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SOME CHARACTERISTICS OF CAMPYLOBACTER FETUS SUBSP. JEJUNI ISOLATED FROM PIGS, BIRDS AND MAN

By

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ROSEF, OLAV and MAGNE YNDESTAD: *Some characteristics of Campylobacter fetus subsp. jejuni isolated from pigs, birds and man.* Acta vet. scand. 1982, 23, 9—15. — One hundred and two strains of *Campylobacter fetus subsp. jejuni* isolated from pigs, various avian species and man were subjected to biochemical characterization including hydrolysis of hippurate, tolerance of 3.5 % saline, H₂S production in TSI medium, growth on TTC agar and antibiotic sensitivity. On this basis the porcine strains could be classified into 4 groups, while the avian and human strains could each be divided into 2 groups. The avian and human strains had the same biochemical characteristics as 2 of the porcine groups.

Campylobacter; biochemical characteristics;
pig; bird; man.

The thermophilic microaerophilic *Vibrio* which do not grow at 25°C have been classified by *King* (1957) as "related vibrios". *Campylobacter* was classed as a genus distinct from *Vibrio* by *Sebald & Véron* (1963). The thermophilic *Campylobacter* were later divided into 2 main groups, *Campylobacter coli* and *Campylobacter jejuni* (*Véron & Chatelain* 1973). *Smibert* (1978), however, reunited these 2 groups as *Campylobacter fetus subsp. jejuni*. *Skirrow & Benjamin* (1980a) classified the enteropathogenic thermophilic *Campylobacter* into 4 biotypes, namely NARTC, *Campylobacter jejuni* biotype 1 and 2, and *Campylobacter coli*.

Campylobacter fetus subsp. jejuni has been reported in recent years as a cause of enteritis both in animals (*Hastings* 1978) and man (*Skirrow* 1977), and several cases of transmission from the animal population to humans have been described (*Svedhem*

& Norkrans 1980, Skirrow *et al.* 1980). Pigs, poultry and wild birds are considered to be the natural reservoir since the bacterium has been isolated from a large number of clinically healthy animals (Jørgensen 1979, Grant *et al.* 1980, Rosef 1981).

It is therefore of epidemiological significance to investigate whether *Campylobacter* isolated from healthy animals are distinguishable from the strains isolated from humans with clinical symptoms of *Campylobacter* infection.

In the present investigation, a comparative study was carried out to determine whether strains isolated from animals and man could be classified into groups, and whether there were any biochemical differences between the strains isolated from the faeces of human patients with diarrhoea, and those isolated from clinically healthy animals.

MATERIAL AND METHODS

A total of 102 isolates of *Campylobacter* was examined. These included 52 strains from pigs, 30 from various birds and 20 human strains. The latter were isolated from patients with diarrhoea, while the animal strains were isolated from clinically healthy animals.

The ability to grow under microaerophilic conditions (5 % O₂, 10 % CO₂, 85 % N₂) was assessed by incubation at 25°C (5 days), 30.5°C (3 days) and 45.5°C (48 h). In addition, the isolates were incubated at 42°C for 48 h under both aerobic and anaerobic conditions.

The catalase reaction was tested on microscope slides with 1 drop of 3 % H₂O₂, while the oxidase reaction was carried out on filter paper with a 1 % aqueous solution of N.N-dimethyl-p-phenylene diamino HCl.

H₂S production and saccharolytic activity were recorded in TSI medium (Triple Sugar Iron).

The ability to grow in a 3.5 % salt (NaCl) solution was determined using the medium described by Rosef (1981), modified by removing the antibiotic components and adjusting the salt concentration. Hydrolysis of hippurate was determined by the rapid method described by Hwang & Ederer (1975) with the modifications of Skirrow & Benjamin (1980a).

The *Campylobacter* strains were tested for growth on blood agar containing 2,3,5 triphenyltetrazolium chloride (TTC) at a concentration of 400 µg/ml agar.

Sensitivity testing was carried out using discs (Neo-Sensitabs Roscoe) which contained 130 µg nalidixic acid, 78 µg erythromycin, 100 µg streptomycin and 16 µg metronidazole. The bacteria were added to physiological saline and spread on blood agar plates which were left to dry for ½ h before incubation. *Campylobacter* showing inhibition zones of ≥ 28 mm diameter after 24 h incubation at 37°C were classed as sensitive.

RESULTS

The strains investigated did not cause haemolysis of bovine blood after incubation under microaerophilic conditions at 42°C for 2 days. Some of the strains gave bacterial colonies with a metallic lustre to the surface. The bacteria produced a yellow to yellow-brown pigment which could be seen by taking a generous amount of the culture on an inoculation needle. The bacteria had a characteristic smell. The strains did not grow aerobically or at 25°C under microaerophilic conditions. The anaerobic growth consisted of very small colonies.

Incubation at 30.5°C resulted in growth of 67.3 % of the porcine strains, 33.3 % of the avian and 50 % of the human strains. At 45.5 °C these figures were 76.9 %, 43.3 % and 20 %, respectively.

None of the isolates grew in 3.5 % saline or showed saccharolytic activity in TSI medium. All the strains investigated were catalase positive. Table 1 shows some other important characteristics of the tested strains of *Campylobacter fetus* subsp. *jejuni*. All were sensitive to erythromycin, whereas streptomycin-resistant strains were found predominantly in pigs (69.3 %). Four

Table 1. Some characteristics of *Campylobacter fetus* subsp. *jejuni* isolated from pigs, birds and man in Norway.

	Number of strains	Growth and biochemical reactions				Number of sensitive strains			
		TTC	Hipp.	Ox.	H ₂ S	Nal.	Eryt.	Metr.	Str.
Pigs	52	49	1	51	2	50	52	51	16
Birds	30	30	26	30	0	29	30	16	20
Man	20	20	15	20	0	20	20	16	17

TTC = growth on 2,3,5, triphenyltetrazolium chloride agar, Hipp. = splitting of hippurate, Ox. = oxidase reaction, H₂S = production of H₂S in TSI medium, Nal., Eryt., Metr., Str. = sensitive to nalidixic acid, erythromycin, metronidazole and streptomycin.

(13.3 %) of the avian strains and 3 (15 %) of the human strains were resistant to streptomycin.

Sensitivity testing for metronidazole revealed that 1 of the 52 porcine strains was resistant, whereas 14 (46.6 %) of the avian strains and 3 (15 %) of human strains showed resistance. One of the avian strains was, in addition, resistant to nalidixic acid.

All the tested strains were oxidase positive with the exception of 1 porcine strain. In addition, this strain did not grow on TTC agar. Two of the porcine strains were H₂S positive on TSI medium; these did not grow on TTC agar. On bloodagar and Skirrow's medium, they produced smaller and less glossy colonies than the other *Campylobacter* tested. They were resistant to nalidixic acid. Whereas only 1 of the 52 tested porcine strains split hippurate, the corresponding figure for all the strains isolated from birds (crows, pigeons, turkeys and hens), was 26 (83.3 %). However, when the bird species were considered separately it was found that 3 of the 4 turkey strains, and 1 of the 15 strains from hens, were not capable of hydrolysing hippurate. The above biochemical characteristics enabled the bacteria to be grouped as shown in Table 2. The strains in Groups I and II were isolated from pigs, birds and man. Group I strains were predominantly found in pigs, whereas strains from birds

Table 2. Grouping of *Campylobacter fetus* subsp. *jejuni* isolated from pigs, birds and man, based on a range of biochemical characteristics.

	Number of strains	TTC	Hipp.	Ox.	H ₂ S	Nal.
<i>Pigs</i>						
Group I	48	48	0	48	0	48
Group II	1	1	1	1	0	1
Group III	1	0	0	0	0	1
Group IV	2	0	0	2	2	0
<i>Birds</i>						
Group I	4	4	0	4	0	4
Group II	26	26	26	24	0	25
<i>Man</i>						
Group I	5	5	0	5	0	5
Group II	15	15	15	15	0	15

For explanation of abbreviations, see Table 1.

and man were found largely to fall within Group II. Three *Campylobacter* strains isolated from pigs were found to have biochemical characteristics that placed them in 2 additional groups (III and IV).

DISCUSSION

According to *Butzler & Skirrow* (1979), 2 biotypes of *Campylobacter fetus* subsp. *jejuni* can be distinguished, one being associated with pigs and the other with wild birds. The porcine strains are said to grow at 30.5°C and to be resistant to TTC. In our investigation, 17 (22.7 %) of the strains isolated from pigs did not grow at this temperature. Three strains (5.8 %) were sensitive to TTC, and 1 of these was oxidase negative. These strains differed from those described by *Butzler & Skirrow*.

The frequency of streptomycin-resistance was greater among the porcine strains than among those from birds and man, a finding which may be explained by the fact that, in Norway, it is common practice to treat diarrhoea in pigs with dihydrostreptomycin.

Swine dysentery is treated with metronidazole, which is active against anaerobic bacteria (*Treponema hyodysenteriae*). *Fernie et al.* (1977) undertook sensitivity testing for metronidazole and found that 44 *Campylobacter* strains isolated from pigs were all sensitive. In the present study, 1 of the 52 strains was resistant. In England *Skirrow & Benjamin* (1980b) found that 85 % of *Campylobacter* strains from pigs were resistant to metronidazole. They associated this high frequency with the widespread use of nitromidazoles. The avian and human strains in our investigation showed a higher frequency of resistance than those from pigs.

Butzler & Skirrow have described a biotype of thermophilic *Campylobacter* associated with wild birds, especially seagulls. This biotype grew at 45.5°C, was resistant to nalidixic acid, metronidazole and to NaCl but sensitive to TTC. This biotype did not split hippurate. *Skirrow & Benjamin* (1980a) referred to this as the NARTC biotype.

In the present investigation, 1 of the strains isolated from hens was resistant to nalidixic acid, but the fact that this strain was resistant to TTC, sensitive to metronidazole and split hippurate, distinguishes it from the NARTC strain described above.

Erythromycin is recommended for use against human Cam-

pylobacter infections (*Butzler & Skirrow*). Reports from several countries, however, have revealed resistant strains in up to 10 % of human isolates (*Walder & Forsgren 1978*). Erythromycin-resistant strains were not found in the present study. The rapid hippurate hydrolysis test was used by *Harvey (1980)* to distinguish between *Campylobacter fetus* subsp. *jejuni* and *Campylobacter fetus* subsp. *intestinalis*. The former is said to hydrolyse hippurate, while the latter does not. *Skirrow & Benjamin (1980a)* used, among other tests, the hippurate hydrolysis test to divide the thermophilic *Campylobacter* into 4 biotypes. In the present investigation, only 1 of the porcine strains of *Campylobacter fetus* subsp. *jejuni* was able to split hippurate, whereas the majority of the avian and human strains did so.

In this study, 2 groups of *Campylobacter* found in pigs differed from the 4 biotypes described by *Skirrow & Benjamin (1980a)*. In one of these groups the bacteria grew more slowly and with less mucoid colonies (Table 2, Group IV). They were probably isolated because a special enrichment technique was employed (*Rosef*). These strains are probably lost when inoculating directly onto selective agar plates. The oxidase negative strain (Table 2, Group III) did not differ from the other *Campylobacter* bacteria in its mode of growth.

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SAMMENDRAG

Noen karakteristika til *Campylobacter fetus* subsp. *jejuni* isolert fra gris, fugl og menneske.

Det ble foretatt en undersøkelse av noen egenskaper til 102 stammer *Campylobacter fetus* subsp. *jejuni* isolert fra gris, fugl og menneske. Undersøkelsen omfattet hippurat hydrolyse, 3,5 % salttoleranse, dannelselse av H₂S og spalting av sukker i TSI-mediet, vekst på TTC-agar og antibiotikasensitivitetstester. Ut fra de påviste egenskaper kunne stammer fra gris plasseres i 4 grupper. Stammene isolert fra fugl og menneske kunne inndeles i 2 grupper. De 2 gruppene fra fugl og menneske hadde de samme egenskaper som 2 av gruppene fra gris.

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