

PRODUCTION OF HAEMOLYSINS BY *YERSINIA* *ENTEROCOLITICA* STRAINS

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Haemolysins are one of the factors of aggression of bacteria. In the present study we demonstrated the results from the analysis of the haemolytic activities of 10 Yersinia enterocolitica strains of different origin and serotypes. Erythrocyte suspension prepared from human blood group "O", sheep and calf blood was used. Most strains isolated from clinically ill patients and belonging to serotype O3 produced haemolysins which was manifested on sheep blood agar. A conclusion was made that proof of haemolysins from strains isolated in epidemics could serve as a marker for epidemic study.

Key-words: *Yersinia enterocolitica*, haemolysins, temperature of civilization, serotypes, origin of strains

In pathogenesis of infection all the "factors of aggression" are important as they induce complex and irreversible changes in host-cell. Haemolysins belong to them, too. In the first studies of *Yersinia enterocolitica* their ability was mentioned to produce thermolabile substance of enterotoxin similar by composition to haemolysin. It was reported that this production did not depend on origin of strains and did not correlate with the production of thermostable enterotoxin (3). It was established that *Yersinia enterocolitica* serotype O3 produced haemolytic substance that could be

separated by sound disintegration. It could not dialyse, it was thermolabile and sensitive to influence with trypsin. It was identified with β -haemolysin. Chemically, it was a protein of high molecular weight and had the properties of thermolabile antigen (6).

The aim of the present study was to investigate the production of haemolysins from *Yersinia enterocolitica* cultures isolated from patients in the town of Varna and referent strains as cultivation was performed at 28°C and 37°C in erythrocyte suspension from different origin.

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MATERIAL AND METHODS

We studied ten *Yersinia enterocolitica* strains. Seven from them were isolated in our laboratory from patients with diarrhea syndrome and were identified by the conventional methods (1). The strains were from serotype 03. Three referent cultures from serotype 03, 09 and 08 kindly granted to us by Military Medical Academy - Sofia, were included in the study. Production of haemolysins was established by the following way: in 24-hour broth culture from strain sterile paper disks were soaked and then they were put in a Petri bowl with double layer agar-simple agar and 10 % erythrocyte suspension with halfsolid agar. The experiment was performed after twice-cultivation at 28°C and at 37°C. We read the changes in blood agar after cultivation after 48 hours. The erythrocyte suspension was prepared from human blood group "0", sheep and calf blood (5). For each strain the experiment was performed three times.

RESULTS AND DISCUSSION

Fig. 1 demonstrates a clear haemolytic zone produced by two strains cultivated at 28°C. They were strains from serogroup 03 isolated from patients with clinical signs and the result was demonstrated on sheep

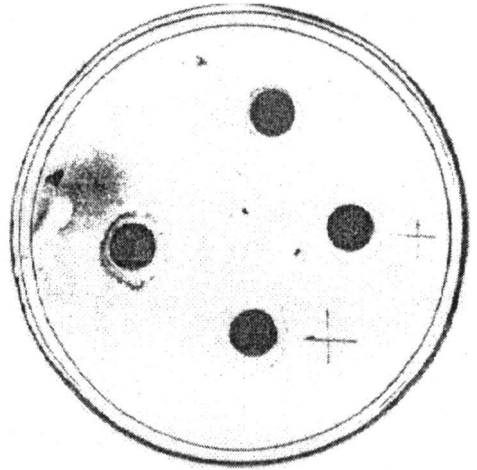


Fig. 1. Haemolytic zone produced by two *Yersinia enterocolitica* strains

blood agar. Another five strains isolated from clinically ill patients showed haemolytic zone under the same conditions. None from the other cultures studied showed haemolysis, nevertheless the temperature of cultivation, the antigen structure or the composition of blood agar. The data from the literature available showed that similar studies demonstrated different results. Pederson (6) had investigated 15 strains of *Yersinia enterocolitica* serotype 03, "wild and referent" and had established production of haemolytic suspension when strains were cultivated at 22°C. Strains from serogroups 08 and 09 did not produce haemolysins. According to other authors (2,7) up to 99 % of the strains excreted haemolysins and that property did not correlate with other

characteristics of the cultures. The study of 14 strains of *Yersinia enterocolitica* serotype 03 isolated from clinically ill children did not prove any production of haemolysins (4). The results from our study showed that when cultivated at 28°C most strains of *Yersinia enterocolitica* serotype 03 isolated from clinically ill patients produced haemolysins. Having in mind the above mentioned and the literature data we supposed

that, most probably, excretion of haemolysins was a plasmid determined quality. Our study concerned strains isolated in sporadic cases which made their inadequate production of haemolysins clearly explicable. Identical results could be expected for strains isolated in epidemics. In those cases proof of haemolysins could be used as an important marker for epidemic study.

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Продукция на хемолизини от щамове на *Yersinia enterocolitica*

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Резюме: Хемолизините са един от факторите на агресия на бактериите. В настоящото проучване представяме резултатите от анализа на хемолитичните активности на 10 щамове на *Yersinia enterocolitica* с различен произход и серотипове. Използвана е еритроцитна суспензия, получена от човешка кръв от група "0", както и от овнешка и телешка кръв. Повечето от щамовете, изолирани от болни с клинично изразени заболявания и принадлежащи към серотип 03, продуцират хемолизини, които се проявяват върху агар от овнешка кръв. Прави се заключение, че това доказване на хемолизините от щамове, изолирани по време на епидемия, може да послужи като маркер за епидемиологично изследване.