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RESEARCH COMMUNICATION

# THE USE OF SENSITIVITY DISCS IN THE IDENTIFICATION OF *CAMPYLOBACTER* SPECIES

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ABSTRACT

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Filter paper discs were impregnated with a solution containing 20 mg of triphenyltetrazolium chloride per millilitre, and used in the typing of catalase-positive *Campylobacter* species. Also used were filter paper discs impregnated with cephalothin at 30 µg/mℓ, 60 µg/mℓ and 3 mg/mℓ and nalidixic acid at the same concentrations, as well as commercially available discs containing 30 µg of, respectively, cephalothin and nalidixic acid. Results obtained proved the technique to be reliable and easier to interpret than previously used methods, and laboratory prepared filter paper discs compared favourably with commercial discs.

## INTRODUCTION

Catalase-positive *Campylobacter* species are important pathogens in both human and veterinary medicine (Roop, Smibert, Johnson & Krieg, 1984). Epidemiological studies are directly dependent on the correct identification of catalase-positive campylobacters isolated from the bovine prepuce, and bovine and ovine aborted foetuses. Thus, it is important to differentiate the *C. fetus* subspecies from one another, and from other catalase-positive campylobacters such as *C. jejuni, C. coli* and *C. fecalis*. *C. fetus* is associated with bovine infertility, abortions and endometritis (Garcia, Eaglesome & Rigby, 1983). *C. fetus* subspecies *fetus* is an important cause of ovine abortions. *C. jejuni,* while capable of causing abortions in sheep, does not cause venereal disease in cattle. Nevertheless, it is frequently isolated from cattle, as is *C. fecalis,* a non-pathogenic catalase-positive *Campylobacter* (Garcia *et al.,* 1983).

Sensitivity to triphenyltetrazolium chloride (TTC), nalidixic acid and cephalothin are amongst the tests on which the typing of catalase-positive campylobacters is based (Roop *et al.*, 1984; Lander & Gill, 1985). They are useful in the differentiation of *C. fetus* from other catalase-positive campylobacters. At Onderstepoort, these tests have always been performed by assessing the growth of the campylobacters on media containing 20 mg/mℓ of TTC, 60 µg/mℓ of cephalothin and 60 µg/mℓ of nalidixic acid. These media are expensive and the interpretation of the results is often subjective.

The use of nalidixic acid discs, cephalothin discs and strips, and TTC strips have been described (Roop *et al.,* 1984; Lander & Gill, 1985; Pefanis, Venter & Herr, 1989). A trial was undertaken to compare results obtained with commercial and laboratory prepared filter paper discs at different concentrations with both cephalothin and nalidixic acid. The trial also introduced the use of laboratory prepared TTC discs. The purpose was to assess the reliability and usefulness of the method in the identification of *Campylobacter* species.

## MATERIALS AND METHODS

### *Media*

The only medium used during the trial was tryptose agar[1] with 10 % sterile citrated horse blood (BTA). The medium was poured into standard 90 mm diameter Petri dishes to give a depth of 3 mm once solidified. Four plates were used for each *Campylobacter* strain.

### *Discs*

Large strips of grade 140 g/m$^2$ filter paper[2] were soaked in sterile solutions of TTC[3] (20 mg/mℓ), cephalothin[4] (30 µg/mℓ, 60 µg/mℓ and 3 mg/mℓ) and nalidixic acid[5] (30 µg/mℓ, 60 µg/mℓ and 3 mg/mℓ) for approximately 2 min and allowed to dry in a sterile cabinet. Discs with a 5,5 mm diameter were then cut out of the strips. TTC and nalidixic acid discs were autoclaved at 121 °C for 15 min in glass bottles, and cephalothin discs were stored in sterile glass bottles without being autoclaved. All the discs were stored at 4 °C and a representative sample was incubated at 37 °C for 48 h on BTA to check for contaminants. Commercial discs[6] containing 30 µg of cephalothin and 30 µg of nalidixic acid were also used.

### *Strains*

Reference strains NCTC 10842 (*C. fetus fetus*), NCTC 1980 (*C. fetus venerealis*), NCTC 1284 (*C. fetus venerealis* biotype *intermedius*), *C. jejuni* biotype 1 strain 702 and *C. coli* strain 7080 were used. One isolate that typed as *C. fecalis* at the Veterinary Research Institute, Onderstepoort, using the methods described by Roop *et al.* (1984) was also used.

### *Culture techniques*

Seventy-two hour cultures of each strain were harvested from BTA plates and suspended in phosphate buffered saline (PBS; pH 7,2) to a density of $10^9$ organisms per millilitre. One hundred µℓ of the suspension was placed onto each of 4 BTA plates, spread evenly over the whole surface of the plates with a glass spreader and allowed to dry. TTC, cephalothin (3 mg/mℓ) and nalidixic acid (3 mg/mℓ) filter paper discs, as well as cephalothin and nalidixic acid commercial discs were placed onto each of 2 plates (Fig. 1 & 2). Two cephalothin (30 and 60 µg/mℓ) and 2 nalidixic acid (30 and 60 µg/mℓ) filter paper discs were placed onto each of the other 2 plates. Cultures were incubated at 37 °C in an atmosphere containing 5 % $O_2$, 10 % $CO_2$ and 85 % $N_2$

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[1] Difco Laboratories, Detroit, Michigan, USA

[2] Penpoint Stationers, P.O. Box 1457, Pretoria 0001

[3] Tetrazolium salt, BDH Chemicals Ltd, Poole, England

[4] Keflin, Eli Lilly (S.A.) (Pty) Ltd

[5] Sigma Chemical Company, St. Louis, USA

[6] Oxoid Ltd, Basingstoke, Hampshire, England

TABLE 1 Sensitivity of various *Campylobacter* strains in the presence of TTC, cephalothin and nalidixic acid discs

| Strain | Species | TTC discs 20 mg/mℓ | Cephalothin discs (30 µg and 3 mg/mℓ) | Nalidixic acid discs (30 µg and 3 mg/mℓ) |
|---|---|---|---|---|
| NCTC 10842 | *C. fetus fetus* | S | S | R |
| NCTC 1980 | *C. f. venerealis* | S | S | R |
| NCTC 1284 | *C.f.v. intermedius* | S | S | R |
| 702 | *C. jejuni* | S | R | S |
| 7080 | *C. coli* | R | R | S |
| 553/21 | *C. fecalis* | R | S | R |

S = Sensitive (clear zone of inhibition of at least 3 mm)
R = Resistant (no zone of inhibition)

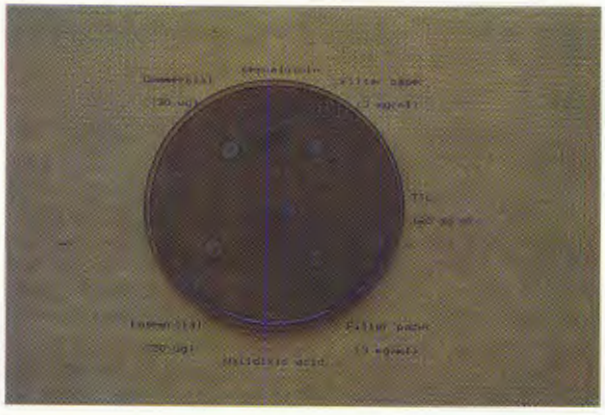

FIG. 1 *C. fetus fetus* inhibited by cephalothin and TTC discs on BTA

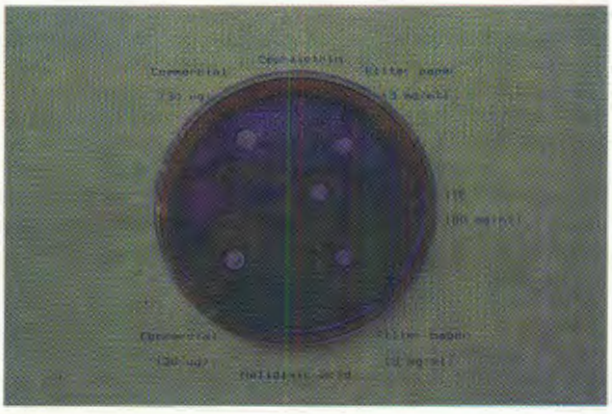

FIG. 2 *C. jejuni* inhibited by TTC and nalidixic acid discs on BTA

(Smibert, 1978), and examined after 48 and 96 h. After 96 h the growth was uniformly dense. Each strain was tested in duplicate on 3 separate occasions.

*Interpretation*

A clear zone of inhibition of at least 3 mm around a disc was regarded as indicative that the strain concerned was sensitive.

## Results and Discussion

The main reason leading to the present trial was the unsatisfactory results obtained with the *Campylobacter* typing methods presently used at Onderstepoort. Thus, all the *Campylobacter* strains tested in the past were found resistant to nalidixic acid when these methods were used. Equally, sensitivity tests to cephalothin and TTC have given inconsistent results, emanating from the subjective nature of their interpretation. The introduction of TTC and cephalothin impregnated filter paper strips by Pefanis *et al.* (1989) was an improvement on previously used methods, but left the nalidixic acid testing problems unsolved. On the other hand, the strips as described are difficult to standardise and cumbersome to work with. Thus, it was decided to perform all 3 tests by the use of discs, which are easier to standardise and less expensive to produce. Also, by being smaller than strips, discs can be more evenly spaced on the plates, giving clearer, more uniform and independent zones of inhibition. The 3 discs can be placed on a single plate for sensitivity testing of each *Campylobacter* isolate, therefore replacing 3 separate plates containing different media.

Good results were reported by Roop *et al.* (1984) and Lander & Gill (1985) when using discs containing 30 µg of either cephalothin or nalidixic acid. To obtain approximately the same reagent concentration on the laboratory prepared filter paper discs, 3 mg/mℓ solutions were used. It was calculated that each disc would absorb approximately 10 µℓ of solution, containing 30 µg of reagent (Ribeiro, unpublished data). The 30 and 60 µg/mℓ solutions were the same as those used by Pefanis *et al.* (1989) for the preparation of cephalothin impregnated filter paper strips.

The filter paper discs prepared with the 30 and 60 µg/mℓ cephalothin and nalidixic acid solutions gave poor results, the zones of inhibition being unclear, small or absent. When using the commercial discs, TTC discs, and the 3 mg/mℓ cephalothin and nalidixic acid filter paper discs, all *Campylobacter* strains used reacted as described by Roop *et al.* (1984) and Lander & Gill (1985) (Table 1). The zones of inhibition around the laboratory made filter paper discs were slightly smaller than those around the commercial discs, seen with both cephalothin and nalidixic acid. This may indicate that the filter paper discs contained less than 30 µg of the respective reagents. Nevertheless, the zones of inhibition were clear enough with both types of discs (Fig. 1 & 2). These results seem to indicate that the discs can be reliably used for the typing of *Campylobacter* species. The method is inexpensive and the results are easy to read.

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