NORMAL CONJUNCTIVAL FLORA AS SEEN IN ADULT PATIENTS AT KIGALI UNIVERSITY TEACHING HOSPITAL

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ABSTRACT

The aim of this study was to assess the pattern of normal ocular bacterial flora isolated from patients attending the Department of ophthalmology at the Kigali University Teaching Hospital and to evaluate their in vitro susceptibility to common antimicrobial agents. From June to October 2011, collection of specimen was performed by rotating a sterile cotton swab on the lower conjunctival sac from the temporal to the medial fornix. Gram stain and culture was performed and antibiotic sensitivity determined in case of bacterial growth. Of the 120 collected samples, 74 (61.6%) showed bacterial growth and all were gram positive. 48.6% were Staphylococcus aureus, while 51.4% were Staphylococcus epidermidis.
There was high sensitivity of Staphylococcus aureus to chloramphenicol (100%), clindamycine (92%), oxacilline (86.7%), ciprofloxacine (76.7%) and norfloxacine (71.9%). However, there was a high resistance of Staphylococcus aureus to penicilline G (11.1%) and tetracycline (52.8%). Staphylococcus epidermidis was highly sensitive to chloramphenicol (71.9%) and oxacilline (71.1%) while it was resistant to erythromycine (28.6%), norfloxacine (35.3%) and penicilline G (40.6%). In this study, all of the isolated pathogens were revealed to be gram-positive bacteria. Chloramphenicol, clindamycine and oxacilline showed good activity against normal flora of the ocular surface and should be used in prevention of post-operative end ophtalmitis.

Keywords: Normal conjunctival flora - antibiotic - sensitivity - bacteria

RESUME

Cette étude a duré cinq mois, de Juin à Octobre 2011, et avait comme objectif l'évaluation du modèle de la flore normale conjonctivale isolée chez les patients ayant consulté le département d'ophtalmologie du Centre Hospitalier et Universitaire de Kigali. La collecte des échantillons a été effectuée en tournant la tige de coton stérile à travers le sac conjonctival inférieur en partant de l'extrémité temporal jusqu'à l'extrémité médial. La coloration Gram et la culture ont été effectuées et l'antibiogramme a été réalisé pour les cultures qui ont montré une croissance bactérienne.
Parmi les 120 échantillons recueillis, il y avait 74 (61.6%) avec croissance bactérienne; et tous étaient des Gram positifs. 48.6% étaient des Staphylocoques dorés tandis que 51.4% étaient des Staphylocoques epidermidis. Le Staphylocoque doré a eu une sensibilité élevée au chloramphénicol (100%), clindamycine (92%), oxacilline (86.7%) et norfloxacine (71.9%). Cependant, il y a eu une forte résistance à la pénicilline (11.1%) et une résistance modérée aux tétracyclines (52.8%). Le Staphylocoque epidermidis était sensible au chloramphénicol (71.9%) et à l'oxacilline (71.1%) tandis qu'il était résistant à la pénicilline G (40.6%). Les germes retrouvés étaient sensibles au chloramphénicol, clindamycine et oxacilline et ces derniers pourraient donc être utilisés pour la prévention des endophtalmites postopératoires

Mots-clés: : Flore conjonctivale normale - antibiotique - sensibilité - bactéries

INTRODUCTION

The body surface supports growth of a variety of bacteria and fungi which collectively are called normal flora. Viruses and parasites are not considered as normal flora; although they are present in asymptomatic individuals. These bacteria may be present and playing an important role in normal body functions and health as they prevent the fixation of invading microorganisms. The normal flora microbes do not produce infections and play no significant role till the epithelial surfaces are injured [1, 2, 3].
Conjunctiva, eyelids and lacrimal apparatus protect the eye. Because of its constant exposure to external media, the conjunctiva is subject to intense microbial contamination[4,5].Most microorganisms are removed by lacrimation, with only a relatively low density microbiota being left behind, consisting of a reduced number of species. The control of ocular invasion by fungi, Gram-negative rods and bacterial of low pathogenicity depends largely on mechanism of immunity [6,7,8].It has been understood for some time that the infection in the wake of surgery, ocular injection, or minor trauma is likely linked to infectious agents resident on the normal ocular surface. The worldwide incidence of post-operative endophthalmitis ranges from 0.05% to 0.30%.Even though post-operative endophthalmitis is rare; it is a devastating complication of intraocular surgery due to its poor vision prognosis % [9].
A clear delineation of interactions between normal flora and the ocular surface is of great benefit in understanding ocular health and pathology. Conjunctival flora is a prime suspect in searching for the cause of post-traumatic or post-operative ocular infections. Better information on its individual composition is therefore desirable.
The Gram positive organisms; particularly coagulase negative Staphylococci are the main residents of the normal eye and cause post-operative infections [4, 10,11]. Recent studies revealed that Gram-positive species predominate 89.8%, and Staphylococcus epidermidis is the most frequently isolated organism, accounting for 60.6%[12].
Newer generation fluoroquinolones, chloramphenicol and tetracycline are widely used as prophylaxis to prevent post-operative endophthalmitis. Assessment of their in vitro sensitivity will help surgeons to prevent and treat post-operative endophthalmitis [13].
The aim of this study is to assess the pattern of conjunctival flora isolated from patients attending the Ophthalmology

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METHODS

This is a cross sectional study that lasted for five months from June to October 2011.The study was conducted after obtaining approval from the Kigali University Teaching Hospital Scientific Research and Ethical Committee. Informed consent was obtained from each participant after giving a detailed explanation of the study purpose. Study population was made of 120 participants, who attended the Ophthalmology Department of Kigali University Teaching Hospital during the study period. All subjects were aged between 16 and 85 years, without any sign of local or systemic infection, and none had used topical or systemic medication over the recent 30 days. Those with ocular trauma or those that had undergone intraocular surgery or wore contact lenses were not included. Were also excluded subjects with eyelid or eyeball deformity, or those with dry eye syndrome. Socio-demographic and clinical data were collected by filling out a questionnaire with information got from patients after the patient's consent. Collection of specimen was performed from both eyes, after application of a topical anaesthetic medication by rotating a sterile cotton swab through the lower conjunctival sac from the temporal to the medial fornix. Touching the eyelashes and the skin of the eyelids was avoided to avoid contamination. The cotton swab was kept in normal saline 0.9% while transporting the sample to the laboratory. Once in the laboratory, the sample was enriched with thioglycolate broth for 24 hours, then the gram stain was performed. Thereafter, culture inoculation to agar plate and antibiotic sensitivity was performed. Quantitative data concerning the results from Gram stain culture and sensitivity were collected from the bacteriology section of the laboratory by filling out the data collection sheets. All data were kept confidentially.

RESULTS

240 eyes of 120 participants were reviewed in this study. 43.3% of these participants were male, while 56.4% were female. This study was carried out in adult subjects, with mean age of 39.7, SD= 17.16. 65.8% of participants came from urban areas while 34.2% came from rural areas. 17.5% of the subjects were farmers, while 82.5% were non farmers.Within people enrolled in the study, 46.7% came from hot weather, 20.8% from cold weather, and 32.5% from moderate weather.

Table 1: Outcome of collected samples

			B.G: 148	
		G. +: 204		
	G.S: 204		**N.B.G: 56**	
C.S: 240		**G.-:** 0		
	G.S.N.D: 36			

C.S: Collected sample	**G.S:** Gram Stain	**G.S.N.D:** Gram Stain Not Done
G.+: Gram Positive	**G.-:** Gram negative	**B.G:** Bacterial Growth
NBG: No Bacterial Growth		

From a total 240 collected specimen, gram stain was performed for 85% and all of them were Gram-positive. Of these 240 specimens, cultures were positive in 61.7%.

Table 2: Pathogen found

Germs	**N**	**%**
Staphylococcus aureus	72	48.6
Staphylococcus epidermidis	76	51.4
Total	**148**	**100**
N= Number with bacterial growth, **%**= percentage		

Of 148 specimens with bacterial growth, all were Gram positive pathogens and 51.4% were Staphylococcus epidermidis, while 48.6% were Staphylococcus aureus.

Table 3: Antibiotic sensitivity pattern of isolatesfound

	Staphylococcus aureus					*Staphylococcus epidermidis*				
Antibiotics	**Number tested**	**Sensitive**	**%**	**Resistant**	**%**	**Number tested**	**Sensitive**	**%**	**Resistant**	**%**
Chloramphenicol	6	6	100	0	0	32	23	71.9	9	28.1
Tetracycline	36	19	52.8	17	47.2	38	23	60.5	15	39.5
Norfloxacine	32	23	71.9	9	28.1	17	6	35.3	11	64.7
penicilline G	36	4	11.1	32	88.9	32	13	40.6	19	59.4
Oxacilline	30	26	86.7	4	13.3	38	27	71.1	11	28.9
Ciprofloxacine	30	23	76.7	7	23.3	17	10	58.8	7	41.2
Clindamycine	25	23	92.0	2	8.0	38	21	55.3	17	44.7
Erythromycin	2	2	100	0	0	21	6	28.6	15	71.4

There was high sensitivity of Staphylococcus aureus to chloramphénicol (100%), clindamycine (92%), oxacilline (86.7%), and ciprofloxacine (76.7%). However, it was found a high resistance of Staphylococcus aureus to penicilline G (11.1%) and tetracycline (52.8%). Staphylococcus epidermidis was highly sensitive to chloramphenicol (71.9%) and oxacilline (71.1%) while resistance to erythromycin was (28.6%), norfloxacine (35.3%) and penicilline G (40.6%).

Table 4: Residence and bacteria found Cross tabulation

		Bacteria found			
		Staphylococcus aureus	*Staphylococcus epidermidis*	No growth	Total
Residence	Urban	29	21	29	79
	Rural	9	15	17	41
Total		38	36	46	120

Staphylococcus epidermidis was found in 21participants coming from urban areas and 15 participants from rural areas. Staphylococcus aureus was observed in 29 peoples from urban areas and 9 peoples from rural areas. This distribution did not show any statistically significant difference (p = 0.09).

DISCUSSION

Conjunctival sac is characterized by a normal flora that is changing dynamically through our lifetime because of its long term exposure to the environment. This diverse and large number of microbes has been regarded as important organisms that are part of the defense mechanism of the eye in preventing colonization by more pathogenic microorganisms, especially after surgery or injury [14].
We reviewed 240 eyes of 120 patients who attended the ophthalmology department of Kigali University Teaching Hospital, from all regions of Rwanda. The age of patients varied between 16 and 81years, with a mean age of 39.75years, and a female to male ratio of 1.3.This gender

distribution could be due to the general population distribution in which female are more prevalent than male.This result is comparable to that found in the study carried out by Arantes TE where male patients were 44% and female were 56%. However, the mean age in this study was high compared to that described by Jing Liu who found a mean age of 21.23 years among young people [14].

This study was performed on participants from both urban and rural areas (65.8% and 34.2% respectively). KUTH is a referral hospital receiving patients from all regions of Rwanda, both rural and urban. Alemayehu et al. found comparable findings with 74.3% of enrolled patients coming from urban areas [4]. Even though this study was a hospital based one, these figures gave opportunity of determining the types of microbes from different categories of populations referred from all regions of Rwanda.46.7% of all enrolled patients came from hot weather, while 32.5% were from moderate weather and the remaining 20.8% came from cold weather. This could be related to the geographic situation of KUTH located in a tropical region.

Gram stain was performed in 85% of all collected specimens and all were gram positive. Cecilia Moeller et al. described similar figures in their study where gram positive was described as the most frequently isolated bacteria [15].

Cultures were positive in 61.6% of all collected specimens and in 23.3% of the collected samples, the culture and antibiotic sensitivity were not performed due to the absence of microbe on the direct exam.

Bacterial growth was low (61.6%) compared to the study performed by Chung et al, where the number of patients with bacterial growth was 81.9%[17].This figure is high compared to the study performed by Saleem et al, with only 10.1% of bacterial growth, while Staphylococcus epidermidis was isolated in 54.5%[18]. Of 148 samples with bacterial growth, all were gram positive germs and 48.6% were Staphylococcus aureus, while 51.4% were Staphylococcus epidermidis. Arantes TE and al found 88.9%of gram positive and 54.0% of coagulase negative staphylococci in a study on normal ocular flora[11]. Shin Haeet al found also similar results and described Staphylococcus epidermidis as the most frequent isolated germ, with a prevalence of 60.6%[16].Staphylococcus aureus was mostly found in the age group between 18 and 25 years (22.8%), while Staphylococcus epidermidis represented 20% in the age group between 26 and 35 years.

This study revealed a high susceptibility rate of Staphylococcus aureus to Chloramphenicol (100%), Rifampicine (100%), Erythromycine (100%) and Clindamycine (92%). However, there was a high resistance of Staphylococcus aureus to Penicilline G (11.1%) and Tetracycline (52.8%). This high resistance of Staphylococcus aureus to penicillin and tetracycline is not a good news for eye care professional given the fact that tetracycline is widely used in prevention and treatment at primary eye care level. Concerning Staphylococcus epidermidis, high susceptibility was seen to chloramphenicol (71.9%) and oxacilline (71.1%); while erythromycine (28.6%), norfloxacine (35.3%) and penicilline G (40.6%) had low responsiveness. These results are in accordance with those described by Arantes et al (6) and CN Ta et al[7] who found that isolated pathogens were sensitive to chloramphenicol and broad-spectrum fluoroquinolones and resistant to penicilline.

CONCLUSION

This study described Staphylococcus epidermidis and Staphylococcus aureus as the most common organism of the normal conjunctival flora at Kigali University Teaching Hospital.

Chloramphenicol and oxacilline showed good response to the normal ocular flora and should be used in prevention and treatment, while antibiotics like penicilline are less effective.

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