■ ***Original article***

Bacteriology and antimicrobial susceptibility of adult chronic dacryocystitis

Chaudhary M[1], Bhattarai A[2], Adhikari SK,[3] Bhatta DR[4]
[1]Assistant professor, B.P Koirala Lions Centre for ophthalmic Studies, Institute of Medicine
[2]Central Department of Microbiology, [3]Professor, Central Department of Microbiology
[4] Associate Professor, Central Department of Microbiology
Tribhuvan University, Kathmandu, Nepal

Abstract

Introduction: Chronic dacryocystitis results in chronic infection and inflammation of the lacrimal sac.

Objective: To identify the aetiology of chronic dacryocystitis and to determine their antimicrobial susceptibility pattern.

Materials and methods: A cross - sectional study was undertaken including 120 lacrimal swab materials collected from patients aged above 15 years suffering from chronic dacryocystitis.

Statistics*:* Data analysis was done by using software "Win pepi" ver 7.9.

Results: The bacteria of eight different species were isolated from 76.66 % (92/120) culture positive samples. 85.86 % showed a single and 14.13 % showed a mixed growth pattern. Coagulase negative staphylococci were the most common bacteria (P= 0.018) accounting for 33.96 % followed by Staphylococcus aureus (25.46 %), Streptococcus pneumoniae (19.81 %), Streptococci viridans (5.66 %), Escherichia coli (5.66 %), Haemophilus spp (4.71 %), Streptococcus pyogenes (3.77 %) and Bacillus spp (0.94 %). Staphylococcus aureus were the most predominant bacteria in mixed growth. Rate of infection was higher in males 81.39 % than in females 74.02 %. Infection was higher in the age group of above 31 years. In the antimicrobial susceptibility test, except staphylococcus aureus, all the Gram positive isolates were 100 % sensitive to chloramphenicol and were least sensitive to tobramycin, but Gram negative isolates were equally sensitive to Chloramphenicol and Nalidixic acid.

Conclusion: Coagulase negative staphylococci are the most frequently isolated bacteria. Staphylococcus aureus is predominantly found in mixed growth. Chloramphenicol is the most effective drug of choice for chronic dacryocystitis.

Key words: chronic dacryocystitis, nasolacrimal duct, lacrimal sac, epiphora, mucopurulent

Introduction

Dacryocystitis is an inflammation of the lacrimal sac which usually occurs because of the obstruction of the nasolacrimal duct. The obstruction may be an idiopathic inflammatory stenosis (primary acquired nasolacrimal duct obstruction) or may be secondary to trauma, inflammation, neoplasm or mechanical obstruction (secondary acquired lacrimal drainage obstruction). Obstruction of the nasolacrimal duct

Received on: 15.02.2010 Accepted on: 10.06.2010
Correspondence and reprint request to: Dr Meenu Chaudhary, MD Assistant Professor, BP Koirala Lions Center for Ophthalmic Studies, Kathmandu, Nepal

leading to stagnation of tears in a pathologically-closed drainage system can result in dacryocystitis.

The microbiology of chronic dacryocystitis may differ from acute dacryocystitis. Few studies done on the bacteriology of chronic dacryocystitis in the past years have shown the most frequent isolation of Coagulase-negative staphylococci (CONS) and staphylococcus aureus in lacrimal sac infections. There are distinct patterns of geographical variation in terms of aetiology according to the local climate in infective keratitis and also in microbial conjunctivitis. Hence, an understanding of the regional etiological agents is important in the management of this disease. The purpose of this study was to identify the bacterial aetiology and the in-vitro antimicrobial susceptibility and resistance patterns of bacterial pathogens to commonly used antimicrobial agents. Knowledge of bacteriology of chronic dacryocystitis would contribute to the choice of effective antimicrobial agents and would help to reduce the unnecessary load of anti-microbial agents.

Materials and methods

A cross-sectional study was carried out from August 2008 till January 2009 at B.P.Koirala Lion's Center for ophthalmic Studies, Tribhuvan University, Teaching Hospital. The patients of chronic dacryocystitis above 15years of age were included in this study.

Sample collections were done after punctum dilatation with a punctum dilator and then syringing was done with a 25 gauze curved canula. Duplicate (2) sterile cotton swabs dipped in physiological saline were used for collection of discharge from the lacrimal punctum of infected lacrimal sac, ensuring that the lid margin or conjunctiva was not touched. One swab was used for Gram's staining and the second one for inoculation into culture media like BHI broth, Blood Agar and Chocolate Agar plates. The inoculated BHI broth and Blood Agar were incubated at 37º C for 24 to 48 hours. Chocolate Agar plates were incubated at 37º C for 24 to 48 hours in the presence of 5-10 % carbon dioxide. After 24 hours of incubation, the plates were taken out from incubator and the colonies were examined for isolation and identification of organisms.

In case of mixed growth, the Gram's stain was done separately from the morphologically-different colonies and the colonial characteristics were studied. The different colonies from which Gram's staining was done were further sub-cultured according to the Gram's staining nature and characteristics. If the colonies were smooth, round and white/cream in color and Gram positive cocci in clusters then it was inoculated on Mannitol Salt Agar. If the colonies were rough, Gram positive and in short or long chains and haemolysis was produced, it was sub-cultured on Blood Agar. If the colonies were Gram negative, then it was sub-cultured on Mac-Conkey Agar.

Identification of the micro-organisms was done using various biochemical as well as routine tests. Biochemical tests were included in order to identify Gram positive (Catalase, Coagulase, VP etc) and Gram negative bacteria (Catalase, Oxidase, SIM, TSI, Urease, O/F etc). The culture plates incubated for aerobic organisms were examined after overnight incubation. Identification of organisms was done by the standard microbiological technique which involves colony morphology, staining reaction and different biochemical properties (Cheesebrough, 2000; Forbes et al 2008).

The antibiotic susceptibility pattern was done by Kirby-Bauer disc diffusion method. Antibiotic discs were placed aseptically on a Muller-Hinton Agar (MHA) plate swabbed with test bacteria pre-grown to Mac-Farland Standard in nutrient broth (NB). The plate was then incubated at 37º C for 24 hours. The zone of inhibition (ZOI) around the disc was measured with a ruler and compared to standard interpretation charts. The quality of each test was maintained by using standard procedures. The quality of each agar plate prepared was ensured by incubating one plate of each lot in the incubator. Control strains of E. coli (ATCC 25922) and staphylococcus aureus (ATCC 25923) were used for the standardization of the Kirby-Bauer test and also for correct interpretation of the zone of diameter. The quality of the sensitivity tests was maintained by maintaining the thickness of MHA at 4 mm and the pH at 7.2-7.4. Strict aseptic conditions were maintained while carrying out all the procedures.

Statistics: Data Analysis was done by using software "Win pepi'' ver 7.9.

Results

Out of 120, 92 (76.66 %) samples showed positive growth. From 92 positive samples, 106 micro organisms of eight different species were isolated. The growth of Gram positive organisms was higher than that of Gram negative. Single growth of organisms was higher than mixed growth. Single to mixed growth ratio was found to be 6:1.

Table 1
Gender distribution of patients and percentage of positive samples

Gender	No. of collected samples	Organisms isolated (%)	P value
Female	77	57 (74.02)	0.360
Male	43	35 (81.39)	
Total	120	92	

77 samples were collected from females and 43 from males. A higher rate of infection was observed in males than females but there was no significant difference of infection between males and females (P= 0.360).

Table 2
Pattern of bacterial isolates among male and female patients

S. No.	Organisms isolated	Organisms from females (%)	Organisms from males (%)	P value
1	CONS	25(23.58)	11(10.38)	0.018
2	Staphylococcus aureus	12(11.32)	15(14.15)	
3	Streptococcus pneumoniae	16(15.O9)	05(4.72)	
4	Viridans Streptococci	03(2.83)	03(2.83)	
5	Escherichia coli	04(3.77)	02(1.87)	
6	Haemophillus influenzae	02(1.87)	03(2.83)	
7	Streptococcus pyogenes	01(0.94)	03(2.83)	
8	Bacillus spp.	01(0.94)	_	
	Total	64(60.38)	42(39.62)	

CONS were the most frequently isolated organisms and the Bacillus spp. was least commonly isolated. There was significant growth of CONS among all isolates (P=0.018).

Table 3
Pattern of organisms isolated from single and mixed growth

S. No.	Organism isolated	No. of org. in mixed growth(%)	No. of org. in single growth	Total positive sample
1	Staphylococcus aureus	10 (37.03)	17	27
2	Streptococcus pneumoniae	06 (22.22)	15	21
3	Streptococcus pyogenes	03 (11.11)	01	04
4	Escherichia coli	03 (11.11)	03	06
5	Haemophilus influenzae	02 (07.40)	03	05
6	Viridans streptococci	02 (07.40)	04	06
7	CONS	01 (03.70)	35	36
8	Bacillus spp.	-	01	01
	Total	27 (25.47)	79 (74.52)	106

Staphylococcus aureus was found to be the most commonly isolated organism in mixed growth, followed by Streptococcus pneumoniae; and CONS were rarely found in mixed growth. Bacillus spp was not found in mixed growth.

Table 4
Age distribution of the sample providing patients

S. No.	Age (years)	Female (%)	Male (%)	Total (%)	P value
1	< 30	13 (10.83)	12 (10)	25 (20.83)	0.513
2	31-40	13 (10.83)	10 (8.33)	23 (19.16)	
3	>40	51 (42.50)	21 (17.50)	72 (60.00)	
	Total	77(64.16)	43(35.83)	120(100)	

The highest percentage of infection was seen in the age group of above 40 years. The mean age calculated was 43.40 years and 50.02 years in male and female respectively.

Table 5
Distribution pattern of organisms between two racial groups

S.No.	Racial groups	Samples collected (%)	Organisms isolated (%)
1	Mongoloids	59 (49.16)	40 (43.47)
2	Aryans	61 (50.83)	52 (56.52)
	Total	120	92

A higher growth of organisms was seen in Aryans than in Mongoloids.

Tables 6
Growth pattern in unilateral and bilateral cases

S.No.	Infected eye	Total	Growth	Growth %	P-value
1	Left eye	56	42	45.65	0.397
2	Right eye	45	34	36.95	
3	Both eyes	19	16	17.39	
	Total	120	92	100	

Out of 92 growth-positive samples, a higher percentage of growth was seen in samples taken from the left eyes.

Table 7
Growth pattern of bacteria in different type of discharges

S.No.	Microorganisms	Types of discharges			
		Mucopurulent (57)	Extensive Purulent(23)	Mucoid (23)	Clear fluid (17)
1	CONS	18	07	05	06
2	Staphylococcus aureus	08	08	08	03
3	Streptococcus pneumoniae	08	05	07	01
4	Viridans streptococci	04	01	00	01
5	Streptococcus pyogenes	01	02	00	01
6	Bacillus spp.	00	00	01	01
7	Escherichia coli	01	04	00	00
8	Haemophilus influenzae	04	01	21	00
	Total	44	28	21	13
	Growth %	41.50	26.41	19.81	12.26

The highest percentage of positive samples was found in the form of mucopurulent discharges, followed by extensive purulent, mucoid, and the lowest was in clear fluid.

Table 8
Correlation of growth pattern with history of epiphora

S.No.	Duration of tearing	No. of samples	Growth pattern		Total org. growth	percentage %
			Single	mixed		
1	Upto 6 months	30	25	1	26	86.66
2	1to 3 years	64	37	8	45	70.31
3	4 to 10 yeas	18	11	2	13	72.72
4	>10 years	8	6	2	8	100
	Total	120	79	13	92	

Among 120 samples the highest number of samples belonged to tearing for 1 to 3 years and the highest % of growth was found from tearing cases of >10years.

Table 9
Antibiotic sensitivity pattern among Gram-positive isolates

S. No.	Antibiotics	Susceptibility of microorganisms						Total%
		CONS (%)	Staphylococcusaureus (%)	Streptococcus pneumoniae (%)	Viridans Streptococci (%)	Streptococcus pyogenes (%)	Bacillus spp. (%)	
		(36)	(27)	(21)	(6)	(4)	(1)	(95)
1	Chloramphenicol	36 (100)	24 (88.88)	21 (100)	6 (100)	4 (100)	1 (100)	92 (96.84)
2	Gentamycin	34 (94.44)	10 (37.03)	20 (95.23)	5 (83.33)	3 (75)	1 (100)	73 (76.84)
3	Ciprofloxacin	35 (97.22)	23 (85.18)	21 (100)	6 (100)	3 (75)	1 (100)	89 (93.68)
4	Ofloxacin	35 (97.22)	24 (88.88)	21 (100)	5 (83.33)	3 (75)	1 (100)	89 (93.68)
5	Cephazolin	34 (94.44)	26 (96.29)	21 (100)	5 (83.33)	4 (100)	1 (100)	91 (95.78)
6	Cephalexin	34 (94.44)	24 (88.88)	20 (95.23)	5 (83.33)	4 (100)	1 (100)	88 (92.62)
7	Tobramycin	17 (47.22)	14 (51.85)	11 (52.38)	5 (83.33)	2 (50)	1 (100)	50 (52.63)
8	Vancomycin	34 (94.44)	22 (81.48)	18 (85.71)	5 (83.33)	3 (75)	1 (100)	82 (87.36)

Out of 93 Gram-positive isolates, Chloramphenicol was the most sensitive drug (96.84 %), followed by Cephazolin (95.78 %), Ciprofloxacin and Ofloxacin (93.68 %), Cephalexin 92.63 %, Vancomycin 87.36 %, Gentamycin 76.84 % and Tobramycin was the least sensitive (52.63 %).

Table 10
Antibiotic sensitivity pattern among Gram-negative isolates

S.No.	Antibiotics	Susceptibility of microorganisms		Total
		Escherichia coli (%) (6)	Haemophilus influenzae (%) (5)	(%) (11)
1	Chloramphenicol	6 (100)	4 (80)	10 (90.90)
2	Gentamycin	5 (83.33)	3 (60)	8 (72.72)
3	Ciprofloxacin	4 (66.66)	2 (40)	6 (54.54)
4	Ofloxacin	4 (66.66)	4 (80)	8 (72.72)
5	Cephazolin	4 (66.66)	3 (60)	7 (63.63)
6	Cephalexin	4 (66.66)	3 (60)	7 (63.63)
7	Nalidixic acid	5 (83.33)	5 (100)	10 (90.90)

Out of 11 (10.37 %) Gram-negative isolates, Chloramphenicol was the most effective (90.90 %) followed by Ofloxacin, Gentamycin, Cephalexin and Cephazolin. Ciprofloxacin was the least sensitive to Gram-negative isolates.

Discussion

The most common infection of the lacrimal apparatus is dacryocystitis. The lacrimal excretory system is prone to infection and inflammation for various reasons. This mucous membrane-lined tract is contiguous with two surfaces (conjunctival and nasal mucosa) that are normally colonized with bacteria. The functional purpose of the lacrimal excretory system is to drain tears from the eye into the nasal cavity. Obstruction of the nasolacrimal duct from whatever source results in stasis with accumulation of tears, desquamated cells, and mucoid secretions superior to the obstruction. This creates a fertile environment for secondary bacterial infection. Chronic dacryocystitis is significantly more common in the age above 30 years.

Worldwide, chronic dacryocystitis has been reported to be much more common in females than males, having a sex ratio 3.99:1 (Bharathi et al 2008). Female predominance with a 3:1 ratio (due to obliteration of lumen) was reported (Iliff, 1996). In this study also ,female predominance was seen with the female to male ratio being 2:1, probably because Nepalese women work in smoky kitchens and use different types of cosmetics especially gajal in their eyes which may lead to partial or complete blockage of the drainage system. The growth percentage of microorganisms was found to be higher in males than in females probably due to more exposure of males to the external environment, but statistically, there was no significant difference of infection between male and female patients (P= 0.360).

Altogether, 120 samples from the outdoor patients department were processed, of which 92 (76.66 %) showed positive growth and 28 (23.33 %) samples showed no growth. From 92 positive samples, 106 microorganisms of eight different species were isolated. This result was similar to the result of Malla et al (2007) who reported 13 different species from 199 cases.

The spectrum and proportion of bacterial pathogens as well as antibiotic susceptibility may differ from region to region (Bharati et al 2008). In this study, single bacterial growth was noted in 85.86 % of cases and mixed bacterial growth in 14.13 %. In mixed growth, Staphylococcus aureus was the most predominant bacteria representing 37.03 %, which might be due to the ability of Staphylococcus aureus to grow with other organisms. The majority of organisms cultured were Gram-positive bacteria accounting 89.62 %, with a significant growth of CONS (P=0.018), accounting for 33.96% of all isolates. Gram-negative bacteria were isolated in

10.37 % of specimens with isolation of Escherichia coli in 5.66 % and Haemophilus influenzae in 4.71 % specimens, which was similar to Upadhyay et al (1981) and Cameron et al (2006). Bharathi et al (2008), Coden et al (1993) and Hartikainen et al (1999) reported 69.7 %, 65 % and 69 % of Gram-positive cocci from dacryocystitis patients respectively. Staphylococococci were frequently isolated organisms in acquired dacryocystitis in the older patients (Bareja & Ghosh, 1990; Pollard, 1991). CONS were reported as the most common isolates representing 32.9 %, followed by Pseudomonas spp. 24.2 %, Staphylococcus aureus 12.9 % and Streptococcus pneumoniae 1.4 % and Enteric Gram-negative bacilli were reported as 8.6 % by Khosravl et al (2007). Gilliand (2007) reported Staphylococcus epidermidis as the most common isolate from lacrimal sac infection followed by Staphylococcus aureus; and the most common Gram-negative bacteria isolated was Escherichia coli. Coden et al (1993) reported isolation of 27 % of Gram-negative organisms which was higher than that of this study (10.38 %) with the predominance of Pseudomonas spp, but in this study, Pseudomonas spp. was not isolated.

Chronic dacryocystitis was reported to be more common in the age group of above 30 years of age (Bharathi et al, 2008). Premenopausal women were reported as the predominant age group in chronic dacryocystitis by Badhu et al (2005). In this study, more samples were collected from patients aged above 30 years, but statistically there was no significant difference of infection among any age groups (P = 0.513).

Anatomical factors are also responsible for blockage of the tear drainage system. It was reported that Negros are rarely infected compared to the Whites, as in the former NLD is shorter, wider and less sinuous (Babar et al 2004). A higher growth of organisms was found in Aryans than in Mongoloids in this study. It is assumed that variation can be present due to gross difference in the facial structures of the two major racial groups or may be due to differences in living conditions and sanitation.

In this study, 84.16 % patients showed unilateral infection and 15.83 % patients showed bilateral infection. In unilateral cases, 45.65 % organisms were isolated from the left eye, 36.95 % organisms were isolated from the right eye and 17.39 % organisms were isolated from bilateral infection, but statistically there was no significant difference of infection between unilateral and bilateral cases. Hartikainen et al (2005) reported cases of unilateral and bilateral dacryocystitis 93 % and 7 % respectively. In another study related to keratitis, 99.9 % of infections were unilateral and 0.09 % were bilateral (Bharathi et al 2003).

In this study, the discharges of lacrimal sacs processed for culture were divided into four groups as extensive purulent, mucopurulent, mucoid and clear fluid. All purulent (even extensive purulent) discharges were not found to be culture-positive and all clear fluid discharges were not found to be culture-negative, which indicated the presence of purulent discharges might not always indicate infection. Similarly, Bharathi et al (2008) reported that there was no relation between type of discharge and growth of microorganisms, and purulent discharge may not confirm infection.

Stagnation of tears due to obstruction and resultant accumulation of debris in the lacrimal sac together act as the potential nidus for the organisms to propagate within the sac causing inflammation, hyperemia, edema and hypertrophy of the mucosal epithelium. Accumulation of mucoid and mucopurulent exudates causes the sac to dilate, ultimately leading to a pyocele (Nayak et al 2003). The infective element is a primary factor in most of the cases of dacryocystitis causing mucosal edema and thereby contributing to a vicious circle for further obstruction (Dayal et al 2009). In this study, 100 % of the samples showed positive culture in the cases of tearing for greater than 10 years. Mixed growth was also predominant in cases of epiphora of greater than 10 years duration. This might be due to stagnation of tear for a longer time providing a better environment for pathogenic organisms to get established by suppressing the normal flora. Many patients with chronic dacryocystitis use different antimicrobial agents for a long duration, due to which organisms show resistance to them enhancing the establishment of mixed flora.

Indiscriminate use and widespread prescription of antimicrobial agents may result in the emergence of resistant strains. In this study, except Staphylococcus aureus, all other isolates tested showed the highest percentage of sensitivity to Chloramphenicol and least sensitivity to Tobramycin. Streptococcus pneumoniae was found to be 100 % sensitive to Chloramphenicol, Ciprofloxacin, Ofloxacin and Cephazolin, 95.23 % to Gentamycin and Cephalexin, 90.47 % to Vancomycin and only 52.38 % to Tobraycin. In this study, a single species of Bacillus was isolated and was found to be 100 % sensitive to all tested antibiotics. The coverage of Chloramphenicol against Escherichia coli was 100 %. Escherichia coli were found 83.33 % sensitive to Gentamycin and Nalidixic acid, 66.66 % to Ciprofloxacin, Cephazolin, and Cephalexin. Overall 95 % of sensitivity was seen to Ciprofloxacin. 100 % Streptococcus pneumoniae were sensitive to Ciprofloxacin, although Pseudomonas and Staphylococcus aureus isolates were found to be resistant to Ciprofloxacin in 50 % and 10 % cases respectively. Gentamycin had good coverage against 74.5 % Gram-positive cocci of all tested isolates and was most effective against Gram negative bacilli, 82.6 % of the total tested Gram-negative isolates. All the tested isolates of Gram-positive cocci and Gram-negative bacilli showed resistance to Tobramycin 73.9 % and 60.8 % respectively. The coverage of vancomycin against CONS was 100%, but all the isolates of Staphylococcus aureus were vancomycin resistant (Malla et al 2007).

Overall, Chloramphenicol and Nalidixic acid were found to be effective drugs of choice for Gram-positive and Gram-negative isolates in chronic dacryocystitis patients. In this study, the limitations were time and the number of patients. For better outcomes, a larger study population for a longer period of time should be undertaken to know the bacteriology and to select effective drugs of choice for chronic dacryocystitis. A comparative study of the bacteriology and anti-microbial susceptibility of both chronic and acute dacryocystitis would be very fruitful.

Conclusion

The frequency of isolation of Gram-positive organisms is higher than that of Gram-negative organisms. Coagulase-negative Staphylococci are the most frequently isolated bacteria among all isolates and Staphylococcus aureus is the most predominant bacteria in mixed growth. Chloramphenicol is the most sensitive drug with a susceptibility of 96.4 % against Gram-positive and 90.90 % against the Gram-negative bacteria. Among all antibacterials tested, Chloramphenicol, Cephazolin and Nalidixic acid showed greater efficacy against bacterial isolates from chronic dacryocystitis.

References

Appelbaum P.C., Spangler S.K., Pankuch G.A et al (1994). Characterization of a beta-lactam from Clostridium clostridioforme. J Antimicrobe Chemother; 33: 33-40

Arbuthnott J.P. (1975). Staphylococcal Toxins; In Schlessinger D (Ed): Microbiology, Washington DC. American Society of Microbiology; 267-71

Babar T.F., Saud M.Z., Saeed N et al (2004). An analysis of patients with chronic dacryocystitis. Medical complex; 18 (3): 424-431

Badhu B, Dulal S, Kumar S et al (2005). Epidemiology of chronic dacryocystitis and success rate of external dacryocystorhinostomy in Nepal; 24 (2):79-82

Baleshwar P, Ram D and Gaya P (2009). Bacterial flora in chronic dacryocystitis. Indian Journal of Ophthalmology; 6(4): 68-70

Bareja U, Ghosh S (1990). Clinico-Bacteriological correlation of congenital dacryocystitis. Indian Journal Opthalmol; 28: 66-69

Bharathi MJ, Ramakrishnan R, Maneksha V, Shivakumar C , Nithya V, Mittal S (2008). Comparative bacteriology of acute and chronic dacryocystitis. Journal of Aravind eye care System; (8):20-28.

Cheesbrough M (2000) Medical laboratory manual for tropical countries. Vol 2: Mecrobiology. Cambridge University Press, India; 196-274.

Dayal Y (2009). Medicinal trials in chronic cacryocystitis. Indian J Ophthalmol; 11(3):55-57.

Forbes BA, Sahm DF and Weissfeld AS (2008). Bailey and Scott's Diagnostic Microbiology, 12th ed. Mosby Inc, USA.

Hartikainen J, Lehtonen OP,Saari KM (1997. Bacteriology of lacrimal duct obstruction in adults. Br J Ophthalmol; 81:37-40.

Iliff NT (1996). Infections of the lacrimal drainage system. Ocular infection and Immunity. Mosby; St Louis MO; 1346-55.

Khosravl AD, Mehdinejad M and Heldari M (2007) Bacteriological findings in patients with ocular infection and artibiotic susceptibility patterns of isolated pathogens. Singapore Med J;48(8): 741.

Malla S, Dumre SP, Ghmire GR et al., (2008). Bacterial etiology and antimicrobial susceptibility pattern of ophthalmic infections in Nepal. JNAMLS;9(1):31-35.

Nayak N (2008). Fungal, infection of the eye-laboratory diagnosis and tretment. Nepal Medical college J;60(1): 48-63.

Pollard ZF (1991). Tretment of acute dacryocystitis in neonates. Padiatric Ophthalmol J Strabis;28:342-43.

Upadhyay MP, Karmacharya PC, Koirala S et al (1981). The Bhaktapur eye study: Ocular trauma and antibiotic prophylaxis for prevention of corneal ulceration in Nepal. Br J Ophthalmol 85 (4): 388-92.