Original article

Acute bacterial conjunctivitis – antibiotic susceptibility and resistance to commercially available topical antibiotics in Nepal

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Abstract

Introduction: There is a shifting trend in susceptibility and resistance of the bacteria towards available antibiotics in the last decade. Therefore, periodic studies to monitor the emerging trends in antibiotic susceptibility and resistance are crucial in guiding antibiotic selection. **Objectives:** The aim of this study was to determine the most common pathogens causing bacterial conjunctivitis, and to find the in vitro susceptibility and resistance of these pathogens to commercially available topical antibiotic eye drops in Nepal. **Subjects and methods:** Conjunctival smears and antibiotic sensitivity tests were performed for 308 patients presenting to the Eye Care Center, Padma Nursing Home, Pokhara, Nepal from 11th December 1012 to 4th October 2013 with clinical signs and symptoms of acute infective conjunctivitisin in a hospital based cross-sectional study. Antibiotic sensitivity tests were performed for thirteen commercially available topical antibiotics- Chloroamphenicol, Moxifloxacin, Ofloxacin, Ciprofloxacin, Gentamycin, Tobramycin, Neomycin, Bacitracin, Polymyxin-B, Methicillin, Cephazoline, Amikacin and Vancomycin. **Results**: Acute infective conjunctivitis and viral conjunctivitis was more common in adults and in males. Bacterial conjunctivitis was present in about one third (32.47% to 36.04%) of the patients with acute infective conjunctivitis, and it was more common in children. Bacteria were highly sensitive (93-98%) to most commercially available antibiotics but significant resistance was found against three antibiotics-Bacitracin (9.0%), Neomycin (16.0%) and Polymyxin-B (24.0%). MRSA infection was found in 7.0% of the bacterial isolates. Rest of antibiotics, showed variable resistance (14.3% to 100.0%). All cases of Ophthalmia neonatorum were bacterial. **Conclusion:** The best commercially available antibiotic for bacterial conjunctivitis was Moxifloxacin.

Keywords: Bacterial conjunctivitis - Acute infective conjunctivitis - Antibiotic sensitivity

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Introduction

Conjunctivitis is the most common ocular disease worldwide (Adebayo et al., 2011; Hovding, 2008; McDonell, 1988; Sheikh & Hurwitz, 2005). The disease can be divided into acute, hyperacute and chronic conjunctivitis according to the mode of onset and the severity of the clinical response (Mannis & Plotnic, 2006).Acute infective conjunctivitis may be viral or bacterial. Acute bacterial conjunctivitis is a common infection of the ocular surface that affects persons of all ages (DeLeon et al, 2012; Sheikh & Hurwitz, 2005). Most bacterial conjunctivitis are characterized by a self-limited course of inflammation of the conjunctiva with mucopurulent discharge. But in some cases it has the potential for significant ocular morbidity. Rapid destruction of the eye may be enhanced by the presence of purulence in some instances (Adebayo et al, 2011; Hovding, 2008). Although most cases of conjunctivitis are self limited, treatment with antibiotics has been shown to decrease the discomfort and duration of the symptoms. Antibiotic treatment also reduces contagious spread (Hutnik, Mohammad & Shahi, 2010; Lietman et al, 1984; Morrow & Abbott, 1998; Tarabishy & Jeng, 2008).Incidence of bacterial conjunctivitis, common pathogens causing it and their susceptibility to existing antibiotics differ from country to country (Adebayo et al, 2011; Azari & Barney, 2013; Haas, Gearinger, Hesje, Sanfilippo, & Morris, 2012; Pichichero, 2011; Sthapit, Tuladhar, Marasini, Khoju, & Thapa, 2011). Also there is a shifting trend in susceptibility and resistance of the bacteria towards available antibiotics in the last decade (Adebayo et al, 2011). Therefore, periodic studies to monitor the emerging trends in antibiotic susceptibility and resistance are crucial in guiding antibiotic selection. The aim of this study was to determine the most common pathogens causing bacterial conjunctivitis, and to find the in vitro susceptibility and resistance of these pathogens to commercially available topical antibiotic eye drops in Nepal.

Materials and methods

Study design and participants

It was a hospital based cross-sectional study conducted among 308 patients presenting to the Eye Care Center, Padma Nursing Home, Pokhara, Nepal from 11th December 1012 to 4th October 2013 with clinical signs and symptoms of acute infective conjunctivitis. When there were follicles in the lower fornix and lower tarsal conjunctiva along with congestion and secretions/discharges, patients were diagnosed to be suffering from acute follicular conjunctivitis. If there was conjunctival congestion and discharge but no follicles, then a diagnosis of acute infective conjunctivitis was made. Patients with copious mucopurulent discharge were diagnosed to be suffering from mucopurulent conjunctivitis, and children who were less than 1 month of age were diagnosed to be suffering from Ophthalmia neonatorum.

Ethical approval for the study was obtained from the institutional review board at Padma Nursing Home, Pokhara, Nepal. Informed consent was taken from all adult patients and from parents of children included in this study. Patients who were taking systemic antibiotics and steroids for other concurrent systemic diseases and those patients who were already using topical antibiotics or steroids at the time of presentation were excluded from the study.

Investigations

Using aseptic precautions, sterile swabs dipped in Brain heart infusion (BHI) broth were used to collect discharges from lid margins and inferior fornices of affected eyes. These were smeared on two slides for Grams and Giemsa staining. The conjunctival discharges were also inoculated in blood and chocolate agar. Once the bacterial growth was observed, their identification was done with the help of Gram's stain and biochemical tests. Thereafter, the antibiotic sensitivity tests were performed by disc diffusion (Kirby- Bauer) method on Mueller Hinton Agar supplied by Hi media laboratories. Zone of inhibition around the antibiotic impregnated discs on Mueller Hinton Agar were measured and the bacteria were classified as sensitive, intermediate sensitive or resistant to a particular antibiotic according to the guideline provided by the manufacturers. Antibiotic sensitivity tests were performed for thirteen antibiotics. These were Chloroamphenicol, Moxifloxacin, Ofloxacin, Ciprofloxacin, Gentamycin, Tobramycin, Neomycin, Bacitracin, Polymyxin-B, Methicillin, Cephazoline, Amikacin and Vancomycin. The rationale for choosing these antibiotics was that the first nine antibiotics were commercially available as topical medications in Nepal. Methicillin was used to detect methicillin resistant Staphylococci, last three antibiotics are easily available as injection in Nepal and topical eye drops can be made from these if required for treatment if organisms were resistant to commercially available eye drops. The commercial availability of these topical antibiotics is similar to other neighboring countries in the Indian subcontinent.

Data entry, analysis and sample size

Patient's demographic data, clinical findings and results of microbiological examinations were entered into Microsoft Excel Sheet in which the first row was for variables. All variables were later converted to tab-limited text files and analyzed with the commercial Stata-12 statistics package(*Stata Corp 2011. Stata Statistical software: Release 12. College Station, TX: StataCorp LP*). Some analyses were conducted using Epi-info 7 package.

In a pilot study conducted prior to original study with ten patients of Acute Infective Conjunctivitis due to bacterial infection showed proportion of Staphyloccus aureus to be 0.70. So, with significance level 5% and 95% level of confidence required, sample size calculated was 100 (Sathian et al, 2010). Out of 308 consecutive acute infective conjunctivitis patients examined, 100 had positive bacterial culture.

Results

Out of 308 patients in this study, there were more males 167(54.2%) than females 141(45.8%). Five (1.6%) patients were below 1 month of age, 53(17.2%) were >1 month to ≤2 years of age, 76(24.7%) were >2 years to ≤15 years of age and 174(56.5%) patients were adults(>15 years).About 43.5% of the patients were children(Table -1.).

Similarly, 178(57.79%) patients were diagnosed with acute follicular conjunctivitis, 122(39.61%) were diagnosed with acute infective conjunctivitis, 3(0.97%) diagnosed with mucopurulent conjunctivitis and 5(1.62%) were diagnosed to be suffering from ophthalmia neonatorum (Table -2).

There was no bacterial growth in culture of 208(67.5%) patients. Out of 100 (32.5%) patients in whom bacterial culture was positive, most common organisms were *Staph. aureus*, which was grown in 60(60.0%) of patients followed by *Strept. pneumoniae* in 27(27.0%) patients, Methicillin resistant *Staphylococcs aureus* (MRSA) in 7(7.0%) of patients and *E. coli* in 4(4.0%) of patients. The least common bacteria grown were *Diptheroid bacilli* and *H. influenzae* both of which, were present in 1(1.0%) of the patients with positive bacterial culture (Table 2,3).

A total of 111(36.04%) patients demonstrated organisms in gram's stain as compared to 100 (32.5%) patients in whom, bacterial culture were isolated. All of these 100 patients demonstrated organisms in Gram's stain(Table 3,4).

No abnormalities were seen in 189(61.36%) of Giemsa staining from conjunctival swabs of patients in this study. 110(35.71%) of patients showed only PMN. While 9(2.93%) patients showed Polymorph nuclear (PMN) cells, Vacuolated giant epithelium (VGE), Inclusion bodies, lymphocytes, and vacuolated granulocytes in various combinations (Table-5).

Both *H. influenzae* and *Diptheroid bacilli* isolated in 1.0% (Table 3) of culture positive patients were sensitive to all 13 antibiotics tested. *Staph. aureus*, *Strept. pneumoniae*, MRSA and *E. coli*isolates were highly sensitive to most antibiotics tested except Neomycin, Polymyxin-B and Bacitracin towards which, significant amount of resistance was encountered (Tables 6-11).

Table 1: Participants Age and Sex distribution (n=308)

Sex	Age Category				
	<1 month	>1mth-2yrs	>2 yrs-15yrs	>15yrs	Total
Female	1	19	23	98	141
Row%	0.7	13.5	16.3	69.5	100.0
Col%	20.0	35.8	30.3	56.3	45.8
Males	4	34	53	76	167
Row%	2.4	20.4	31.7	45.5	100.0
Col%	80.0	64.2	69.7	43.7	54.2
Total	5	53	76	174	308
Row%	1.6	17.2	24.7	56.5	100.0
Col%	100.0	100.0	100.0	100.0	100.0
Cumulative%	1.6	18.8	43.5	100.0	

Table 2: Clinical Diagnosis and Culture Report(n=308)

Clinical Diagnosis	Diptheroids	E.coli	H. influenzae	MRSA	NG	Staph.au	Strept. pneum.	Total
AFC	0	1	0	4	147	20	6	178
	0.00	0.56	0.00	2.25	82.58	11.24	3.37	100.00
	0.00	25.00	0.00	57.14	70.67	33.33	22.22	57.75
AIC	1	0	1	3	59	38	20	122
	0.82	0.00	0.82	2.46	48.36	31.15	16.39	100.00
	100.00	0.00	100.00	42.86	28.37	63.33	74.07	39.61
MPC	0	0	0	0	2	0	1	3
	0.00	0.00	0.00	0.00	66.67	0.00	33.33	100.00
	0.00	0.00	0.00	0.00	0.96	0.00	3.70	0.97
ON	0	3	0	0	0	2	0	5
	0.00	60.00	0.00	0.00	0.00	40.00	0.00	100.00
	0.00	75.00	0.00	0.00	0.00	3.33	0.00	1.62
Total	1	4	1	7	208	60	27	308
	0.32	1.30	0.32	2.27	67.53	19.48	8.77	100.00
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

AFC: Acute follicular conjunctivitis AIC: Acute infective conjunctivitis MPC: Mucopurulent conjunctivitis
ON: Ophthalmia neonatorum NG: No growth

Table 3: Frequency of bacterial culture

Culture Report	Freq	Percent	CI
Diptheroid bacilli	1	1.00%	CI(0.0%, 5.4%)
E.coli	4	4.00%	CI(1.1%, 9.9%)
H.influenza	1	1.00%	CI(0.0%, 5.4%)
MRSA	7	7.00%	CI(2.9%, 13.9%)
Staph. Aureus	60	60.00%	CI(49.7%, 69.7%)
Strept. Pneumonia	27	27.00%	CI(18.6%, 36.8%)
Total	100	100.00%	

Table 4: Gram Staining findings

Gram Stain	Freq.	Percent
Pus Cells	33	10.71
GPC Pus Cells	102	33.12
GP Rods Pus Cells	1	0.32
GNB Pus Cells	4	1.30
GNB GPC Pus Cells	4	1.30
NAD	164	53.25
Total	308	100.00

Table 5: Giemsa Staining findings

Giemsa Stain	Freq.	Percent
PMN	110	35.71
VGE PMN	2	0.65
Lymphocytes PMN	2	0.65
Lymphocytes VGE PMN	2	0.65
Vacuolated granulocytes Lymphocytes PMN	1	0.32
Inclusion bodies Lymphocytes VGE PMN	1	0.32
Inclusion bodies Vacuolated granulocytes VGEPMN	1	0.32
NAD	189	61.36
Total	308	100.00

Table 6: Antibiotic sensitivity test for Staphylococcus aureus (n=60)

	Sensitive			Intermediate Sensitive			Resistant		
AB	No	%	(CI)	No	%	(CI)	No	%	(CI)
AK	60	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
G	60	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
M	60	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
MFX	60	100.0	CI(“-”)	0	0.0	CI(“-”0%)	0	0.0	CI(“-”)
CZ	59	98.3	CI(91.1%, 100.0%)	1	1.7	CI(0.0%, 8.9%)	0	0.0	CI(“-”)
CIP	58	96.7	CI(88.5%, 99.6%)	2	3.3	CI(0.4%, 11.5%)	0	0.0	CI(“-”)
TOB	58	96.7	CI(88.5%, 99.6%)	2	3.3	CI(0.4%, 11.5%)	0	0.0	CI(“-”)
C	57	95.0	CI(86.1%, 99.0%)	2	3.3	CI(0.4%, 11.5%)	1	1.7	CI(0.0%, 8.9%)
OF	57	95.0	CI(86.1%, 99.0%)	3	5.0	CI(1.0%, 13.95%)	0	0.0	CI(“-”)
VA	57	95.0	CI(86.1%, 99.0%)	3	5.0	CI(1.0%, 13.9%)	0	0.0	CI(“-”)
N	55	91.7	CI(81.6%, 97.2%)	2	3.3	CI(0.4%, 11.5%)	3	5.0	CI(1.0%, 13.9%)
PB	46	76.6	CI(64.0%, 86.6%)	12	20.0	CI(10.8%, 32.3%)	2	3.3	CI(0.4%, 11.5%)
B	44	73.3	CI(60.3%, 83.9%)	12	20.0	CI(10.8%, 32.3%)	4	6.7	CI(1.8%, 16.2%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table-7:Antibiotic sensitivity test for Streptococcus pneumoniae(n=27)

	Sensitive			Intermediate Sensitive			Resistant		
AB	**No**	**%**	**(CI)**	**No**	**%**	**(CI)**	**No**	**%**	**(CI)**
CZ	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
M	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
MFX	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
OF	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
TOB	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
VA	27	100.0	CI(100.00%)	0	0.0	CI(0.00%)	0	0.0	CI(0.00%)
C	26	96.3	CI(81.0%, 99.9%)	1	3.7	CI(0.1%, 19.0%)	0	0.0	CI(0.00%)
CIP	26	96.3	CI(81.0%, 99.9%)	1	3.7	CI(0.1%, 19.0%)	0	0.0	CI(0.00%)
AK	25	92.6	CI(75.7%, 99.1%)	2	7.4	CI(0.9%, 24.3%)	0	0.0	CI(0.00%)
G	24	88.9	CI(70.8%, 97.6%)	3	11.1	CI(2.4%, 29.2%)	0	0.0	CI(0.00%)
B	21	77.8	CI(57.7%, 91.4%)	5	18.5	CI(6.3%,38.1%)	1	3.7	CI(0.1%, 19.0%)
N	12	44.4	CI(25.5%, 64.7%)	5	18.5	CI(6.3%, 38.1%)	10	37.0	CI(19.4$, 57.6%)
PB	8	29.6	CI(13.8, 50.2%)	4	14.8	CI(4.25, 33.7%)	15	55.6	CI(35.53%, 74.5%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table 8: Antibiotic sensitivity for MRSA (Methicillin Resistant Staph.aureus) (n=7)

	Sensitive			Intermediate Sensitive			Resistant		
AB	**No**	**%**	**(CI)**	**No**	**%**	**(CI)**	**No**	**%**	**(CI)**
AK	6	85.7	CI(42.1%,99.6%)	0	0.0	CI("-")	1	14.3	CI(0.4%, 57.9%)
C	6	85.7	CI(42.1%,99.6%)	1	14.3	CI(0.4%, 57.9%)	0	0.0	CI("-")
CZ	6	85.7	CI(42.1%,99.6%)	1	14.3	CI(0.4%, 57.9%)	0	0.0	CI("-")
TOB	6	85.7	CI(42.1%,99.6%)	0	0.0	CI("-")	1	14.3	CI(0.4%, 57.9%)
MFX	5	71.4	CI(29.0%, 96.3%)	1	14.3	CI(0.4%, 57.9%)	1	14.3	CI(0.4%, 57.9%)
VA	5	71.4	CI(29.0%, 96.3%)	2	28.6	CI(3.7%, 71.0%)	0	0.0	CI("-")
CIP	4	57.1	CI(18.4%, 90.1%)	2	28.6	CI(3.7%, 71.0%)	1	14.3	CI(0.4%, 57.9%)
G	4	57.1	CI(18.4%, 90.1%)	1	14.3	CI(0.4%, 57.9%)	2	28.6	CI(3.7%, 71.0%)
OF	4	57.1	CI(18.4%, 90.1%)	1	14.3	CI(0.4%, 57.9%)	2	28.6	CI(3.7%, 71.0%)
B	3	42.9	CI(9.9%, 81.6%)	1	14.3	CI(0.4%, 57.9%)	3	42.9	CI(9.9%, 81.6%)
N	1	14.3	CI(0.4%, 57.9%)	3	42.9	CI(9.9%, 81.6%)	3	42.9	CI(9.9%, 81.6%)
M	0	0.0	CI("-")	0	0.0	CI("-")	7	100.0	CI("-")
PB	0	0.0	CI("-")	1	14.3	CI(0.4%, 57.9%)	6	85.7	CI(42.1%,99.6%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table 9: Antibiotic Sensitivity for E. coli (n=4)

	Sensitive			Intermediate Sensitive			Resistant		
AB	No	%	(CI)	No	%	(CI)	No	%	(CI)
AK	4	100.0	CI(“-”)	0	*0.0*	CI(“-”)	0	0.0	CI(“-”)
C	4	100.0	CI(“-”)	0	*0.0*	CI(“-”)	0	0.0	CI(“-”)
CIP	4	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
M	4	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
MFX	4	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
OF	4	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
TOB	4	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
CZ	3	75.0	CI(19.4%, 99.4%)	0	0.0	CI(“-”)	1	25.0	CI(0.6%, 80.6%)
G	3	75.0	CI(19.4%, 99.4%)	0	0.0	CI(“-”)	1	25.0	CI(0.6%, 80.6%)
N	3	75.0	CI(19.4%, 99.4%)	1	25.0	CI(0.6%, 80.6%)	0	0.0	CI(“-”)
VA	3	75.0	CI(19.4%, 99.4%)	0	0.0	CI(“-”)	1	25.0	CI(0.6%, 80.6%)
PB	2	50.0	CI(6.8%, 93.2% %)	1	25.0	CI(0.6%, 80.6%)	1	25.0	CI(0.6%, 80.6%)
B	1	25.0	CI(0.6%, 80.6%)	0	0.0	CI(“-”)	3	75.0	CI(19.4%, 99.4%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table 10: Antibiotic sensitivity in ophthalmia neonatorum patients (n=5)

	Sensitive			Intermediate Sensitive			Resistant		
AB	No	%	(CI)	No	%	(CI)	No	%	(CI)
AK	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
C	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
CIP	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
M	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
MFX	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
OF	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
TOB	5	100.0	CI(“-”)	0	0.0	CI(“-”)	0	0.0	CI(“-”)
N	4	80.0	CI(28.4%, 99.5%)	1	20.0	CI(0.5%, 71.6%)	0	0.0	CI(“-”)
PB	4	80.0	CI(28.4%, 99.5%)	1	20.0	CI(0.5%, 71.6%)	0	0.0	CI(“-”)
CZ	4	80.0	CI(28.4%, 99.5%)	0	0.0	CI(“-”)	1	20.0	CI(0.5%, 71.6%)
G	4	80.0	CI(28.4%, 99.5%)	0	0.0	CI(“-”)	1	20.0	CI(0.5%, 71.6%)
VA	4	80.0	CI(28.4%, 99.5%)	0	0.0	CI(“-”)	1	20.0	CI(0.5%, 71.6%)
B	2	40.0	CI(5.3%, 85.3%)	0	0.0	CI(“-”)	3	60.0	CI(14.7%, 94.7%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table 11:Antibiotic sensitivity in culture positive bacterial conjunctivitis(n=100)

AB	Sensitive			Intermediate Sensitive			Resistant		
	No	%	(CI)	No	%	(CI)	No	%	(CI)
MFX	98	98.0	CI(93.0%, 99.8%)	1	1.0	CI(0.0%, 5.4%)	1	1.0	CI(0.0%, 5.4%)
AK	97	97.0	CI(91.5%, 99.4%)	2	2.0	CI(0.2%, 7.0%)	1	1.0	CI(0.0%, 5.4%)
CZ	97	97.0	CI(91.5%, 99.4%)	2	0.0	CI(0.2%, 7.0%)	1	1.0	CI(0.0%, 5.4%)
TOB	97	97.0	CI(91.5%, 99.4%)	2	2.0	CI(0.2%, 7.0%)	1	1.0	CI(0.0%, 5.4%)
C	95	95.0	CI(88.7%, 98.4%)	4	4.0	CI(1.1%, 9.9%)	1	1.0	CI(0.0%, 5.4%)
CIP	94	94.0	CI(87.4%, 97.8%)	5	5.0	CI(1.6%,11.3%)	1	1.0	CI(0.0%, 5.4%)
OF	94	94.0	CI(87.4%, 97.8%)	4	4.0	CI(1.1%, 9.9%)	2	2.0	CI(0.2%, 7.0%)
VA	94	94.0	CI(87.4%, 97.8%)	5	5.0	CI(1.6%,11.3%)	1	1.0	CI(0.0%, 5.4%)
G	93	93.0	CI(86.1%,97.1%)	4	4.0	CI(1.1%, 9.9%)	3	3.0	CI(0.6%, 8.5%)
M	93	93.0	CI(86.1%,97.1%)	0	0.0	CI("-")	7	7.0	CI(2.9%, 13.9%)
N	73	73.0	CI(63.2%, 81.4%)	11	11.0	CI(5.6%, 18.8%)	16	16.0	CI(9.4%,24.7%)
B	71	71.0	CI(61.1%, 79.6%)	20	20.0	CI(12.7%, 29.2%)	9	9.0	CI(4.2%, 16.4%)
PB	58	58.0	CI(47.7%, 67.8%)	18	18.0	CI(11.0%,26.9%)	24	24.0	CI(16.0%, 33.6%)

AB= Antibiotics AK=Amikacin B= Bacitracin C= Chloroamphenicol CIP= Ciprofloxacin CZ= Cephazoline G= Gentamycin

M=Methicillin MFX= Moxifloxacin N=Neomycin OF=Ofloxacin PB= Polymyxin-B TOB= Tobramycin & VA= Vancomycin

Table 12: Age and Culture Report

Final Diagnosis	Age Category				
	≤1 month	>1mth-≤2yrs	>2 yrs-≤15yrs	>15yrs	Total
Culture positive Bacterial conjunctivitis	5	24	23	48	100
Row%	5	24	23	48	100
Col%	100.0	45.3	30.3	27.6	32.5
Culture negative Acute Conjunctivitis	0	29	53	126	208
Row%	0.0	13.9	25.5	60.6	100.0
Col%	0.0	54.7	69.7	72.4	67.5
Total	5	53	76	174	308
Row%	1.6	17.2	24.7	56.5	100.0
Col%	100.0	100.0	100.0	100.0	100.0

Discussion

In this study, adults were more affected with infective conjunctivitis. Bacterial conjunctivitis was more common in children as compared to viral conjunctivitis which was more common in adults. (Tables 1, 12). This finding was similar to those of previous studies (Azari & Barney, 2013; Fitch, Rapoza & Owens, 1989; Harding, Mallinson, Smith & Clearkin, 1987; Hørven, 1993; Rönnerstam, Persson, Hansson, & Renmarker, 1985; Stenson, Newman, & H., 1982; Uchio, Takeuchi, & N, 2000; Woodland, Darougar, & Thaker, 1992).

Out of 178(57.75%) patients, clinically diagnosed as acute follicular conjunctivitis (AFC) or viral, 147(82.58%) had no growth in bacterial culture. But 31(17.42%) patients diagnosed to have AFC showed positive bacterial culture. In the similar manner, 48.36% of patients diagnosed to have acute infective conjunctivitis(AIC), and 66.67 patients diagnosed clinically as mucopurulent conjunctivitis had negative bacterial culture. These findings support the fact that diagnosis of viral or bacterial conjunctivitis cannot be made on the basis of clinical signs and symptoms only. These findings are similar to previous studies (Azari & Barney, 2013; Rietveld, Ter Riet, Bindels, Sloos & van Weert, 2004; Rietveld, Van Weert, Ter Riet & Bindels, 2003; Tarabishy & Jeng, 2008).

In this study, 32.47% patients had positive bacterial culture and 36.04% showed organisms in Grams stain (Tables 2-4) . This was in contrast with the earlier study conducted by Sthapit et al in Nepal in 2009-10 in which, they had found organisms in grams stain as well as bacterial growth in 16.9% of their acute infective conjunctivitis patients(Sthapit et al., 2011).

The most common bacteria causing acute infective conjunctivitis (Table 3) in our study was *Staph. aureus* (60%) followed by *Strept. pneumonia*(27%). Less common organisms were E. *coli*(4%), *Diptheroid bacilli*(1.0%) and *H influenzae*(1.0%). It was alarming to note that Methicillin resistant *Staph. aureus*(7.0%) was on the rise as compared to similar study done few years back in Nepal by Sthapit et al in which the incidence of MRSA was 3.1%. Sthapit et al reported *Strept. pneumonia* as most common organisms followed by *Staph. aureus*. The findings of the present study is similar to the findings of Azari and Barney, who after searching literature published through March 2013, using Pubmed, the ISI web of Knowledge database and the Cochrane Library reported that the most common pathogens for bacterial conjunctivitis in adults are *Staphylococcal species*, followed by *Streptococcus pneumoniae* and *Haemophilus influenzae* (Azari & Barney, 2013). Abedayo et al reviewed records of all conjunctival bacterial cultures performed at New York Eye and Ear Infirmary from 1997 to 2008. They found that *Staphyloccus aureus* was the most common gram positive pathogen isolated, and also the most commonly isolated pathogen overall. *Haemophilus influenzae* was the most common gram-negative pathogen isolated (Adebayo et al, 2011). While their findings was similar to the present study in case of gram positive organisms, in the present study, most common gram negative organism was *E. coli*. In 2010, Hutnik et al analyzed systematic reviews, meta analyses and randomized controlled trials for bacterial conjunctivitis from 1990 to 2010. Their search sources were from ovid MEDLINE, PubMed, Cochrane Library, NHS evidence and Clinical Evidence. They concluded that bacterial conjunctivitis is caused by *Staphyloccus species* in adults and by *Streptococcus pneumoniae* and the Gram-negative organisms *Haemophilus influenxae* and *Moraxella catarrhalis* in children (Hutnik et al, 2010). However, Haas et al (2012)from the USA reported that the most prevalent species in their study was H. influenzae, followed by *Staphylococcus epidermidis*, *Staphylococcus aureus*, the *Streptococcus mitis* group, and *Streptococcus pneumoniae*. One species identified in this study, which was not previously noted as a common cause of bacterial conjunctivitis was *Dolosigranulum pigrum* (Haas et al, 2012).

Haas and his colleagues conducted a randomized, double masked, vehicle-controlled, parallel group study in the United States with besifloxacin ophthalmic suspension 0.6% dosed twice daily. They had 496 bacterial isolates. They found that Ampicillin resistance was common among *H. influenzae* isolates, while macrolide resistance was high among *Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Staphyloccus aureus*. The latter two species also included a number of isolates resistant to methiciillin and ciprofloxacin.

Abedayo and his colleagues from New York Eye and Ear Infirmary after analyzing 20,180 conjunctival bacterial cultures (Adebayo et al, 2011) concluded that conjunctival bacterial isolates demonstrated high levels of resistance to tetracycline, erythromycin and TMP/SMZ. According to their study, Moxifloxacin and gatifloxacin appeared to be currently the best choice for empirical broad-spectrum coverage and vancomycin was the best antibiotic for MRSA coverage.

Bacterial resistance to antibiotic therapy can result from a number of factors. Nationwide surveillance studies such as the Ocular Tracking Resistance in US Today (TRUST) survey, and The Surveillance Network (TSN) have documented emerging resistance among ocular pathogens to ocular anti-infectives (Asbell, Colby, et al, 2008; Asbell, Sahm, Shaw, Draghi & Brown, 2008; Pichichero, 2011).Survey of Ocular TRUST, describing data collected from October 2005 through June 2006 showed 65.3% resistance among *S. pneumoniae* isolates to tobramycin. Tobramycin was active against MSSA, but 63.6% of MRSA were resistant to tobramycin. Additional analysis of archived isolates of *S. pneumoniae* and *H. influenzae* obtained between 1999 and 2006 further showed 59.9%of penicillin-sensitive *S pneumoniae* (PSSP) isolates were resistant to tobramycin compared with 73.1% of penicillin-non susceptible *S. pneumoniae* (PNSP) isolates. Of note, little to no aminoglycoside resistance was seen in *H influenzae*. Most strains of *H. influenzae* remained susceptible to polymyxin B alone or in combination with neomycin or trimethoprim regardless of β-lactamase status. Ocular TRUST 1 data reported 100% resistance among *S pneumoniae* and MSSA to polymyxin B, but no resistance by *H influenzae*. Surveillance data thus far has failed to show resistance of *S pneumoniae* or *H influenzae* isolates to either the older or newer fluoroquinolones. In contrast, there is documented resistance to both older and newer ophthalmic fluoroquinolones among *S aureus*. From 2004 to 2006 it was reported that 90% to 92% of MSSA isolates, but only 27% to 32% of MRSA isolates, were susceptible to the fluoroquinolones tested (ciprofloxacin, levofloxacin and moxifloxacin), and a consistent annual 2.5% increase in MRSA as a cause of ocular infections was identified (Wald, Greenberg & Hoberman, 2001). Another study reported an increase in resistance to ciprofloxacin by *S aureus* isolates from 13.3% to 36.0% and the prevalence of methicillin resistance among these isolates increased concurrently from 4.4% to 42.9% (Cavuoto, Zutshi, Karp, Miller & Feuer, 2008). More recently, a study of bacterial conjunctivitis isolatesfound that 65% of MRSA isolates were resistant to ciprofloxacin (Pichichero, 2011).

In the present study, 1.7%, 3.3%, 5.0% and 6.7% of MSSA isolates were resistant to Chloroamphenicol, Polymyxin-B, Neomycin and Bacitracin respectively(Table-6). In contrast, plenty of resistances were encountered for MRSA. About 14.% of MRSA were resistant to Amikacin, Tobramycin, and Ciprofloxacin, 28.6% were resistant to Gentamycin and Ofloxacin, 42.9% were resistant to Bacitracin and Neomycin and 85.7% were resistant to Polymyxin-B (Table 8). *Strept. pneumoniae* was sensitive to most antibiotics except for three– Bacitracin(.7%), Neomycin(37.0%) and Polymyxin-B(55.6%)(Table 7). About 25% of *E. coli*, which were the predominant gram negative isolates in this study, were resistant to Cephazoline, Gentamycin, Vancomycin and Polymyxin-B and 75% were resistant to Bacitracin (Table 9).One of the isolates *Diptheroids bacillus* and the other *H. influenzae,* were sensitive to all thirteen antibiotics tested.

Collectively, most (93 to 98%) bacterial isolates were highly sensitive to most antibiotics used. But these isolates demonstrated significant resistance to three antibiotics- Bacitracin(9.0%), Neomycin(16.0%) and Polymyxin-B(24%) (Table 11). The best commercially available antibiotic for bacterial conjunctivitis was Moxifloxacin. Therefore, this antibiotic seems to be best choice for patients with bacterial conjunctivitis for all ages. Although resistance is developing with older fluorquinolones (Ciprofloxacin, Ofloxacin), it is less common with newer fluorquinolones (moxifloxacin, gatifloxacin, besifloxacin) because, the newer fluoroquinolones exhibit balanced dual binding of these enzymes and require multi step mutations, whereas resistance to the older fluoroquinolones which typically target one enzyme in preference to the other, may require only a single such mutation (Asbell, Colby et al, 2008; Pichichero, 2011).

In the present study, five (1.62%) patients of acute infective conjunctivitis were below 1 month of age and were suffering from Ophthalmia neonatorum (Table 1,2,10). Among these five patients, *E. Coli* was isolated in 3, and *Staph. aureus* were isolated in 2 patients (Table 2). All of them(100.0%) were sensitive to seven antibiotics namely Amikacin, Chloroamphenicol, Ciprofloxacin, Methicillin, Moxifloxacin, Ofloxacin, and Tobramycin. About 20% of them were resistant to Cephazoline, Gentamycin and Vancomycin while, 60% patients in this group were resistant to Bacitracin (Table 10).

Ophthalmia neonatorum is caused by *C. trachomatis*, *N. gonorrheae*, herpes simplx virus (typically HSV-2) and less commonly by *Staphylococci*, *Streptococci*, *H. influenzae*, and various gram negative bacteria (Kanski & Bowling, 2011). It may be also due to chemicals used at birth. Infections by first three organisms can be serious with disastrous consequences. Therefore, it was good to know that all the cases of ophthalmia neonatorum in this study was caused by bacteria *E. coli* and *Staph. aureus* which were sensitive to many antibiotics tested.

Treatment of acute bacterial conjunctivitis with a broad-spectrum, preferably bactericidal, antibacterial is often initiated empirically because the rapid kill of bacteria shortens the time to recovery; limits the spread of disease; relieves a financial burden by speeding up a patient's return to day care or school or work; and reduces the risk of sight-threatening complications.

Conclusions

Acute infective conjunctivitis and viral conjunctivitis was more common in adults and in males. Bacterial conjunctivitis was present in about one third(32.47% to 36.04%) of the patients with acute infective conjunctivitis more common in children than in adults in whom viral conjunctivitis was more common. Bacteria were highly sensitive (93-98%) to most commercially available antibiotics but significant resistance was found against three antibiotics-Bacitracin(9.0%), Neomycin(16.0%) and Polymyxin-B(24.0%). MRSA infection was found in 7.0% of the bacterial isolates. These were sensitive to three antibiotics-chloroamphenicol, Cephazoline and Vancomycin. Rest of antibiotics, showed variable resistance (14.3% to 100.0%). All cases of ophthalmia neonatorum in this series were bacterial and sensitive to most antibiotics tested. The best commercially available antibiotic for bacterial conjunctivitis was Moxifloxacin (99.0% sensitivity).

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