



ORIGINAL ARTICLE

Microbial safety implications of in-use topical diagnostic ophthalmic medications in eye clinics in Ghana



Samuel Kyei^a, Eric Appiah^b, Eunice Ampadubea Ayerakwa^b, Clara Bemmah Antwi^a, Kofi Asiedu^{c,*}

^a Department of Optometry and Vision Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

^b Department of Biomedical Sciences, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

^c Eye Clinic, Twumasiwaa Medical Center, ARS Junction, East Legon Accra, Ghana

Received 28 August 2018; accepted 16 February 2019

Available online 29 August 2019

KEYWORDS

Diagnostics;
Ocular infections;
Clinical setting;
Atropine;
Pilocarpine

Abstract

Purpose: To determine the microbial contaminants and its clinical importance in topical diagnostic ophthalmic medications (cycloplegics/mydriatics and miotics) in eye clinics in Ghana.

Method: A cross-section of eye clinics was sampled for the diagnostic agents (Atropine, Phenylephrine, Tropicamide and Cyclopentolate, Pilocarpine). 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 testing was also performed to ascertain the clinical importance of the isolated microbes.

Results: A total of 113 samples were obtained, from which 334 bacteria were isolated which included *Bacilli spp.* 91(27.25%), Coagulase Negative *Staphylococci spp.* 59(17.66%), *Moraxella spp.* 47(14.07%), *Staphylococcus aureus* 41(12.27%), *Streptococcus spp.* 21(6.29%), *Klebsiella spp.* 20(5.99%), *Pseudomonas spp.* 13(3.89%), *Proteus spp.* 12(3.59%), *Escherichia coli.* 12 (3.59%), *Serratia spp.* 10(2.99%), *Shigella spp.* 7(2.09%), *Salmonella spp.* 1(0.3%). There were 96 isolated fungal contaminants mainly *Penicillium spp.* 41(42.71%), *Cephalosporium spp.* 19(19.79%), *Cladosporium spp.* 15(15.63%), *Aspergillus spp.* 13(13.54%), *Cercospora spp.* 8(8.33%). The diagnostic agent with the most bacteria contamination was Phenylephrine 90 (26.95%) and the least being Pilocarpine 49 (14.67%). Also, the diagnostic agent with the most fungal contamination was Cyclopentolate 29 (30.2%) and the least was Tropicamide and Pilocarpine with 15 (15.63%) each. Gentamicin and Ciprofloxacin were the only antibiotics that showed 100% activity against all the bacterial isolates. Fungal contaminants were more susceptible to Ketoconazole as compared to Fluconazole.

* Corresponding author at: Eye Clinic, Twumasiwaa Medical Center, ARS Junction, East Legon Accra, Ghana.
E-mail address: Kasiedu60@yahoo.com (K. Asiedu).

PALABRAS CLAVE

Diagnósticos;
Infecciones ocular;
Entorno clínico;
Atropina;
Pilocarpina

Conclusion: Topical diagnostic ophthalmic preparations used in clinical settings in Ghana are contaminated with clinically important bacteria and fungi.

© 2019 Spanish General Council of Optometry. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Implicaciones sobre seguridad microbiana de los fármacos oftálmicos diagnósticos tópicos utilizados en las clínicas oftalmológicas de Gana

Resumen

Objetivo: Determinar los contaminantes microbianos y su importancia clínica en los fármacos oftálmicos diagnósticos tópicos (ciclopléjicos/midriáticos y mióticos) en clínicas oftalmológicas de Gana.

Método: Se realizó una muestra transversal de clínicas oftalmológicas para los agentes diagnósticos (Atropina, Fenilefrina, Tropicamida y Ciclopentolato, Pilocarpina). Se observaron procedimientos y protocolos de laboratorio estándar en cuanto al cultivo de muestras en diferentes soluciones de Agar. Se realizaron diversas pruebas microscópicas y bioquímicas para identificar las especies microbianas. También se realizó la prueba de susceptibilidad antimicrobiana para comprobar la importancia clínica de los microbios aislados.

Resultados: Se obtuvieron un total de 113 muestras, de las cuales se aislaron 334 bacterias que incluyeron Bacilli spp. 91(27,25%), Staphylococci spp. Coagulasa negativos 59(17,66%), Moraxella spp. 47(14,07%), Staphylococcus aureus 41(12,27%), Streptococcus spp. 21(6,29%), Klebsiella spp. 20(5,99%), Pseudomonas spp. 13(3,89%), Proteus spp. 12(3,59%), Escherichia coli. 12(3,59%), Serratia spp. 10(2,99%), Shigella spp. 7(2,09%), Salmonella spp. 1(0,3%). Se encontraron 96 contaminantes fúngicos aislados, principalmente Penicillium spp. 41 (42,71%), Cephalosporium spp. 19 (19,79%), Cladosporium spp. 15 (15,63%), Aspergillus spp. 13 (13,54%), Cercospora spp. 8 (8,33%). El agente diagnóstico con mayor contaminación bacteriana fue Fenilefrina 90(26,95%), siendo Pilocarpina 49 (14,67%) el que reflejó una menor contaminación bacteriana. De igual modo, el agente diagnóstico con mayor contaminación fúngica fue Ciclopentolato 29 (30,2%), siendo Tropicamida y Pilocarpina, con 15 (15,63%) cada uno, los que reflejaron menos contaminación fúngica. Gentamicina y Ciprofloxacina fueron los únicos antibióticos que reflejaron un 100% de actividad frente a todos los aislados bacterianos. Los contaminantes fúngicos fueron más susceptibles a Ketoconazol, en comparación con Fluconazol.

Conclusión: Los preparados oftálmicos diagnósticos tópicos en entornos clínicos en Gana están contaminados por bacterias y hongos clínicamente importantes.

© 2019 Spanish General Council of Optometry. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Infectious diseases remain an important issue globally with Sub-Saharan Africa presenting a high proportion of the global burden. Several factors including poor personal hygiene, contaminated surgical theaters, diagnostic agents and other health care material contribute to the high burden of infectious diseases of which eye infections form a part.¹ Some recalcitrant infectious diseases of the eye such as microbial keratitis and endophthalmitis have been named to contribute to the increased incidence of ocular morbidity and blindness worldwide.²

Currently, many researchers have stated that ophthalmic solutions contain pathogenic contaminants associated with ocular infections in clinical settings.³ A contaminated topical ophthalmic agent serves as a potential cause of avoidable ocular diseases and/or aggravation of pre-existing

infections. Apart from the risk of infection, bacterial contamination of ocular diagnostic agent may alter the pH of the solution and therefore reduce the efficacy of the medication.⁴ The following has been suggested as the probable factors for this contamination; cross-contamination from diagnostic ophthalmic equipment, use on multiple patients, white coats of clinicians, the duration of use, unsterile hospital environment.⁵ Plastic bottles which is the most common container for eye drops have been reported to be more contaminated near the bottle cap.⁴ The type and concentration of preservatives in most of these diagnostic agents have been reported to cause disruptions of the ocular surface and this has the potential of enhancing microbial attachment and penetration as well.⁶ Despite the intent of preservatives in multiple-use bottles (eye drops meant for multiple usages beyond a day) to prevent contaminations, some microbial contaminations have still been recorded.⁷

It is therefore not surprising that most eye infections are attributed to exogenous microbes.⁷

Ocular diagnostic drugs are pharmaceutical agents that are used to test for the normal functioning and impairment of the eye. Thus, they aid in the effective diagnosis of disorders associated with the eyes. These agents are also useful in the examination of the ocular media and fundus, to improve retinal photography and in techniques where anesthesia is required. They are also used in cycloplegic refraction and for the detection of lesions in the visual pathway.⁸ These medications are very essential in ophthalmic practice, therefore, should they serve as a means of transmitting infectious pathogens would have public health implications. In response to this problem, there is the need to investigate specific microbial contaminants of these agents so as to inform policy on patient safety and nosocomial infections control. This study was carried out to investigate the prevalence of microbial contamination of diagnostic pharmaceutical agents (DPAs) used in eye clinics in Ghana and to ascertain the clinical importance of these microbes through antimicrobial susceptibility testing. These commonly used diagnostic agents included; cycloplegics and mydriatics i.e. Atropine, Phenylephrine, Tropicamide and Cyclopentolate, miotics i.e. Pilocarpine.

Materials and methods

Sampling procedure

A List of 188 eye clinics was obtained from the Ghana Eye Secretariat of which fifty (50) eye clinics were proportionately selected across the 10 regions of the country. The criterion for the selection was based on what the researchers considered as a major clinic. Thus, only clinics with the full complement of eye care staff and referral centers were selected. These were deemed to perform procedures that would require the use of most of these diagnostics. In each of the selected clinics, the DPAs were collected based on their availability once access was granted to the researchers.

Sample collection procedure

These clinics were not aware they had been selected to participate in the study and were also not given prior notice of our visit to collect samples. This was to avoid any changes and modifications to their normal protocol on the use of these agents. Sterile airtight bottles (one for each sample) wrapped in a pre-sterilized plastic zip bags were used to collect 2 ml each of the samples on the clinicians' desk meant for routine use. Each sample was drawn with a separate sterile 2.5 ml syringe fitted with a 25 gauge needle. In drawing the samples, a sterile surgical glove was worn to avoid the introduction of hand to sample contamination. Once the samples were collected they were placed into a different labeled sterilized plastic zip bag which was firmly sealed for transportation to the laboratory. All the samples collected had been opened and had been in use for at least 2 weeks and at most 8 weeks. Only one facility had their sample stored at 4°C, all others were stored at room temperature. Another set of the diagnostic ophthalmic medications as per their availability were obtained from the dispensaries of these

facilities to serve as controls for those opened and in use. The controls were unopened unexpired diagnostic eye drops in multiple use bottles on the shelves (kept at room temperature) of the various dispensary units of the facilities where the samples were collected. They were as well transported under aseptic conditions to the laboratory for the same investigations. All samples were processed within 12 h of collection.

Culturing and identification of microbes

Serial dilution of the sample was done to the fifth dilution using peptone water. The 4th and 5th dilution were plated with nutrient agar using pour plating method and incubated for 18–24 h at 37°C. Growth on nutrient agar we examined qualitatively and quantitatively and the findings were recorded. Each colony of bacteria isolated from the nutrient agar was inoculated in nutrient broth and incubated overnight under an 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 obtain pure colonies by incubating overnight under an aerobic condition at 37°C. Loop flaming was always done per plate streaking to avoid contamination of other samples. After incubation, morphological examination of colonies was recorded and further identification was done. Microscopy and Gram staining were done for pure colonies obtain from both Blood and MacConkey agars to differentiate Gram-positive bacteria from Gram-negative bacteria. Gram-positive bacteria obtained were further identified 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.

Prepared Sabouraud Dextrose Agar, SDA (Oxoid Limited, Thermo Fisher Scientific Inc., UK) in a conical flask and Petri dishes were autoclaved. SDA was allowed to cool to 45–50°C, and 50 mg of Chloramphenicol was added to 1 L agar and swelled to mix. The SDA media was then poured into Petri dishes to solidify. Diagnostic agents (Phenylephrine, Atropine, Cyclopentolate, Pilocarpine, and Tropicamide) 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 fungi growth were examined and recorded. Lactophenol cotton staining preparation was done for each fungus growth, for microscopic examination. Morphological characteristics of stained fungus under the microscope were examined and recorded.

Antimicrobial sensitivity testing

Identified Subculture organisms from both blood and MacConkey agar were picked using sterile loops and emulsified pure colonies in 3 ml of sterile normal saline. Its turbidity was checked by comparing it to 0.5 McFarland standard solutions. An adequate lighting was ensured to visually compare the inoculum tube and the 0.5 McFarland standards against 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 antibiotics 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 pressed gently with the help of the forceps to ensure a complete contact with the agar surface and proper diffusion of the drugs into the agar and cover the plate. The plate was inverted and incubated for 24 h at 37°C. Measurement of inhibition zone size was done from the center of the disk to a point on the circumference of 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 5–15 mg/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

Using one-way ANOVA the mean bacterial contaminations were compared among the regions. One-way ANOVA followed by Bonferroni *posthoc* test was used to compare

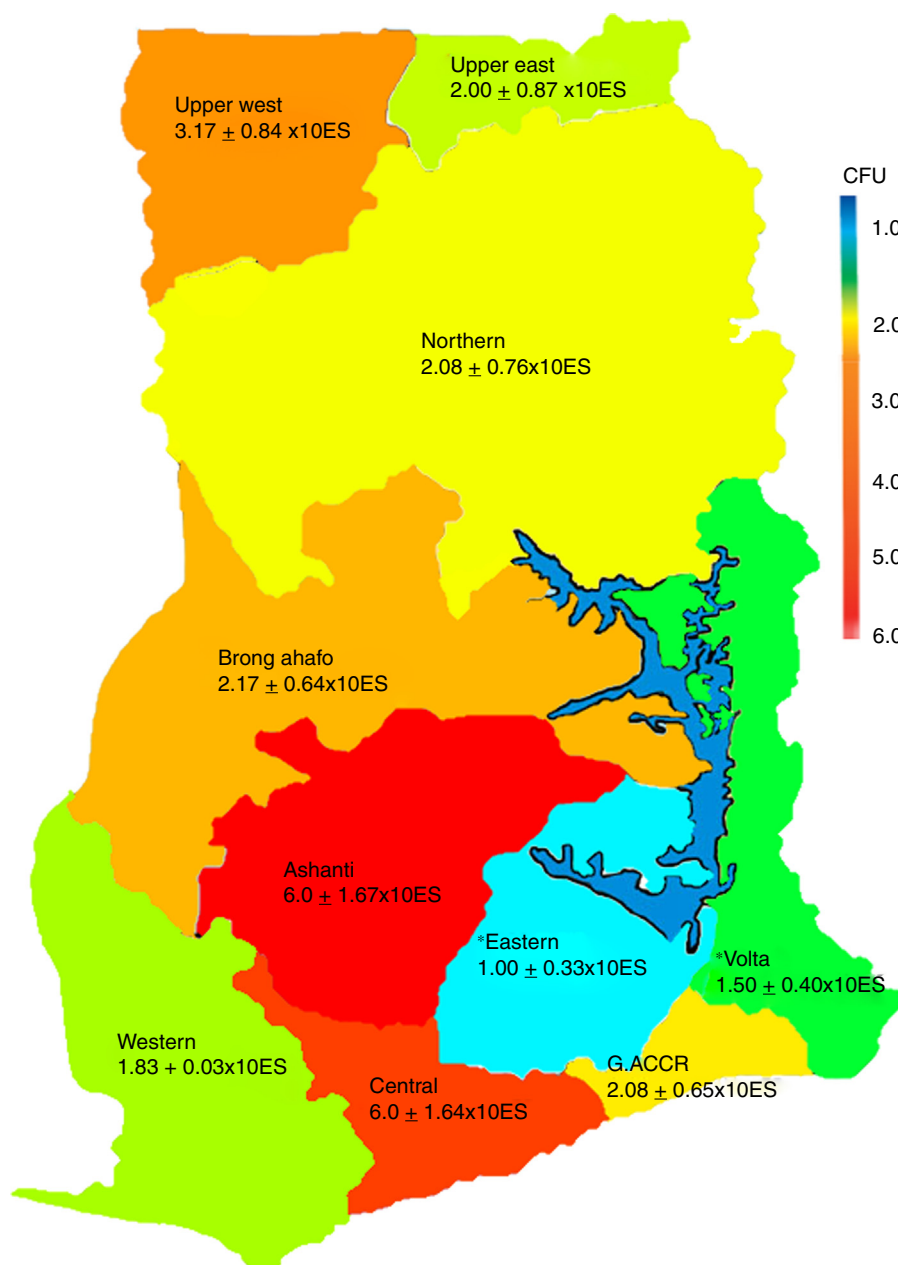


Figure 1 A map showing the means \pm SEM levels of contamination by region by with blue indicating the region with the least contamination and the red the region with the most contamination. One-way ANOVA (multiple comparisons) indicated Volta and Eastern Region had less contaminations compared to Ashanti and Central Regions. *Indicates $p \leq 0.005$.

Table 1 A table showing the number of topical ophthalmic diagnostic agents sampled from the various regions in Ghana.

Regions	No. of agents obtained	Atropine	Phenylephrine	Cyclopentolate	Pilocarpine	Tropicamide
Central	16	4	4	3	2	3
Ashanti	22	7	5	5	2	3
Brong Ahafo	11	1	2	3	2	3
Northern	8	0	2	2	2	2
Upper East	8	1	2	3	1	1
Western	9	2	2	1	0	4
Upper West	12	2	3	2	3	2
Volta	11	1	2	2	1	5
Greater Accra	11	2	2	2	1	4
Eastern	5	0	3	0	1	1
Total	113	20	27	23	15	28

Table 2 The number and percentages of isolated bacteria from the various diagnostic agents.

Diagnostic agents	No. of isolated bacteria	% of isolated bacteria
Atropine	54	16.18
Phenylephrine	90	26.95
Cyclopentolate	71	21.26
Pilocarpine	49	14.67
Tropicamide	70	20.95
Total	334	100

the mean values of the various fungi treated with Ketoconazole and Fluconazole. Values were expressed as the mean \pm standard error of the mean. $p \leq 0.05$ was considered to be statistically significant. GraphPad Prism (version 7; GraphPad, La Jolla, CA, USA) was used in the data analysis.

Ethical considerations

The study protocol was approved by the Department of Biomedical Science Ethics Committee, University of Cape

Coast. Permission to access the facilities was sought from their respective managements on arrival of the research team to the facility. Biosafety guidelines for protection of personnel in the laboratory were observed.

Results

Profile of collected samples

Out of the 50 facilities proportionately selected from the list obtained from the Ghana Eye Secretariat, 40 of them granted access and therefore participated in the study. In all 113 samples were obtained across the 10 regions, of which sixteen (16) diagnostic agents were from the Central region, eleven (11) from Brong-Ahafo, twenty-two (22) from Ashanti, eleven (11) from Volta, eleven (11) Greater Accra, five (5) from Eastern, nine (9) from Western, eight (8) from Northern, eight (8) from Upper East, and twelve (12) from Upper West region. Volta and Eastern regions were the regions with the least contamination compared to Ashanti and Central regions (Fig. 1).

Table 3 A table showing details of the number of bacteria isolated from the various topical ophthalmic diagnostic agents.

Isolated Gram (+) bacteria	Atropine	Phenylephrine	Cyclopentolate	Pilocarpine	Tropicamide	Total
<i>Escherichia coli</i>	2	5	2	2	1	12 (3.59%)
<i>Klebsiella spp.</i>	2	4	5	4	5	20 (5.99%)
<i>Moraxella catarrhalis</i>	9	16	12	4	6	47 (14.07%)
<i>Proteus spp.</i>	1	5	2	2	2	12 (3.59%)
<i>Pseudomonas spp.</i>	2	3	4	1	3	13 (3.89%)
<i>Serratia spp.</i>	2	1	2	1	4	10 (2.99%)
<i>Shigella spp.</i>	3	2	1	1	0	7 (2.09%)
<i>Bacilli spp.</i>	16	28	21	11	15	91 (27.25%)
<i>Cogulaes negative Staphylococcus. spp.</i>	10	15	14	8	12	59 (17.66%)
<i>Staphylococcus aureus</i>	6	5	6	10	14	41 (12.27%)
<i>Streptococcus spp.</i>	1	6	2	5	7	21 (6.29%)
<i>Salmonella spp.</i>	0	0	0	0	1	1 (0.3%)
Total	54	90	71	49	70	334 (100%)

Table 4 The antibiotic susceptibility pattern of the isolated bacteria.

Isolated bacteria	AS	BA	CF	TZP	CH	CP	CI	TE	OF	GM	AK	LE	CX	PR	PF	LM	RF
<i>Klebsiella spp.</i>	R	R	R	S	R	S	R	S	S	S	S	S	n/a	n/a	n/a	n/a	n/a
<i>Pseudomonas spp.</i>	R	R	I	S	R	S	R	S	S	S	S	S	n/a	n/a	n/a	n/a	n/a
<i>Escherichia coli</i>	R	R	R	I	I	S	R	S	I	S	S	I	n/a	n/a	n/a	n/a	n/a
<i>Moraxella catarrhalis</i>	R	R	I	S	I	S	R	S	S	S	S	R	n/a	n/a	n/a	n/a	n/a
<i>Proteus spp.</i>	R	S	R	S	R	S	R	S	S	S	S	I	n/a	n/a	n/a	n/a	n/a
<i>Shigella spp.</i>	R	S	R	S	R	S	R	R	S	S	S	S	n/a	n/a	n/a	n/a	n/a
<i>Cogulaes negative Staphylococcus spp.</i>	R	S	R	n/a	n/a	S	n/a	I	S	S	n/a	n/a	R	R	I	R	R
<i>Bacillus spp.</i>	R	R	R	n/a	n/a	S	n/a	R	R	S	n/a	n/a	R	R	I	R	R
<i>Staphylococcus aureus</i>	R	R	R	n/a	n/a	S	n/a	R	S	S	n/a	n/a	R	R	R	R	R
<i>Streptococcus spp.</i>	R	R	R	n/a	n/a	S	n/a	S	I	S	n/a	n/a	R	R	R	R	R
<i>Salmonella spp.</i>	R	R	R	I	I	S	R	S	I	S	S	S	n/a	n/a	n/a	n/a	n/a

GM: gentamicin, OF: ofloxacin, AK: amikacin, LE: levofloxacin, TE: tetracycline, AS: ansamycin, BA: bacitacin, CF: cephalothin, TZP: tabzobactam/piperacillin, CH: chloramphenicol, LM: lactam; RF: rifampicin, CX: cefotaxime, PR: primaxin.

R – resistant; S – sensitive; I – intermediate; N/A – not applicable.

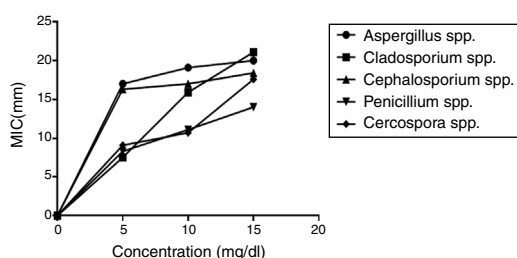


Figure 2 A plot the area under the curve of the concentrations of Ketoconazole against zones of inhibition. One-way ANOVA indicated that Ketoconazole at all dose levels had a comparable effect on all 5 species isolated (*Aspergillus spp.*, *Cladosporium spp.*, *Cephalosporium spp.*, *Penicillium spp.*,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

Of the 113 multi-dose diagnostics agent were sampled, 28 (24.78%) of the agents were Tropicamide and pilocarpine 15 (13.27%) being the one that was collected in a smaller quantity (Table 1). All the medications collected were preserved either with Benzalkonium chloride, Chlorobutanol or Sodium perborate.

Profile of cultured samples

A total of 109 diagnostic agents which constitute 96.46% indicated a positive culture with only 4 samples with no

contamination. Bacteriological analysis indicated 334 bacterial isolates from 109 diagnostic agents of which the most, 90 bacteria were isolated from Phenylephrine and least 49 from Pilocarpine contaminated (Table 2). The most occurring bacteria were *Bacillus spp.* and the least were *Salmonella spp.* (Table 3). The numbers of Gram-positive and Gram-negative bacteria isolated from the diagnostic agents were also 212 and 112 respectively. On the Sabouraud Dextrose Agar, 96 fungi were isolated of which 16 (16.67%) were from Atropine, 21 (21.87%) from Phenylephrine, 29 (30.2%) from Cyclopentolate, 15 (15.63%) were from Pilocarpine (15.63%) and 15 from Tropicamide. Five different species of fungi were isolated namely, *Aspergillus spp.*, *Cladosporium spp.*, *Cephalosporium spp.*, *Penicillium spp.*, *Cercospora spp.* The diagnostic agents with the most fungal contamination were Cyclopentolate and the least were Tropicamide and Pilocarpine.

Antimicrobial susceptibility test

Table 4 is a summary of these results for antimicrobial susceptibility test of isolated bacteria for bacteria found. Fig. 2, table 5 and fig. 3, table 6 are also the summaries of the minimum inhibition concentration (MIC) of Ketoconazole and Fluconazole on identified fungi species. Ketoconazole and Fluconazole at doses of 5–15 mg/ml did show a comparable inhibitory effect for all fungal isolates ($p > 0.05$).

Table 5 A table showing details of the mean \pm SD MICs of Ketoconazole for the fungal isolates.

Fungal isolate	5 mg Mean MIC \pm SD mm	10 mg Mean MIC \pm SD mm	15 mg Mean MIC \pm SD mm
<i>Penicillium spp.</i>	8.267 \pm 0.252	11.067 \pm 0.153	14.00 \pm 0.200
<i>Cephalosporium spp.</i>	16.300 \pm 0.300	17.00 \pm 0.000	18.267 \pm 0.153
<i>Aspergillus spp.</i>	17.00 \pm 0.200	19.067 \pm 0.058	20.033 \pm 0.950
<i>Cercospora spp.</i>	9.067 \pm 0.153	10.467 \pm 0.208	0.208 \pm 0.153
<i>Cladosporium spp.</i>	7.33 \pm 0.153	15.633 \pm 0.252	21.369 \pm 0.643

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.*)

MIC: minimum inhibition concentration; SD: standard deviation.

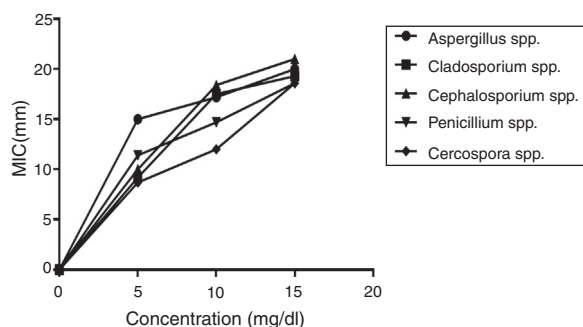


Figure 3 A plot the area under the curve of the concentrations of Fluconazole against zones of inhibition. One-way ANOVA indicated that Fluconazole at all dose levels had a comparable effect on all 5 species isolated (*Aspergillus spp.*, *Cladosporium spp.*, *Cephalosporium spp.*, *Penicillium spp.*, *Cercospora spp.*).

Discussion

It is unfortunate to visit the clinic to seek remedy and to return home with a different disorder or an aggravation of your existing condition. Such is the experience of many who visit healthcare facilities especially in low resource settings of the world.⁹ It has been established that contaminated ophthalmic solutions could be an important conduit for such infections.^{10,11} More so, the multidose form which is impregnated with preservatives such as benzalkonium chloride is known to alter ocular surface integrity thereby aiding the penetration of the drug and its accompanying contaminants.¹² From literature, Chlorobutanol also alters the normal cellular movement and proliferative activity resulting in conjunctival and corneal cytotoxicity.¹³ With long-term used of ocular agents containing Chlorobutanol as a preservative, significant keratitis has been noted due to degeneration of human corneal epithelial cells.¹⁴ These alterations caused by Chlorobutanol to the ocular surface could rather fester the invasion by microbes in cases where contaminated solutions are used.

The contaminated topical diagnostic preparations could have resulted from the clinician's hand contact with the nozzle while opening for usage, or it comes in contact with ocular tissue, multi-user handling, duration of use and other factor involving environmental dynamics.³ Caps of these agents usually harbors airborne microbes which may contaminate these agents. These factors have the potential

to overwhelm the agents meant for preservation due to their limited concentrations in these eye drops rendering them ineffective. The extent of contamination recorded in this study far outweighs those recorded in other several studies involving ophthalmic solutions.¹⁵⁻¹⁹ None of the regions meet the strict sterility criteria required of ophthalmic solutions²⁰ despite a statistically significant lower level of contaminations recorded for Volta and Eastern Regions relative to Ashanti and Central regions ($p \leq 0.05$). The microbes found in this study further augment the possible causes of the contamination (use on multiple patients, white coats of clinicians, the duration of use, unsterile hospital environment, etc.) as most of the isolated microbes were either associated with normal flora of humans (from the skin e.g. *Streptococcus spp.*, nasopharynx e.g. *Moraxella catarrhalis* and the ocular tissues e.g. *Staphylococcus aureus* and *Streptococcus spp.*), gastrointestinal tract pathogens e.g. *Escherichia coli*, and environmental factors by airborne e.g. *Penicillium spp.*, *Aspergillus spp.* etc. Bacteria isolates from the Enterobacteriaceae family such as *E. coli.*, *Serratia spp.*, *Shigella spp.*, *Proteus spp.* indicates possible fecal contamination of these diagnostic agents. *Klebsiella spp.* which are microbiota found in the nasopharyngeal and gastrointestinal has been reported to cause endophthalmitis.²¹ *Pseudomonas spp.* a common microbial associate of ocular infections are even capable of traversing uncompromised corneal epithelium.²²

Previous studies have suggested white coats, hospital objects, clinicians mobile, bags and wallet in the transmission cycle of pathogenic organisms in healthcare facilities.²³ *Streptococcus spp.* and *M. catarrhalis* which are nasopharynx commensal bacteria may contaminate topical diagnostic agent by talking and breathing.²⁴ Other *Staphylococcus spp.* (*Coagulase-negative Staphylococcus spp.*) and *Streptococcus spp.* which are normal flora of the skin are indicative of contamination from human contact mainly due to poor handling. The predominance of *Bacilli spp.* in the diagnostic agents is because of the high survival rate of this spore-forming bacteria even in preserved ophthalmic solutions.⁶ The constant introduction of non-native bacteria such as *Serratia spp.*, *Klebsiella spp.*, *Salmonella spp.*, and *Shigella spp.* into the eye may have future consequences for the emergence of recalcitrant ocular infections.

The incidence of airborne fungal contamination varied widely, depending on the season, temperature and relative humidity. According to literature, fungal contaminations to

Table 6 A table showing details of the mean \pm SD MICs of Fluconazole for the fungal isolates.

Fungal isolate	5 mg Mean (MIC) \pm SD mm	10 mg Mean (MIC) \pm SD mm	15 mg Mean (MIC) \pm SD mm
<i>Penicillium spp.</i>	11.40 \pm 0.100	14.467 \pm 0.252	15.60 \pm 0.200
<i>Cephalosporium spp.</i>	10.033 \pm 0.058	18.267 \pm 0.115	21.000 \pm 0.000
<i>Aspergillus spp.</i>	18.00 \pm 0.100	17.20 \pm 0.000	20.167 \pm 0.153
<i>Cercospora spp.</i>	8.767 \pm 0.115	12.033 \pm 0.058	18.50 \pm 0.100
<i>Cladosporium spp.</i>	9.167 \pm 0.058	17.330 \pm 0.153	19.267 \pm 0.058

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.*).

MIC: minimum inhibition concentration; SD: standard deviation.

diagnostic agents are largely due to airborne flora with *Aspergillus spp*, *Penicillium spp*, *Cephalosporium spp*, and *Cladosporium spp*.²⁵

This suggests that contamination of topical diagnostic preparations was airborne due to geographical, environmental factors and unhygienic clinical settings. The most recalcitrant form of ocular surface infections are of fungal etiologies and could have dire consequence for patients who are innocently infected.²⁶ This is even much more so; since patients infected with these conditions may present with an unrelated history of contact with vegetative matter leading to misdiagnosis or delayed detection. *Penicillium spp* has been reported to be a common cause of conjunctival infections.²⁷ *Cephalosporium spp* does not only cause scleral infections but affects corneal sensitivity.²⁸ *Cladosporium spp*, causes *Cladosporium Keratitis*²³ and *Cercosporium spp* is also a known cause of ocular infections.²⁷

Ciprofloxacin and Gentamicin showed high activity against all bacteria isolates but the organisms proved resistant against most of the conventional antibiotics. However, all the fungal isolates were susceptible to Fluconazole and Ketoconazole.

Conclusion and recommendations

Conclusion

Topical diagnostic ophthalmic preparations (Atropine, Phenylephrine, Cyclopentolate, Pilocarpine, and Tropicamide) used in clinical settings in Ghana were contaminated with clinically important microbes. These clinically important bacteria isolated are pathogenic and would cause serious secondary eye infection if they come in contact with the eye of immunocompromised persons. Ciprofloxacin and Gentamicin were active against Gram-negative and positive bacteria isolated but most bacteria were resistant or intermediate to multidisc antibiotic susceptibility testing used (from Axiom laboratories). The isolated fungi were susceptible to Ketoconazole and Fluconazole at concentrations of minimum concentration of 5 mg/dl.

Disclosure of funding

This study was solely funded by the authors.

Conflicts of interest

The authors have no conflicts of interest to declare..

Acknowledgment

The authors are grateful to Mrs Esther Duncan Kyei for proof-reading this article and Dr Carl Halladay Abraham for his assistance with the diagram in the work.

References

- Lindahl JF, Grace D. The consequences of human actions on risks for infectious diseases: a review. *Infect Ecol Epidemiol.* 2015;5, <http://dx.doi.org/10.3402/iee.v5.30048>. Nov 27:30048.
- Sherwal BL, Verma AK. Epidemiology of ocular infection due to bacteria and fungus. Prospective study. *JK Sci.* 2008;10:127–131.
- Nentwich MM, Kollmann KHM, Meshack J, et al. Microbial contamination of multi-use ophthalmic solutions in Kenya. *Br J Ophthalmol.* 2007;91:1265–1268.
- Landivar ME, Jara PF, Hernandez E, et al. The most important sensitizing drug in ocular examination: 1-year study. *J Allergy Clin Immunol.* 2011;127:AB189, <http://dx.doi.org/10.1016/j.jaci.2010.12.751>.
- Clark PJ, Ong B, Stanely CB. Contamination of diagnostic ophthalmic solutions in primary eye care settings. *Mil Med.* 1997;162:501–506.
- Rahman MQ, Tejwani D, Wilson JA, et al. Microbial contamination of preservative free eye drops in multiple application containers. *Br J Ophthalmol.* 2006;90:139–141.
- Feghhi M, Mahmoudabadi ALIZ, Mehdinejad M. Evaluation of fungal and bacterial contaminations of patient-used ocular drops. *Med Mycol.* 2008;46:17–21.
- Brown L. Resistance to ocular antibiotics: an overview. *Clin Exp Optom.* 2007;90:258–262.
- Allegranzi B, Storr J, Gerald Dziekan G, et al. The first global patient safety challenge ‘‘clean care is safer care’’: from launch to current progress and achievements. *J Hosp Infect.* 2007;65:115–123.
- Micallef C, Cuschieri P. Ocular infections due to contaminated solutions. *Ophthalmologica.* 2001;215:337–350.
- Vaughan DG. Contamination of fluorescein solutions. *Am J Ophthalmol.* 1955;39:55–61.
- Pisella PJ, Fillacier K, Elena P, et al. Comparison of the effects of preserved and unpreserved formulations of timolol on the ocular surface of albino rabbits. *Ophthalmic Res.* 2000;32:3–8.
- Tripathi BJ, Tripathi RC. Cytotoxic effects of benzalkonium chloride and chlorobutanol on human corneal epithelial cells in vitro. *Lens Eye Toxic Res.* 1989;6:395–403.
- Epstein SP, Ahdoot M, Marcus E, et al. Comparative toxicity of preservatives on immortalized corneal and conjunctival epithelial cells. *J Ocul Pharmacol Ther.* 2009;25:113–119.
- Geyer O, Bottone EJ, Podos SM, et al. Microbial contamination of medications used to treat glaucoma. *Br J Ophthalmol.* 1995;79:376–379.
- Hamard P, Blondin C, Debbasch C, et al. In vitro effects of preserved and unpreserved antiglaucoma drugs on apoptotic marker expression by human trabecular cells. *Graefes Arch Clin Exp Ophthalmol.* 2003;241:1037–1043.
- Oldham GB, Andrews V. Control of microbial contamination in unpreserved eyedrops. *Br J Ophthalmol.* 1996;80(7):588–591.
- Pisella PJ, Fillacier K, Elena P, et al. Comparison of the effects of preserved and unpreserved formulations of timolol on the ocular surface of albino rabbits. *Ophthalmic Res.* 2000;32:3–8.
- Kenyon KR, Starck T, Hibberd PL, et al. Microbial contamination of in-use ocular medications. *Pharmakother Aug.* 1992;540:209–214.
- Madigan M, Martinko J, eds. *Brock Biology of Microorganisms.* 13th ed. London, UK: Pearson Education; 2006:1096.
- Lee SS, Chen YS, Tsai HC, et al. Predictors of septic metastatic infection and mortality among patient with *Klebsiella pneumoniae* liver abscess. *Clin Infect Dis.* 2008;47:642–650.
- Alarcon I, Tam C, Mun JJ, et al. Factors impacting corneal epithelial barrier function against *Pseudomonas aeruginosa* traversal. *Invest Ophthalmol Vis Sci.* 2011;52:1368–1377.
- Fayyad DS, Touveneau S, Sauvan V, et al. Bacterial contamination of the hands of hospital staff during routine patient care. *Arch Intern Med.* 1999;159:81–826.

24. García-rodríguez JA, Martínez MJF. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother.* 2002;50(suppl S2): 59–73.
25. Martins-Diniz JN, da Silva RA, Miranda ET, et al. Monitoring of airborne fungus and yeast species in a hospital unit. *Rev Saude Publica.* 2005;39:398–405.
26. Leck AK, Thomas PA, Hagan M, et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. *Br J Ophthalmol.* 2002;86:1211–1215.
27. Chou FF, Kou HK. Endogenous endophthalmitis associated with pyogenic hepatic abscess. *J Amer Coll Surg.* 1996;182:33–36.
28. Chee SP, Ang CL. Endogenous Klebsiella endophthalmitis – a case series. *Ann Acad Med Sing.* 1995;24:473–478.