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Full Length Research Paper

Prevalence of *Streptococcus pyogenes* Among Pre-School Children Ages 4 to 6 in Makah city, Saudi Arabia

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Group A Streptococcus (GAS) is a one of the most common bacterial pathogens. It causes pharyngitis among pre-school aged children. The aim of this study is to determine the prevalence of transmission of Group A streptococci among pre-school children in the city of Makah, Saudi Arabia. The study included 2370 Saudi and non-Saudi children four to six years from 19 kindergarten schools from seven different districts in Makah. The sampling was conducted from February to May 2014. GAS were isolated from 36 (1.5%) of 2370 asymptomatic children. Among the isolates, 16 (44.4%) were from male subjects whereas 20 (55.6 %) were from females. The highest colonization of *S. pyogenes* was found in the 5-year age group 17 (1.6 %). The prevalence was higher in boys (1.6%) as compared to girls (1.5%). All of the isolates of GAS were sensitive to penicillin (100%) and the highest resistance was (33 %) for trimethoprim. This study demonstrated low prevalence of GAS was observed in our present study when compared to studies conducted on other countries, which reflect relatively favorable conditions of generally hygiene and environmental factors in Saudi Arabia.

Keywords: Group A streptococcus, Makah, GAS, pharyngitis, hygiene

INTRODUCTION

Streptococci are gram positive cocci arranged in chains or pairs and are part of normal flora of humans and animals. Some of them are human pathogens. The most Prevalent of them is *Streptococcus pyogenes*, which is the cause of pyogenic infections (Kushwaha et al., 2014). It belongs to Lancefield Group A, is beta-hemolytic, and is called Group A beta-hemolytic Streptococci (GABHS). Group A Streptococcus (GAS) is a single species, *Streptococcus pyogenes* (Khan et al., 2013). *Streptococcus pyogenes* or Lancefield Group A beta hemolytic streptococci is one of the most common bacterial pathogens that causing pharyngitis among school aged children living in lower socioeconomic conditions (Chand et al., 2013). Approximately 7.3 million outpatient visits attributable to sore throats occur each year among children in the United States. *S. pyogenesis* responsible for 16%- 38% of these cases (Linder et al.,

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2013). Reports show that 5-17% of school children have *S. pyogenes* in their throats and are at the risk of contracting the disease (Charmaine et al., 2006).GAS colonization of the upper respiratory tracts of children plays a role in the spread of infection in households and community settings such as schools and day care centers (Vincent et al., 2004). Asymptomatic carriers of S. *pyogenes*is able to transmit the disease and are often the sources of bacterial dissemination and are able to transmit the disease (Nabipour and Tayarzadeh, 2005). Upper respiratory tract infections are the most common infectious disease among children (Ameen et al., 1997; Bell and Smith, 1976; Sevinc et al., 2008). Pharyngeal infection rates among healthy school children vary according to geographic location and season of the year (Elifozltuk et al., 2004). GAS is the ninth major infectious cause of deaths of humans globally (WHO, 2004). There is evidence that asymptomatic throat infections caused by GAS may lead to acute rheumatic fever (ARF) (Gordis et al., I969). Group A streptococci are bacterial pathogens that cause suppurative and non-suppurative infections in humans. Suppurative infections occur in mucous membranes of tonsils and pharynx. If GAS is allowed to progress, acute rheumatic fever may occur, which is an non-suppurative condition and a precursor to acquired heart disease in children, particularly in developing countries (Charmaine et al., 2006). Manifestations of GAS range from mild infectious, such as pharyngitis and tonsillitis, to more serious complications such as toxic shock syndrome. These conditions are often followed by complications of rheumatic fever, rheumatic heart disease, and acute glomerulonephritis (Capoor et al., 2006). Although rheumatic fever is uncommon in most developed countries, however it remains the leading cause of acquired heart disease among children in many resource-poor areas such as sub Saharan Africa, India, parts of Australia and New Zealand (Carapetis et al., 2005). The economic burden of the treatment of streptococcal pharyngitis among children in USA has been estimated at $226 million to $540 million per year (Lee, 2008). Streptococci are readily transmitted when a large number of people share common environments and personal contact with either infected persons or healthy care (Arguelles et al., 2004). GAS infection is spread by direct person to person contact via drops of saliva, nasal secretions, and contaminated fingers (Arguelles et al., 2004). *S. pyogenes* can cause infections when they are introduced or transmitted to vulnerable tissues such as pharyngitis, characterized by severe sore throat, mild fever, enlarged tonsils and tonsillar exudates. The incubation period for streptococcal pharyngitis is short (3 to 5 days) (Pichichero et al., 1999). More recent studies have suggested that throat culture is still considered the gold standard method for GABHS detection, giving accurate results in about 90-95% of the cases (Fatima, 2013). With screening and antibiotic treatment, pharyngeal carriers can be prevented from spreading respiratory infections in their communities.

Aim of work

The aim of this study is to evaluate the streptococcal throat carriage rate amongst preschool children, ages four to six years in Makah, Saudi Arabia, in order to assist in the creation of a database of disease transmission rates among children in this age group, to encourage precautionary actions that will help to reduce the spread of infection, as well as to avoid complications that result from delays in accurate diagnosis and treatment.

MATERIALS AND METHODS

In this study, preschool children from various parts of Makah, Saudi Arabia were screened to detect pharyngeal infections resulting from GAS. The study included 2370 Saudi and non-Saudi children aged four to six years from 19 kindergartens school from seven different districts of Makah. The total number of children screened from each district was as follows: 572 from the Azizah district, 460 from Mansour Street vicinity, 886 from Al-Sateen Street vicinity, 194 from Al-Shisha district, 104 from Umm Al-Jood vicinity, 62 from Al- Awali district and 92 from Alkhansa district. The children were questioned about their ages and clinical symptoms such as sore throat, fever, rheumatic fever and whether or not they were taking antibiotics during the previous week. The study was carried out between February and May 2014, in the microbiology research laboratory of the Faculty of Medicine at Umm Al-Qura University.

Samples collection

The specimens were collected via sterile cotton swabs and preserved in an Amie's Transport Medium, swabbing the upper tonsil and posterior pharyngeal areas. Each sample was labeled with a code number. Other information including age, sex and locations was also recorded. The samples were transported to the microbiology research laboratory within one to two hours or acquisition for processing.

Samples processing

The swab was rolled over a small portion of a sheep blood agar plate. The inoculum was streaked over the remainder of the plate with a sterile loop to obtain well isolated colonies and incubated at 37°C for 24 hours in a

Table 1. Distribution of GAS Isolates from Throats of Children According to Age and Gender

Ages	Boys			Girls			Total		
	Swabs No.	GAS No.	GAS %	Swabs No.	GAS No.	GAS %	Swabs No.	GAS No.	GAS %
4	292	4	1.4	250	3	1.2	542	7	1.3
5	530	10	1.9	514	7	1.4	1044	17	1.6
6	198	2	1.0	586	10	1.7	784	12	1.5
Total	1020	16	1.6	1350	20	1.5	2370	36	1.5

carbon dioxide incubator (3-5% CO2). After 24 hours, small grayish white colonies surrounded by clear zones of hemolysis were selected and subjected to Gram's staining and catalase test. Bacteria which exhibited negative catalase and appeared in chain were sub cultured on a 10% sheep blood agar with low concentration bacitracin (0.04 IU) and incubated at 37°C for 24 hours. *S pyogenes* was further identified by observing sensitivity to bacitracin.

Antibiotic susceptibility test

All the identified GAS isolates from throat swabs were subculture onto sheep blood agar to subject antimicrobial susceptibility tests were performed using AST-STO1 card on the VITEK II instrument (bioMerieux). Two clear sterile plastic tubes containing 3 ml sterile saline aqueous 0.45% Na CL, P1-I 7.0 in each tube were prepared. In one of these tubes (table 1). Five colonies from the subculture were transferred using a sterile stick and mixed to produce a homogenous organism suspension, and the density was adjusted to be equivalent to a McFarland number of 0.50 to 0.63 using a calibrated VITEK 2 Densicheck. A volume of 280 µl of the suspension was transferred from the first tube to the second tube. All inoculated cards were placed in the instrument within 30 mm of inoculation. Data were analyzed using software version VT2-R4.0l. The results were available after approximately 8 -12 hours. The antibiotic sensitivity test (AST) measured the minimum inhibitor concentrations (MIC) of the following antibiotics against GAS: penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin, levofloxacin, linezoid, tetracycline, trimethoprim/ sulfamethoxazole, vancomycin and ceftriaxone.

Ethical approval

The protocol for this study was approved by the director of the board of education of Makah, Saudi Arabia before sampling. Approval was also obtained from the director of each pre-school. The children were given some gifts as an incentive.

RESULTS

Among the total 2370 children,1020 (43%) were boys and 1350 (57%) were girls, as shown above.

Table 1 Showed that 542 (21.9%) were four years of age, 1044 (44%) were 5 years of age and 784 (33.1%) were six years of age. In the study, a total of 36 (1.5%) isolates of beta hemolytic *Streptococci pyogenes* were obtained from healthy children. Among the isolates, 16 (44.4%) were from males whereas 20 (55.6 %) were from females. The highest colonization of and pyogenes was found in the five-year age group 17(1.6 %). The prevalence was higher in boys (1.6 %) as compared to girls (1.5%).

Table 2 Showed that among the total isolates of GAS, 35(97%) were Saudi children and 1 subject (3%) was non Saudi. The highest percentage of isolation of GAS was in the Khansa district (3.3%), followed by the Mansour Street vicinity (2%) (table 2) and the lowest percentage of isolation of GAS was from Aziziah district (0.7) followed by the Shisha district (1.1%). The carrier rate of GAS varied in residential areas from one to another. Differences in prevalence may be due to varying socio-economic conditions

Table 3 Showed that the results of antimicrobial susceptibility testing are summarized in (Table 3). Streptococcus pyogenes showed sensitivity to penicillin's including ampicillin and amoxicillin, cephalosporin's (third generation) including cefotaxime and ceftriaxone, lincosamides including clindamycin and lincomycin, glycopeptides including vancomycin and quinolones including levofloxacin. *Streptococcus pyogenes* exhibited varying resistance to sulfonamides including, trimethoprim, tetracycline's including tetracycline and macrolides including erythromycin.

Table 2. Distribution of GAS Isolates According to Residential Districts Included in the in the study and Nationality.

Residential Districts	Nationality								
	Saudi			Non- Saudi			Total		
	Swab No.	GAS No	GAS %	Swab No.	GAS No	GAS %	Swab No.	GAS No	GAS %
Aziziah	569	4	0.7	3	0	0	572	4	0.7
Al-Awali	62	1	1.6	-	-	-	62	1	1.6
AL-MansourStreet	460	9	2.0	-	-	-	460	9	2.0
Al-Siteen	886	14	1.6	-	-	-	886	14	1.6
Umm Al-Jood	96	2	2.0	8	1	12.5	104	3	2.9
Al-Khansa	92	3	3.3	-	-	-	92	3	3.3
Al-Shisha	188	2	1.1	6	0	0	194	2	1.1
Total number	2353	35	1.5	17	1	5.9	2370	36	1.5

Table 3. Antibiotic Susceptibility tests of 36 GAS Isolates

Antibiotics	Sensitive		Resistant	
	No.	%	No.	%
Penicillin-G	36	100	0	0
Ampicillin	36	100	0	0
Cefotaxime	36	100	0	0
Ceftriaxone	36	100	0	0
Levofloxacin	36	100	0	0
Erythromycin	32	89	4	11
Clindamycin	36	100	0	0
Linezolid	36	100	0	0
Vancomycin	36	100	0	0
Tetracycline	30	83	6	17
Trimethoprim/Sulfamethoxazole	24	67	12	33

DISCUSSION

Healthy carriers of GAS are sources for bacterial dissemination, able to transmit the disease, and may even cause severe epidemics. In this study, throat cultures were taken to investigate the carrier rate of *Streptococcus pyogenes* in healthy children. The prevalence of GAS in throat swabs of asymptomatic children was 1.5%. The highest prevalence of *Streptococcus pyogenes* was 1.6% in the five to six-year age group. A low prevalence of GAS was observed in our present study when compared to studies conducted in other countries. The prevalence rates of *Streptococcus pyogenes* in other countries are in a range of 0% to 32.7% (Cimolai et al., 1990; Odigwe et al., 2002; Gur et al., 2002; Ameen et al., 1997). The isolation rate of GAS in children five to seven years old at nine schools in the United Arab Emirates as 10% during the winter of 1995 (Gunnarsson et al., 1997). The study implied that the prevalence of *Streptococcus pyogenes* in healthy individuals was highest in the three to 15 year (Christenson et al., 1997). Reports was 2% in Stockholm, Sweden. The prevalence of *Streptococcus pyogenes* was 2.7% in infants and 8.4% and 17.8% in toddlers in day care facilities in Israel, reaching 8.5% and 17.8% in the two groups, respectively (Yagupsky et al., 1995). *Streptococcus pyogenes* was 36% of children in daycare centers in USA (Smith et al., 1989). Factors affecting the carrier state in individual children include differences in climatic condition, geographical region and socioeconomic status. Attendance of preschool, housing standards, previous tonsillectomies, family size and structure as well as the number of students per-classroom may also be important factors affecting the transmission of *Streptococcus pyogenes*. There was a suggestion that throat infections were more common among the children from large families than among those from small numbers. The 0% incidence rate may be due to factors such as improvements in the living conditions of families and developments within the community. In the present study, there was no observed difference in the carrier rates between boys (1.6 %) and girls (1.5 %).

Other studies conducted in different parts of the world showed a higher prevalence of GAS among girls as compared to boys may be due to certain cultural practices of parents giving less care to girls than boys (Rijal et al., 2009). This is not the case in all countries, however, especially in Saudi Arabia. All of the isolates of *Streptococcus pyogenes* were sensitive to penicillin (100%) therefore, Penicillin remains effective in treatment of *Streptococcus pyogenes* infections and preventing rheumatic fevers. In our study, 89% of GAS isolates demonstrated sensitivity to erythromycin. In some parts of Europe, resistance rates of erythromycin greater than 30% have been reported. The resistance rate of erythromycin in USA considered to be low (<3%)' (Michael and Gerber, 1996). The highest resistance was (33 %) for trimethoprim.

CONCLUSION

In our study, the prevalence of GAS throat carriage amongst pre-school children in the city of Makah was (1.5%). This low prevalence rate compared to studies of other countries may be attributed to improvements in hygiene and health care as well as improvements in the living conditions of families. The results of the present study are encouraging. They reflect the beneficial results that arise from improvements in hygiene and health care in communities. This study also indicates the importance of regular surveillance programs to keep *Streptococcus pyogenes* infections and transmissions under control. Continuous evaluation is recommended by conducting similar studies in other parts of the country to further describe the epidemiology of GAS activity for this age group and increase control of the development of complications such as rheumatic fever.

Conflict of interest

The authors have no conflicts of interest.

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REFERENCES

Ameen AS, Nsanze H, Dawson KP, Othman, Mustafa N, Johnson DR, Kaplan EL (1997). Serotypes of group A streptococciisolated from healthy schoolchildren in the United Arab Emirates. Bull World Health Organ. 75:355-359.

Ameen AS, Nsanze H, Dawson KP, Othman, Mustafa N, Johnson DR, Kaplan EL (1997). Serotypes of group A Streptococci isolated from healthy schoolchildren in the United Arab Emirates. Bull World Health Organ. 75:355-359.

Arguelles KP, Naguit RM, Cabrera BC (2004). Incidence of Streptococcus pyogenes as Pharyngeal Flora among Children Ages 5 to 12 of Barangay 708, Leveriza, Malate, Manila. Philipp J. Microbiol. Infect. Dis. 33: 149-151.

Bell SM, Smith DD (1976). Quantitative throat-swab culture in the diagnosis of Streptococcal pharyngitis in children. Lancet; 2:62-3.

Capoor MR, Nair D, Deb M, et al (2006). Resistance to erythromycin and rising penicillin MIC inStreptococcus pyogenes in India. Japanese J. Infect. Dis. 59: 334-336.

Carapetis JR, Steer AC, Mulholland EK, Weber M (2005). The global burden of group A Streptococcal diseases. Lancet Infect. Dis. 5:685-694.

Chand P, Arvind N, Vishrutha Ky, Vidyalakshmi K, Shalini S (2013). Int. J. Biol. Med. Res. 4(4): 3585-3589

Charmaine AC, Jacob SE, Menon T (2006). Pharyngeal carriage of group A Streptococci in school children in Chennai. Indian J. Med. Res. 124: 195-198.

Christenson B, Sylvan SP, Noreen B (1997). Carriage of multi resistant Streptococcus pneumonia arnong children attending day-care centers in the Stockholm area. Scand. J. Infect. Dis. 29:555-558.

Cimolai, Mac C, Damm S (1990). he epidemiology of beta hemolytic non Group AStreptococci isolated from the throats of children over a one year period. Epid. infect. 104:119-126.

Elifozltuk C, Yavuz T, Kaya D, Muhteremyucel (2004). The Rate of Asymptomatic Throat Carriage of Group A Streptococcus School Children and Associated ASO Titers in Duzce, Turkey. Jpn. J. Infect. Dis. 57: 271-272.

Fatima F, Shaba DS (2013). Prevalence Survey for Assessing Intensity of Group a Beta 1-lemolytic Streptococci (GABHS) Subclinical Infection Rate in School Children: A Cross Sectional Study. Glob. J. Med. Res. Dis. 13(3):Verl.0

Gordis L, Lilienfeld A, Rodriguez R (I969). Studies on the epidemiology and prevention of rheumatic fever. JIT. Evaluation of the Maryland rheumatic fever registry. Pub. Health Rep. 84:333-339.

Gunnarsson RK, Holme SE, Soderstrom M (1997). The prevalence of beta-hemolytic Streptococci in throatspecimens from healthy children and adults.Implications for theclinical value of throat cultures. Scand. J. Prim . Health Care. I5:149-155.

Gur E, Akkus , Arvas A, Guzeloz , Can G, Diren S, Ercan, Cifcili, Ilter O (2002). Prevalence of positive throat cultures for group A beta-hemolytic Streptococci amongschool children in Istanbul. Indian Pediatr. 39:569-573.

Khan I, Ullah H, Ahmad , Saqib M, Nisa , Irshad M, Idrees M, Saeed A (2013). Susceptibility of treptococcusPyogenes against various antibiotics. App. Sci. Report. 4: 181-183

Kushwaha N, Kamat M, Bijendra B, Sah J (2014). Prevalence of Group A Streptococcal Infection Among School Children of Urban Community A Cross Sectional Study. IJIMS. 1:249- 256.

Lee GM (2008). Burden and economic cost of group A Streptococcal pharyngitis.Pediatrics. 121:229-234.

Linder JA, Bates DW, Lee GM, Finkelstein JA (2013). Antibiotic treatment of children with sore throat. JAMA. 23: 15-2322.

Michael A, Gerber MD (1996). Antibiotic Resistance: Relationship to persistence of Group A Streptococci in the upper respiratory tract. Pediatrics. 97: 1-975.

Nabipour F, Tayarzadeh M (2005). Prevalence of beta hemolytic Streptococcus carrier state and its sensitivity to different antibiotics among guidance school children in Kerman, Iran. Amer. J Infect. Dis. 1: 128-131.

Odigwe C, Udo, Mereinikwu M, Ohia N (2002). Association of Acute Sore Throat with Group A Beta HemolyticStreptococci Carriage among Children in a Public School in Calabar - Implications for the Control of Rheumatic Fever/Rheumatic Heart Disease (R1-IIRHD) in Developing Countries. Global. J. Med. Sci. 1: 55-59.

Pichichero ME, Steven MM, Marie Lynal, et al (1999). Incidence of Streptococcal carriers in Private Pediatric Practice. Arch. Pediatr. Aldoses Med. 153: 624-628.

Rijal KR, Dhakal N, Shah RC, Timilsina, Mahato P, et al (2009). Antibiotic susceptibility of Group AStreptococcus isolated from throat swab culture of school children in Pokhara, Nepal. Nepal Med. CoII. J. 11:238-240

Sevinc I, Enoz M (2008). The Prevalence of Group A Beta-hemolytic Streptococcus in Healthy Turkish Children in Day-care Centers in Ankara. Chang. Gung. Med. J. 31:554-558.

Smith TD, Wilkinson V, Kaplan EL(1989): Group A streptococcus-associated upper respiratory tract infections in a daycare center. Pediatrics. 83:380-384.

Vincent MT, Celestin N, Hussain AN (2004). Pharyngitis. Amer. Famp. Pys. 69: M65-70.

WHO (2004). The World Health Report. Geneva: WHO.

Yagupsky P, Landau D, Beck A, Dagan R (1995). Carriage of Streptococcus pyogenes among infants and toddlers attending day-care facilities in closed communities insouthern Israel. Eur. J. Clin. Microbiol. Infect. Dis. 14:54-58.