THE USE OF CEPHALOTHIN AND TRIPHENYLTERAZOLOM CHLORIDE IMPREGNATED FILTER PAPER STRIPS IN THE IDENTIFICATION OF CAMPYLOBACTER SPECIES

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ABSTRACT


Filter paper impregnated strips using cephalothin at 30 and 60 µg/ml and triphenyltetrazolium chloride at 20 mg/ml were prepared and used in the typing of catalase-positive Campylobacter species. There was no variation in sensitivity of campylobacters to cephalothin at 30 µg/ml and 60 µg/ml. Results were as reported by other workers except for a C. jejuni strain which was resistant to the triphenyltetrazolium. The technique is nevertheless inexpensive and the results are consistent and easy to interpret.

INTRODUCTION

Catalase-positive campylobacters are important pathogens in both human and veterinary medicine (Roop, Smibert, Johnson & Krieg, 1984). C. fetus is the cause of an important venereal disease of cattle. The correct identification and typing of catalase-positive campylobacters is based, to a large extent, on physiological characteristics, amongst which are sensitivity tests to cephalothin and triphenyltetrazolium. The technique is nevertheless inexpensive and the results are consistent and easy to interpret.

Materials and Methods

Media

The media used for the trial were serum dextrose agar (SDA) (Corbel, Gill & Thomas, 1983) and brucella agar with 10% sterile citrated horse blood (BBL). The media was poured into standard 90 mm diameter Petri dishes to give a final agar depth of 3 mm.

Sensitivity strips

TTC² strips were made by soaking strips of grade 140 g/m² filter paper² (approximately 4 x 20 mm) in a TTC solution (20 mg/ml) for 1 h, then drying and autoclaving the strips as described by Lander & Gill (1985). Cephalothin¹ was made up in sterile solutions at 60 µg/ml and 30 µg/ml. The filter paper strips (approximately 4 x 12 mm) were soaked in these solutions, hung up to dry in a sterile cabinet and stored in sterile glass bottles. The strips were then incubated for 48 h on BBL to check for contaminants. At no stage were the cephalothin strips autoclaved. Elongated filter strips instead of discs were used in order that costs could be even further reduced by testing a series of isolates across a single strip as described by Lander & Gill (1985).

Strains used

Reference strains NCTC 10842 (Campylobacter fetus fetus), NCTC 1980 (Campylobacter fetus venerealis), NCTC 1284 (Campylobacter fetus venerealis bio intermedius) were used. Also included were isolates typed at the Veterinary Research Institute, Onderstepoort, using the methods described by Roop et al. (1984), as Campylobacter coli, C. jejuni, C. faecalis, C. f. venerealis, C. f. v. intermedius (Table 1).

Culture technique

Seventy-two hour cultures of each strain were harvested from the brucella agar plus blood plates and suspended in phosphate buffered saline (pH 7.2) to a density of 10⁶ organisms/ml which was determined at this laboratory to be in the region of Unigalvo² 90. One hundred µl of the suspension was dropped onto each of a plate of SDA and BBL, spread evenly with a glass spreader and allowed to dry. A 30 µg and 60 µg cephalothin strip as well as a TTC strip was put onto each plate, equidistant from each other (Fig. 1 & 2).

Each strain was tested in quadruplicate on 2 separate occasions. All cultures were incubated in an atmosphere containing 5% O₂, 10% CO₂ and 85% N₂ at 37 °C (Smibert, 1978), and examined at 48 h and 96 h. The growth after 96 h was uniformly dense as can be seen on the photographs (Fig. 1 & 2).

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¹ Biolab Chemicals, P.O. Box 15849, Lynn East 0039
² Tetrazolium salt. BDH Chemicals Ltd., Poole, England
³ Filter paper 140 g/m². Penpoint Stationers, P.O. Box 1457, Pretoria 0001
⁴ Keflin, Eli Lilly (S.A.) (Pty) Ltd.
⁵ Diffusion Systems Ltd., 43 Rosebank Road, London W7, England

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**FIG. 1** C. fetus fetus inhibited by cephalothin and TTC on BBL plate

**FIG. 2** C. fetus fetus inhibited by cephalothin and TTC on SDA plate

**TABLE 1** Sensitivity of various Campylobacter strains in the presence of cephalothin (30 µg and 60 µg/ml) and TTC strips (20 mg/ml)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Species</th>
<th>SDA 30 µg</th>
<th>SDA 60 µg</th>
<th>SDA TTC</th>
<th>BBL 30 µg</th>
<th>BBL 60 µg</th>
<th>BBL TTC</th>
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<tbody>
<tr>
<td>NCTC 10842</td>
<td>C. fetus fetus</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>NCTC 1980</td>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>NCTC 1284</td>
<td>C. fetus intermedius</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>7080</td>
<td>C. coli</td>
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<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
</tr>
<tr>
<td>702</td>
<td>C. jejuni</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>553/21</td>
<td>C. jejuni</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>AQ 350</td>
<td>C. jejuni</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>AQ 451</td>
<td>C. jejuni</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>5396</td>
<td>C. fetus</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>S</td>
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</tr>
</tbody>
</table>

*S = Sensitive (zone of inhibition more than 3 mm)

*R = Resistant (no zone of inhibition)

**Interpretation**

A strain was regarded as sensitive if a clear zone of inhibition of 3 mm or more was observed around the strip.

**RESULTS AND DISCUSSION**

There was no difference in sensitivity to cephalothin between the 30 or 60 µg/ml strips (Table 1). There was also no difference in the size of the zones of inhibition around the 2 strips (Fig. 1 and 2). There was no difference between the SDA and BBL plates. The NCTC cultures and the other cultures all reacted to both cephalothin and TTC as described by Roop et al. (1984) and Landers & Gill (1985) except for the culture typed as C. jejuni which was resistant to the TTC but gave a positive hippurate hydrolysis test as described by Harvey (1980).

The TTC strips tend to develop a pink colour about 2 days after they are autoclaved. This pink colour diffuses out into the medium. Where there is a zone of inhibition it tends to accumulate at the border of the zone of inhibition. This phenomenon can best be seen on the SDA plates (Fig. 2). Where there is no zone of inhibition, the pink colour tends to dissolve uniformly in the medium around the strip.

It is concluded that, although sensitivity to cephalothin and TTC are not important in the differentiation of the C. fetus strains, they are useful in differentiating C. fetus from other catalase-positive campylobacters. The use of the strips is also inexpensive and the results are easy to read and reproducible.

**REFERENCES**


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