Characteristics of a strain of *Clostridium carnis* causing septicaemia in a young infant

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SYNOPSIS  *Clostridium carnis* is a species which is only rarely isolated from man or animals and is occasionally found in the soil. This paper is an account of a single isolate found in blood cultures obtained from an 8-week-old boy who was suffering from gastroenteritis.

*Clostridium carnis* is a rare organism which is known to be an animal pathogen (Sompolinsky, 1950; Gianforte and Brown, 1958). In man it has occurred as a contaminant in war wounds (Hamilton, 1945; Greenberg, 1945; Lindberg, *et al*, 1955) and on occasions it has been recovered from the soil.

In this paper we describe the features of a strain isolated from blood cultures of an 8-week-old boy who had severe gastroenteritis.

**Clinical data**

The patient was an 8-week-old boy whose mother had awakened to find him suffering from diarrhoea and nearly moribund in his cot.

When admitted to hospital a few hours later he was dehydrated and in severe shock. The abdomen was not distended, bowel sounds were present although somewhat reduced, and it was estimated that he was 15% dehydrated. Rectal examination produced a quantity of pinkish, yellowish, watery faeces, some of which was submitted for bacteriological examination. Blood cultures were taken and inoculated into Trypticase soy medium and thioglycollate broth.

Biochemical data indicated that he was haemocentrated, severely acidotic, and hypernatraemic. The white blood cell count (WBC) was 26 000 (26% neutrophils, 16% band forms) and the haemoglobin was 10.2 g/dl. Gram-stained smears of the faeces showed many Gram-positive organisms, both bacilli and cocci, as well as the usual Gram-negative flora.

Fluid therapy was begun shortly after admission and cloxacillin, 200 mg/kg, carbenicillin, 200 mg/kg, and gentamicin, 4 mg/kg, per day were given intravenously. The following day a clostridium species was found in the anaerobic blood cultures and gentamicin therapy was discontinued. Although the child's condition had improved significantly, fluid therapy was continued for several more days. Barium studies of the gastrointestinal tract were carried out during the convalescent period and showed no abnormality. The patient was discharged well on the 20th day.

**Bacteriology**

After overnight incubation there was no growth in the aerobic blood culture but the thioglycollate broth contained quantities of gas and a heavy growth of Gram variable bacilli. Unfortunately the faeces were not cultured anaerobically so that these organisms were not recognized in this material.

Subcultures made on blood agar, McConkey agar, and an egg yolk half-antitoxin Nagler plates were incubated under aerobic and, where appropriate, anaerobic conditions (Gaspak—BBL, Division of Becton, Dickinson and Co, Cockeysville, MD 21030, USA). Aerobic cultures on horse-blood agar produced small (0-5-1·0 mm) dome-shaped colonies after 48 hours' incubation. After overnight incubation the anaerobic blood agar plate showed isolated, non-haemolytic, flattened colonies with well-defined outgrowths from the well of the inoculum. The egg yolk agar plate, incubated anaerobically for 48 hours, showed only a light growth with no evidence of a lecithinase activity. Growth on MacConkey agar showed the yellowish fluorescent growth characteristic of many clostridia (Willis, 1969).

Smears from anaerobic cultures on blood agar showed that it was a Gram-positive bacillus with.
oval subterminal spores, and electron micrographs showed peritrichate flagella.

When grown anaerobically in peptone water (BBL), the organism produced acid from glucose, lactose, sucrose, salicin, maltose, xylose, and arabinose, but not from raffinose, trehalose, mannitol, dulcitol, adonitol, inositol, glycerol or sorbitol. It split aesculin but not arbutin, and there was no reaction with starch or inulin, but acid was produced in litmus milk. H2S or indole was not produced and no urease activity was demonstrated. Gelatin was not hydrolysed and there was no digestion of cooked meat. No haemolysin was demonstrated on horse-blood agar. Nitrates were not reduced when the organism was grown in inodle nitrate broth (BBL).

On the basis of these findings we have classified this organism as Clostridium carnis. Because of the rarity of the organism, it was referred to the National Collection of Type Cultures (NCTC), Colindale, where it was examined by Mr K. Phillips and later referred to Dr A. T. Willis, both of whom confirmed these results. The strain has now been added to the Collection under the number NCTC 10913.

Discussion

The microaerophilic clostridia include Clostridium histolyticum, Clostridium tertium, and Clostridium carnis. Of these, Clostridium histolyticum is proteolytic and thus easily classified; the other two are not and are difficult to distinguish from each other. Key points on which we based the classification of this strain were its inability to reduce nitrates to nitrites (Sompolinsky, 1950) and its failure to ferment starch, mannitol, and sorbitol (Buchanan and Gibbons, 1974).

According to some authors, it produces subterminal oval spores (Cowan and Steel, 1965; Holdeman and Moore, 1972). Others, however, indicate that the spores are terminal (Prevot, 1966; Smith and Holdeman, 1972). Sompolinsky (1950), reporting on his original observations, noted that both types of spores occurred although terminal ones are more frequent.

Recently, Smith and Holdeman (1972) have drawn attention to the value of gas chromatography of fermentation end products for the classification of the clostridia. As yet there are few data on the classification of Clostridium carnis by this method, and the distinction between the two organisms does not appear to be well-defined.

The nomenclature for Clostridium carnis is confusing. Bergey's Manual (Breed et al, 1957) appears to follow Hall and Duffett (1935) and traces the organism back to von Hibler (edited by Hall and Duffett (1935)) who isolated a clostridium from a rabbit after inoculating it with garden soil named it 'bacillus VI'. Klein (1904) recovered another organism from putrefying meat and called it, appropriately enough, Bacillus carnis. Hall and Duffett (1935), who were able to study cultures from the collection of these early workers, concluded that the two organisms were the same and proposed the name Bacillus carnis. Prevot (1966), on the other hand, has cautioned against accepting this view and regards Plectridium carnis of his classification as synonymous with B. carnis (Klein, 1904) but not with bacillus VI (von Hibler, 1899). While Prevot's classification is not universally accepted, there now seems to be agreement that the Clostridium carnis of the modern Anglo-American literature and Plectridium carnis of the French literature are synonymous (Buchanan and Gibbons, 1974).

Previous isolates of Clostridium carnis have come from various sources. Prevot (1966) records that it was found in samples of soil collected in the Ivory Coast, and Lindberg et al (1955) found it in a number of soil samples collected in Korea.

If the doubt about von Hibler's bacillus is accepted, then the earliest report of its isolation from animals is probably that of Sompolinsky (1948), who found it in mink dying on a mink farm in Denmark. Since then, Gianforte and Brown (1958) have reported a similar outbreak in the United States, and Lord and co-workers (1956a, b) have suggested that it is the aetiological agent for Errington's disease, a condition which has been described in muskrats. Experimental work (Sompolinsky, 1950) has shown that it is pathogenic for many animals, including axolotl, frog, sparrow, fox, cat, dog, mink, ferret, rabbit, and mouse.

So far the present case appears to be the only one in which Clostridium carnis was isolated in pure culture from human blood although there are several previous reports of its isolation from battle injuries, particularly those caused by explosive missiles (Greenberg, 1945; Hamilton, 1945, Lindberg, et al, 1955). In all of these, the organism was isolated along with other clostridia, and until now there has always been ground for questioning its pathogenicity for man.

We thank Miss Candace MacDonald for her studies of the original isolate; Dr S. P. Lapage and Mr K. D. Phillips, of the National Collection of Type Cultures, and Dr A. T. Willis and Mrs Kate Williams, of the Luton Public Health Laboratory, for their confirmatory studies; Mr J. Boutlier for the electron micrograph; Dr A. G. Stewart for helpful comments; and Miss Marlene Ward for secretarial services.
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doi: 10.1136/jcp.29.11.1011

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