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RESEARCH COMMUNICATION

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

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ABSTRACT

PEFANIS, S. M., VENTER, CATHARINA G. & HERR, S., 1989. The use of cephalothin and triphenyltetrazolium chloride impregnated filter paper strips in the identification of *Campylobacter* species. *Onderstepoort Journal of Veterinary Research*, 56 143–144 (1989).

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 difference in the 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 isolated from bulls, and cattle and sheep foetuses is of epidemiological importance. It is important to differentiate the *C. fetus* group of campylobacters from other catalase-positive campylobacters like *C. jejuni, C. coli* and *C. faecalis* which are also frequently isolated from bulls but do not cause venereal disease. The typing of the catalase-positive campylobacters is based, to a large extent, on physiological characteristics, amongst which are sensitivity tests to cephalothin and triphenyltetrazolium chloride (TTC) (Roop *et al.*, 1984; Lander & Gill, 1985). Although the use of cephalothin discs and TTC strips has been documented elsewhere (Roop *et al.*, 1984; Lander & Gill, 1985), this laboratory has always used media with cephalothin and TTC incorporated into the media at 60 µg/mℓ and 20 mg/mℓ respectively. The media is expensive to make up and the interpretation of the results was frequently subjective and difficult. It was thus decided to do a trial using filter paper impregnated with cephalothin and TTC, made up in the laboratory to compare the results reported by Roop *et al.* (1984) and Lander & Gill (1985) as well as to compare the ease of interpretation and repeatability of the results. As a matter of interest a comparison of the sensitivity of campylobacters to cephalothin at 30 µg/mℓ and 60 µg/mℓ was also made to see if there was any variation in sensitivity.

MATERIALS AND METHODS

Media

The media used for the trial were serum dextrose agar (SDA) (Corbel, Gill & Thomas, 1983) and brucella agar[1] 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

TTC[2] strips were made by soaking strips of grade 140 g/m^2 filter paper[3] (approximately 4 × 20 mm) in a TTC solution (20 mg/mℓ) for 1 h, then drying and autoclaving the strips as described by Lander & Gill (1985). Cephalothin[4] was made up in sterile solutions at 60 µg/mℓ and 30 µg/mℓ. The filter paper strips (approximately 4 × 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 10^9 organisms/mℓ which was determined at this laboratory to be in the region of Unigalvo[5] 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 % O_2, 10 % CO_2 and 85 % N_2 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/m^2. 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

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

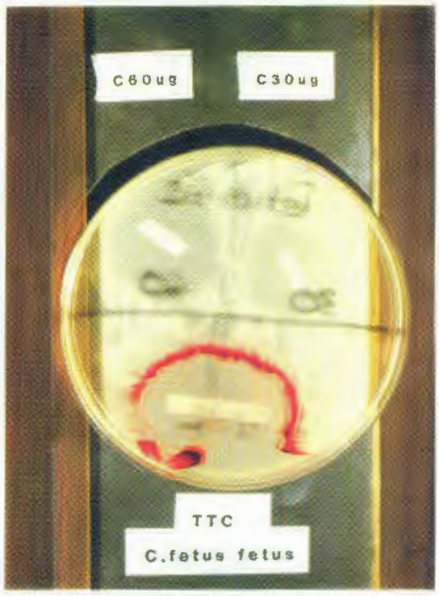

FIG. 2 *C. fetus fetus* inhibited by cephalothin and TTC on SDA plate

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

Strain	Species	SDA			BBL		
TTC		30 µg	60 µg	TTC	30 µg	60 µg	TTC
NCTC 10842	*C. fetus fetus*	S	S	S	S	S	S
NCTC 1980	*C.f. venerealis*	S	S	S	S	S	S
NCTC 1284	*C.f.v. intermedius*	S	S	S	S	S	S
7080	*C. coli*	R	R	R	R	R	R
702	*C. jejuni*	R	R	R	R	R	R
553/21	*C. faecalis*	S	S	R	S	S	R
AQ 390	*C.f. venerealis*	S	S	S	S	S	S
AQ 451	*C.f.v. intermedius*	S	S	S	S	S	S
5396	*C.f. fetus*	S	S	S	S	S	S

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.

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