JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1980, p. 94-96
0095-1137/80/01-0094/03$02.00/0

Vol. 11, No. 1

NOTES

Hyaluronidase Production by Type B *Pasteurella multocida* from Cases of Hemorrhagic Septicemia

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Seventy-four cultures of *Pasteurella multocida* representing all four capsular types, A, B, D, and E, from various animal species and diseases were examined for the production of hyaluronidase by two procedures. In one, hyaluronidase production was determined by the depolymerization of streptococcal capsular hyaluronic acid, and in the other, production was determined by degradation of sodium hyaluronidate in a solid culture medium. Hyaluronidase production was only demonstrated in the 13 type B cultures that had been recovered from cases of hemorrhagic septicemia.

A number of gram-positive bacteria, including some species of staphylococci, streptococci, and clostridia, produce the enzyme hyaluronidase (11); however, no reports could be found of the production of this enzyme by gram-negative bacteria. While studying cultures of *Pasteurella multocida* for bacteriocin production (5), it was noted that a type B hemorrhagic septicemia culture elaborated an appreciable amount of hyaluronidase. The purpose of this note is to record the production of hyaluronidase by a number of type B hemorrhagic septicemia cultures, and its apparent lack of production by other varieties of this serologically heterogeneous species.

Strains of *P. multocida* comprise a number of different serological varieties on the basis of differences in capsular and somatic antigens (2, 3, 6-8). Four different capsular types have been recognized, viz., types A, B, D, and E (2, 3). Type B cultures cause the important disease of principally cattle and water buffaloes called hemorrhagic septicemia (1). Type E cultures cause a severe disease in cattle in Africa that appears to be identical to hemorrhagic septicemia (9). Based upon the examination of a number of type B and E cultures, it has been concluded that those that cause hemorrhagic septicemia possess the same somatic antigen, designated 6 (7).

The type B hemorrhagic septicemia cultures are highly virulent for cattle, water buffaloes, rabbits, and mice (1). Type A cultures with different somatic antigens cause most fowl cholera and are associated with respiratory disease in cattle, sheep, swine, and other animals (4). Type D cultures, again with differing somatic antigenic composition, are associated with respiratory infections in swine and occasional infections in other animals (4). Only one type B culture has been found to have a different somatic antigen than that of the type B and E hemorrhagic septicemia cultures (8). This culture (no. 14; Table 1) was isolated from a bovine wound (8).

The animal origin, capsular type, and associated disease of the cultures examined for hyaluronidase are listed in Table 1. All of the type B cultures except culture no. 14 were isolated from cases of hemorrhagic septicemia. The seven type E strains had all been recovered from cases of what was described as hemorrhagic septicemia. Two procedures were employed to test for hyaluronidase. In the first, a hyaluronic acid-producing *Streptococcus equi* from a blood plate culture was streaked across the center of a blood agar plate (100 by 50 mm) (tryptic soy agar [Difco Laboratories] and 5% bovine blood). The *Pasteurella* cultures to be tested were then streaked across the *S. equi* streak at right angles. Six *Pasteurella* cultures could be conveniently streaked in this manner on one plate. Freshly prepared blood agar plates and a humidified incubator were employed to assure adequate production of hyaluronic acid by *S. equi*. The inoculated plates were incubated at 37°C for 18 h.

Hyaluronidase activity was indicated by the diminution in size of the streptococcal growth adjacent to the *Pasteurella* streak (Fig. 1) due to degradation of capsular hyaluronic acid. The loss of capsule (hyaluronic acid) was confirmed by negative staining. Only the 13 type B hem-

orrhagic septicemia cultures produced observable hyaluronidase by this "cross-over streak" method. Culture no. 14 was negative.

In the second procedure for the demonstration of hyaluronidase activity, *Pasteurella* cultures were spotted on a medium containing sodium hyaluronidate (Sigma Chemical Co.) and bovine albumin fraction V (Miles Laboratories, Inc.). This medium was prepared precisely as described previously (10), and 20 ml was dispensed in each petri plate. The reliability of each lot of the sodium hyaluronidate medium used was tested with a *Staphylococcus aureus* culture that produced hyaluronidase and one that did not.

The *Pasteurella* cultures (Table 1) examined were grown in brain heart infusion broth (Difco Laboratories) for 18 h at 37°C. Each quadrant or half of plates containing the sodium hyaluronidate medium was inoculated with a loopful (0.01 ml) from a brain heart infusion broth culture. These plates were incubated for 18 h at 37°C, after which each was flooded with 2 N acetic acid for 10 min. It was subsequently observed that hyaluronidase production could be demonstrated equally well if growth from a plate culture was used for spotting.

The nondegraded substrate precipitated with the albumin, leaving a clear zone around the *Pasteurella* growth that produced hyaluronidase (Fig. 2). No clearing around the *Pasteurella* growth was interpreted as being negative for hyaluronidase activity. As in the aforementioned procedure, only the 13 type B hemorrhagic septicemia cultures provided evidence of hyaluron-

TABLE 1. *Identity of cultures of P. multocida examined for hyaluronidase activity*

Culture no.	Capsular type	Animal origin and disease
1 to 13 inclusive	B	Bovine, hemorrhagic septicemia
14	B	Bovine, wound
15 to 27 inclusive	A	Avian, fowl cholera
28 to 39 inclusive	A	Swine, pneumonia
40	A	Bovine, sepsis
41 to 49 inclusive	A	Bovine, pasteurellosis
50 to 53 inclusive	A	Rabbit, pasteurellosis
54	A	Human, peritonitis
55 to 62 inclusive	D	Swine, pneumonia
63, 64	D	Bovine, pasteurellosis
65	D	Ovine, pneumonia
66, 67	D	Human, wound
68 to 74 inclusive	E	Bovine, hemorrhagic septicemia

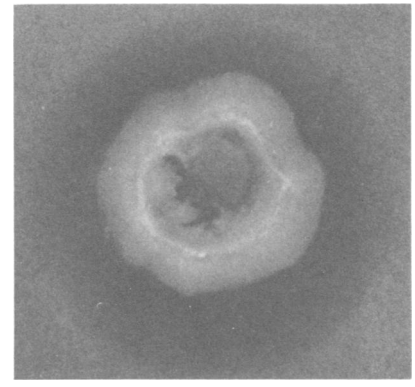

FIG. 2. *Plate medium containing sodium hyaluronidate. Clear zone around P. multocida is due to hyaluronidase production.*

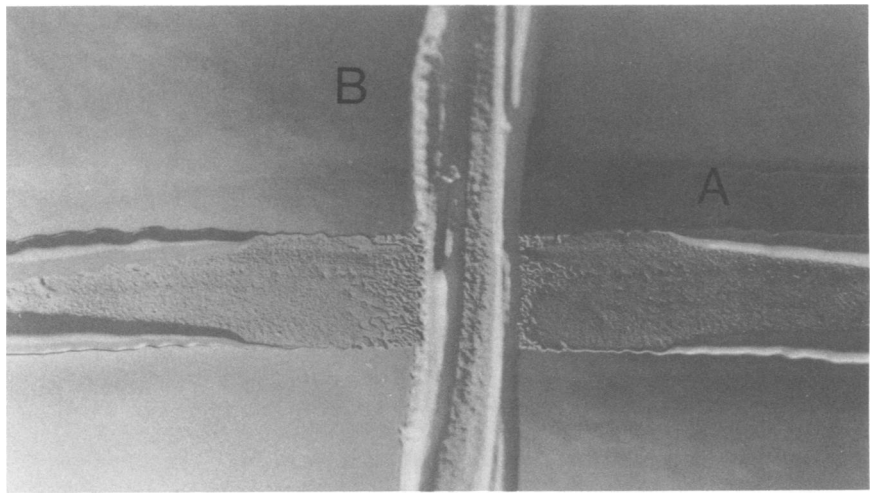

FIG. 1. *Diminution of hyaluronic acid capsules of S. equi (A) due to hyaluronidase production by adjacent growth of P. multocida (B).*

idase activity. The type B culture no. 14 did not produce hyaluronidase.

The fact that only the type B hemorrhagic septicemia cultures produced hyaluronidase is particularly interesting in that these cultures are known to be highly virulent. The bovine type B culture no. 14 which had not been associated with hemorrhagic septicemia and possessed a distinct somatic antigen did not produce hyaluronidase. It was surprising that the seven type E strains did not produce hyaluronidase in that it has been claimed that this serological variety causes hemorrhagic septicemia in cattle in Africa (9). However, before one can conclude that only the type B hemorrhagic septicemia cultures produce hyaluronidase, more type B and E cultures should be examined. Unfortunately, the type E cultures, which have only been isolated in Africa, are difficult to obtain. If it is ultimately confirmed that only the type B hemorrhagic septicemia strains produce hyaluronidase, this characteristic may be of value in their identification. Whether or not this enzyme has a role in the pathogenesis of hemorrhagic septicemia seems worthy of investigation.

The production of hyaluronidase by this gram-negative species demonstrates that this characteristic is not confined to gram-positive species. That it may be elaborated by other gram-negative species would seem very likely.

We gratefully acknowledge the assistance of Paul C. Watkins in the preparation of the figures and of Aung Myint in performing many of the tests. Some of the type B and E cultures were kindly provided by M. C. L. de Alwis (Sri Lanka) and M. T. H. Shigidi (Sudan).

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