CORRESPONDENCE

Arcanobacterium hemolyticum: identification and susceptibility to nine antimicrobial agents

Arcanobacterium hemolyticum is a β-hemolytic Gram-positive, pleomorphic, facultative anaerobic, non-acid-fast rod that has been implicated as an etiologic agent of non-streptococcal pharyngitis, especially in adolescents and young adults, as well as a cause of skin and wound infections, osteomyelitis, sepsis, and central nervous system infections.

We describe 19 strains of *A. hemolyticum* that were isolated during a period of two years (1998–2000) from human sources, their microbiological features, and their antimicrobial susceptibility.

The samples were cultured on Columbia agar (Difco, Detroit, Michigan, USA) with 5% human blood for 24–48 h at 35 °C in an aerobic atmosphere.

Identification was achieved using morphology of colonies, Gram stain and biochemical tests, following the scheme of von Graevenitz and Funke [1], API Coryne (bioMérieux SA, Marcy L′ Etoile, France), and additional tests such as PYR, production of α-mannosidase (Rosco Diagnostic, Taastrup, Denmark), and bacitracin susceptibility (0.04 U) (Laboratory Britania, Argentina). Any strain with a zone of inhibited growth around the bacitracin disk was considered to be susceptible for identification purposes.

Antigenic characteristics were determined by extraction of cells by the Lancefield extraction procedure and reaction with antisera for groups A, B, C, D, F and G (bioMérieux SA).

The CAMP reaction was performed with a β-hemolytic strain of *Staphylococcus aureus* (ATCC 25923) and with a group B β-hemolytic streptococcus.

The following antimicrobial agents were tested: penicillin, cephalothin cefuroxime, erythromycin, azithromycin, tetracycline, clindamycin, ciprofloxacin, and vancomycin. The MICs were determined by an agar dilution method on Mueller–Hinton agar supplemented with 5% sheep blood. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.

Because of the absence of accepted breakpoints for *A. hemolyticum*, those established by the NCCLS for organisms that grow aerobically were used [2].

Thirteen strains were isolated from the throat, and others from diabetic foot ulcers, bone, urethral discharge, peritonsillar abscess, pleural fluid, and lochia. Three of the infections were mixed, with the following organisms being simultaneously isolated: from pleural fluid of a patient with traumatic hemothorax, *Streptococcus agalactiae* and group G *Streptococcus dysgalactiae* subsp. *equisimilis*; from urethral discharge, *Gardnerella vaginalis* and anaerobic flora; and from lochia from spontaneous abortion, *Escherichia coli*.

None of the throat samples yielded β-hemolytic streptococci, including *A. hemolyticum*.

Antimicrobial susceptibility results are shown in Table 1.

Discrepant PYR results were obtained between API Coryne and PYR tests based on commercial disks. Whereas 13 strains gave positive PYR tests

Table 1 Antimicrobial susceptibility of 19 strains of *Arcanobacterium hemolyticum*

Antimicrobial agent	No. of susceptible strains/Total (% susceptible strains)[a]	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Penicillin	18/19 (94.7)[b]	0.004–0.25	0.016	
	19/19 (100)[c]			0.064
Cephalothin	19/19 (100)	0.004–0.064	0.016	0.032
Cefuroxime	19/19 (100)	0.032–0.5	0.125	0.25
Erythromycin	19/19 (100)	0.004–0.25	0.004	0.032
Azithromycin	19/19 (100)	< 0.004	< 0.004	< 0.004
Tetracycline	13/19 (68)	0.25–64	0.5	32
Clindamycin	19/19 (100)	0.004–0.032	0.008	0.016
Ciprofloxacin	19/19 (100)	0.25–0.5	0.25	0.5
Vancomycin	19/19 (100)	0.25–0.5	0.25	0.5

[a]According to NCCLS interpretative standards (2000). [b]The category for staphylococci was applied. [c]The category for *Listeria monocytogenes* was applied.

with disks, only six gave positive PYR tests with API Coryne.

Bacitracin inhibition zone diameters around the disks were between 8 and 14 mm.

The codes obtained by the API Coryne identification system were 2010360, 2110360, 2130360, 2210360, 2230360, 2310360, 2510360, 2530360, 6010360, 6110360, 6130360, 6330360 and 6530361, giving good, very good or excellent identification as *A. hemolyticum*.

The clinical picture of *A. hemolyticum* is indistinguishable from that of streptococcal pharyngitis, and since all strains were susceptible to bacitracin, and 13 of 19 were PYR test positive using commercial disks, we consider that a Gram stain should always be performed on throat samples to differentiate *Streptococcus pyogenes*. Furthermore, all the strains showed cross-agglutination with antisera for groups A, B, C, D, F or G.

All strains were α-mannosidase positive, as reported by Carlson and Kontiainen [3].

All the strains were CAMP positive when the test was performed by streaking *S. agalactiae* according to the method of Lammer and Blobel [4], and reverse CAMP positive when using *S. aureus* ATCC 25923.

A. hemolyticum was susceptible to penicillin (MIC_{90} 0.064 mg/L).

Even though most of the strains were inhibited by $\leq$0.064 mg/L, the MIC was 0.25 mg/L for one strain. Clinical failures that have been reported could be associated with penicillin tolerance [5].

All clinical isolates were susceptible to cephalosporins. The MICs of cephalothin were lower than those of cefuroxime. The MIC_{90}s were 0.032 mg/L and 0.25 mg/L, respectively.

The strains were susceptible to vancomycin (MIC_{90} 0.5 mg/L), as has been reported by Carlson et al. [5]. However, a single *A. hemolyticum* strain carrying the *vanA* gene conferring resistance to vancomycin has been reported by Power et al. [6].

Six of 19 strains were resistant to tetracycline, as has been described in other reports [5].

Virtually all *A. hemolyticum* strains studied so far have been susceptible to erythromycin, azithromycin and clindamycin, but one macrolide-resistant *A. hemolyticum* strain has been reported by Carlson et al. [5].

In conclusion, a Gram-positive rod that exhibits β-hemolysis on human blood agar, that is catalase negative, and gives a positive result with the CAMP test and reverse CAMP test using *S. agalactiae* and *S. aureus* ATCC 25923, respectively, may be identified as *A. hemolyticum*. Only tetracycline showed variable activity against the strains.

ACKNOWLEDGMENTS

This article was presented (in part) at the 11th European Congress of Clinical Microbiology and Infectious Diseases, Istambul, Turkey, 1–4 April 2001.

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