

Prevalence of streptococcal hemolytic beta from group a in asymptomatic children

Abstract

Objective: This study aimed to determine the prevalence of Group A β -hemolytic streptococcus in oropharynx of asymptomatic children who attend a public school and also the sensitivity and resistance profile of these bacteria to antibiotics commonly tested

Methods: It was used sterile swabs to collect samples from the oropharynx of asymptomatic children for the accomplishment of microbiological culture. These children should not be using any antibiotic. For the differentiation of samples between others beta hemolytic species, bacitracin and L-Pyrrolidonyl- β -Naphthylamide tests were performed. All Group A β -hemolytic streptococcus samples were submitted to antibiogram test to establish the sensitivity and resistance profile.

Results: From the 229 samples analyzed the etiological agent of study was detected in 4.8%. Antibiogram test was made and despite of the high sensitivity observed, there were cases of intermediate and/or total resistance to the antibiotics tested: beta-lactams, microclines and cephalosporin of the 3rd and 4th generation. In addition, only one of the positive cases for the bacterium under study was sensitive to all antibiotics.

Conclusion: These results are relevant considering that bacteria belonging to Group A Beta-hemolytic Streptococcus are one of the microorganisms of major clinical interest, mainly when diagnosed in children. Thus, in order to reduce bacterial resistance to antibiotics it is important to use these drugs rationally.

Keywords: streptococcus, oropharynx, children

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Abbreviations: EBHGA, group a streptococcus beta-hemolytic; RF, rheumatic fever; AP, acute pharyngitis; REC, research ethics committee

Introduction

Upper airway infections are one of the most common causes of medical appointment in clinical practice mainly faced by pediatric population.¹ According to a study conducted by Moraes et al.² the incidence of respiratory tract infections due to β -hemolytic streptococcus can be more than 18 times higher in children attending some day care center or environments where they are close to others sharing belongings. However, individuals of all ages are susceptible to propagation of this microorganism under conditions of overcrowding, because the interpersonal dissemination of these bacteria occurs through aerosolized microdroplets and/or by direct contact, mainly in environments where there are clusters of people. Among the diseases that affect the upper airways, acute pharyngitis (AP) is one of the most frequent in children. Although most of the AP is of viral origin, Group A Streptococcus Beta-hemolytic (EBHGA) presents as a significant bacterial etiological agent diagnosed in about 15-30% of acute pharyngitis.³ EBHGA or Streptococcus pyogenicus is gram-positive aerobic bacteria that causes a variety of infectious syndromes⁴ and is considered of great clinical interest due to its high pathogen city, usually involving oropharynx and skin, as a consequence these bacteria may cause several inflammatory diseases. One of the main laboratorial characteristics this pathogen presents to be identified and classified is the ability to shatter completely red blood cells in 5% sheep blood culture, causing a transparency in the culture medium, which is called Beta-hemolytic.⁵ Streptococcus pyogenicus is important

when compared to groups B, C and G, not only because it is the main etiological agent of streptococcal pharyngitis,⁶ but also because it causes suppurative complications such as, cervical adenitis, peritonsillar abscess, retropharyngeal or cervical abscess.⁷ In addition, EBHGA is related to other diseases such as streptococcal toxic shock syndrome, necrotizing fasciitis, scarlet fever and skin infections.⁸ According to Areas et al.⁹ its main complications are acute rheumatic fever, rheumatic heart disease and acute streptococcal disease, with a higher incidence especially in developing countries. Therefore, the early diagnosis of this condition, followed by adequate antimicrobial treatment, is extremely important not just for the prevention of rheumatic fever (RF) and other pathologies, but also for the reduction of EBHGA transmission among patients.³ Living in restricted settings, such as day care centers and schools, can greatly influence the dissemination of these bacteria, placing the health of children and adolescents at risk,¹⁰ considering that children and teenagers oropharynx is very sensitive to EBHGA infections, especially among 5 and 15 years of age.¹¹ Besides that, they can carry pathogenic microorganisms without presenting symptoms, characterizing the asymptomatic carriers. The epidemiological profile of this pathogen shows the relevance of its diagnosis due to the existence of complications it may cause and their ease of transmission.² Therefore, a precise etiological diagnosis contributes to avoid the abusive use of antibiotics and its consequences as the increase of bacterial resistance.¹² This study aimed to determine the prevalence of Group A β -hemolytic streptococcus in oropharynx of asymptomatic children who attend a public school and also the sensitivity and resistance profile of these bacteria to main antibiotics tested.

Materials and methods

It was a cross-sectional and descriptive study, which was performed between February and October, 2016 in a public school in the city of Caruaru-PE. The study was approved by the Research Ethics Committee (REC) of the Tabosa de Almeida University (Asces-Unita) under the opinion n° 49273815.4.0000.5203. This study has begun after parents and/or Responsible for each child sign the Free and Informed Consent Form. The main inclusion criteria to participate of the research includes, asymptomatic children aged 5 to 15 years who were duly enrolled in the school chosen to carry out the study. In order to be classified as "asymptomatic", they could not present previous signs or symptoms of infection, for example: sore throat, fever, increased sensitivity of the cervical ganglia, hyperemia, edema, etc. Moreover, it is necessary not to use any kind of antibiotics trying to avoid changes in the examination or false negative results. The pharynx samples were collected in the morning, by using sterile swabs and tongue depressors. It was performed twists of 360°C on the right and left palatine tonsils. Then, these samples were transported into a Stuart medium in an isothermal container to the Laboratory of Microbiology at Taboos de Almeida University (Asces-Unita).

At the laboratory, the material was seeded by depletion in Petri dish containing Mueller Hinton agar medium (KASVI) plus 5% sheep defibrinated blood. Then, the plates were incubated in a stove at 36-37°C during 24 hours in order to visualize possible beta hemolytic formed. All beta hemolytic colonies were isolated again in 5% sheep blood agar to obtain pure colonies and after 24 hours of incubation, these colonies were stained by the Gram technique. Samples that presented as Gram-positive cocci were firstly tested for the production of the enzyme catalase, to characterize microorganism as belonging to the genus *Streptococcus*. Subsequently, its differentiation between the other beta hemolytic species was performed through complementary tests as bacitracin and L-Pyrrolidonyl- β -Naphthylamide (PYR), confirming the bacterium EBHGA. EBHGA strains were evaluated for their susceptibility profile and resistance to antibiotics by in vitro antibiogram test. The technique performed was disc diffusion method as recommended by Kirby & Bauer also, the antibiotics tested were selected according to the CLSI (Clinical and Laboratory Standards Institute) 2016. In order to present statistical data collect from this study, tables were made with the variables tested, the resistance

and sensitivity profile were described using percentages. The tables of results were created and stored by using Excel 2010 (Microsoft Office®).

Results and discussion

From 297 children whose parents and/or guardians signed the Free and Informed Consent Term, 157 were male and 140 were female. From this quantitative, 10 children left the study and 58 do not applied to inclusion criteria proposed. At the end, 229 children participate of this clinical research. The results obtained from the analyzed samples, as shown in Table 1, determined that 11 (4.80%) of the children were colonized by EBHGA. Regarding antibiotic test, from 11 samples analyzed, 10 showed intermediate or total resistance to at least one antibiotic tested and only one bacteria showed total sensitivity (Table 2). The antimicrobials which bacteria were resistant to the isolated EBHGA samples were: Penicillin and Cefepime (54.54%), Ceftriaxone (45.45%), Clindamycin and Ampicillin (27.27%) and Vancomycin and Erythromycin (%). Although the prevalence of EBHGA was relatively low in our study (4.80%), the values coincide with a study carried out by Maciel et al.⁶ in which from the 753 samples analyzed in asymptomatic children, only 6 presented positivity for the same pathogen of this study. In Spain, a similar work verified a prevalence of 6% in asymptomatic schoolchildren.¹³ Another study carried out by Scalabrín et al.¹⁴ presented higher results compared with the above mentioned, which 15 (25.90%) from the 58 participants had positive culture for EBHGA collected in pharyngotonsillitis. Following the recommendations of the CLSI 2016 it is noted that, one of the beta-lactam antibiotics tested, penicillin, remains one of the main drugs of choice for the treatment of infections caused by EBHGA, considered universally sensitive to penicillin's.⁷ For this reason, it is important to test penicillin through in vitro analysis by using the antibiogram, in order to obtain relevant data about the efficiency of this drug. Regarding resistance and sensitivity to antimicrobials, Scalabrín¹⁴ reports that all the samples tested showed a high sensitivity to penicillin. Other studies, such as those by Lopardo et al.¹⁵ also show their efficacy. This study, however, showed five resistant strains and one intermediate to penicillin. Similar information was found in a study made by Antunes et al.⁴ where penicillin no longer proved to be as efficient in invasive EBHGA infections.

Table 1 Isolation of EBHGA in asymptomatic children, according to age and positivity

Age	Collections held	Positive cases	Prevalence EBHGA
	N°	N°	%
5	6	0	-
6	17	1	0,44
7	30	3	1,31
8	20	1	0,44
9	39	1	0,44
10	46	3	1,31
11	22	2	0,87
12	22	0	-
13	16	0	-
14	9	0	-
15	2	0	-
Total	229	11	4,80

EBHGA, group a streptococcus beta-hemolytic

Table 2 Sensitivity and resistance to tested antimicrobials

Antibiotic	SIGLA	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
CLINDAMYCIN	CLI	S	I	S	S	S	R	I	S	S	S	S
CEFTRIAZONE	CRO	S	I	S	S	I	I	S	S	R	R	S
PENICILIN	PEN	S	R	S	S	S	R	I	R	S	R	R
CEFEPIME	COM	I	I	S	S	I	R	R	S	S	S	R
VANCOMYCIN	VAN	S	R	I	S	S	S	S	S	S	S	S
AMPICILLIN	AMP	S	S	S	S	S	S	R	R	S	I	S
ERYTHROMYCIN	ERY	S	S	S	S	I	S	S	S	R	S	S

PC, positive case; S, sensitive; I, intermediate; R, resistant.

Ferreira et al.¹⁶ demonstrated that of the 20 strains tested, 19 samples were resistant to penicillin, but none showed resistance to 3rd and 4th generation cephalosporins, as evidenced by Marot et al.¹⁷ where the tested cephalosporins were 100% effectiveness. In the present study, from eleven positive cases, five presented intermediate or total resistance to Ceftriaxone and six to Cefepime. In the case of microcline-class antibiotics, the incidence of resistance in 120 patients with EBHGA in Portugal¹⁸ was 13.5%, equivalent to 16 children. In this study, from 11 positive samples, 5 presented partial or total resistance to macrolides (erythromycin and clindamycin), corresponding to 45.45%. In Brazil, this data resembles a study by Weber¹⁹ where significant resistance to erythromycin was found.

Conclusion

From these results, the prevalence of EBHGA in asymptomatic children and the pattern of resistance found are worrisome, since this pathogen is considered one of the main pathogens of clinical interest in the oropharynx. In this way, attention to Group A Beta-hemolytic *Streptococcus* in childhood is of paramount importance, as well as the awareness of the rational use of antibiotics by children. Thus, adequate orientation regarding antimicrobial therapy is important in order to contribute to the reduction of bacterial resistance.

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Conflict of interest

The author declares no conflicts of interest.

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