Haemolytic differential identification of *Arcanobacterium haemolyticum* isolated from a patient with diabetic foot ulcers

Hyesook Kang, Gyunam Park, Hyeran Kim and Kyungsoo Chang

**Introduction:** *Arcanobacterium haemolyticum* (formerly known as *Corynebacterium haemolyticum*) is the causative agent of sore throat and also causes skin and soft tissue infections in diabetes patients. *A. haemolyticum* is a Gram-positive, catalase-negative, *β*-haemolytic bacillus. *A. haemolyticum* poses a diagnostic challenge in the hospital laboratory because most coryneform bacilli are considered as normal flora or contaminants, and it is therefore difficult to differentiate from *β*-haemolytic streptococci by colony characteristics.

**Case presentation:** *A. haemolyticum* was isolated from a diabetic patient with foot ulcers and the isolate was identified by using a VITEK-2 system, CAMP inhibition test, reverse CAMP test and a 23S rRNA gene sequence analysis. The isolated *A. haemolyticum* inhibited haemolysis of *Staphylococcus aureus* in the CAMP test and enhanced haemolysis of *Streptococcus agalactiae* in the reverse CAMP test. The diabetic patient was treated with teicoplanin and imipenem, and the ulcers healed within 2 weeks.

**Conclusion:** The present study suggests that a haemolytic differential method using the CAMP inhibition and reverse CAMP tests can be useful for differentiating *A. haemolyticum* from *β*-haemolytic streptococci.

**Keywords:** *Arcanobacterium haemolyticum*; CAMP inhibition test; diabetic foot ulcers; haemolytic differential method; reverse CAMP test.
under conditions of 5 % CO₂ for 24 h. Very small grey colonies without observable β-haemolysis were found on the SBA plate (Fig. 1b, left panel), but no colonies were found on the MacConkey agar plate (data not shown). The isolates from the colonies were identified as Gram-positive rods by Gram staining and a catalase reaction was negative (data not shown). The colonies became more distinct and displayed a small degree of β-haemolysis after 48 h of culture (Fig. 1b, middle panel). Small smooth grey colonies with distinct β-haemolysis were observed after 72 h of culture (Fig. 1b, right panel). The isolate was identified as *A. haemolyticum* (99 %) by the VITEK-2 automated microbiology system (BioMérieux) (Table 1). Blood samples were incubated four times in the BACTEC 9240 blood culture system (BD) for 5 days, but all blood cultures were negative (data not shown).

A CAMP inhibition test was then performed with the isolated *A. haemolyticum* in order to confirm its identity. *Staphylococcus aureus* ATCC 25923 was streaked in a straight line across the centre of the SBA plate (Fig. 1a, indicated as ‘a’). The isolated *A. haemolyticum* was streaked in a straight line perpendicular to the *Staphylococcus aureus* streak (Fig. 1a, indicated as ‘b’) in the lower left. *Streptococcus agalactiae* ATCC 13813 was streaked (Fig. 1a, indicated as ‘c’) similarly in the upper right as a positive control for haemolysis.

The characteristic haemolysis inhibition by *A. haemolyticum* was not observed after 24 h of culture (Fig. 2a, yellow arrow in left panel) but became apparent after 48 h (Fig. 2a, yellow arrow in right panel). However, the arrow shape of haemolysis by *Streptococcus agalactiae* (ATCC 13813) occurred against *Staphylococcus aureus*.

**Table 1.** Comparison of biochemical properties and haemolysis activities between the *A. haemolyticum* isolate and reference strain ATCC 9345 by VITEK-2 and CAMP tests

<table>
<thead>
<tr>
<th>Biochemical reaction</th>
<th><em>A. haemolyticum</em> ATCC 9345</th>
<th><em>A. haemolyticum</em> clinical isolate</th>
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<tbody>
<tr>
<td>Catalase</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
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<tr>
<td>Maltose</td>
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<td>Sucrose</td>
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<tr>
<td>Xylose</td>
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<td>–</td>
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<tr>
<td>Aesculin hydrolysis</td>
<td>–</td>
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<tr>
<td>CAMP reaction</td>
<td>I</td>
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<td>Reverse CAMP</td>
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+, Positive; –, negative; V, variable; I, inhibition.

**Fig. 1.** Diabetic ulcers and colony changes. (a) Diabetic ulcers on the feet of the patient, who has been suffering from chronic diabetes. (b) Colony changes of *A. haemolyticum* on an SBA plate over time.
ATCC 25923 after 24 h (Fig. 2a, blue arrow in left panel) and became more apparent after 48 h (Fig. 2a, blue arrow in right panel). In addition, we used haemolytic-sensitive strain Staphylococcus aureus ATCC 29213 in Fig. 2(b). The isolated A. haemolyticum haemolysis inhibition using Staphylococcus aureus ATCC 29213 was apparent within 24 h (Fig. 2b, yellow arrow in left panel) and increased after 48 h (Fig. 2b, yellow arrow in right panel). In contrast to the result in Fig. 2(a), haemolysis by Streptococcus agalactiae was not observed (Fig. 2b, blue arrow in both panels) due to enhanced haemolysis in Staphylococcus aureus ATCC 29213.

**Fig. 2.** CAMP inhibition and reverse CAMP reaction of A. haemolyticum. (a, b) A. haemolyticum isolate in the lower left and Streptococcus agalactiae in the upper right were streaked perpendicularly to vertically streaked Staphylococcus aureus (ATCC 25923) (a) and Staphylococcus aureus (ATCC 29213) (b) on an SBA plate. (c) Reverse CAMP reaction of the isolated A. haemolyticum in the upper left and A. haemolyticum (ATCC 9345) in the lower right against Streptococcus agalactiae (ATCC 13813), which developed crescent-shaped haemolysis. ‘a’, Staphylococcus aureus (ATCC 25923); ‘b’, A. haemolyticum isolated from the patient; ‘c’, Streptococcus agalactiae (ATCC 13813); ‘d’, Staphylococcus aureus (ATCC 29213); ‘e’, A. haemolyticum (ATCC 9345).
In the reverse CAMP test, the isolate A. haemolyticum was streaked in the upper left (Fig. 2c) perpendicular to the Streptococcus agalactiae streak. The waxing crescent-shaped haemolysis on the contact surface was observed clearly after 24 h. The control A. haemolyticum ATCC 9345 was streaked in the lower right (Fig. 2c), perpendicular to the Streptococcus agalactiae streak. Similar to the isolate A. haemolyticum, the waning crescent-shaped haemolysis on the contact surface was observed clearly after 24 h. Finally, the isolated A. haemolyticum was confirmed by genotyping using 23S rRNA gene PCR and sequencing. The sense and antisense primers were 5′-TAACGGTCTTA AGGTACCGA-3′ and 5′-GATAGGGACCGAACTGTCTC-3′, respectively. The sequencing result revealed that the isolated A. haemolyticum shared 99.9 % identity with A. haemolyticum DSM 20595 (data not shown).

Discussion

Although A. haemolyticum isolated from respiratory samples typically produces rough colonies without β-haemolysis, the isolate from the wound skin infection produced smooth colonies with haemolysis (Carlson et al., 1994). As expected, the isolated A. haemolyticum was identified as a β-haemolytic coryneform bacillus. Because coryneform bacilli are considered part of the normal flora or a contaminant, they are more difficult to identify (Meyer & Reboli, 2005).

The morphological characteristics of A. haemolyticum should be distinguished from the irregular shape of Corynebacterium spp. as Gram-positive rods and from Streptococcus spp. The differences between A. haemolyticum and Corynebacterium spp. are their colony shape on an agar plate and catalase positivity. However, comparisons between A. haemolyticum and catalase-negative Streptococcus, particularly β-haemolytic Streptococcus spp., do not yield distinct differences in colony shape when grown on 5 % SBA plate or in the catalase test. Thus, the methods in previous studies cannot differentiate between A. haemolyticum and catalase-negative Streptococcus.

In order to correctly identify A. haemolyticum, a CAMP inhibition test and reverse CAMP test should be used in conjunction with commercially available biochemical property test kits, colony size, morphology and haemolysis according to culture time. In this case, the haemolysis pattern of the A. haemolyticum isolate changed with culture time (from unclear β-haemolysis to weak β-haemolysis to distinct β-haemolysis after 24, 48 and 72 h of culture, respectively). A. haemolyticum inhibited the β-haemolysis of Staphylococcus aureus (ATCC 25923 and 29213). In addition, the CAMP inhibition and reverse CAMP tests using Streptococcus agalactiae were positive. Several previous studies have confused CAMP inhibition (β-haemolysis inhibition) with reverse CAMP (β-haemolysis enhancement) (Kim et al., 2004; Bae et al., 2010).

This report sought to redefine the role of the CAMP inhibition and reverse CAMP tests in identifying and differentiating A. haemolyticum from β-haemolytic streptococci using Staphylococcus aureus and Streptococcus agalactiae. In the CAMP inhibition test, Staphylococcus aureus ATCC 29213 caused more haemolysis inhibition than Staphylococcus aureus ATCC 25923 (Fig. 2a, b). The reverse CAMP test with the isolated A. haemolyticum from the diabetic patient exhibited a waxing crescent-shaped haemolysis, and the reference A. haemolyticum ATCC 9345 exhibited a waning crescent shape haemolysis (Fig. 2c).

In conclusion, this report suggests that haemolytic differential identification using CAMP and reverse CAMP tests might be a more effective method to differentiate A. haemolyticum from other Gram-positive coryneform bacilli and β-haemolytic streptococci.

Acknowledgements

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References


