowed to cool to 45-50 ˚C, and 50mg of Chloramphenicol was added to 1L agar and swelled to mix. The SDA media was then poured into autoclaved petri dishes to solidify. Some 2ml of the 5^{th} dilution of each sample were plated on the prepared SDA using streaking method and incubated at room temperature for 5-7 days under aerobic condition. Morphological characteristics of various fungal growths were examined and recorded. Lactophenol cotton staining was completed for each fungal growth, for microscopic examination. Morphological characteristics of stained fungus under the microscope were examined and documented.

Antimicrobial sensitivity testing

Identified sub-cultured organisms from both Blood and MacConkey agar were picked using sterile loops and emulsified in 3ml of sterile normal saline. Turbidity was verified by comparing it to 0.5 McFarland standard solutions. There was a visual comparison between the inoculum tube and the 0.5 McFarland standard using a card with a white background and contrasting black line. An already prepared Mueller Hinton agar plates with dried surface were inoculated by streaking the prepared colony in the saline solution by swabbing over its entire surface. Placement of the antibiotic's disks (A multi-disk for antimicrobial susceptibility testing from Axiom laboratories) using sterilized forceps aseptically was used to place the antimicrobial impregnated disk on the surface of the agar. The disk was press gently using sterile forceps to ensure a complete contact with the agar surface and adequate diffusion of the drugs into the agar. The plate was inverted and incubated for 24 hours at 37˚C after which measurement of inhibition zone size was determined from the center of the disk to a point on the circumference on the zone where a distinct edge is to the nearest millimeter.

The method of well diffusion was employed in the fungal sensitivity testing which assessed the efficacy of Ketoconazole and Fluconazole at dose levels of 5 to 15mg/ml. A sterile swab was dipped into the standardized inoculum and used to inoculate evenly the surface of already prepared Sabouraud Dextrose Agar. The wells were created in the plated media and then incubated at 25°C for 5 days.

Statistical Analysis

A one-way ANOVA was used to determine if the effect of treatment at different dose levels differ on the isolated group of species and the Bonferroni post-test was used to determine which groups were statistically significantly different from each other. Values were expressed as frequencies and percentages. At $P \leq 0.05$, there was statistically significant difference between variance of the groups. GraphPad Prism (GraphPad, version 5.03; La Jolla, CA, USA) was used in the data analysis.

Ethical considerations

The study protocol was approved by Department of Biomedical Science Ethics Committee, University of Cape Coast. Permissions were sought from their respective managements the facilities on arrival of the research team. Biosafety guidelines for protection of personnel in the laboratory were observed.

Results

Profile of collected samples

Forty (40) out of fifty (50) facilities selected from the list obtained from Ghana Eye Secretariat granted access to their facility and therefore participated in the study. Across the 10 regions, 27 samples were obtained with at least a sample from each region. Of the 27 multi dose anesthetics agent sampled, 15(55.56%) of the agents were Proparacaine and 12(44.44%) were Amethocaine being the least (Table 1). All the drug samples collected were preserved either with Benzalkonium chloride, Chlorobutanol or Sodium perborate.

Profile of cultured samples

Bacteriological analysis indicated 87 bacterial isolates from 27 anesthetic agents of which the most, 44 bacteria were isolated from Proparacaine and least 43 from Amethocaine. *Bacillus* spp. was the most encountered and the least was *Pseudomonas* spp. (Table 2). Of these 53 were Gram positive and 34 Gram negative respectively. On the Sabouraud Dextrose Agar, 22 fungi were isolated of which 11(50.0 %) were isolated from Amethocaine and another 11 (50.0 %) from Proparacaine. Five different species of fungi were isolated namely, *Aspergillus* spp., *Cladosporium* spp., *Cephalosporium* spp., *Penicillium* spp., *Cercospora* spp.

Antimicrobial susceptibility test

Table 3 shows the result of antimicrobial susceptibility test of the isolated species of bacteria. Gentamicin proved effective in inhibiting bacterial growth as compared to the other conventional antibiotics. Figures 1&2 are also the summary of the minimum inhibition concentration (MIC) of Ketoconazole and Fluconazole on identified fungi species. Ketoconazole and Fluconazole at doses of 5 to 15mg/ml did show comparable inhibitory effect for all fungal isolates ($p>0.05$).

Discussion

As a rule of thumb ophthalmic preparations are expected to meet a strict requirement of sterility.[17] For this reason, preservatives are added to these ophthalmic preparations as an important component to provide antimicrobial activity in their bottles.[18] Anesthetics in particular have been documented to possess antimicrobial activities whether preserved or unpreserved.[11,19-21] Nevertheless, a recent study has reported microbial contamination of local anesthetics in a clinical setting in Ethiopia.[7] In this study Amethocaine (Teteracaine) and Alcaine (Proparacaine) were found to be contaminated with clinically important microbes as these were resistant strains of bacteria (Table 3). This might have been accounted for by their ability to overwhelm the anesthetic solutions' antimicrobial potency. Microbial activity in contaminated ophthalmic preparations is known to alter pH of the preparation affecting its efficacy.[22] The antimicrobial

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activity of anesthetics is dependent on other factors such as its concentration and temperature as they have been reported to bactericidal at 37^{0}C and not at room temperature.[11] All the stocks from which samples were collected were noted to be kept under room temperature. This might have influenced the antimicrobial efficacy of the anesthetic solutions.

The least contaminant found was *Pseudomonas* spp. and unexpectedly low *Staphylococcus aureus* contamination (Table 3). It would have been expected that *S. aureus* being the most ubiquitous commensal organisms of the conjunctiva, eyelids and skin should have abound. This is no surprise as the limited microbial activity of Amethocaine and Proparacaine are remarkable against these two specific microbes (i.e *Pseudomonas* spp and *Staphylococcus aureus*).[23,24] Microbial contamination of these drugs could worsen a patient's existing condition or result in a different disorder rather than aiding in the diagnostic process. Research has identified ophthalmic solutions which include topical ocular anesthetics as an important conduit for these contaminations despite its espoused supplemental antimicrobial effect .[25, 26, 11]

Considering the kind of microorganisms that were isolated from the drugs, the contaminations could be due to contact with hands of clinicians during usage, issues of personal hygiene, contact with ocular tissues of patients who reported with eye infections, storage conditions and other environmental factors.[27] For drugs that were contaminated with microbes that form part of the normal flora, such as *bacillus* spp., *staphylococcus* spp., *coagulase-negative staphylococcus* spp., and *streptococcus* spp., contact with skin or fingers of clinicians may be implicated. Also, some of the fungi isolates (*Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp.) form part of the nail flora or may get trapped under the nails [28] through dust and can contaminate drugs during use.

Contamination of topical ocular anesthetic drugs could also be due to contact with ocular tissues since the drugs were kept in the eye clinics and used on multiple patients. Most of these bacteria and fungi isolates have been implicated in most eye infections such as Endophthalmitis, Keratitis and Conjunctivitis. *Pseudomonas* spp. has been identified to be associated with microbial ocular infections perforating uncompromised corneal epithelium.[29] *Penicillium* spp., *Cladosporium* spp., *Cercosporium* spp., and *Cephalosporium* spp., have also been implicated a number of ocular infections such as conjunctival infections, fungal keratitis and also affects corneal sensitivity.[30-32]

It is quite alarming to isolate *E coli and shigella spp*. from ophthalmic preparations since it signifies contamination of the drug with faecal matter. In this study, the percentage of E coli contamination in both drugs was found to be 3.30%. In this instance, issues with personal hygiene of clinicians require attention. From the antimicrobial test results, most of the isolated microbes were resistant to almost all the antibiotics that were used except Gentamicin which could inhibit the growth of the microbes also, both ketoconazole and fluconazole were effective at inhibiting fungal growth.

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Conclusion

Topical ophthalmic anesthetic preparations (Amethocaine and Proparacaine) despite their acclaimed supplemental antimicrobial action were contaminated with potential pathogenic microbes of clinical importance. Gentamicin was potent against both Gram negative and positive bacteria isolated. Ketoconazole and Fluconazole were susceptible to the isolated fungi at minimum concentration of 5mg/dl.

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Conflict of Interest

The Authors declare that there is no conflict of interest

Table 1

The number of topical anesthetics (Amethocaine and Proparacaine) sampled from the various regions in Ghana.

Region	No. of anesthetics obtained	No. of amethocaine	No. of proparacaine
Central	3	1	2
Ashanti	4	2	2
Brong-Ahafo	2	2	0
Northern	3	0	3
Upper East	4	2	2
Western	5	3	2
Upper West	2	2	0
Volta	1	0	1
Eastern	1	0	1
Greater Accra	2	0	2
TOTAL	**27**	**12**	**15**

Table 2

Details of bacteria isolated from the various topical anesthetic agents.

Isolated bacteria	Ameth-ocaine	Propara-caine	Total(%)
Moraxella catarrhalis	10	7	17(19.54)
Coagulase negative Staph. spp.	10	7	17(19.54)
Bacilli spp.	10	16	26(29.89)
Proteus spp.	6	1	7(8.04)
Staphylococcus aureus	2	6	8(9.19)
Klebsiella spp.	1	2	3(3.44)
Shigella spp.	2	1	3(3.44)
Streptococcus spp.	1	2	3(3.44)
Escherichea coli.	1	1	2(3.30)
Pseudomonas spp.	0	1	1(1.15)
Total	**43**	**44**	**87(100)**

Table 3

Result of antimicrobial susceptibility test of isolated bacteria

Name of sample	Gen	Pen	Amp	Flx	Ery	Tet	Cot	Crx	Ctr	Chl	Ctx
Bacillus spp.	S	R	R	R	I	I	I	R	n/a	n/a	n/a
Staphylococcus aureus	S	R	R	R	R	R	R	S	n/a	n/a	n/a
Cogulase negative Staphylococcus. spp.	S	R	R	R	I	R	I	R	n/a	n/a	n/a
Streptococcus spp.	S	R	R	R	R	I	R	R	n/a	n/a	n/a
Moraxella catarrhalis	S	n/a	R	n/a	n/a	R	R	R	R	R	R
Pseudomonas spp.	S	n/a	R	n/a	n/a	R	R	R	R	R	R
Klebsiella spp.	S	n/a	R	n/a	n/a	I	R	R	R	R	R
Proteus spp.	S	n/a	R	n/a	n/a	R	R	R	R	R	R
EscherichIa coli.	S	n/a	R	n/a	n/a	I	R	R	R	R	R
Shigella spp.	S	n/a	R	n/a	n/a	S	R	R	R	R	R
Serratia spp.	S	n/a	R	n/a	n/a	R	R	R	R	R	R

AMP: Ampicillin, COT: Cotrimoxazole, CRX: Cefuroxime, CTX: Cefotaxime, PEN: Penicillin,CHL: Chloramphenicol, ERY: Erythromycin, GEN: Gentamicin, TET: Tetracycline, FLX: Fleroxacin, CTR: Ceftriaxone

R-Resistant; S-Sensitive; I-intermediate; n/a-not applicable.

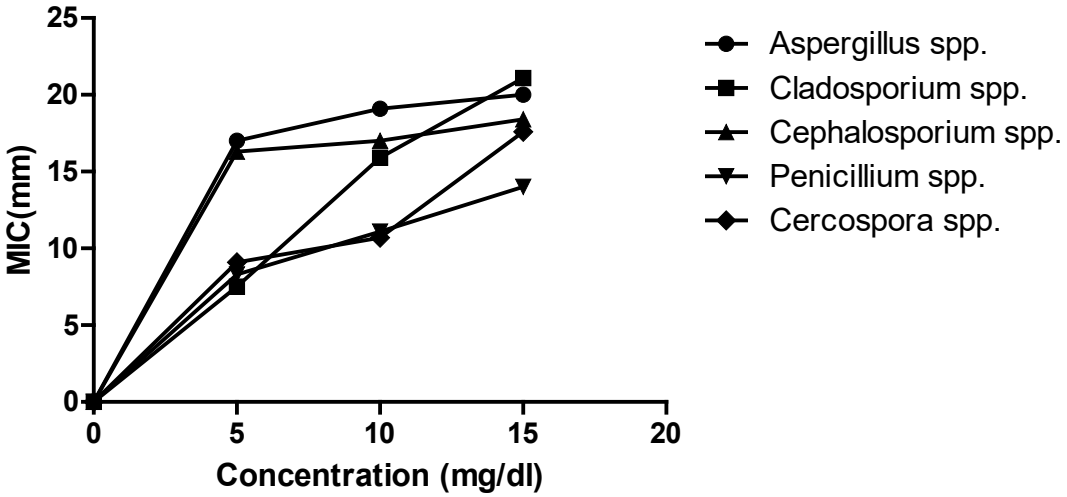

Figure 1: A plot the area under the curve of the concentrations of Ketoconazole against zones of inhibition. One-way ANOVA indicated that Ketoconazole at all dose levels had a comparable effect on all 5 species isolated (*Aspergillus* spp., *Cladosporium* spp., *Cephalosporium* spp., *Penicillium* spp., *Cercospora* spp.) ($p>0.05$).

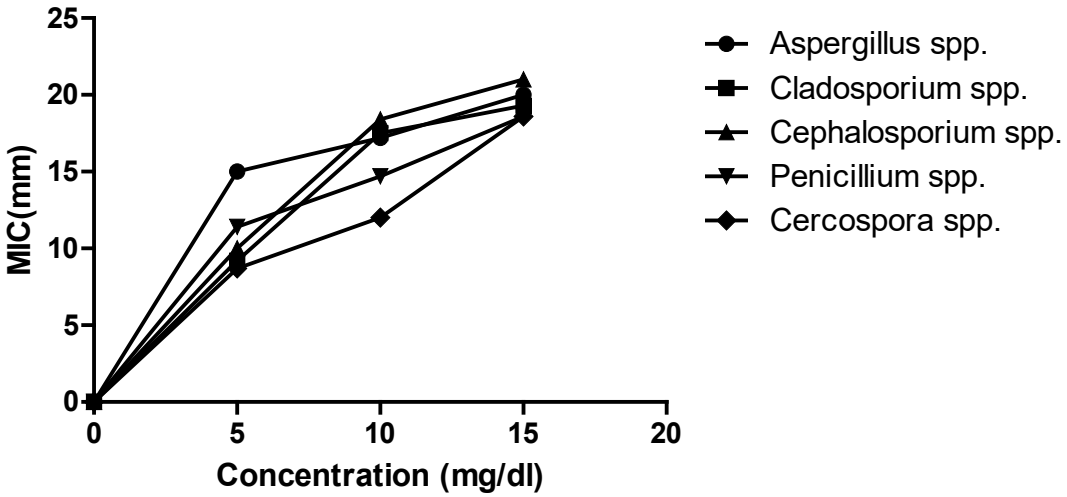

Figure 2: A plot the area under the curve of the concentrations of Fluconazole against zones of inhibition. One-way ANOVA indicated that Fluconazole at all dose levels had a comparable effect on all 5 species isolated (*Aspergillus* **spp., *Cladosporium* spp., *Cephalosporium* spp., *Penicillium* spp., *Cercospora* spp.) ($p>0.05$).**