



Original Contribution

ATTEMPTS FOR IDENTIFICATION OF *CLOSTRIDIUM PERFRINGENS* TYPE A AND SOME HAEMOLYTIC BACTERIAL SPECIES USING THE CAMP TEST

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ABSTRACT

With regard to the most convenient laboratory diagnostics of *C. perfringens* infections and those caused by some weakly haemolytic bacteria, attempt to determine their relationships in the CAMP test in different combinations were carried out.

For this purpose, agar supplemented with 10% defibrinated sheep blood was used as nutrient medium. The incubation was carried out in aerobic, microaerophilic and anaerobic conditions.

C. perfringens type A showed a clear CAMP test reaction with *S. agalactiae* and *R. equi*, but not with *S. aureus*, whereas *R. equi* exhibited a positive reaction with *S. aureus* and vice versa (*S. aureus* showed a positive reaction with *R. equi*, when the latter was used as haemolysis-enhancing factor).

The attempts to use *C. perfringens* as haemolysis-enhancing factor showed that it exhibited a positive CAMP test result with *R. equi*, but there was no reaction with *S. aureus*. When used as a haemolysis-enhancing factor with regard to *L. monocytogenes* type ¹/₂ a, 4b and *L. ivanovi*, *C. perfringens* showed almost equal reaction with a slightly stronger haemolysis with *L. ivanovi*. This reaction became more detectable when the incubation time was prolonged up to 48 h.

Key words: CAMP test *C. perfringens* *R. equi* *L. monocytogenes*

INTRODUCTION

In order to differentiate streptococci group B of other Lancefield groups of the same species, in 1944 Christie et al. (1) have developed a test based upon the synergistic haemolysis phenomenon between *Staphylococcus aureus*, producing beta-toxin and the streptococcal factor, called later CAMP factor. The test is made only on agar containing defibrinated sheep or bovine blood, but not human blood. The zone of enhanced haemolysis is similar in shape to an arrowhead pointing at the *Staphylococcus* streak (1). Despite the high sensitivity of this test, further studies have shown that some strains of Lancefield streptococcal groups A, C and D also yielded a synergistic reaction in the CAMP test although weaker than that of group B (2, 3, 4, 5).

After acknowledging that *Clostridium perfringens*, producing an alpha toxin, exhibited a synergistic activity with *Streptococcus agalactiae*, which belongs to the streptococcal group B, experiments were carried out to prove that it could replace *S. aureus* in this test with a higher specificity in the differentiation of group B streptococci from those belonging to groups A, C and D. Later, the haemolytic synergism between *S. agalactiae* and *C. perfringens* was used in a reverse variant to differentiate alpha toxin-producing *C. perfringens* (6). It was also evidenced that the test could be run in anaerobic conditions, as confirmed by Naydenov too (7).

The CAMP test mechanism is explained by the synergism between haemolysins of *Corynebacterium pseudotuberculosis* and *Rhodococcus equi*. The exotoxin of *Corynebacterium pseudotuberculosis* represents a phospholipase D that acts as sphingomyelinase, which hydrolyzes cell

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membrane sphingomyelin. The hydrolysis is however not complete and therefore, complete haemolysis is not occurring. A synergistic effect is observed when phospholipase C is also participating. This toxin is produced by *R. equi*. A similar reaction exists between *R. equi* and *Listeria ivanovi* that is used to differentiate *L. ivanovi* from *L. monocytogenes* (8, 9).

Taking into consideration the difficulties encountered in the identification and differentiation of *C. perfringens*, requiring in almost all cases biological samples, as well as in order to differentiate some weakly haemolytic bacteria, we have performed attempts to improve the laboratory diagnostics of such microbial agents using the CAMP test.

MATERIAL AND METHODS

The experiments were carried out with *Cl. perfringens* type A, *L. monocytogenes* type 1/2a, *L. monocytogenes* 4b, *L. ivanovi*, *R. equi*, *S. agalactiae*, *S. aureus*, *P. trhalosi*, *Manheimia haemolytica*.

The nutrient media for isolation and maintenance of microorganisms were selected according to their requirements and the routine practice. The CAMP test was performed with agar containing 10% defibrinated sheep blood. The incubation was made in aerobic, microaerophilic and anaerobic conditions. The Gas pak system (Oxoid) was used to create microaerophilic conditions. Microbial combinations that showed a positive result in the CAMP test were utilized in a reverse variant too, in order to find the most appropriate combinations to identify the respective microorganisms. In CAMP test variants, a profuse amount of fresh agar cultures of the tested microorganism was inoculated. The source of CAMP factor was inoculated as a horizontal streak on blood agar Petri dish. Of the tested microorganisms were done as perpendicular streaks ending at a distance of 1-2 mm from the horizontal streak. The incubation was performed under various oxygen content for 24 to 48 h at 37°C. The following variants were tested:

- horizontal *S. agalactiae* streak and perpendicular *C. perfringens* streak;
- horizontal *C. perfringens* streak and perpendicular streaks of *P. trhalosi*, *M. haemolytica*, *L. monocytogenes* 4b, *L. monocytogenes* 1/2a, *L. ivanovi*, *R. equi*, *S. aureus*;

- horizontal *S. aureus* streak and perpendicular *C. perfringens* streak;
- horizontal *R. equi* streak and perpendicular *C. perfringens* and *S. aureus* streaks.

Immediately after the end of incubation period, Petri dishes and the respective results were photodocumented.

RESULTS

The CAMP test in which *S. agalactiae* was a source of a synergistic factor, showed a clear haemolytic zone around the *C. perfringens* streak adjacent to staphylococcal culture, with the typical crescent shape lying in a hat-like manner on the top of *C. perfringens* streak. The tested microorganism's haemolysis shape was similar to a mushroom with a cap on the streak line (**Fig. 1**). A similar appearance is seen when *R. equi* is used as a synergistic factor. The enhanced haemolysis resulting from the growth of *C. perfringens* near to the *Rhodococcus* streak was equal to that resulting from the *S. aureus* growth. The shape, however, was not of a crescent but of a shovel in both microorganisms (**Fig. 2**). In the reverse CAMP test, when *C. perfringens* was tested as a source of synergistic factor, enhanced haemolysis was observed only with *R. equi* but not with *S. aureus*. Here, the shape of enhanced haemolysis zone was the same as in the original test with these bacteria (**Fig. 3**). The CAMP test was not positive with respect to *C. perfringens* when *S. aureus* was used as a source of synergistic factor. The haemolysis zone along the *C. perfringens* streak was unchanged, whereas that of *R. equi* acquired the shovel-like shape nearby the staphylococcal streak (**Fig. 4**).

When the CMP test was performed with *Listeria*, with *C. perfringens* as a source of synergistic factor, no growth was observed by the 24th hour of incubation and this required a longer incubation up to 48 hours. Nevertheless, no profuse growth of *Listeria* has been observed. There was neither a haemolytic zone around the streaks. Weak haemolytic zones were demonstrated in all three cultures with a slightly stronger zone in *L. ivanovi*. The enhanced haemolysis shape was also with the shape of a shovel, but was inferior in both size and intensity to that of other tested microorganisms. When *C. perfringens* was used as a source of synergistic factor, no changes occurred in the haemolysis of *P. trhalose* and *M. haemolytica*.

The experiments performed in aerobic, anaerobic and microaerophilic conditions of incubation revealed that the environment with lower oxygen content was the most suitable for performing the test, taking advantage of

the circumstance that *C. perfringens* is not an obligate anaerobe and that other tested microorganisms were, to some extent, facultative anaerobes.

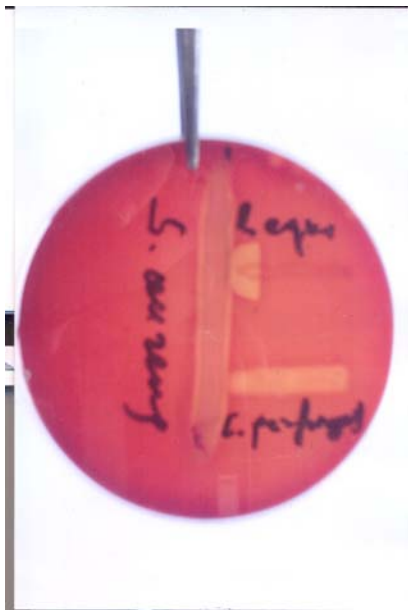


Fig. 1

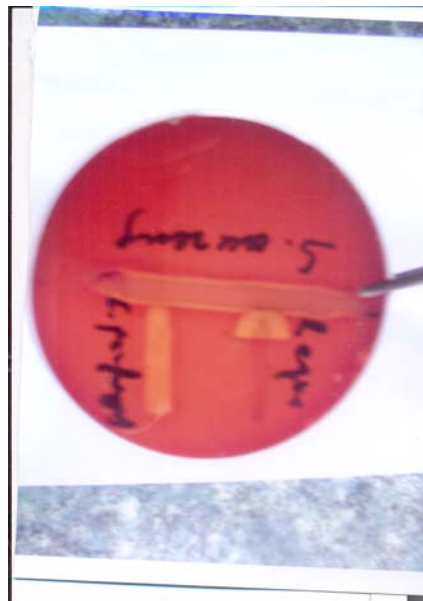


Fig. 2



Fig. 3



Fig. 4

DISCUSSION

The identification of microbial isolates is essential for diagnostics of bacterial diseases. Therefore, continuous efforts are made to speed up and ease the performance of microbiological tests. Recently, modern methods to identify the species of microbial agents at a genetic level were introduced. In most cases, these techniques are not available in small laboratories requiring the use of other test based on some phenotype traits of bacteria. One of them is the CAMP test, which uses a factor in some haemolytic bacteria that enhances the haemolysis of other haemolytic organisms when they are cultured together, in

close vicinity on media containing erythrocytes. A subject of interest among microorganisms containing this factor is *C. perfringens* type A. In our view, it is very appropriate for this test because it is not fastidious, could be easily maintained in every microbiological laboratory and contains a lot of alpha toxin. That is why Gubash (10) utilizes it as a source of alpha toxin for identification of streptococci containing the CAMP factor. In previous studies of ours (7) we have confirmed this trait of *C. perfringens* type A alpha-toxin and also, have identified *C. perfringens* by this test (6). As none of known haemolysis tests is consistent enough to

identify microorganisms, we aimed to verify the utility of the CAMP test with other microorganisms as well. Therefore, the finding that *R. equi* could serve as a source of CAMP factor for *C. perfringens* is important because it could be used instead of *S. agalactiae*. In the reverse CAMP test variant, *C. perfringens* could be used to identify *R. equi*. We could not provide an explanation why in both CAMP test variants with *S. aureus* and *C. perfringens*, no synergism was found between these bacteria similarly to what was observed between *R. equi* and *S. aureus*. Possibly, this could be attributed to relationships between the toxins sphingomyelinase and phospholipase, but additional studies are necessary to confirm this hypothesis. In attempts to differentiate the tested *Listeria* species, we observed an almost similar enhancement of haemolysis in *L. monocytogenes* type 1/2a and type 4b, and *L. ivanovi*. Therefore, in diagnostic practice, these organisms could be identified as a genus and for species identification; the test with *R. equi* should be performed (8). The rather weak haemolysis observed in CAMP test with *Listeria* could be explained by the opinion of Gubash (10) that a profuse growth of tested microorganisms is needed for complete haemolysis to occur, as the amount of produced enzymes and their ability to diffuse in the nutrient medium are directly related to growth. In our experiments, there was not a dense growth of tested *Listeria* strains that probably accounted for the weaker haemolytic zone. It is reported (11) that the *Listeria* toxin is somewhat similar to the alpha-toxin of *C. perfringens* and acts in the presence of reducing agents, whereas lecithinase acts in the presence of calcium ions and is responsible for a more intensive haemolytic activity. Better results could be obtained when the test is performed with erythrocytes of other animal species or with human erythrocytes that is attributed by some authors (12, 13) to the different amount of sphingomyelin in the red blood cells of the different mammalian species and the different ion content. Perhaps, this could explain the lack of positive CAMP test with *P. trehalosae* and *M. haemolytica*. These circumstances could however be made clear in additional experiments.

CONCLUSIONS

1. *Clostridium perfringens* type A could serve as a source of CAMP factor to identify *Rhodococcus equi* and vice versa;

Rhodococcus equi could be a source of CAMP factor to identify *Clostridium perfringens*.

2. A positive CAMP test was observed in both variants using *Rhodococcus equi* and *Staphylococcus aureus*, therefore these bacteria could be identified this way.

3. The use of *Clostridium perfringens* in the CAMP test to prove *Listeria* allows the genus, but not species identification of *Listeria monocytogenes* type 1/2a, type 4b and of *Listeria ivanovi*.

4. No synergistic effect of *Clostridium perfringens* was shown in the CAMP test with regard to *Staphylococcus aureus*, *Manheimia haemolytica* and *Pasteurella trehalosi*.

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