Nepal Journal of Science and Technology Vol. 14, No.2 (2013) 137-142

Identification of β-Haemolytic Streptococci among Pharyngitis Cases at Bir Hospital, Kathmandu

A. K. Shrestha[1], B. Lekhak[1], J. Shrestha[2] and D. R. Bhatta[1]
[1]Central Department of Microbiology, Tribhuvan University, Kathmandu
[2]Pathology Department, Bir Hospital, Kathmandu
e-mail: atmazshrestha@gmail.com

Abstract
Respiratory tract infection is one of the major reasons of patients seeking medical care. Among infections pharangitis is most common in the upper respiratory tract, which is seen most frequently in children and adolescents and less frequently among adults. The objective of this study was to analyze the underlying bacterial pathogens in pharyngitis cases and antibiotic susceptibility of various isolates. During February to August, 2010, a total of 134 patients visiting E.N.T. OPD of Bir Hospital complaining sore throat were studied. The throat swabs of the patients were cultured in 5.0% sheep blood agar plates and incubated at 37^{0}C for 24-48 hrs. Clear transparent β-haemolytic colonies of the germs were selected and identified. Group A streptococcus was identified by β-haemolytic colonies with bacitracin and penicillin sensitivity, gram staining, catalase negativity and growth on crystal violet blood agar. In this study, 23 (17.2%) patients were positive for β-haemolytic streptococci. Among them, 12 (9%) were identified as Group A streptococcus. The culture positive for BHS was highest (56.5%) among the age group of 21-40 years. Pathogens other than β-haemolytic streptococci were isolated from 9 patients, of which 4 cases showed mixed bacterial growth. Other bacterial isolates reported in the study were *Staphylococcus aureus* 6 (4.5%), *Klebsiella pneumoniae* 5 (3.7%), *Pseudomonas aeruginosa* 2 (1.5%), *Citrobacter freundii* 1 (0.7%), and yeast cells 1 (0.7%). It was found that infections caused by β-haemolytic streptococci was significantly higher p=0.0084 i.e. (p<0.05) than infections caused by other bacteria. This study demonstrated that young adults of age group 21-40 were affected by streptococcal pharyngitis. The isolates of GAS were 100% sensitive to amoxycillin, cephalexin, cefotaxime, ciprofloxacin and azithromycin followed by cotrimoxazole (91.7%) and erythromycin (91.7%).

Key words: β-haemolytic streptococci, bacitracin, group A streptococcus, pharyngitis, sore throat

Introduction
Streptococcus pyogenes (group A streptococcus, GAS) is an important species of gram-positive bacterial pathogen which colonizes the throat of human beings and causes pharyngitis (Cunningham 2000, Pfoh *et al*. 2008). Streptococcal pharyngitis continues to be one of the most common childhood illnesses throughout the world (Bisno 1996, Linder *et al*. 2005). Rheumatic fever (RF) is a delayed sequel to GAS pharyngitis (Dale & Beachey 1986, Adderson *et al*. 1998).

It is estimated that over 600 million cases of symptomatic GAS pharyngitis occur annually among people aged over 4 years, and that over 550 million of these occur in less developed countries. Additional cases occur in children aged under five years, although GAS pharyngitis becomes less common in younger children. Therefore, it seems reasonable to conclude that approximately 15% of school-age children suffer symptomatic episode of GAS culture-positive pharyngitis each year in more developed countries, and that 4–10% of adults are similarly affected. The incidence of pharyngitis cases in less developed countries, may be five to ten times greater than more developed countries on basis of the data from India, Kuwait, the New Zealand, Maori and Pacific Islander populations (Carapetis *et al*. 2005). To initiate control programme of RF/RHD in less developed countries data on the prevalence and incidence of â-haemolyic streptococci, especially GAS pharyngitis are required.

However, only scanty information is available in Nepal regarding the magnitude of this problem.

The aim of this study was to explore the current situation of streptococcal infection as well as other bacterial pathogens in the throat of symptomatic patients visiting Bir Hospital. Moreover, to study antibiotic sensitivity pattern of isolated pathogens.

Methodology

Altogether 134 throat swab samples were processed for culture, isolation and identification of causative organisms of pharyngitis/tonsillitis. Patients with sore throat visiting E.N.T. Out-patient Department of Bir Hospital, recommended by medical practitioners for throat swab culture were included in the study. The throat swabs obtained were cultured in sheep blood agar (BA) and MacConkey agar (MA) plates within 30 minutes upon receipt at the laboratory. Standard protocols of Vandepitte *et al*. (2003) and Cheesbrough (2006) were followed for culture and identification of bacteria. The cultures inoculated on blood agar plates were incubated at 37^{o}C in 5-10% carbon dioxide and examined after overnight incubation and again after 48 hours. All the plates with â-haemolytic colonies identified by conventional methods (colony morphology, haemolysis pattern, catalase test, gram staining). *Streptococcus pyogenes* was further identified by observing its sensitivity towards 0.04 units of bacitracin disc and 10 units of penicillin disc on blood agar plate. Isolates from aerobically incubated MacConkey agar plates were identified using colony morphology, gram-staining and biochemical tests. All the pathogenic microorganisms isolated from throat swab samples were subjected for antimicrobial susceptibility test by Kirby-Bauer disk diffusion method as recommended by CLSI. *S. pyogenes* ATCC 19615 was taken as control for susceptibility test of low concentration of bacitracin for identification of GAS. For the standardization of Kirby-Bauer test and for performance testing of antibiotics and MHA, control strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were tested primarily. Antibiotics discs containing penicillin 10 units, amoxycillin 10 mcg, chloramphenicol 30 mcg, ciprofloxacin 5 mcg, cotrimoxazole 25 mcg, cephalexin 30 mcg, cefotaxime 30 mcg, erythromycin 15 mcg, azithromycin 15 mcg, amikacin 30 mcg, gentamicin 10 mcg, polymyxin B 300 units were tested for isolates.

Results and Discussion

Out of 134 samples, 17.2% (23) were found to be â-haemolytic streptococci (BHS), Bacterial pathogens other than BHS accounted for 6.7% (9/134) cases and no pathogenic organisms were reported in 76.1% (102/134) cases. Group A â-haemolytic streptococcus (GAS) constituted 52.2% (12).

On the basis of agewise distribution of the â-haemolytic streptococci, 56.5% (n=13) BHS were isolated from the age group 21-40 and 21.8% (n=5) BHS were isolated from age group of 16-20. Similarly 13% (n=3) BHS from age group <15 and 8.7% (n=2) BHS from age group of 41-65 were found. There was no significant association between â-haemolytic streptococci cases and the age group of the patients (p>0.05), (Table 1).

Table 1. Age and gender wise distribution of the patients with positive cases of BHS

Age group	Number			%
	Male	Female	Total	BHS
< 15	3	0	3	13.0
16-20	4	1	5	21.8
21-40	5	8	13	56.5
41-65	0	2	2	8.7
Total	12	11	23	100

Microbiological pattern

Out of 134 throat swabs that were cultured, 23 BHS were isolated and nine cases showed pathogenic microorganisms other than BHS. Out of nine cases 4 cases had mixed bacterial growth. The other pathogenic microorganisms isolated were *S. aureus* 6 (4.5%), *K. pneumoniae* 5 (3.7%), *P. aeruginosa* 2 (1.5%), *C. freundii* 1 (0.7%), and Yeast Cells.1 (0.7%), (Table 2).

Table 2. Microbiological pattern of throat isolates

Organisms	Male		Female		Total %	Total no. of isolates
	No. of isolates	(%)	No. of isolates	(%)		
β Haemolytic streptococci other than Group A	3	2.2	8	6.0	8.2	11
Group A β Haemolytic streptococcus	5	3.7	7	5.2	9.0	12
Staphylococcus aureus	4	3.0	2	1.5	4.5	6
Klebsiella pneumoniae	2	1.5	3	2.2	3.7	5
Pseudomaonas aeruginosa	2	1.5	0	0	1.5	2
Citrobacter freundii	0	0	1	0.7	0.7	1
Yeasts	1	0.7	0	0	0.7	1

Antibiotic susceptibility test (AST) of the â-haemolytic streptococci isolates

The antibiotic sensitivity pattern of GAS showed that most sensitive drugs for GAS were amoxycillin, cephalexin, cefotaxime, ciprofloxacin and azithromycin, which were 100% sensitive followed by cotrimoxazole (91.7%) and erythromycin (91.7%). Most sensitive drugs for BHS were amoxycillin, cefotaxime, ciprofloxacin and azithromycin, which were 100% sensitive followed by cotrimoxazole (90.9%), erythromycin (72.7%) and cephalexin (63.6%), (Table 3).

Table 3. Antibiotic sensitivity pattern of â-haemolytic streptococci isolates

Antibiotics	BHS Other than Group A					GAS				
	Sensitive		Intermediate	Resistant		Sensitive		Intermediate	Resistant	
	No.	%	No.	No.	%	No.	%	No.	No.	%
Amoxycillin	11	100	0	0	0	12	100	0	0	0
Cephalexin	7	63.6	0	4	36.4	12	100	0	0	0
Cefotaxime	11	100	0	0	0	12	100	0	0	0
Cotrimoxazole	10	90.9	0	1	9.1	11	91.7	0	1	8.3
Ciprofloxacin	11	100	0	0	0	12	100	0	0	0
Erythromycin	8	72.7	0	3	27.3	11	91.7	0	1	8.3
Azithromycin	11	100	0	0	0	12	100	0	0	0

Antibiotic sensitivity pattern of bacteria other than â-haemolytic streptococci isolates

The antibiotic sensitivity pattern of bacterial isolates other than BHS showed highest sensitivity to ciprofloxacin (78.6%), gentamycin (75%) and amikacin (75%), followed by chloramphenicol (71.4%), cefotaxime (57.1%), azithromycin (66.7%), penicillin (33.3%) and cephalexin (42.9%) and least sensitivity to amoxycillin (28.6%) and cotrimoxazole (21.4%). Polymyxin B was used only for *P. aeruginosa*, (Table 4).

Table 4. Antibiogram sensitivity pattern of bacteria other than â-haemolytic streptococci isolates

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ciprofloxacin	11	78.6	0	0	3	21.4	14
Amoxycillin	4	28.6	1	7.1	9	64.3	14
Cephalexin	6	42.9	1	7.1	7	50.0	14
Cefotaxime	8	57.1	0	0	6	42.9	14
Gentamicin	6	75	0	0	2	25.0	8
Amikacin	6	75	0	0	2	25.0	8
Chloramphenicol	10	71.4	0	0	4	28.6	14
Cotrimoxazole	3	21.4	0	0	11	78.6	14
Polymxin B	2	100	0	0	0	0.0	2
Azithromycin	4	66.7	0	0	2	33.3	6
Penicillin	2	33.3	0	0	4	66.7	6

In this study, 134 throat swab samples were processed for detection of bacterial pathogens causing pharyngitis. Out of 23 (17.1%) cases that were found to be positive to BHS and 12 cases were accounted for GAS. This study showed that the prevalence of GAS was 9% in patients. Similar studies conducted by Bista *et al*. (2005), Shrestha *et al*. (2006), Rijal *et al*. (2009) and Gurung *et al*. (2010) showed the prevalence of GAS being 18%, 58.8%, 9.2% and 5% respectively. However, in the present study more samples were obtained from adults of age >15 years. Only Bista *et al*. (2005) in above mentioned study included adult population. In this study among 23 positive â-haemolytic cases, 88.46% (20/23) patients were of >15 years of age. Data obtained in this study correlate with the studies of Poses *et al*. (1985), Komaroff *et al*. (1986) and Ebell *et al*. (2000) that the prevalence of GAS was reported to be around 5% to 10% in adults. The high prevalence of BHS in adult population in patients in our study may be due to hygienic and socioeconomic conditions the adult population serves as carrier of BHS. In this study, the frequency of BHS was not similar in all age groups. It was slightly higher in adults aged 21-40 years, however, it was statically insignificant ($p>0.05$). In this study it has been seen that the age of the patients coming for treatment shifted from school aged children to young adults (21-40 years of age). This is more likely because Bir Hospital entraps more of adult population and children that usually visit children's hospital located nearby, thus eluding attention.

In this study highest number of pathogenic organisms in throat swab was represented by BHS, then followed by *S. aureus* 6 (4.5%), *K. pneumoniae* 5 (3.7%), *P. aeruginosa* 2 (1.5%), *C. freundii* 1 (0.7%) and yeast 1 (0.7%) respectively (Table 2). According to Vandepitte *et al*. (2003) these microorganisms do not cause pharyngitis, except in association with granulocytopenia. It is advisable to report such isolates to the clinicians, as they occasionally indicate the existence of (or may sometimes give rise to) a lower respiratory tract infection (e.g. pneumonia) or bacteraemia. Isolation of these organisms in the throat

may be due to transient colonisation of these organisms in adults secondary to repeated use of antibiotics (Longanathan *et al*. 2006). In this study it was found that infections caused by BHS in tonsillitis/pharyngitis patients is significantly higher than infections caused by other pathogenic bacteria (p=0.0084 i.e. p<0.05). Thus, according to the data obtained it can be said that BHS were the major pathogens in pharyngitis cases.

In present study, *S. aureus* and *K. pneumoniae* were reported in 4.5% and 3.7% cases respectively. Bista *et al*. (2005) in comparative study of core and surface culture of tonsillectomy cases in Tribhuvan University Teaching Hospital (T.U.T.H.) reported *S. aureus* in 21% of cases. Likewise, *K. pneumoniae* on 10% cases, both organisms were more significantly seen in >20 age group than others. According to Longanathan *et al*. (2006), *S. aureus* was reported in 45.5% of cases and was the most common organism in adults with sore throat followed by *K. pneumonia* in 26.0% of cases, and was the second most common organism. The low prevalence of these organisms in the present study may be due to pharyngeal swab cultures which do not reliably reflect the presence of pathogens in the tonsilar core as shown in the study carried out by Al-roosan *et al.* (2008).

It was observed that most sensitive drugs for GAS were amoxycillin, cephalexin and cefotaxime, ciprofloxacin and azithromycin, which were 100% sensitive, followed by cotrimoxazole (91.7%) and erythromycin (91.7%). The most sensitive drugs for BHS other than Group A are amoxycillin, ciprofloxacin and azithromycin, which were 100% sensitive. The result of sensitivity pattern of macrolide used in present study correlates with study carried out by Rijal *et al*. (2009), which showed 97.8% isolates were sensitive to azithromycin and 84.4% were sensitive to erythromycin. Dumre *et al*. (2009) reported 94.8% isolates were sensitive to erythromycin. According to Gurung *et al*. (2010) erythromycin showed 100% sensitivity. The present study also revealed that macrolide antibiotics were sensitive towards GAS. Hence, azithromycin is a drug of choice for pharyngitis patients, who are allergic to penicillin.

In the present study, GAS isolates showed 91.7% sensitivity to ciprofloxacin and cotrimoxazole. This finding is in agreement with that of Dumre *et al*. (2009), which reported 94.8% isolates sensitive to ciprofloxacin but 29.0% were sensitive to cotrimoxazole.

In this study amoxycillin inhibited 80% of *S. aureus*, cotrimoxazole inhibited only 40% of the isolates whereas azithromycin and penicillin inhibited 80% and 60% of isolates respectively. In the study carried out by Shrestha *et al*. (2006), amoxycillin and cotrimoxazole inhibited 76.3% of isolates of *S. aureus*, erythromycin and penicillin inhibited 65.8% and 44.7% isolates, respectively.

The results showed that BHS was one of the major causes of bacterial pharyngitis among adult population. It has been demonstrated that antibiotic resistance of *S. pyogenes* is not a clinically significant problem. The results of this study also highlighted the importance of regular programs of monitoring the rate of GAS carriage and the antibiotic susceptibility of GAS isolates in adult population.

In the treatment of clinical pharyngitis, it is known that oral antibiotics do not give as much coverage as benzyl penicillin to patients who may later go on to develop RF/RHD due to lack of adequate antibiotic protection. However, over treatment with penicillin may pave way to increased penicillin resistance. Finally, one may encounter larger number penicillin anaphylaxis, which can significantly reduce patient's compliance to antibiotic treatment. Thus, scientifically, it is better to manage treatment after confirming a GAS etiology which would overcome the above problems (Gurung *et al*. 2010).

This study concludes that information of GAS susceptibility pattern is crucial for monitoring the development of antibiotic resistance in *S. pyogenes*. During this study, 76.1% culture results showed the growth of non pathogenic microorganisms, which may be either due to viral infection (Cheesbrough 2006) in patients or allergic reactions. Immunohistochemical findings Modrzyñski *et al.* (2003) hypothesized that allergic sensitization takes place in the adenoid and tonsils. Further, Modrzyñski *et al.* (2005) confirmed that tonsil dendritic cells, macrophages, eosinophils, and mast cells are important in allergic tonsillitis. Therefore, viruses along with quantitative analysis of bacterial molecular biology together with allergic reactions in consideration will be necessary in the future research.

Our present findings demonstrated that young adults (age group: 21-40) are affected by BHS tonsillitis/ pharyngitis, so further long term study is needed in this matter.

Acknowledgments

The authors would like to acknowledge all the staff and management committees of the Bir hospital as well as the faculty and staff of Central Department of Microbiology, Kirtipur for their kind cooperation during the study period.

References

Adderson, E.E., A.R. Shikhman, K.E. Ward and M.W. Cunningham. 1998. Molecular analysis of polyreactive monoclonal antibodies from rheumatic carditis: human anti-N-acetylglucosamine/anti-myosin antibody V region genes. *Journal of Immunology* **61**: 2020-2031.

Al-roosan, M.,N. Al-khtoum and H. Al-said. 2008. Correlation between surface swab culture and tonsillar core culture in patients with recurrent tonsillitis. *Khartoum Medical Journal* **01**: 129-132.

Bisno, A.L. 1996. Acute pharyngitis: etiology and diagnosis. *Pediatrics* **97**: 949-954.

Bista, M., B.K. Sinha, R.C.M. Amatya, N.R. Tuladhar and B.M. Pokharel. 2005. Comparison of core and surface cultures in recurrent tonsillitis. *Journal of Institute of Medicine* **27**: 60-65.

Carapetis, J.R., A.C. Steer, E.K. Mulholland and M. Weber. 2005. The global burden of group A streptococcal diseases. *Lancet Infectious Disease* **5:** 685-694.

Cheesbrough, M. 2006. *District laboratory practice in tropical countries*. 2nd ed. Cambridge University Press.

Cunningham, M.W. 2000. Pathogenesis of group A streptococcal infections. *Clinical Microbiology Reviews* **13**: 470-511.

Dale, J.B. and E.H. Beachey. 1986. Sequence of myosin-crossreactive epitopes of streptococcal m protein. *Journal of Experimental Medicine* **164**: 1785-1790.

Dumre, S.P., K. Sapkota, N. Adhikari, D. Acharya, M. Karki, S. Bista and S.R. Basanyat. 2009. Asymptomatic throat carriage rate and antimicrobial resistance pattern of *Streptococcus pyogenes* in Nepalese school children. *Kathmandu University Medical Journal* **7**: 392-396.

Ebell, M.H., M.A. Smith, H.C. Barry, K. Ivesand M. Carey. 2000. The rational clinical examination. Does this patient have strep throat? *Journal of the American Medical Association* **284**: 2912-2918.

Gurung, R., S. Budhathoki, R. Amatya, N. Poudyal, S. Shrestha and R. Baral. 2010. Group A â-haemolytic streptococcus infection presenting with sore throat at paediatric OPD. *Health Renaissance* **8**: 90-92.

Komaroff, A. L., T.M. Pass, M.D. Aronson, C.T. Ervin, S. Cretin, R.N. Winickoff, W.T Branch, and B. Brigham. 1986. The prediction of streptococcal pharyngitis in adults. *Journal of General Internal Medicine* **1**: 1-7.

Linder, J.A., D.W. Bates, G.M. Lee and J.A. Finkelstein. 2005. Antibiotic treatment of children with sore throat. *Journal of American Medical Association* **294**: 2315-2322.

Longanathan, A., U.D. Arumainathan and R. Raman. 2006. Comparative study of bacteriology in recurrent tonsillitis among children and adults. *Singapore Medical Journal* **47**: 271-275.

Modrzyñski, M., P. Grochowski, E. Zawisza and A. Lipiec. 2003. Histopathological and immunological analysis of hyperplastic palatine tonsils and adenoids in children with coexisting atopic dermatitis. *Przegl Lek* **60**: 317-321.

Modrzyñski, M., H. Mazurek and E. Zawisza. 2005. Allergic tonsillitis: myth or reality. *Postepy Hig Med Dosw* **59**: 450-456.

Pfoh, E., M.R Wessels, D. Goldmann and G.M. Lee. 2008. Burden and economic cost of group A streptococcal pharyngitis. *Pediatrics* **121**: 229-34.

Poses, R.M., R.D. Cebul, M. Collins and S.S. Fager. 1985. The accuracy of experienced physicians' probability estimates for patients with sore throats: implications for decision making. *Journal of American Medical Association* **254**: 925–929.

Rijal, K.R., N. Dhakal, R.C. Shah, S. Timilsina, P. Mahato, S. Thapa and P. Ghimire. 2009. Antibiotic susceptibility of group A streptococcus isolated from throat swab culture of school children in Pokhara, Nepal. *Nepal Medical College Journal* **11**: 238-240.

Shrestha, A., J.B. Sherchand, P.R. Sharma and D.R. Bhatta. 2006. Bacteriological study of upper respiratory tract infection in pediatric patients. *Journal of Institute of Medicine* **28**: 28-31.

Somro, A., M. Akram, I.M. Khan, H.M. Asif, A. Sami, S.M.A. Shahi, G. Shahen, K. Ahmed and R.U. Rehman. 2011. Pharyngitis and sore throat: A review. *African Journal of Biotechnology* **10**: 6190-6197.

Stillerman, M. and S. Bernstein. 1961. Streptococcal pharyngitis: evaluation of clinical syndromes in diagnosis. *American Journal of Diseases of Children* **101**: 476–489.

Vandepitte, J., J. Verhaegen, K. Engbaek, P. Rohner and C.C. Heuck. 2003. *Basic laboratory procedures in clinical bacteriology*. 2nd ed. Geneva: World Health Organization.