Ribosomal RNA Gene Restriction Fragment Diversity amongst Penner Serotypes of *Campylobacter jejuni* and *Campylobacter coli*

S. I. Smith[a], D. K. Olukoya[a], A. J. Fox[b] and A. O. Coker[c]

[a] Genetics Division, Nigerian Institute of Medical Research, P. M. B. 2013, Yaba, Lagos
[b] Public Health Lab, Withington Hospital, Nell Lane, Manchester, M20 2LR
[c] Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, P. M. B. 12003, Idi-Araba, Lagos

Z. Naturforsch. **53c,** 65–68 (1998); received June 2/September 29, 1997

Ribotypes, *Campylobacter*, Hae III, Ribosomal RNA and Penner Serotype

Diversity based on ribosomal RNA gene-restriction endonuclease digest patterns was detected amongst forty-seven strains of *Campylobacter* made up of 38 strains of *Campylobacter jejuni* and 9 strains of *Campylobacter coli.* Restriction digests of chromosomal DNA prepared by treating with Hae III were probed with an oligonucleotide specific for *Campylobacter* 16S ribosomal RNA genes. Seventeen distinct hybridization patterns, each indicating the presence of 2–4 copies of the 16S rRNA gene are encoded in *Campylobacter* DNA. Differences in fragment patterns were observed not only between members of two species, but also between individual strains of the same species. Ribopattern fragments of 8.71, 7.56, 2.81 and 1.0 kb were characteristic of the majority of *C. jejuni*, whereas 7.59 and 4.68 kb fragments were commonly present in *C. coli.*

In conclusion, Hae III ribotyping was even more discriminatory than the Penner serotyping of *C. jejuni* and *C. coli*, as strains of the same serotype were distinguished.

Introduction

Campylobacter jejuni and to a lesser extent *C. coli* are major cause of acute diarrhoeal disease in man throughout the world (Healing *et al.,* 1992). The majority of infections are sporadic, but large outbreaks often occur from milk and environmental sources such as water. For over 15 years, many phenotypic methods have been described for identifying strains of *C. jejuni* and *C. coli* in epidemiological investigations. However, biotyping and serotyping are the most widely used (Patton and Wachsmuth, 1992). Such schemes when used even in conjunction have several disadvantages which include, lack of reproduciblity, low typability, the requirement of a battery of antisera, which might not be available and poor resolution. In contrast, DNA fingerprints measure relatively stable chromosomal differences and provide consistent and reproducible results between individual *Campylobacter* strains as well as between *C. jejuni* and *C. coli* (Patton and Wachmuth, 1992).

In this study, rRNA gene restriction patterns (ribopatterns) of different Penner serovars of *C. jejuni* and *C. coli* were investigated to determine which typing method was most discriminatory.

Reprint requests to Dr. S. I. Smith.

Materials and Methods

Bacterial strains and growth conditions

Forty-seven strains of *Campylobacter*, made up of 38 strains of *C. jejuni* and 9 strains of *C. coli* were analysed by the Penner serotyping scheme (Penner and Hennessy, 1980) and ribotype. The human strains were made up of twenty strains, while the chicken strains were twenty-seven. All bacteria were cultivated at 42 °C for 48 h on 7% (v/v) defibrinated sheep blood agar under microaerophilic conditions in a candle jar (Blaser *et al.*, 1980).

DNA isolation, restriction digestion, southern blotting and RNA gene hybridization

Chromosomal DNA was isolated and purified using the SDS lysis method of Marmur (1961). All DNA samples (5 μg) were digested for 3 h at 37 °C with 5 units of Hae III (Appligene, France) according to the manufacturer's instruction . The reaction was stopped at 65 °C for 10 min. The di-

0939–5075/98/0100–0065 $ 06.00

gested DNA was electrophoresed at 150 volts/cm for 3 hours in 0.8% horizontal agarose gels, with ice-cold TBE running buffer. Gels were photographed after staining in 0.5 mg / ml ethidium bromide for 30 min. After electrophoresis the DNA fragments were denatured in the gel using a vacuum-assisted device (Vacugene 2516 blotting unit, Pharmacia-LKB Biotechnology, Uppsala, Sweden) and then transferred to membranes by the method of Southern (1975) modified by the use of nylon membranes (Hybond-N, Amersham International, U. K.) by vacuum transfer blotting. The membranes were rinsed briefly in 2×SSC (1×SSC is 0.15 M NaCl plus 0.015 M trisodium citrate), air-dried and the baseline was marked with λ DNA and the DNA was fixed by ultraviolet (312 nm, 4 min) irradiation.

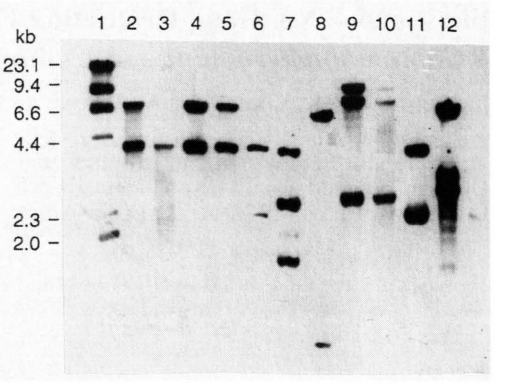

Fig. 1 B. Ribopatterns obtained by Southern blot analysis of an agarose gel (Hae III digest) hybridized with a 16S rRNA probe of *E. coli.* Lane 1, Hind III digest fragment of bacteriophage λ DNA; Lane 2, CD 72; Lane 3, CD 71; Lane 4, CD 69; Lane 5, CD 70; Lane 6, CD 2; Lane 7, CD 37; Lane 8, CD 45; Lane 9, CD 23; Lane 10, CD 27; Lane 11, CD 5; Lane 12, 13.

Hybridization

Nylon membranes with immobilized DNA restriction fragments were pretreated with Enhanced Chemiluminiscence (ECL, Amersham International, U. K) hybridization buffer at 38 °C for 1 h. After which the rRNA probe was labelled and hybridized according to manufacturer's instruction (ECL, Amersham International, U. K.). After two washes at 38 °C and two stringent washes at room temperature, the filters were subjected to autoradiography.

Results

Serogroup and their distribution by host

A total of 17 distinct ribopatterns were identified from 47 *Campylobacter* strains. Seven strains (15%) belonged to ribotype 03 and 07 (Fig 1A, B), while five strains (11%) belonged to ribotype 14 and 16 (Table I). Ribotype 07 was unique for the *C. jejuni* chicken strains and were unique to Penner 53 (Fig 1A, B), while ribotype 05 and 14 were unique to the human *C. coli* strains. Ribotypes 03, 04 and 09 were common to both the chicken and human strains. The three Penner 4 serotypes were subtyped into three ribopatterns, 01, 02 and 12 (Table I). The nine Penner 53's were subtyped into 4 ribopatterns. All Penner 24 and Penner 27 strains had the same ribopattern, ll are human strains (Table I). The five Penner 4 complex (4, 13, 16, 50), were subtyped into 2 ribopatterns, 02 and 03.

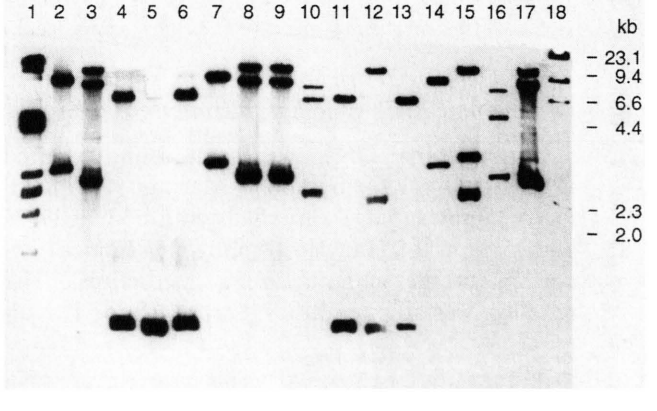

Fig. 1 A. Ribopatterns obtained by Southern blot analysis of an agarose gel (Hae 111 digest) hybridized with a 16S rRNA probe of *E. coli.* Lane 1, Pst 1 digest fragment of bacteriophage λ DNA; Lane 2, CD 31; Lane 3, CD 25; Lane 4, CD 44; Lane 5, CD 33; Lane 6, CD 48; Lane 7, CD 30; Lane 8, CD 26; Lane 9, CD 24; Lane 10, CD 21; Lane 11, CD 41; Lane 12, CD 20; Lane 13, CD 46; Lane 14, CD 28; Lane 15, CD 29; Lane 16, CD 43; Lane 17, CD 22; Lane 18, Hind 111 digest fragment of bacteriophage λ DNA.

Table I. Details of *C. jejuni* and *C. coli* used and typing results.

Ribotype	Strain code no.	Serotype	Source
01	CD 15	1	Human
01	CD 12	4	Human
02	CD 22	4	Chicken
02	CD 24	4, 13, 16, 50	Chicken
02	CD 25	4, 13, 16, 50	Chicken
02	CD 26	4, 13, 16, 50	Chicken
03	CD 64	27	Human
03	CD 65	27	Human
03	CD 66	27	Human
03	CD 67	27	Human
03	CD 68	27	Human
03	CD 23	4, 13, 16, 50	Chicken
03	CD 27	4, 13, 16, 50	Chicken
04	CD 21	NT	Chicken
04	CD 18	5	Human
05	CD 69	NT	Human
05	CD 70	NT	Human
05	CD 71	NT	Human
05	CD 72	NT	Human
06	CD 28	NT	Chicken
06	CD 30	53	Chicken
06	CD 31	38	Chicken
07	CD 33	53	Chicken
07	CD 41	53	Chicken
07	CD 44	53	Chicken
07	CD 45	53	Chicken
07	CD 46	53	Chicken
07	CD 48	53	Chicken
08	CD 43	53	Chicken
09	CD 16	2	Human
09	CD 37	NT	Chicken
10	CD 29	21	Chicken
11	CD 20	NT	Chicken
12	CD 17	4	Human
13	CD 42	53	Chicken
14	CD 2	24	Human
14	CD 3	24	Human
14	CD 4	24	Human
14	CD 5	24	Human
14	CD 6	24	Human
15	CD 14	31	Human
16	CD 35	NT	Chicken
16	CD 36	NT	Chicken
16	CD 37	NT	Chicken
16	CD 38	NT	Chicken
16	CD 39	NT	Chicken
17	CD 40	NT	Chicken

Ribotype analysis of non-typable (NT) strains

The 14 NT strains were subtyped into 6 ribopatterns. Five strains represented type 16, 4 types were of ribopattern 05 (Fig 1A, B), while 1 each belonged to 04, 06, 09, 11 and 17 (Table I).

Molecular weight of the C. jejuni and C. coli strains by ribotype

Generally, the *C. jejuni* strains ranged from 0.06 to 13.49 kb, while the *C. coli* strains ranged from 0.5 kb to 7.59 kb in molecular weight (Table II).

Discussion

A Southern blot analysis using a probe complementary to the 3′ end of 16S rRNA enabled the discrimination of *C. jejuni* and *C. coli*. This approach was first carried out by Grimont and Grimont (1986). In their approach, they used total 16S and 23S rRNA as probes, but they couldn't speciate their *Campylobacter* isolates. From this study, all the Hae III restriction patterns appeared to be species- specific and therefore allowed for speciation of the *Campylobacter* strains. The results clearly demonstrated that a high degree of ribotype diversity exists between sporadic strains of the same serotype, with four distinct ribotype patterns amongst the serogroup 53 strains and three distinct ribotype patterns amongst three Penner serogroup 4 strains. A previous study by Fayos *et al.* (1993), confirmed the above view. In their study, six distinct ribotypes existed amongst the serogroup 01 and 02 of *C. jejuni*. They then concluded that overall ribopattern similarities broadly reflect the taxonomic affinity of strains within the thermophilic campylobacters. Another report by Fraser *et al.* (1992), confirmed diversity amongst *C. coli* serogroup 20 (Lior). Interestingly, this technique was able to sub-type the Penner 4 complex. Results of this study show that some ribopatterns are common to both the chicken and human hosts. The common ribopatterns are 03, 04 and 09. This supports the view by Segreti *et al.* (1992), that poultry are an important source of *Campylobacter* infection to humans. This technique is also able to speciate the campylobacters, this is because the ribopatterns common to *C. coli*

Table II. Common *C. jejuni* and *C. coli* ribopatterns and identification of ribotypes.

Ribotypes	No. of strains	Mean fragment size (kb)
03	7	8.71, 7.56, 2.81, 1.0
07	6	7.01, 0.06
14	5	3.8, 2.24
05	4	7.59, 4.68

were 05 and 14, while 01 was common to *C. jejuni* human strains. Fourteen non-typable strains were subtyped into six distinct ribopatterns. This shows that for each strain, there is a definite rRNA fingerprint and there is always a fragment pattern irrespective of whether the strains are nontypable by the Penner scheme. Of interest is the fact that one nontypble strain by Penner scheme (strain CD 40 , *C. jejuni*), could have been classified as *C. coli* or *C. lari* as a result of its hippurate reaction (negative), but from the banding patterns observed between the *C. jejuni* and *C. coli* strains it was confirmed to be *C. jejuni*. This is because the *C. coli* strains band lower than the *C. jejuni* strains and this strain was found to band in the same region as other *C. jejuni* strains by this ribotype technique. This further shows the usefulness of this technique in correctly classifying the strains. The ribotype analysis thus shows that extensive heterogeneity exists in the restriction enzyme sites in the rRNA genes amongst *C. jejuni* and *C. coli*. This technique proves to be useful in species identification, particularly for strains that are difficult to analyze phenotypically or to cultivate in large quantities enough for DNA - DNA hybridization. The data also indicates that each species of *C. jejuni* and *C. coli* has more than one copy of the 16S rRNA gene. This is apparent from the fact that each pattern has more than one band, hybridizing to the probe (Fig 1A and B). In this study, the *C. coli* strains had mainly two band patterns, while the *C. jejuni* strains had more than two hybridizing bands. The ribopatterns are therefore generally easier to interprete than the RFLP, as they have only two to four bands. The *C. coli* strains were recognized by their conserved 4.68 kb hybridizing fragment. Similar reports have been given by other workers (Moureau *et al.,* 1989; Romanuik and Trust, 1987). Four nontypable strains isolated from three geographical locations in Lagos area had the same ribopattern number,10. This suggests that the *C. coli* strains in Lagos, Nigeria are of a clonal nature. Restriction endonuclease analysis is therefore very efficient in the classification of campylobacters especially at species and sub-species level. The level of discrimination, however, depends on the choice of the enzyme.

Acknowledgement

We would like to acknowledge the European Union grant number 6100.52.41.032 for the support of this work.

Blaser, M. J., Glass, R. I., Huq, M. I., Stoll, B., Kibriya, G. M. and Alim, A. R. M. A. (1980), Isolation of Campylobacter fetus subsp. jejuni from Bangladeshi children, J. Clin. Microbiol. **12**, 744–748.

Fayos, A., Owen, R. J., Hernandez, J., Jones, C. and Lastovica, A. (1993), Molecular subtyping by genome and plasmid analysis of *Campylobacter jejuni* serogroups 01 and 02 (Penner) from sporadic and outbreak cases of human diarrhoea, Epid. Infect. **111**, 415–427.

Fraser, A. D. E., Chandan, V., Yamazaki, H., Brooks, B. W. and Gracia, M. M. (1992), Simple and economical culture of *Campylobacter jejuni* and *C. coli* in carbon-dioxide in moist air. Int. J. Food Microbiol. **15,** 377–382.

Grimont, F. and Grimont, P. A. D. (1986), Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools, Ann. Inst. Pasteur/Microbiol. (Paris) **137B**, 165–175.

Healing, T. D., Greenwood, M. H. and Pearson, A. D. (1992), Campylobacters and enteritis, Rev. Med. Microbiol. **3,** 159–167.

Marmur, J. (1961), A procedure for the isolation of deoxyribonucleic acid from microorganisms, J. Mol. Biol. **3**, 208–218.

Moureau, P., Derclaye, I., Gregoire, D., Janssen, M. and Cornelis, G. R. (1989), *Campylobacter* species identification based on polymorphism of DNA encoding rRNA, J. Clin. Microbiol. **27,** 1514–1517.

Patton, C. M. and Wachsmuth, K. I. (1992), Typing schemes: Are current methods useful?: In *Campylobacter jejuni* : Current status and future trends,eds Nachamkin, I., Blaser, M. J. and Tompkins, L. S., published by ASM, Washington, DC. pp 110–128.

Penner, J. L. and Hennessy, J. N. (1980), Passive haemagglutination technique for serotyping *Campylobacter* fetus subsp jejuni on the basis of soluble heat-stable antigens, J. Clin.Microbiol. **12** , 732–737.

Romonuik, P. K. and Trust, T. J. (1987), Identification of *Campylobacter* species by Southern hybridazation of genome DNA using an oligonucleotide probe for 16S rRNA genes. FEMS Microbiol. Lett. **43,** 331–335.

Segreti, J., Gootz, T. D., Goodman, L. J., Parkhurst, G. W., Quinn, J. P., Martin, B. A. and Trenholme, G. M. (1992), High level quinolone resistance in clinical isolates of *Campylobacter jejuni*, J. Infect. Dis. **165**, 667–670.

Southern, E. M. (1975), Detection of specific sequences among DNA fragments separated by gel electrophoresis, J. Mol. Biol. **98,** 503–517.