

ISOLATION OF *ARCANOBACTERIUM HAEMOLYTICUM* FROM PATIENTS WITH PHARINGITIS

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Abstract. *Arcanobacterium haemolyticum* has been described as a rare etiologic agent in acute pharyngotonsillitis in pediatric and young population. Four strains of *A. haemolyticum* were isolated from throat swabs of 3584 patients (10-26 years) with acute pharyngitis. The samples, collected in January 1997-January 2001, were plated on Mueller-Hinton agar with 10% sheep blood and incubated 24-48 hours at 37 °C.

In three cases *A. haemolyticum* was the only one etiologic bacterial agent and in the fourth case association of *A. haemolyticum* with *S. aureus* was present.

The identification of *A. haemolyticum* strains was based on cells and colonies' morphology, cultural and biochemical characters.

Key-words: *Arcanobacterium haemolyticum*, isolation, pharyngitis.

Rezumat. *A. haemolyticum* a fost semnalat, cu frecvență redusă, ca agent etiologic al faringoamigdalitelor acute la copii și tineri. Autorii descriu 4 tulpini de *A. haemolyticum* izolate din exudate faringiene de la bolnavi cu faringoamigdalită acută. 3484 probe, recoltate în perioada ianuarie 1997-ianuarie 2001, au fost însămânțate pe agar Mueller-Hinton suplimentat cu 10% sânge de berbec și incubate la 37 °C timp de 24-48 ore.

La 3 din cazuri *A. haemolyticum* a fost unicul agent izolat iar la 1 caz a fost prezent în asociație cu *S. aureus*.

Tulpinile de *A. haemolyticum* au fost confirmate pe baza morfologiei celulelor bacteriene și a caracteristicilor culturale și biochimice.

Cuvinte – cheie : *Arcanobacterium haemolyticum*, izolare, faringită.

A. haemolyticum (*Corynebacterium haemolyticum*) is an aerobic gram – positive rod that was first described by MacLean et al., in 1946, isolated in persons with pharyngitis and skin infections. Recently, many studies point out that *A. haemolyticum* has been isolated from patients with pharyngitis and various other infections in Europe, USA and Asia (1,2).

The taxonomical position of *A. haemolyticum* caused many confusions because it is a close phenotypical similarity

to *Actinomyces pyogenes* (*C. pyogenes*), an animal bacterial pathogen (1). From 1982 this species was classified as *A. haemolyticum* by Collins et al., in a new genus *Arcanobacterium* composed of this single species (1,3, 4).

In the infection with *A. haemolyticum* clinical most cases involve pharyngitis and / or tonsillitis and approximately 50% are exudative. Throat infections are often accompanied by cervical lymphadenopathy (1,2,3,5). Symptoms

resemble those of beta – haemolytic streptococci or viral infection.

An erythematous morbilliform or scarlatinal rash of trunk, neck or extremities were associated with the presence of *A. haemolyticum*. In addition, central nervous system infections, sepsis, endocarditis, osteomyelitis, dermatologic and other infections were described (1,5).

The aim of this study were to determine the presence of *A. haemolyticum* in acute pharyngitis in children and young population and to characterize the *A. haemolyticum* isolated strains.

MATERIAL AND METHODS

3584 throat culture samples were collected during January 1997–January 2001 from patients with acute pharyngitis. The patients of 5–26 years of age were seen in districtual or Children and Students Hospital of Iasi city.

The samples were plated on Mueller – Hinton agar added with 10% sheep blood and incubated for 24–48 hours at 37 C in aerobic conditions.

The *A. haemolyticum* strains were identified by the following tests : the small colonies (< 1 mm diameter) with incomplete beta–haemolysis on sheep blood agar, Gram stain, catalase, nitrate reduction, urease, gelatin hydrolysis, reverse CAMP, DNase test, fermentation of glucose, lactose, sucrose, maltose, mannitol and xylose – table 1. The tests Voges–Proskauer and nitrate reduction were read after 24 and 48 hours.

The *A. haemolyticum* strains susceptibility was tested *in vitro* by

diffusion method. They were tested to penicillin, erythromycin, tetracycline, gentamycin, cephalotin, ceftazidime, ceftriaxone, chloramphenicol, and thrimethoprim– sulphamethoxazole.

RESULTS AND DISCUSSION

A. haemolyticum was isolated in 4 (0.1%) cases of all investigated patients (10–26 years). In 3 cases this species was the only bacterial pathogen and in one case was associated with *S. aureus*.

Clinical manifestations of *A. haemolyticum* pharyngitis were similarly with those of streptococcal pharyngitis but the severity of the disease varied. Sore throat and pharyngeal erythema were always present. Additional symptoms and signs included fever, non–productive cough and headache. Another symptoms and signs such as skin rash, tonsillar exudates, lymphadenopathy have also been described. Erythema multiforme and urticarial rash have also been found in patients with *A. haemolyticum* pharyngitis (1,6,7). In our investigation the frequency of *A. haemolyticum* isolation was very low. The isolation rate of this pathogen from clinical throat specimens varies from 0.07% to 1.3% in non-age selected material (1). In Romania were described 24 cases confirmed with *A. haemolyticum* (6,7). Epidemiological data suggest that *A. haemolyticum* pharyngitis is primarily a disease of adolescent and young adults. Carlson P.; Coman et al., reported paediatric patients with *A. haemolyticum* pharyngitis (1,6).

The laboratory diagnosis of *A. haemolyticum* infections was based

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on classical culture techniques. This organism grows slow, with little (<1 mm diameter) and smooth/rough colonies on blood agar and it is easily overlooked in the bacteriological laboratory. Around of the colonies appears a narrow incomplete haemolysis zone. Banck et al., considered that the detection of beta- haemolysis produced by this bacteria can be facilitated by using special double-layered human blood agar plates (3). The identification of *A. haemolyticum* was made by classical biochemical tests presented in table 1 and 2. The catalase and gelatin hydrolysis differentiated this species of *Corynebacterium* sp. and *Actinomyces pyogenes*. A catalase negative, gram-positive rods which is reverse CAMP and DNase positive tests is very important for the identification of *A. haemolyticum*. The inclusion of bacteria in genus and species have been confirmed by the acid production test from maltose, lactose, sucrose, xylose and mannitol . Many authors successfully used the API Staph and API Coryne systems for testing carbohydrate fermentation by *A. haemolyticum* (1,6,8). This species can be divided in smooth and rough biotypes by biochemical tests. The majority smooth biotype strains fermented sucrose and/or trehalose in API Staph but not

produced beta-glucoronidase. The rough biotype strains produced beta-glucoronidase but not fermented sucrose and trehalose.

All our *A. haemolyticum* strains were susceptible to penicillin, erythromycin, gentamycin, chloramphenicol, cephalotin, cephtazidime, cephtriaxone. 2 strains were resistant to tetracycline and the thrimethoprim-sulphamethoxazole had no activity *in vitro*. In majority studies, the *A. haemolyticum* strains were susceptible to the antibacterial agents recommended for treatment of streptococcal tonsillitis, such as penicillin, oral cephalosporins, erythromycin and clindamycin (1,3,6, 8).

CONCLUSIONS

- *A. haemolyticum* was isolated from 0.1% of the throat specimens of 10-26 year old patients with acute pharyngitis, using sheep blood agar on which this species grows as little smooth or rough colonies.
- The identification of this organism was possible using: Gram stain, catalase, reverse CAMP, gelatin hydrolysis and the capacity of acid formation from glucose, maltose, sucrose, xylose, mannitol .
- All *A. haemolyticum* strains were susceptible to penicillin, cephalosporins, macrolides, tetracycline and resistant to thrimethoprim-sulphamethoxazole.

Table 1. Differential characteristics of *A. haemolyticum* and other bacterial groups

Bacterial group	Catalase	Motility	Nitrat reduction	Urease	Gelatin hydrolysis	Carbohydrate fermentation	Glucose	Esculin hydrolyzed	Acid from				
									Maltose	Lactose	Sucrose	Xylose	Mannitol
<i>Corynebacterium sp.</i>	+	-	V	V	V	+	+	-	V	-	V	-	-
<i>Arcanobacterium haemolyticum</i> (<i>C. haemolyticum</i>)	-	-	-	-	-	+	+	-	+	+	V	-	-
<i>Actinomyces pyogenes</i> (<i>C. pyogenes</i>)	-	-	-	-	+	+	+	-	V	+	V	+	V
<i>Listeria monocytogenes</i>	+	+	-	-	-	+	+	+	+	V	V	-	-

+, positive; -, negative; V, variable reaction.

Table 2. Principal characteristics of *A. haemolyticum*

Test	Reaction	Test	Reaction
β-haemolysis	+	Reverso CAMP	+
Catalase	-	DNase	+
Nitrate reduction	-	Glucose	+
Urease	-	Maltose	+
Gelatin hydrolysis	-	Sucrose	V
Esculin hydrolysis	-	Xylose	-
Lipase	-	Mannitol	-

+, positive; -, negative; V, variable reaction.

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