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International Journal of Current Research
Vol. 9, Issue, 11, pp.61113-61117, November, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PREVALENCE OF GROUP A BETA HEMOLYTIC *STREPTOCOCCUS* IN AN ACADEMIC COMMUNITY OF RISARALDA, COLOMBIA

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ARTICLE INFO

Article History:
Received 29th August, 2017
Received in revised form
16th September, 2017
Accepted 10th October, 2017
Published online 30th November, 2017

Key words:

Streptococcus pyogenes,
Bacitracin,
Epidemiology,
Agglutination,
Latex,
Tonsillitis,
Antistreptolysin.

ABSTRACT

Aim: To determine the prevalence of group A beta hemolytic Streptococcus in the academic community of the Fundación Universitaria Autónoma de las Américas sede Pereira.
Methods: Descriptive clinical epidemiological study. A sample of 226 people was obtained from the academic community to which a structured survey type instrument was applied. A throat sample was taken with swab and the material obtained was cultured on 5% sheep blood agar medium in the presence of 10% CO2. Bacitracin susceptibility test was performed at a concentration of 0.04 U. A sample of 5 ml of peripheral blood was taken in a dry tube to obtain serum, in order to perform a rapid test for titration of Antistreptolysin (ASLO) and Measurement of anti-Streptococcus beta hemolytic type a antibodies by the latex agglutination technique.
Results: The prevalence of β-hemolytic Streptococcus was 47.34% (n=107) in subjects of the academic community. The distribution according to the results of the culture for SBHGA of 4.42% (n=10), sensitivity to bacitracin appears in 4.4% of the 226 people under investigation, the ASO TEST of 400 UI/mL, was n=10 represented by 4.42%; for asymptomatics with ASO titre of 200 UI/mL was the 42.92% (n = 97).
Conclusion: This study shows a percentage of positivity of 4.42% (n=10) with tonsilpharyngitis and highlights the problem of asymptomatic patients with a 42.92% of prevalence (n=97) which is considered a risk factor for acute pharyngotonsillitis.

Citation: Beatriz Giraldo, O., Sandra C Garzón, C., Daniela De La Cruz, B. and Maria E Vélez A., 2017. "Prevalence of group A beta hemolytic Streptococcus in an academic community of Risaralda, Colombia", *International Journal of Current Research*, 9, (11), 61113-61117.

INTRODUCTION

Pharyngo-amygdalian infections are very frequent pathological processes in man, occurring at least once throughout life. Group a haemolytic beta-Streptococcus (GABHS) or *Streptococcus pyogenes* (SP), a human-adapted pathogen, reemerging and considered the main bacterial etiologic agent of acute pharyngotonsillitis, is recognized (Guevara *et al.,* 2008; Wong and Yuen, 2012). All human beings are equally susceptible to streptococcal infection, regardless of gender, age and race.

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However, it has been observed that age is an important factor in the microbial etiology of tonsillarpharyngitis; children and adolescents being the most frequently affected. EBHGA causes 37% of acute pharyngotonsillitis in children older than 5 years and 10% in adults (Rodríguez-Herrera and Ramírez-Navarro, 2009). The EBHGA are spherical bacteria cocci, that are grouped in chains, Gram positive, catalase negative, capable of invading the upper respiratory tract as well as other soft tissues tejidos blandos (Gutiérrez *et al.,* 2015; Luis-León *et al.,* 2015), the infection occurs by close contact with infected colonized persons, from spit or runny nose; for this reason it is more frequent among children in schools and kindergarten, and overcrowding favors transmission. Likewise, it has been shown that school-age children colonized by the bacteria become their reservoir. In fact, there has been a coincidence

between the strains that cause tonsillofaringitis in these children and those cases associated with invasive disease in the community (Lozada *et al.,* 2012). This ubiquitous microorganism can lead to a great variety of cutaneous-mucous and systemic infections that tend to cause nonsuppurative sequelae, like acute rheumatic fever and acute post-streptococcal glomerulonephritis. This ubiquitous microorganism can lead to a great variety of cutaneous-mucous and systemic infections that tend to cause nonsuppurative sequelae, like acute rheumatic fever and acute post-streptococcal glomerulonephritis (Nieto Vera *et al.,* 2011), besides clinical presentations that can cause death such as invasive diseases (ID), which are defined by the isolation of the microorganism in normally sterile sites. This group of ID includes three clinically overlapping syndromes: toxic shock syndrome (TSS), necrotizing fasciitis, and invasive diseases not associated with the previous ones: bacteremia, pneumonia, osteomyelitis, necrotizing cellulitis and septic arthritis, which require a diagnosis and early treatment since they produce high morbi-mortality (Vomero *et al.,* 2014). Microorganism has several virulence factors, including its hyaluronic acid capsule, serum opacity factor, F protein, C5a peptidase, O and S hemolysins, SpeB (streptococcal pyrogenic exotoxin B, potent protease), exotoxins pyrogenic streptococci: SpeA and SpeC, the streptococcal superantigen (SSA) and the M protein, but its toxic capacity is given by the erythrogenic toxin and streptolysins O and S (Vomero *et al.,* 2014; Gutiérrez *et al.,* 2010). Infection produces several toxins that can act as antigens so that responds with the production of neutralizing antibodies antistreptolysin O (ASO) which appear between 8-30 days after the start of infection, whose level is directly related to the disease and remains elevated in chronic patients. The suspicion of infection by SP is based mainly on the clinic and its confirmation is through specific tests. Currently, tests such as pharyngeal culture or latex agglutination are used to detect them. The detection of ASO is considered the quantitative reference method that allows following the course of the disease and differentiating chronic cases from those that are in course of remission (Nieto Vera, 2011).

In the world, between 15.6 and 19.6 million people are affected by rheumatic heart disease, each year presenting 282,000 new cases and 233,000-468,000 deaths. The incidence of acute post streptococcal glomerulonephritis varies with time. 472,000 new cases in the world were report in 2005 and the 83% in developing countries. The World Heart Federation recently requested to reduce by 25% by 2025 the burden of rheumatic heart disease in people less than 25 years of age (Minodier *et al.,* 2014). Different studies, the high frequency of tonsilphaningitis due to SP in children has been reported; in the United States, of the 15-36% of cases of sore throat in children are attributable to SP. In Nicaragua, a prevalence of 8.5% was reported in asymptomatic children and in Guatemala 15% was found in children with pharyngotonsillitis. In Chile there aren't prevalence studies in this regard, but it is also one of the main causes of medical consultation. In the last report of basic health indicators for 2009 in Medellín, Colombia; acute tonsillofaringitis accounted for 2.2% of outpatient morbidity, two points more than the percentage reported in 2008; Although the proportion of SP as an etiologic agent is unknown, it is clear that the entity is increasing (Lozada *et al.,* 2012; Gutiérrez *et al.,* 2010). In the last two health indicators reports for Medellín specifically in the 2014 an incidence in under 1 year olds of 11.8%, from 1-4 11.2% and from 15-44 2.9% and in 2015 under 1 year 12.3%, 1- 4 11.6% and from 15-44 of 2.9% (Situación de la salud, libro de indcadores básicos, 2014; Situación de la salud , libro de indicadores básicos, 2015). In Colombia there are few studies on the frequency of infection by this agent; according to different authors, the frequency of colonization by SP is variable; being lower in developed countries and higher in those in the process of development, but epidemiology is incompletely known. In 2005, 90% of symptomatic tonsilpharyngitis, 96% of invasive diseases and 97% of SP deaths were observed (Minodier *et al.,* 2014). The World Health Organization highlights the importance and necessity of epidemiological studies of streptococcal infections in developing countries in order to implement effective prevention programs in primary care, since the recurrence of streptococcal pharyngitis can be explained by lack of adherence to treatment, reinfection from a nearby source or due to an incorrect diagnosis associated with SBHGA (Ralph *et al.,* 2012). Therefore, taking into account the global prevalence of SP portability, the prevalence of Group A beta hemolytic *Streptococcus* was estimated in the academic community of the Fundación Universitaria Auónoma de las Américas Pereira, Risaralda.

MATERIALS AND METHODS

This is a descriptive clinical epidemiological study. Using a stratified simple random sample with the reliability parameters of 95% and a margin of error of 5% with $p<0.5$ for the homogeneity of the study, which was carried out at the Fundación Universitaria Autónoma de las Américas Pereira, Risaralda. The sample calculated through the sample design was 226 people from the academic community between administrative and students of the Program of medicine, odontology and introductory health sciences. A structured survey type instrument was applied, obtaining demographic information, personal history of signs, pharyngeal symptoms and population framework. With prior informed consent, in a fasting were taken, pharyngeal swabs and a sample of 5 mL of peripheral blood in a tube without anticoagulant to obtain serum. Pharyngeal swabs were immediately cultured on 5% lamb blood agar plates, placed in plastic bags with CO_2 >15% generator bags GEN bag microaer, and incubated at 37 ^{o}C for 24 hours, according to the manufacturer's instructions. As a control strain ATCC 19615 of *Streptococcus pyogenes* was used. The analysis of the results was based on the criteria of the National Committee for Clinical Laboratory Standards, NCCLS19. From a concentrated inoculum of microorganisms from the 24 hours the culture was purified by sowing on 5% sheep blood agar; to the suspicious betahemolytic colonies were Gram staining, catalase test, and sensitivity with differential disks with 0.04 IU according to NCCLS standards using the disc diffusion method. The plates were incubated at 37 °C for 24 hours, placed in plastic bags with CO_2>15% generator bags GEN bag microaer, according to the manufacturer's instructions, any halo of inhibition observed was considered positive.

The identification of the microorganisms was carried out in a conventional manner. The samples that showed a β hemolytic growth pattern and positive for bacitracin were classified as group A hemolytic beta *Streptococcus*. The serum samples were used for the confirmation of the strains by agglutination of latex particles, considering positive high or increasing titres higher than 200 IU/mL according to the manufacturer's specifications. In this study, all subjects with positive culture were sensitized to bacitracin and this group of subjects had an

ASO titre of 400 IU/mL, which received treatment through their health service provider.

RESULTS

The Table 1 shown the information of general manner regard to sample, about the gender the female sex was prevailing 63.72% (n=144). The average age for both sexes was 19 years old; the number of patients with ≥ 1 episodes per year of acute tonsilpharyngitis is shown. With 1 episodes 28.76% (n=65), 2 episodes 17.26% (n=39), 3 episodes 4.42% (n=10) and 4 episodes 3.10% (n=7), of which 15 (6.64%) them had laboratory tests.

receive treatment. In this study, the prevalence of *β- hemolytic Streptococcus* (Table 3) was 47.34% (n=107) in the academic community. The distribution of the SBHGA in the sample was the 4.42% (n=10), besides, the 4.4% sensitive to bacitracin is presented in 10 of the 226 people who were the object of the investigation, the ASO test of 400 IU/mL, was n=10 represented in a 4.42%, for the result of asymptomatic 42.92% (n = 97) with ASO titre of 200 IU/mL. No significant association was found between the numbers of episodes of acute tonsillitis tonsilpharyngitis suffered in the last year and the carrier state (p>0.05). In agreement with these results, the carriers had 1.1 times the risk of having ≥ 2 episodes of acute tonsilpharyngitis than those who didn't carry SBGS (OR, 1.1).

Table 1. General descriptive information

Variable	Option	n	%	Confidence interval 95%	
				Lower	Upper
Academic community	Employed (Teacher - administrative)	24	10,62	7,08	14,60
	Student	202	89,38	85,40	92,92
Gender	Masculine	82	36,28	30,09	42,92
	Feminine	144	63,72	57,08	69,91
Regimen	Contributive	219	96,90	94,69	98,67
	Subsided	6	2,65	0,88	4,87
	Without EPS (linked)	1	0,44	0,00	1,33
¿Have you suffered tonsillitis?	Yes	105	46,46	40,27	53,10
	No	121	53,54	46,90	59,73
¿How many episodes of tonsillitis annuals do you have?	0	105	46,46	40,27	52,65
	1	65	28,76	23,01	34,96
	2	39	17,26	12,39	22,12
	3	10	4,42	2,21	7,51
	4	7	3,10	1,33	5,31
¿Have you done laboratory tests?	No	211	93,36	89,83	96,46
	Yes	15	6,64	3,54	10,17

Table 2. Description of clinical data

Variable	Option	n	%	Confidence interval 95%	
				Lower	Upper
In the last 6 months have you had joint pain?	None	178	78,76	73,45	84,07
	Located	17	7,52	4,42	11,06
	Small joints	11	4,87	2,21	7,96
	Great joints	7	3,10	0,88	5,75
	Generalized	13	5,75	2,67	8,85
Have you had heart murmur?	No	225	99,6	98,67	100,00
	Yes	1	0,4	0,00	1,33
Have you had problems in the kidney?	No	217	96,02	93,36	98,23
	Yes	9	3,98	1,77	6,64
Do you suffer from any disease of the bones or joints?	No	222	98,23	96,46	99,56
	Yes	4	1,77	0,44	3,54
Have you operated on the tonsils?	No	214	94,69	91,59	97,35
	Yes	12	5,31	2,65	8,41
Have ever they put you benzathine penicillin G?	No	165	73,01	67,26	79,19
	Yes	61	26,99	20,81	32,74

Table 3. Isolation of SBHGA, sensitive to bacitracin and ASO test

Variable	Option	n	%	Confidence interval 95%	
				Lower	Upper
SBHGA	Negative	216	95,58	92,92	98,23
	Positive	10	4,42	1,77	7,08
Sensitive to bacitracin	No	216	95,6	92,92	98,23
	Yes	10	4,4	1,77	7,08
ASO Test Ul/mL	Negative	119	52,65	46,90	59,29
	200	97	42,92	36,73	49,12
	400	10	4,42	1,77	7,08

Clinical data (Table 2) showed that the 78.76% (n = 178) haven't presented joint pain in the last 6 months, the 99.6% (n = 225) haven't had a heart murmur; 96.02% (n = 225) hadn't problems in the kidney, 98.23% (n = 217) didn't suffer from any disease of the bones or joints, 94.69% (n = 222) hadn't operated on the tonsils, The 26.99% of the cases were treated with benzetacil or benzatinic penicillin, while 73.01% didn't

It is important to note that most of the samples were not positive for SBHGA.

DISCUSSION

The clinical diagnosis of acute streptococcal tonsillofaringitis is difficult to do because it has the same clinical profile with

those of viral origin or unknown cause and is a frequent cause of ambulatory consultation. In practice, the carrier of the SBHGA is defined by the presence confirmed by the culture in an asymptomatic individual, being the best examination for the diagnosis, the results are obtained between 24 to 48 hours, hindering adherence to the treatment causing a problem prevalent in primary care, which leads to indiscriminate use of antibiotic and the consequent emergence of resistant strains in the population. In this study, the prevalence of carrying SBHGA was 47.34%, which was decreasing with age being higher in subjects between 18 and 22 years old. In different studies there are similar, superior and inferior results. A case-control study conducted in children from Egypt, reported a prevalence of 42.2% using the same tests and n=300 of asymptomatic subjects (El-Ghany *et al*., 2015). Silvana M. *et al*., Reported a prevalence of 13% (Giannelli Silvana, 2007). Gastón Delpech1 *et al*., Reported a prevalence of 14.2% (Delpech *et al*., 2017), on the other hand Samson *et al.* In the investigation of the detection made during the investigations. Long-term care centers and military training camps showed the prevalence of colonization of *S. pyogenes* was around 16% - 17% (Wong and Yuen, 2012). These differences in prevalences are explained by the numerous factors that can intervene in the process of colonization of the SBHGA, among others: age, overcrowding, time of year, humidity, environment, geographical area, tonsillectomy, habit of brushing teeth and viral catarrh of upper airways. Incidence of positive culture for SBHGA was 4.42%; with an ASO titre of 400 IU/mL. Bacitracin susceptibility was evident in a prevalence of females with an incidence of 70% in relation to positive ones and with an average age of 21 years, comparing it with the study done in France in adults where they report that the rate of the infection invasive by SBHGA for 2007 in 3.1 cases. The median age of the patients was 60.3 years and the infection was more frequent in women as well as in countries such Denmark, Norway and Sweden, this study also shows that the incidence is higher in women than men (Plainvert *et al*., 2010). No relationship was found between colonization and the number of episodes of acute amygdalpharyngitis suffered in the last year, bearing in mind that specifically SBHGA can cause invasive infections, so it's important to monitor the development of resistant infections. These results emphasize the need for continuous monitoring. It can be evidenced that the people participating in the study don't present basic diseases that affect the large organs, which means that a relationship between the diseases cannot be established with the presence of the *β-hemolytic Streptococcus*. In this study, such as the one conducted by Saddik *et al*. (2014) Irene Nayiga *et al.* (2017) and Manandhar *et al.* (2013) used culture tests for SBHGA, bacitracin sensitivity and ASO test for diagnosis,

Conclusion

This study shows a percentage of positivity of 4.42% (n = 10) with tonsilpharyngitis and highlights the problem of asymptomatic patients with a prevalence of 42.92% (n = 97) which is considered a risk factor for acute pharyngotonsillitis. In the diagnosis of acute pharyngotonsillitis due to SBHGA, it seeks the correct use of antibiotics and thus the prevention of complications of suppurative and non-suppurative diseases, and it is necessary that if there is a strong clinical suspicion of infection with SBHGA, it should be confirmed with a pharyngeal culture.

Financing: The research was funded by Fundación Universitaria Autónoma de las Américas.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgments

We are grateful to directives of Fundación Universitaria Autónoma de las Américas for their support and funding for the development of this research.

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