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A study of the microbial quality and organoleptic properties of ophthalmic preparations obtained from traditional medical practitioners in South Western Nigeria

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Summary

Twenty eight samples of traditional ophthalmic preparations were obtained from various traditional medical practitioners in the South Western states of Nigeria over a one-year period. They were examined for sterility, clarity, presence of particulate contamination and pH. All of them were found to be contaminated with bacteria to the order of 2.00×10^6 to 1.60×10^{11} colony forming units/ml, with *Pseudomonas* species and other potential pathogens such as *Staphylococcus aureus, Yersinia enterocolita, Micrococcus varians* and others being isolated from these preparations. A rotifer, *Rotaria rotatoria*, at the adult stage, was also isolated from one sample, this being an indication of gross faecal contamination. All the samples were also found to contain significant particulate contamination and pH values encountered varied over the range of 2.5-9.2. None of the samples was found to comply with official specifications and all of them may therefore be regarded as being potentially dangerous to the users.

Keywords: *Ophthalmic, microbial organoleptic, traditional medicine, practitioners*

Résumé

Vingt huit échantillons des prépararations ophthamologiques traditionelles étaient obtenues des tradipracticiens aux états du sud ouest du Nigéria durant une période d un an. Ces échantillons étaient examinées pour stérilité, clarité, presence de contaminants et le PH.Tous étaient contaminées des bactéries a l ordre de 2.00×10^6 à 1.60×10^{11} colonies par unité/ml avec les espéces *pseudomena* et d autres pathogénes potentiel tels que les *staphylocoque aureus, yersinia enterocolita, microcoque* variants et d autres isolats des préparations. A rotifer *Rotaria rotatoria* a l étape adulte, était isolé de l un des échantillons, une indication de contamination des selles. Ces échantillons contenaient significativement des contaminants et les valeurs du PH variaient entre 2.5-9.2. Aucune des échantillons ne satisfaisaient les spécifications officielles et pourraient etre vues comme dangereux pour les usagers.

Introduction

The eye is a very delicate organ, which is very susceptible to bacterial attack especially when it has been traumatized, or when the conjunctiva is inflamed [1]. This is because blood supply to the anterior segment of the eye is limited [2] and, this being so, defense against bacteria invasion is minimal. Furthermore, the eye appears to provide a particularly good environment for bacterial growth [3]

Virtually all bacterial species are potentially pathogenic to the eye and ocular infections like conjunctivitis, keratitis, endophthalmitis and hypopyon corneal ulcer must always be regarded as a possibility in the presence of any bacterial cells [4]. There are however some specific pathogens that are frequently involved in ocular infections. *Ps. aeruginosa* has been shown to have the capacity to cause complete loss of sight within 48 hours by its corneal destroying properties [5]. Other eye pathogens like *S.aureus, S.epidermidis, Strept. pneumoniae, H.influenzae, N. gonorrhea, M. tuberculosis, Francisella tularensis, Treponema pallidum, Serratia marcescens, B. subtilis, Corynebacterium spp, Klebsiella spp, Moraxella spp* as well as the fungi *Fusarium solanin* and *Candida albicans* have been identified [6]. The danger that many bacterial species pose to the eyes is the reason why all eye drops are required to be sterile, not only after preparation but also throughout the period of use in the case of multidose preparations. It is therefore also mandatory that a suitable preservative be included in multidose eye drops. These preservatives are required to maintain the sterility of the preparation by killing the organisms that might have been introduced into the product within three hours [7,8].

Aseptic technique is not one of the practices associated with traditional medical practice. Yet traditional medical practitioners also treat eye diseases with a variety of traditional ophthalmic preparations [9]. The use of traditional ophthalmic preparations in the treatment of trivial eye ailments and curable conditions such as cataracts has been observed to give rise to serious complications [10,11,12,13]. For this reason, some ophthalmic preparations which were prepared by traditional medical practitioners or prepared under their direction were examined for sterility, clarity, presence of particulate matter and pH with a view to assessing their suitability for use in the treatment of ocular pathologies.

Materials and methods

Twenty-eight samples of traditional ophthalmic preparations were obtained from various traditional medical practitioners over a one-year period (August 1998-July 1999) in the South Western states of Nigeria, West Africa.

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Samples

Four sets of the traditional ophthalmic preparations were collected.

1. Samples collected at the Trado-medicine Trade Fair at old Osun State House of Assembly secretariat, Osogbo, Osun state in September 1998.
2. Samples collected at the Trade Fair at the Cultural Centre, Mokola, Ibadan, Oyo State in June, 1999.
3. Samples collected at the 4th Trado-medicine Fair '99 at National Television Authority (NTA), Ibadan, Oyo State in June, 1999.
4. Samples prepared extemporaneously following instructions given by traditional medical practitioners.

The first three sets were obtained already packed in the containers by the traditional medical practitioners. For the fourth set, each of the samples was collected in sterile McCartney bottles. Each of the samples was given a number and labeled appropriately. All the samples were then transferred to the laboratory within 48 hours. The properties of the containers in which the ophthalmic preparations were packed were noted.

Number of Colony Forming Units (CFU).

1.00ml of each of the sample was serially diluted to $1.0x10^{-6}$ with sterile distilled water. 1.00ml of each of these dilutions and 1.00ml of each of the sample was transferred in duplicate onto the surface of nutrient agar (Oxoid, England) plates (Sterilin, England) using a 1.00ml pipette. The plates were then incubated for 48hours at 37°C and distinct colonies from the plates for the $1.0x10^{-6}$ dilution were counted and related to the number of colony forming units in the original sample.

Isolation and identification of the organisms

Those distinct colonies from the plates were subcultured onto fresh nutrient agar plates to obtain pure cultures and isolated colonies with organisms of the same genus, showing the same morphological characteristics being regarded as identical isolates. A code number was ascribed to each organism. Colony morphology on the nutrient agar plates was noted and the isolates were stored in cryovials (Nalgene, Rochester, NY, USA) in the medium of Gibson and Khoury [14]. The cultures were kept frozen at -20°C in a Thermocool (PZ Nigeria, Limited) freezer. Biochemical tests were carried out for the identification of isolates according to tests described by Cowan and Steel [15]. The *Escherichia coli* NCTC 8196, *S. aureus* NCTC 6571, *Ps. aeruginosa* ATCC 19429 and *B. subtilis* NCTC 8236 were used as control organisms.

Determination of particles; shape, size and number

Particle shape determination was performed by putting a drop of the sample on a clean grease free glass microscope slide and covered with a clean cover slip. One drop of each sample on a microscope slide, was observed for shape of particles by light microscopy under high power (x400). Other microscopy characters in the samples used were noted.

The particle size determination was performed as described by Adeoye *et al.*, [16]. The first stage in this method was the calibration of the graticule (or eye piece micrometer) division with a stage micrometer under low power (x100). The graticule and the stage micrometer were superimposed so that their zero lines were mutually aligned on the left hand side of the field of view. Then a reading was taken at that point on the graticule scale which most nearly coincides with 100 stage micrometer divisions and from the result, 1 small graticule was calculated to be 14im long. The second stage was the measurement of the diameter of particles under low power objective. The particles to be measured were placed in the centre of the field of view with the long axis of the particle coincident with the axis of the graticule. The sizes of the particles were then calculated from the number of graticule divisions covered by them. A total of ten particles were measured for each sample.

The number of particles in each sample was counted using the light microscope with haemocytometer counting chamber, the improved Neubauer B.S. 748 Hawksley crystalite with a depth of 0.1mm, on a microscope slide. With the area of each small square being $0.04mm^2$, the volume of liquid in each small square is therefore $4.0x10^{-6}ml$

A small drop of each of the samples was placed on the slide and covered with a cover slip, care being taken not to trap air bubbles under the cover slip. The mean number of particles in a small square for each sample was calculated by counting the number of particles in 25 small squares.

pH Determination

The pH of each sample was determined using standard procedure [17] in a pH meter (CORNING MODEL 7).

Results

Samples

The nature of the samples varied. They were comprised of honey preparation (No1), cattle urine (No2), extract of peeled *Manihot utilissima* (No3), decoction of peeled, unripe *Carica papaya* fruit (No4), fresh human urine (No5), fresh human breast milk (Nos 6 and 7) and those preparations with unknown constituents purchased directly from the traditional medical practitioners. The contents varied from nearly clear liquid preparations (Nos 13 and 20),to cloudy suspensions (Nos 11,14,16,17,18,19,21,22,23,24,27,28), to viscous liquid preparations (No1) of various colours ranging from colourless to red, brown, yellow and green. The measured contents of the samples varied from 10ml to 50ml with a mean volume of 19. 52ml.

Table 1: Viable colony count

Sample code	Viable cells per ml(cfu/ml).n=	Sample code	Viable cells per ml(cfu/ml).n=
1	$3.70X10^{7}$	15	$2.20X10^{7}$
2	$3.00X10^{6}$	16	$4.50X10^{7}$
3	$3.10X10^{7}$	17	$3.00X10^{6}$
4	$2.60X10^{8}$	18	$2.70X10^{6}$
5	$4.80X10^{7}$	19	$6.59X10^{10}$
6	$4.96X10^{8}$	20	$3.56X10^{10}$
7	$5.38X10^{8}$	21	$6.36X10^{8}$
8	$1.15X10^{8}$	22	$2.52X10^{9}$
9	$1.00X10^{9}$	23	$1.20X10^{8}$
10	$1.45X10^{8}$	24	$2.20X10^{6}$
11	$3.28X10^{8}$	25	$2.00X10^{8}$
12	$4.01X10^{8}$	26	$3.65X10^{10}$
13	$3.00X10^{6}$	27	$1.60X10^{11}$
14	$1.10X10^{7}$	28	$3.60X10^{8}$

Containers

The containers in which the samples were supplied were found to be of various kinds. Twenty (71.4%) of the total samples were purchased from the traditional medical practitioners. Thirteen of these had containers with a dropper and a screw cap in the container apparatus. Five of these were glass bottles while the others were white plastic containers. Also, five of the total containers did not have a dropper and one was packaged in a paper envelope, only six of the samples were given one kind of label or the other with the information given in a mixtures of languages: English, Arabic and Yoruba.

Sterility of samples

None of the samples was sterile as they were all found to be contaminated with bacterial cells to the order of $2.0x10^{6}$ to $1.6x10^{11}$ cfu/ml.

Identity of the organisms

The isolated colonies were those that grew on nutrient agar plates at 37°c. A total of 60 organisms were isolated from the samples. One third (33.3%) of these isolates were gram negative organisms contained in 16 (57.1%) of the 28 samples. Twenty-two (78.6%) of the samples were found to be contaminated by gram-positive organisms. Seven of the preparations contained one species of organism each, two different species of bacteria were isolated from each of 15 preparations, two were contaminated with three different species each, whilst four different organisms were isolated from each of the other 4 preparations. Seven (25%) of the samples were contaminated with *Staphylococcus* spp. whilst 5 (17.9%) were contaminated with *Pseudomonas* spp. Sample 8 was also found to harbour an organism belonging to *Streptococcus* spp. Eight (28.6%) of the samples were contaminated with *Bacillus* spp. Samples 6 and 20 were found to contain *Corynebacterium* spp. Also samples 9 and 28 were found to be contaminated with *Proteus* spp. Table 2 shows the identity of these isolates.

Tabel 2: Identity of the isolated organisms

Sample Code	Identity
1	*Alcaligenes faecalis*
2	*Pasteurella spp*
3	*Micrococcus varians, Bacillus brevis, B.coagulans, Yersinia enterocolita*
4	*Neisseria caviae, Bacillus. spp*
5	*B. sphearicus, Ps. aeruginosa*
6	*M. varians, Corynebacterium ovis*
7	*Staphylococcus epidermidis, M. varians, Streptococcus faecalis*
8	*M. luteus, Chromobacterium lividum*
9	*Proteus spp, Flavobacterium spp*
10	*Alkaligenes spp, Aerococcus viridans*
11	*Kurthia spp, Ps. fluorescens*
12	*Staph. epidermidis*
13	*Acinetobacter anitratus, B brevis, Bacillus spp. M. luteus.*
14	*Micrococcus Spp, M. megaterium, M. roseus, Anaerobic cocci.*
15	*Ps. stutzeri, Veillonella spp*
16	*Aerococcus spp, Staph. epidermidis*
17	*Enterobacter spp*
18	*Serratia rubidaea, Staph. epidermidis, Pas teurella spp, B. megaterium.*
19	*Unidentified*
20	*Corynebacterium ovis, Staph. epidermidis, M. varians*
21	*Staph. epidermidis, Micrococcus spp*
22	*Aerococcus viridans, B. brevis*
23	*M. varians, Ps. pseudomallei*
24	*Listeria spp, Micrococcus spp*
25.	*Staph. aureus*
26	*B. polymyxa, Listeria spp*
27	*Bacteroides spp*
28	*B. subtilis,Proteus spp.*

Determination of particles and microscopic characters

Limits for particles in eye drops are specified in the official books [2,7,8], on the basis of which the determination of particles in the traditional ophthalmic preparation samples was carried out. Some particles found in the samples were visible to the naked eye while others were only observable under the light microscope.

Different shapes were noted for the particles and these varied from circular, oval and rod shapes to irregular. In samples 19 and 24, the fragmentary remains of insects as well as plant particles were clearly visible under the microscope.

While studying the particles in sample 22 the presence of a living worm-like organism identified as adult *Rotaria rotatoria* was observed. As many as eight worms were observed in each drop of the sample when viewed under the high power (x400) lens of the light microscope.

Table 3 shows the particle size and particle number properties of the samples.

Table 3: Particles properties of the samples

Sample code	Particle size (µm) Smallest	Largest	Particle size (µm) (Average)	Number of particles Per ml of sample
1	7.0	69.9	34.2	3.50×10^{3}
2	7.0	139.9	30.6	6.50×10^{4}
3	1.0	21.0	12.3	1.53×10^{5}
4	28.0	279.7	105.7	9.70×10^{4}
5	13.0	181.8	48.4	2.50×10^{4}
6	0.2	14.8	2.8	8.40×10^{5}
7	0.2	14.8	2.8	2.64×10^{5}
8	13.7	82.2	35.7	1.20×10^{4}
9	20.5	82.2	47.2	2.50×10^{5}
10	6.9	20.5	13.7	2.53×10^{5}
11	34.2	54.8	54.8	2.35×10^{5}
12	13.7	137.0	45.4	1.25×10^{4}
13	13.7	287.7	81.6	1.68×10^{5}
14	27.4	82.2	45.9	6.50×10^{4}
15	6.9	123.3	54.8	1.50×10^{5}
16	1.4	35.0	13.7	1.50×10^{5}
17	14.0	70.0	22.9	9.50×10^{3}
18	13.0	84.0	33.0	2.15×10^{4}
19	1.4	49.0	17.6	4.25×10^{4}
20	28.0	140.0	57.2	5.10×10^{3}
21	1.4	35.0	11.3	8.35×10^{4}
22	7.0	105.0	35.0	6.00×10^{3}
23	12.0	84.0	34.3	3.25×10^{4}
24	8.0	70.0	26.6	1.20×10^{4}
25	6.0	420.0	63.0	4.52×10^{4}
26	13.0	70.0	27.3	6.00×10^{3}
27	0.2	113.4	28.9	5.90×10^{3}
28	2.8	518.0	80.9	2.10×10^{4}

pH Values

The pH values ranged from 2.5 in sample 1 to 9.2 in sample 22. Six of the samples (samples 1,3,6,7,14 and 15) tend toward acidity with their pH ranging from 2.5 to 3.9. Three (samples 22, 25 and 27) gave readings on the side of alkalinity with pH ranging from 8.9 to 9.2. The others tended towards neutrality.

Discussion

The requirement of sterility for all ophthalmic preparations first appeared in 1955 in the United States Pharmacopoeia [18]. This means in effect that the presence of a single viable organism renders an ophthalmic preparation unfit for use. From this point of view therefore, none of the preparations investigated in this study was found acceptable for use as eye drops. This is more so because each of these preparations carried a heavy bacterial load as shown by the presence of 2×10^{6} to 1.6×10^{11} cfu/ml. Contamination at this level increases the possibility of bacterial infection should these preparations be applied to the eyes. This confirms earlier observations [5,10,11,12]. Furthermore, no less than 15 of these eye drops were found to be contaminated by at least two organisms and four were shown to harbour at least four different organisms suggesting that no significant effort was made to protect the preparations from bacterial contamination during the process of preparation.

Several bacterial species have been reported to have the ability to cause blindness all of which were isolated in the course of this study [19,20,21,22,23]. The isolation of *Ps. aeruginosa* is of special significance as this

organism has been associated with injury to the eye tissues and its ability to cause blindness within 48 hours [20,21]. Other organisms encountered included *Corynebacterium* spp which have been linked to conjunctivitis [3]. *Proteus* spp. which are implicated in endopthalmitis [3,6] as well as Bacillus spp which are implicated in wounds to the eye and endophthalmitis [5,6]. These samples therefore pose a significant danger to their users.

The British Pharmacopoea [7] specifies that eye drops that are solutions should be practically free from particles when examined under suitable conditions of visibility. This is because of the ability of particles present in ophthalmic preparations to cause corneal scarring, a condition which has been associated with visual loss [22]. In addition, particulate contamination has also been recognized as a non-infectious etiological agent of endophthalmitis [4]. Similarly, particles may easily get locked up in ocular tissues causing injuries and may introduce a wide range of bacteria and fungi into eye chambers giving rise to hypopyon. These are the bases for the BP specifying limits for particle size in all eye drops. It states that not more than 20 particles should have a maximum dimension greater than 25µm, not more than 2 particles should have a maximum dimension greater than 50µm and no particles having a dimension greater than 90ì m should be found in an eye drop preparation. Only 4 (14.3%) of the samples: samples 3,6,7 and 10 passed this requirement for particles size.

The presence of particulate contamination in these preparations suggests that little attempt was made to protect the products from contact with the environment during the process of manufacture and packaging. This suggestion is further strengthened by the fact that a worm, *Rotaria rotatoria* was isolated from one of the samples tested in this study. Although this worm is free living and not known to be pathogenic, its presence in the preparation suggests that the water used in preparing the specimen was grossly contaminated with faecal material [24].

The containers of simple dose preparations are required to maintain the sterility of the contents and the applicator up to the time of use. Similarly, containers for multidose eye drops should be fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and rubber or plastic teat [7]. Containers must exclude contaminants and evidently, this was not taken into consideration in the packaging of these traditional ophthalmic preparations. All the containers of the preparations having a dropper were used containers of modern pharmaceutically produced eye drops, which were probably washed and recycled by the traditional medical practitioners.

Another requirement of a suitable multidose ophthalmic preparation is that it should be properly preserved using suitable preservatives in order to kill any microorganism, which might be introduced into the preparation during use. The consistent isolation of bacteria from the samples suggests that no preservatives were added to the eye drops investigated in this study.

Suitable pH as a measure of acidity, alkalinity and stability of the ophthalmic product and of comfort to the user is another official requirement as stated in the Pharmaceutical Codex [2]. The Codex states that unbuffered solutions with pH values of between 3.5 and 10.5 can usually be tolerated with little discomfort. Only 4 (14.3%) of the samples: Nos 1,6,7,and 14, fall outside the official range and this is to the acidic side of the pH. This may result in toxicity to the cornea and other structures of the eye causing irritation.

One of the major reasons attributed to the continued increasing patronage of the traditional medical practitioners by consumers is the issue of cost as traditional medicine preparations are believed to be cheaper than the modern pharmaceutical products. The cost of each sample used in this study was found to be quite high so that there is no comparative advantage in the cost of traditional eye drops over the products of modern pharmaceutical industry, which have been prepared in strict compliance with official requirements and are therefore quite safe.

The results of this study suggest that the eye drops prepared by traditional medical practitioners in Nigeria are a source of danger to ocular health from the point of view of microbial damage caused by microorganisms and physical damage caused by solid particles. Apart from the dangers they pose, these preparations are also quite expensive, many of them more so than the much safer products of the modern pharmaceutical industry.

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