

Free-sporing *Cl. welchii* in ordinary laboratory media and conditions

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SUMMARY A strain of *Clostridium welchii* produced spores in ordinary blood agar plates. Investigations confirmed that it was the character of this particular strain and that the laboratory media were not inducing sporulation. During a period of 12 months a total of 100 strains of *Cl. welchii* were studied. None of them produced spores in ordinary laboratory media and conditions when examined microscopically.

The general statement that *Clostridium welchii* produces spores in the gut but not in wounds or in routine media applies mainly to type A, which is chiefly associated with human disease (Willis, 1969). When a sporing bacillus is seen in clinical material or a culture it is assumed that a different species of clostridium is present (either alone or in combination with *Cl. welchii*). This paper describes a strain that did not support this generally accepted belief.

The work was initiated by a surprise finding in a practical demonstration class. Students were given cultures of various clostridia, including *Cl. welchii*, for making Gram and spore stains. After staining many of the students reported finding spores from cultures of *Cl. welchii*. This raised the following questions: (1) Were they spores? (2) If they were real spores was the organism *Cl. welchii*? (3) Did the laboratory medium contain any agent that stimulated spore formation of *Cl. welchii*? The following investigations were done to find the answers to these questions.

Material and methods

From one of the culture plates given to the practical class five colonies were carefully selected and two smears were made on separate clean glass slides from each colony. One smear was Gram stained and the other was stained for spores with malachite green and safranin.

For identification inoculations were made from each of the five colonies into cooked meat media,

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which were incubated at 37°C overnight. Subcultures were then made from these on to blood agar plates, incubating anaerobically at 37°C. A separate inoculated blood agar plate was also incubated aerobically to check that there was no growth in the presence of oxygen. Complete identification of the organism was made by the Nagler reaction on egg-yolk agar and sugar reactions (Cowan, 1974; Willis, 1969). Gram-staining and staining for spores was again done.

To check for any agent in the laboratory media that might have stimulated spore formation a few other strains of *Cl. welchii* were grown in parallel with the original strain (strain O) in identical conditions of media, temperature, and time. Ten different strains of *Cl. welchii* were isolated from different stool specimens, which were selected randomly without knowing the clinical condition of the patients from whom specimens were received. All these new strains and strain O were then sub-cultured on to blood agar plates, incubated anaerobically at 37°C overnight, and examined in the morning. Selected colonies from each culture were then Gram-stained and stained for spores.

Results

Five colonies from the culture plate given in the class showed Gram-positive rods. Many of them had unstained oval areas, most of which were situated subterminally. Spore staining showed spores as green oval areas on the subterminal or terminal positions of red-stained rods. Staining of a few more subcultures from these colonies showed similar spores. When incubation was continued up to the second or the third day abundant free spores

could be seen in the slides after Gram or spore staining.

Colonies on blood-agar were raised, entire, smooth, and haemolytic. Nagler test and sugar reactions identified the strain O to be a *Cl. welchii*. When subcultured in parallel with 10 other strains of *Cl. welchii* in identical conditions strain O continued to show spores while other strains did not. This proved that the laboratory media were not inducing spore formation.

Strain O was then sent to Dr A. T. Willis, Director of the Public Health Laboratory, Luton, to confirm the finding and for typing. He identified the strain as *Cl. welchii* of toxicological type A and confirmed that it was sporulating freely in the ordinary laboratory media.

Further studies

After completing this investigation another 90 strains of *Cl. welchii* were examined over a period of 12 months. Ten of them were isolated from post-operative wounds and the rest from faeces. They were microscopically examined for spores in ordinary laboratory media in conditions exactly similar to those in the earlier investigation. None showed any spores. Thus a total of 100 strains of *Cl. welchii* (including 10 strains which were examined in parallel with strain O) were examined and none showed spores in ordinary laboratory media and conditions.

Discussion

Others have tried to induce sporulation of *Cl. welchii* in various special media (Gibbs and Hirsch,

1956; Ellner, 1956). Rapid and abundant sporulation of *Cl. welchii* was observed by Ellner (1956) in a medium containing peptone, yeast extract, magnesium sulphate, and starch; but profuse sporulation in ordinary laboratory media as produced by this particular strain (strain O) has not been reported. None of the other 100 strains examined showed any spores. Thus the general statement that *Cl. welchii* rarely produces spores in ordinary laboratory media cannot be discredited by the finding of a single atypical strain. Nevertheless, clinical laboratory workers should be aware of the possibility of finding an uncommon character in an otherwise typical strain.

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References

- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edition. Cambridge University Press, London.
- Ellner, P. D. (1956). A medium promoting rapid quantitative sporulation in *Clostridium perfringens*. *Journal of Bacteriology*, **71**, 495-496.
- Gibbs, B. M., and Hirsch, A. (1956). Spore formation by *Clostridium* species in an artificial medium. *Journal of Applied Bacteriology*, **19**, 129-141.
- Willis, A. T. (1969). *Clostridia of Wound Infection*. Butterworth, London.