Bacteriological Profile, Culture, Sensitivity, and Associated Factors of Bacterial Conjunctivitis Among Patients Attending Eye Clinic at a Tertiary Hospital in Uganda

Abdulsalam Ahmed Mohamed
Makerere University
Immaculate Atukunda
Makerere University
Senai Goitom Sereke (nayhersen@gmail.com)
Makerere University
Philip Orishaba
Makerere University
Henry Kajumbula
Makerere University
Lydia Nakiyingi
Makerere University
Claire Lusobya Rebecca
Makerere University

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Abstract

Background: An estimated 30 million people in Sub-Saharan Africa are affected by conjunctivitis and untreated conjunctivitis poses an increased risk of developing keratitis and corneal ulcerations which could result in blindness and visual impairment. Despite the commonality of the disease in this region, there is a paucity of studies on the occurrence and associated factors of bacterial conjunctivitis. This study sought to determine the bacteriological profile, culture, sensitivity, and factors associated with bacterial conjunctivitis among patients seen in the eye clinic at Mulago National Referral Hospital (MNRH).

Methods: This was a cross-sectional study conducted among participants who presented to the eye clinic of MNRH with eye discharge, irritation, tearing, and red eyes, between January and March 2020. Findings of slit-lamp biomicroscopy were recorded. Conjunctival swabs were taken from participants for laboratory analysis to establish the bacterial profiles and antibiotic susceptibility.

Results: A total of 82 participants were enrolled in the study. The median age was 30 years (IQR 16-48). Forty-two (51%) had bacterial conjunctivitis and the majority had unilateral disease (n=72, 87.8%). Participants aged 10-24.9 years had a higher prevalence of bacterial conjunctivitis compared to those aged 25 and above (aPR = 1.89, 95%CI 1.18-3.03, p = 0.008). The commonest bacteria was *S. aureus* (n=24, 57.1%) and were sensitive to ciprofloxacin with resistance percentages less than 20%. Almost 95% of the bacteria were susceptible to ciprofloxacin and 90% were susceptible to chloramphenicol.

Conclusions: Bacterial conjunctivitis was mainly caused by gram-positive bacteria, especially *S. aureus*. Age was significantly associated with bacterial conjunctivitis. Most of the bacteria were sensitive to chloramphenicol, ciprofloxacin, and clindamycin.

Background

Bacterial conjunctivitis is an infection of the eye's mucous membrane (1). The conjunctiva, extends from the back surface of the eyelids (palpebral and tarsal conjunctiva), into the fornices, and onto the globe (bulbar conjunctiva) until it fuses with the cornea at the limbus (2). Bacterial Conjunctivitis is mainly characterized by yellowish-white discharge. Other symptoms might include; itching, irritation, foreign body sensation, and tearing (3).

In a study done in the United States, conjunctivitis occurred in 0.13% of the general population and accounted for 30% of all patient visits to the outpatient clinic (4, 5). Staphylococcal infection was the commonest cause of bacterial conjunctivitis in adults in the U.S, followed by *Streptococcus pneumonia* and *H. influenza* (3). In studies done in Pakistan and Nigeria, the prevalence of bacterial conjunctivitis was estimated at 18.3% and 1.1% respectively (6, 7). Trachoma which is caused by C*hlamydia trachomitis* is the commonest form of bacterial conjunctivitis found in sub-Saharan Africa and it was estimated to affect 30 million people in 2001 (8, 9). Of the global 229 million people living in trachoma endemic areas, about 10.8 million of them live in Uganda (10). The disease contributed to the blindness of many as repeated infection turns eyelashes inwards, scraping the cornea and eventually causing irreversible damage (10). The sequel of bacterial conjunctivitis may include compromised tear production, trauma, immune suppression, and disruption of the epithelial barrier (11). Age is also associated with bacterial conjunctivitis and it mostly affects neonates followed by young adults (12).

In Uganda, an outbreak of bacterial conjunctivitis was reported in 2017 in Gulu district by the Ministry of Health (13). However, the bacteriological profile and factors associated with bacterial conjunctivitis were not established. Moreover, there are limited published studies in our setting. This study, therefore, aimed to establish the bacteriological profile, culture, sensitivity, and factors associated with bacterial conjunctivitis among patients.

Methods

Study design and setting

This was a hospital-based cross-sectional study conducted between January and March 2020. The study was conducted in the two ophthalmology outpatient clinics of Mulago National Referral Hospital (MNRH). It's located on Mulago Hill in the northern part of the city of Kampala. It is approximately 5 kilometers by road, northeast of Kampala's central business district. The Consultants and Ophthalmic clinical officer clinics of MNRH both receive around 50–60 patients per month with a diagnosis of clinical bacterial conjunctivitis based on symptoms and signs listed by the American Academy of Ophthalmology (e.g., mattering and adherence of eyelids, itching, tearing, discharge, irritation, pain, photophobia, blurred vision). On average 3 out of the total patients seen in a day (100 to 120) have conjunctivitis.

Study population

All consenting participants including children (parent's consent) and adults who presented with eye discharge, irritation, tearing, and pink or red eyes at the eye clinic in MNRH were included in the study. Patients with conjunctivitis but had no mucopurulent eye discharge, critically ill patients, and patients with reduced cognition due to mental illness or any other condition were excluded.

Sample size

Using the sample size estimation formula for a single proportion by Scheaffer, Mendenhall III (14)

$$n = deff\ x\ \frac{N\hat{p}\hat{q}}{\frac{d^2}{1.96^2}(N-1)+\hat{p}\hat{q}}$$

Where n = sample size, deff = Design effect = 1, N = population size (Estimated number of patients with diagnosis clinical bacterial meningitis seen at eye clinic in 3 months – 3 x 60 = 180)

Bacteriological profile sample size estimation was done with an assumption that $\hat{p}$ = the estimated proportion = e.g 85.06 for Staphylococcus aureus (15), $\hat{q}$ = 1 - $\hat{p}$ = 14.94, *d =* desired absolute precision or absolute level of precision *= 0.05, n* = **82**.

Sample size estimation for sensitivity pattern was done with the assumption that $\hat{p}$ = the estimated proportion of gram-positive that are sensitive to antimicrobial agent = e.g 92.2 for ciprofloxacin (16); $\hat{q}$ = 1 - $\hat{p}$ = 7.8; *d =* desired absolute precision or absolute level of precision *= 0.05, n* = **69**. Then we took the biggest number (82) as our sample size.

Study procedure

All patients presenting with symptoms of bacterial conjunctivitis were approached and given information about the study. Consenting participants were directed into a private consultation room for an interview and eye examination. Details of diagnosis, age at onset of disease, further history on the details of the disease, and family history were obtained from the parents or guardians when the participant was a child. Blood pressure with pulse rate was measured using an age-appropriate blood pressure cuff. Vision assessment was done by a trained nurse. The nurse tested the distance vision using an illuminated Snellen chart at 6 meters for school-going children, Lea test; the gratings for 0-24months, and Cardiff test for 18–60 months. Those with refractive correction were tested with their available correction. Each eye was occluded in turn and the best Visual acuity (VA) was assessed. Those found with VA worse than 6/6 had their vision with pinhole assessed and any changes recorded. Retinoscopy was done to categorize the refractive errors if found.

A slit-lamp biomicroscopy was done by an ophthalmology resident on all the study participants and findings in the anterior segment were recorded. Conjunctival swabs were taken for culture and sensitivity by adding biochemicals such as coagulase and catalase. The participant was asked to look up and gently pull down the lower lid exposing the conjunctiva. A swab stick was then gently swept along the lower fornix from inner to outer canthus taking care not to touch the eyelids and cornea. For a participant with discharge in both eyes, one sterile swab was taken from one eye. Swabs were collected using aseptic techniques in Amies transport media and immediately sent to the laboratory. The laboratory results of culture and sensitivity were obtained between three to five days.

A routine random blood sugar test and Human Immunodeficiency Virus (HIV) antibody tests were performed on all participants. Pre-and post-test HIV counseling was done by a qualified counselor.

Statistical analysis

Data was entered in Epidata V 4.2 and analyzed using Stata V14. Descriptive statistics such as proportions, means, standard deviations, and medians were used for baseline characteristics and data displayed in tables and graphs. The bacteriological profile was summarized using frequencies and proportions and was displayed in tables and graphs. The numerator was the type of bacteria while the denominator was all the study subjects with culture-positive conjunctivitis. Assumptions of independence, equal variance, normal distribution, and no collinearity were checked. For bivariate analysis, we used logistic regression to obtain unadjusted odds ratios and corresponding 95% confidence intervals if the proportion of the bacterial conjunctivitis is < 10%. Otherwise, Poisson regression was used to obtain unadjusted prevalence ratios and their corresponding 95% Confidence interval. All variables with a p-value less than 0.2 were included for multivariate analysis. At multivariate regression, a binary logistic or modified Poisson regression model was run. The interaction was assessed using the chunk test and confounding variables that had a more than 10% difference between the adjusted and unadjusted odds/prevalence ratios were considered to be confounders. Variables with p-values less than 0.05 at multivariate were considered to be significantly associated with bacterial conjunctivitis.

Results

A total of eighty-two participants were recruited for the study with a median age of 30years (IQR 16- 48). The majority (n=47, 57.32%) of participants were twenty-five years or older. More than half (n=51, 62.2%) of the participants were males. Most of the participants had primary (n=32, 39%) and secondary education (n=31, 37.8%). Most (n=40, 62.5%) of the adults in this study were sexually active. Seven (8.5%) of the participants reported that it was not important to wash hands with soap but the majority (n=62, 75.6%) believe that it was important to wash hands with soap after eating. Only 24 (29.3%) reported that they always wash their hands with soap before eating and 68 (82.9%) participants reported washing their hands with soap after using the toilet. Most of the participants were HIV negative 80 (97.6%) and had no history of ocular medications 68 (82.9%). Beta N eye drop was used in 5 (35.71%) of the participants (Table 1).

All participants presented with mucopurulent eye discharge, eye irritation, and tearing. Other eye complaints included red-eye 79 (97.5%), burn sensation 60 (73.2%), and light sensitivity 80 (97.6%). Unilateral conjunctivitis predominated the cases 72/82 (87.8%). Of all the participants who had clinical bacterial conjunctivitis, 42 (51%) had a bacterial isolate. Similarly, 40 (49%) showed no bacterial growth in the culture and sensitivity test (Table 2) (Figure 1).

Thirty-eight out of 42 (90.5%) of the causative agents were gram-positive bacteria. The Commonest gram-positive bacteria was *Staphylococcus Aureus* 15 (39%), followed by Coagulase-Negative *Staphylococcus* 9 (24%) bringing the total of bacterial conjunctivitis attributable to *Staphylococcus* to 24 (57.1%). Table 3 and Figure 2 show the bacteriological profile of patients with bacterial conjunctivitis.

Staphylococcus aureus showed 100% susceptibility to ciprofloxacin in this study. Coagulase Negative staphylococcus showed 78% susceptibility to chloramphenicol. Streptococcus Pneumonia showed 80% susceptibility to chloramphenicol. In general, 48 % of bacterial conjunctivitis were susceptible to clindamycin and 43% susceptible to chloramphenicol and ciprofloxacin as shown in Table 4. One-third of all bacteria causing conjunctivitis were resistant to Co-trimoxazole and penicillin G (Table 5) and (Figure 3).

At bivariate, participant's age (10-24.9yrs, cPR = 1.84, 95%CI 1.14-2.97, p = 0.012), handwashing after eating (cPR = 1.43, 95%CI 0.92-2.24, p = 0.116) and handwashing after toilet use (cPR = 1.39, 95%CI 0.87-

2.24, p = 0.17) were found to have p values <0.2 so they qualified for multivariate analysis. At multivariate, only the participant's age was significant. Participants aged 10-24.9 years were almost twice as likely to have bacterial conjunctivitis as compared to participants who were 25 years and older (aPR = 1.89, 95%CI 1.18 – 3.03, p = 0.008). The importance of washing hands with soap was not included in the final model due to the small number of outcomes among those who responded in the negative. Washing hands with soap before the toilet was not included in the final model due to collinearity with washing hands with soap after the toilet (Table 6).

Discussion

This study determined the bacteriological profile of patients with bacterial conjunctivitis at MNRH. The findings of the study showed that more than half of the patients at the ophthalmology clinic in MNRH have bacterial conjunctivitis and the most common bacterial pathology was *staphylococcus* which was susceptible to chloramphenicol, ciprofloxacin, and clindamycin. This prevalence is slightly lower than the prevalence seen in a study that enrolled 111 patients with a mean age of 33.2 months (prevalence = 78% 95% CI = 69.6%-85.6%) (17). The lower prevalence found in our study could be because children tend to have more bacterial conjunctivitis compared to their older counterparts (18). This prevalence is though, higher than the one observed in the United States (4) and Pakistan (6) at 18.3% and this could be explained by the fact that the USA and Pakistan, have better sanitation practices compared to Uganda and proper sanitation has been documented to reduce the risk of bacterial conjunctivitis.

Of the 42 bacterial infections confirmed, over 90% were gram-positive bacteria, with *Staphylococcus aureus* accounting for almost forty percent of the gram positives followed by Coagulase Negative staphylococcus, which accounted for almost a quarter of the gram-positive infections. There were only 3 gram-negative isolates, accounting for 10% of the infections and these were *Acinetobacter spp*, *Escherichia coli*, and *Proteus mirabillis*. This trend of the majority of conjunctivitis being caused by gram-positive bacteria and especially *S. aureus* have been observed in other studies (3, 19). This is probably because *S. aureus* is a normal flora on the skin, so it can easily be picked when someone touches their skin and then touches the eyes.

In this study, almost half of the participants with a microbial diagnosis of conjunctivitis had culture-positive bacterial conjunctivitis and this is mainly caused by gram-positive bacteria, in particular *S. aureus* and the coagulase-negative staphylococcus. We also found that most of the gram-positive bacteria were susceptible to the commonly used antibiotics. Almost 95% of the isolates were susceptible to ciprofloxacin and 90% were susceptible to chloramphenicol. Most of the bacteria were found to be resistant to penicillin G, co-trimoxazole, ampicillin, and augmentin. The most common bacteria isolated, *S. aureus* was generally sensitive to the antibiotics with resistance percentages less than 20%. This trend in susceptibility has been shown in other studies (16).

Participants aged 10-24.9 years had a significantly higher prevalence of bacterial conjunctivitis when compared to their older counterparts. Moreover, when compared with those aged 25 and above, participants aged under 10 had a higher prevalence of bacterial conjunctivitis but the difference in the prevalence was not statistically significant. This association has been documented in the literature, with bacterial conjunctivitis prevalence reducing with an increase in age (18). In the Smith et al study, the disease was more prevalent among the pre-school age children than their older counterparts. The most logical explanation from the literature is that bacterial conjunctivitis is mainly caused by poor sanitation behaviors like washing hands, which is better done by older people than the young ones.

Washing hands after eating has been documented (20) as a risk factor for bacterial infections among ophthalmology patients but were not significant in our study even though patients who do not wash their hands after eating had an increased prevalence of bacterial conjunctivitis compared to those who washed the hands. This could be because of the way the information was captured. We used the patient's word of mouth, which may not be true sometimes, leading to information bias.

When people visit the toilet and they do not wash their hands, there is a possibility of certain bacteria especially coliforms remaining on the fingers which can become pathogenic for instance if someone touches the eyes. This variable was not statistically significant although it is a risk factor for bacterial conjunctivitis, especially for trachoma (20). In this study, patients that did not wash their hands after visiting a toilet, had a 13% prevalence of bacterial conjunctivitis compared to those who wash, though it was not statistically significant p = 2.19). This is probably because, in this study, coliforms were not prevalent hence they had not a lot of impact on the disease. Another possible explanation could be because of information bias. Patients could claim to wash their hands and yet they don't. A major limitation is that routine antimicrobial susceptibility testing is based mainly on concentrations we expect to achieve by systemic use of an antibiotic and not the topical application used in conjunctivitis which achieves much higher concentrations and may kill the organisms even when reported as resistant, if the routine Clinical & Laboratory Standards Institute (CLSI) (21) standards are used. Since the study used the word of mouth of participants, this could have introduced information bias.

Conclusion

Among patients diagnosed with microbial infections at ophthalmology clinics in MNRH, nearly 50% have bacterial conjunctivitis, mainly caused by gram-positive bacteria, especially *S. aureus*. Most of the bacteria that caused conjunctivitis were sensitive to chloramphenicol, ciprofloxacin, and clindamycin. Younger age was significantly associated with bacterial conjunctivitis.

Abbreviations

CLSI: Clinical and Laboratory Standards Institute

HIV: Human immunodeficiency virus

MNRH: Mulago National Referral Hospital

VA: Visual acuity

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants to participate in the study. Ethical approval was obtained from the Makerere University School of Medicine Research and Ethics committee (reference number #REC REF 2020-004) and administrative clearance was sought from Mulago National Referral Hospital.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contribution

All authors made a significant contribution to the work, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Tables

Table 1 Demographics of Study participants

Variable	Frequency n=82	Percent
Age categories, median (IQR)		30 (16-48)
≥25	47	57.32
10-24.9yrs	17	20.73
<10yrs	18	21.95
Sex		
Male	51	62.2
Female	31	37.8
Ethnicity		
Bantu	81	98.8
Nilotic	1	1.2
Marital status		
Single	34	41.5
Married	37	45.1
Separated	11	13.4
Education level		
None	9	11.0
Primary	32	39.0
secondary	31	37.8
Tertiary	10	12.2
Occupation		
Employed	40	48.8
Unemployed	20	24.4
Student	22	26.8
Sexually active		
No	24	29.3
Yes	40	48.8
Not applicable*	18	22.0
Condom Use		

No	54	65.8
Yes	10	12.2
Not applicable*	18	22.0
Smoking		
No	64	78.0
Not applicable*	18	22.0
Alcohol Consumption		
No	60	73.2
Yes	4	4.8
Not applicable*	18	22.0

**denotes the participants aged <10years. Questions relating to sexual activity, condom use, smoking, and alcohol consumption did not apply to participants aged <10 years in this study.*

Table 2: Diagnostic Clinical Criteria of Bacterial Conjunctivitis:

	Frequency n=82	percent
Mucopurulent eye discharge		
Yes	82	100
Red eye		
Yes	80	97.6
No	2	2.4
Burn sensation of the eye		
No	22	26.8
Yes	60	73.2
Light sensitivity of the eye		
No	2	2.4
Yes	80	97.6
Irritation of the eye		
Yes	82	100
Tearing of the eye		
Yes	82	100
Nature of symptoms		
Unilateral	72	87.8
Bilateral	10	12.2

Table 3: Bacteriological profile from the different sites

	Eyelids involvement	Eyelashes involvement	Conjunctiva involvement	Cornea involvement
Gram-positive				
Acinetobacter Spp	0	0	1	1
Coagulase - Negative *Staphylococcus*	0	0	9	9
Group C *Streptococcus*	0	0	1	1
Haemophilus influenza	0	0	5	5
Staphylococcus aureus	0	0	15	14
Streptococcus pneumonia	0	0	5	5
Streptococcus Spp	0	0	1	1
Viridans streptococcus	0	0	1	1
Total	**0**	**0**	**38**	**37**
Gram-Negative	**1**	**0**	**3**	**3**
Acinetobacter Spp	0	0	1	1
Escherichia coli	1	0	1	1
Proteus mirabillis	0	0	1	1
Total	**1**	**0**	**3**	**3**

Table 4. Antibiotic Susceptibility

	Isolated species										
drug	Acinetobacter Spp	Coagulase - Negative staphyloco	Escherichia Coli	Group C Streptacoccus	Haemophilus Influemziae	Proteus Mirabillis	Staphylococcus Aureus	Streptococcus Pneumonia	Streptococcus Spp	Viridans Streoptococcus	Total
Ampicillin	0	0	0	0	0	1	0	0	0	0	1
Augmentin	0	0	0	0	0	1	0	0	0	0	1
Cefotaxime	0	0	1	0	0	0	0	0	0	0	1
Ceftazidime	1	0	0	0	0	1	0	0	0	0	2
Ceftriaxone	0	0	1	1	0	1	0	1	1	0	5
Cefuroxime	0	0	1	0	4	0	0	0	0	0	5
Chlorampheni	0	7	0	0	0	0	7	4	0	0	18
Ciprofloxacin	1	0	0	0	0	1	15	1	0	0	18
Clindamycin	0	1	0	1	0	0	14	2	1	1	20
Erythromycin	0	1	0	1	0	0	8	2	1	0	13
Gentamycin	1	7	0	0	0	1	0	0	0	0	9
Imipenem	2	0	0	0	0	0	0	0	0	0	2
Oxacillin	0	1	0	0	0	0	6	1	1	0	9
Penicillin G	0	0	0	0	0	0	0	1	0	0	1
Piperacillintaz	2	0	1	0	0	0	0	0	0	0	3
Rifampin	0	0	0	0	0	0	0	1	0	0	1
Tetracylin	0	0	0	0	0	0	7	1	1	0	9
Vancomycin	0	1	0	1	0	0	2	1	0	1	6

Table 5: Antibiotics Resistance in bacterial Conjunctivitis

	Species Isolated									
Drug	Acinetobacter Spp	Coagulase-Negative staphyloco	Escherichia Coli	Haemophilus Influemziae	Proteus Mirabillis	Staphylococcus Aureus	Streptococcus Pneumonia	Streptococcus Spp	Viridans Streoptococcus	Total
	freq	freq	freq	freq	freq	freq	freq	freq	freq	freq
Ampicillin	0	0	1	5	0	0	0	0	0	6
Augmentin	0	0	1	1	0	0	0	0	0	2
Chloramphenicol	0	0	0	0	1	0	1	0	0	2
Ciprofloxacin	0	1	0	0	0	0	0	0	0	1
Clindamycin	0	0	0	0	0	1	1	1	0	3
Co-trimoxazole	0	0	1	5	1	2	3	0	1	13
Erythromycin	0	2	0	0	0	1	3	0	1	7
Nafcillin	0	1	0	0	0	0	0	0	0	1
Oxacillin	0	7	0	0	0	0	0	0	0	7
Penicillin G	0	1	0	0	0	9	3	0	0	13
Piperacillintaz	1	0	0	0	0	0	0	0	0	1
Tetracylin	0	1	0	0	0	0	3	0	1	5
Trimethoprim	0	0	0	0	0	2	0	0	0	2

Table 6. Multivariable analysis of factors associated with Bacterial conjunctivitis.

Variables	PR (95% CI)	p value	Adjusted PR (95% CI)	p value
Age				
>=25	1 (1.00-1.00)		1	
10-24.9yrs	1.84 (1.14-2.97)	**0.012**	1.89 (1.18-3.03)	**0.008***
<10yrs	1.74 (1.07-2.85)	**0.027**	1.49 (0.83-2.69)	0.184
Hand Washing after eating				
Yes	1 (1.00-1.00)		1	
no	1.43 (0.92-2.24)	0.116	1.34 (0.76-2.37)	0.313
Hand Washing After toilet				
yes	1 (1.00-1.00)		1	
no	1.39 (0.87-2.24)	0.17	1.13 (0.58-2.19)	0.726

Figures

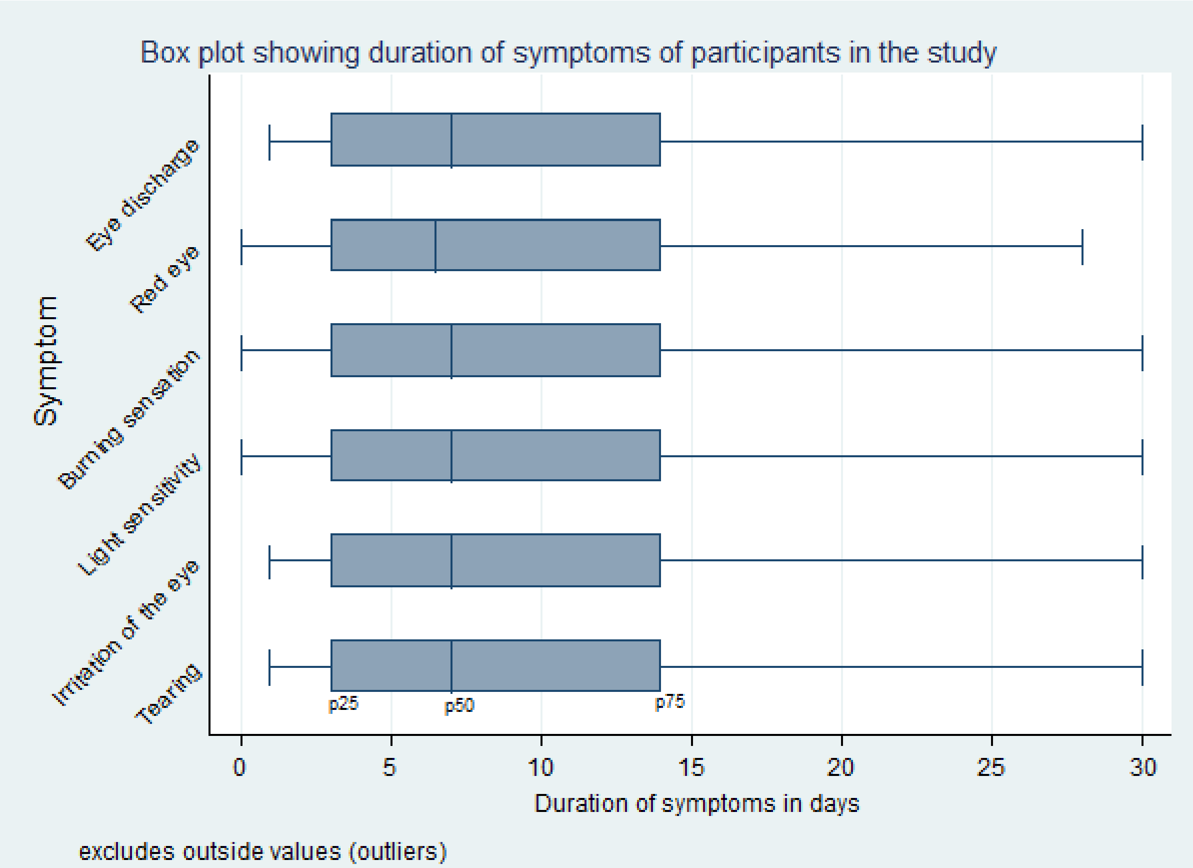

Figure 1

Duration of symptoms of participants in the study

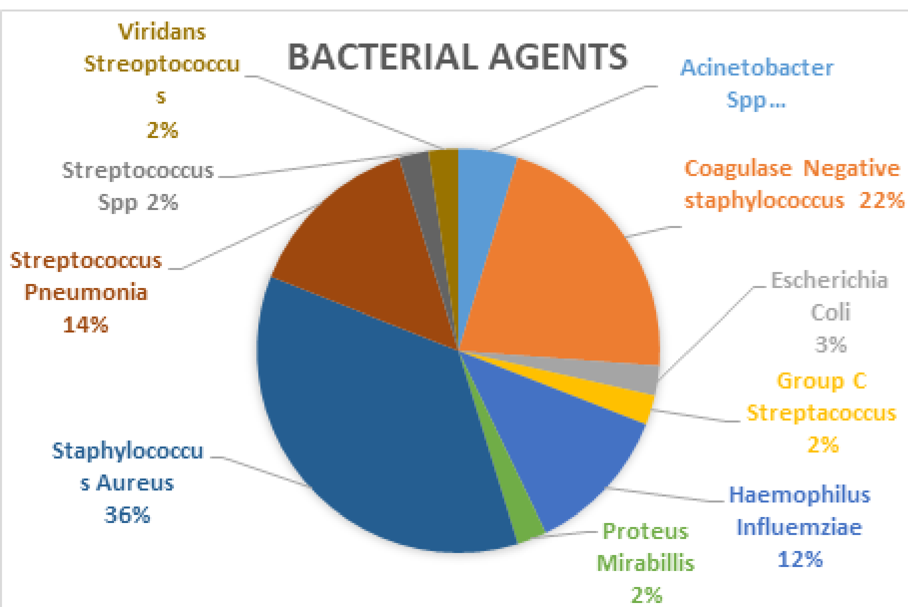

Figure 2

Bacterial Agents

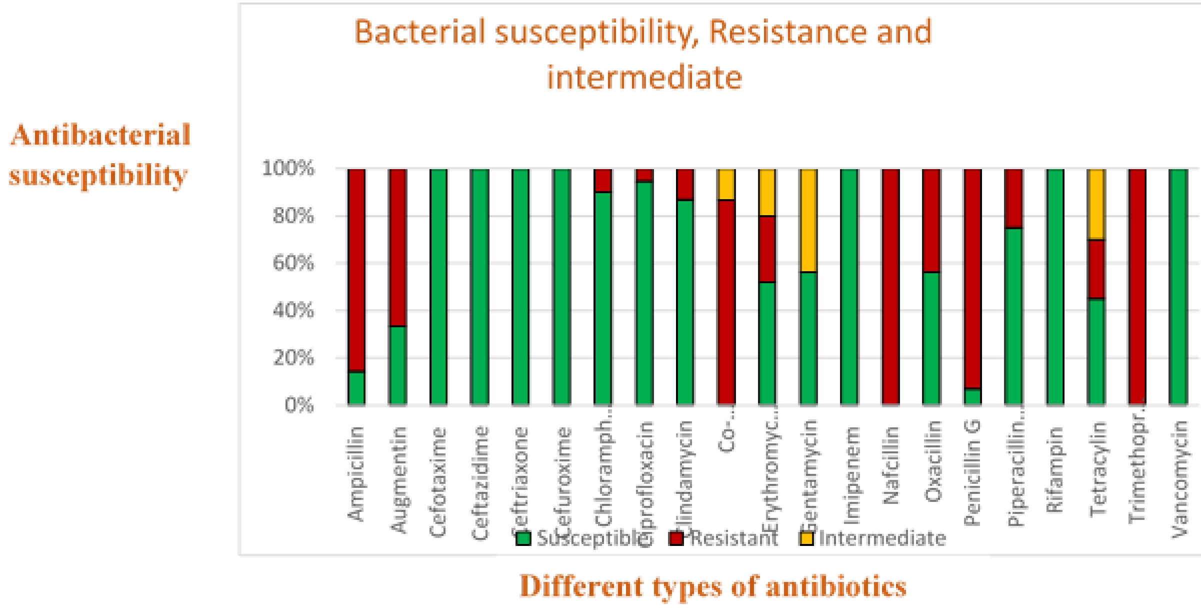

Figure 3

Bacterial susceptibility, Resistance and intermediate