Biotyping of Campylobacter Strains Isolated in Lagos, Nigeria Using the Modified Preston Biotype

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Fifty-eight *Campylobacter* strains were isolated from children with diarrhoea at various health centres in Lagos and from healthy chicken. Twenty – nine strains of *Campylobacter* were isolated from humans, while the same number were isolated from chicken. The strains were biotyped using the modified Preston biotype scheme. The Preston biotyping results have been compared with the results of Penner serotyping. Out of fifty-eight strains studied, the technique identified ten strains (17%) as *C. coli*, three (5%) as *C. lari* and fourty-five (78%) as *C. jejuni*, by the coding system. This technique identified twenty-eight *Campylobacter* species. This method highlights the usefulness of this technique in the biotyping of local strains, however, when the two schemes are used in combination they give excellent typing results suitable for epidemiological purposes.

Introduction

Campylobacter enteritis is caused by the two closely related species, *Campylobacter jejuni* and *C. coli*, but *C. jejuni* is the more predominant of the two. Animal sources have been the main reservoirs for strains infecting humans (Blaser *et al.*, 1983). This has emphasized the need to determine the possible role non- human sources play in human infections and differentiation for epidemiological purposes of outbreak isolates. It is therefore essential to use epidemiological typing methods which are reliable and reproducible, that discriminate between the same strains and different strains.

Campylobacter species can be typed by various techniques. There are three biotyping schemes commonly used for distinguishing campylobacters. Those of Skirrow and Benjamin (1980); Lior (1984) and Bolton *et al.* (1984) (Preston biotyping). The Preston biotyping scheme of Bolton *et al.* (1984) uses 12 tests, including 10 resistotyping tests, that determine resistance to antibiotics, dyes and chemicals for biotyping four *Campylobacter* spp. It differentiates 55 types of *C. jejuni*. On the basis of test arrangement and results, a four-figure code, generated for each strain, indicates both the *Campylobacter* species and biotype. The four – figure code is basically made up of four rows which indicates values assigned to a positive result to differentiate between the *Campylobacter* species. This is called the coding system (Table I). From Table I it can be seen that a strain with code number 6000, simply means that 6 shows positive result for resistance to cephazolin and hippurate hydrolysis (2+4 = 6). The last three zeros represent the last three rows which indicate a negative result for each of the tests. Since the first row is 6, then Table I indicates that it is a *C. jejuni* strain.

The Penner serotyping scheme makes use of tolerance to heat as a means to separate somatic, flagellar and capsular antigens (Penner and Hennessy, 1980). This method involves passive haemagglutination techniques using non-absorbed antisera to detect thermostable antigens.

The objective of thes study is to biotype *Campylobacter* strains using the modified Preston biotype and comparing the results with the Penner serotyping scheme which is commonly used e.g. in England to identify *Campylobacter* species. We are using the modified Preston biotype for the first time in this context.

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Table I. Modified Preston identification and biotyping scheme.

TEST	Value assigned to a + result	*C. fetus* Subsp *fetus*	*C. jejuni* Subsp *jejuni*	*C. jejuni* Subsp *doylei*	*C. coli*	*C. lari*	*C.upsali ensis*
Growth $Mic0_2$ at 25°C	1	1	6	4	2	0	0
Resistance to: Cephazolin (200 mg/U)	2						
Hippurate hydrolysis	4						
Resistance to: Sodium arsenite (0.001%)	1	0	V	V	V	0	0
TTC* (0.1%)	2						
Pyronin (0.002%)	4						
Resistance to: Metronidazole (5 mg/l)	1	V	V	V	V	V	6
5-Fluorouracil(100 mg/l)	2						
Cycloserine (10 mg/l)	4						
Resistance to: Nalidixic acid (60 mg/l)	1	V	V	V	V	V	0
H_2S production in FBP medium	2						
Deoxyribonuclease production	4						

* 2, 3, 5-triphenyl tetrazolium chloride.
V variable according to biotype.
Key: 6 – – – *C. jejuni;*
2 – – – *C. coli;*
00 – – *C. lari;*
10 – – C.*fetus* ssp *fetus;*
0060 *C.upsaliensis;*
4 – – – *C. jejuni* ssp *doylei.*

Materials and Methods

Isolation

The strains were cultured from diarrhoeal patients and from chicken. The isolates were obtained from the *Campylobacter* Research Laboratory, at the College of Medicine, University of Lagos, Idi – Araba, Lagos.

The medium used for the sub- culturing of the strains is the Bultzler's type medium of Coker and Dosunmu- Ogunbi, 1984. This is made up of Brucella agar base (Oxoid code CM 169) with 10% whole sheep blood and Oxoid antibiotic supplement, SR 85 with the following antibiotic supplements, bacitracin (25 i.u./ml), colistin (10 U/ml), cyclohexemide (50 mg/ml), novobiocin (5 mg/ml) and cephazolin (15 mg/ml).

Characterisation

Campylobacter isolates were characterized as follows: catalase, gram stain, oxidase, motility, indoxyl acetate hydrolysis and nitrate reduction tests were performed on *Campylobacter* strains as described by Cowan (1993). Temperature tolerance was performed by inoculating organisms in blood agar and incubating microaerobically at 25 ^{0} C, 37 ^{0} C and 42 ^{0} C for 48 hours.

Resistotyping tests

The culture medium for the resistotyping of the campylobacters consists of charcoal, ferrous sulphate and pyruvate (CFP) blood free medium of Bolton *et al.* (1984), containing (g/l) : nutrient broth no. 2 (Oxoid CM 167), 25; bacteriological charcoal, 4; casein hydrolysate, 3; ferrous sulphate,

0.25; sodium pyruvate, 0.25; New Zealand Agar, 12. The pH is adjusted to pH 7.4. The standardized suspensions of campylobacters were inoculated onto resistotyping medium which consists of charcoal, ferrous sulphate, pyruvate (CFP) (w/v), blood free medium with 20 g/l New Zealand (CFP 2%). Each agar incorporating cephazolin, sodium arsenite, nalidixic acid, sodium chloride (15 & 35 g) and glycine, with concentrations as indicated in Table I. The components were filter – sterilized. The only difference with Bolton et al., (1984), biotyping is that tri – phenyl tetrazolium chloride (TTC) concentration was increased to 0.1%. The plates were incubated microaerobically at 37 ^{0} C for 48 hours. The procedure was repeated thrice to check for accuracy.

Anaerobic growth in the presence of trimethyl – N – oxide (TMAO)

The method of Razi *et al.* (1981) was used.

In all of the above tests, positive and negative control strains were included: NCTC 11168; *C. jejuni*; NCTC 11392; *C. jejuni*; NCTC 5850; *C. fetus*; NCTC 11366; *C. coli* and NCTC 11352; *C. lari.*

Serotyping

The method used was the passive haemagglutination technique of Penner and Hennessey (1980). The serotyping procedures was as described by Jones *et al.* (1985). Briefly, the serotype of each strain of *C. jejuni* and *C. coli* was determined by testing each, in the 64 immune antisera diluted 1/40 in microtitration plates. Positive reactions showed agglutination, while those that did not agglutinate with any of the 64 antisera were regarded as non-typable. The serotype was indicated by listing all reactive antisera in order of strength of titre, with the antiserum giving the strongest reaction listed first.

Results

Out of 58 strains, the technique identified ten strains (17%) as *C. coli*, three strains (5%) as *C. lari* and fourty- five strains (78%) as *C. jejuni* by the coding system. The technique identified twenty- eight *Campylobacter* species (Table II).

Table II. Typing of *Campylobacter* species by the modified Preston biotype.

Code No	Source	Identification	Preston Biotype	Penner Serotype
CD 1–6	human	*C. coli*	2440	24
CD 7	human	*C. jejuni*	6004	13,5
CD 8	human	*C. jejuni*	*6440*	
CD 9	*human*	*C. jejuni*	6015	5
CD 10	human	*C. jejuni*	6031	2
CD 11	human	*C. jejuni*	6004	2
CD 12 and 55	human	*C. jejuni*	6015	4 and 23
CD 13	human	*C. jejuni*	6100	31
CD 14 and 15	human	*C. jejuni*	6000	31 and 1
CD 47	chicken	*C. jejuni*	6000	53
CD 16	human	*C. jejuni*	6130	2
CD 17	human	*C. jejuni*	6014	4
CD 38	chicken	*C. jejuni*	6014	NT
CD 18	human	*C. jejuni*	6114	5
CD 19 and 26	chicken	*C. jejuni*	6304	4,13,16,50
CD 20 and 36	chicken	*C. jejuni*	6112	NT
CD 21	chicken	*C. jejuni*	6006	NT
CD 22 and 27	chicken	*C. jejuni*	6314	4,13,16,50
CD 23 - 25	chicken	*C. jejuni*	6704	4,13,16,50
CD 28	chicken	*C. jejuni*	6706	4,13,16.50
CD 29 and 30	chicken	*C. jejuni*	6002	21 and 53
CD 31	chicken	*C. jejuni*	6002	38
CD 32	chicken	*C. jejuni*	6410	53
CD 33	chicken	*C. jejuni*	6050	53
CD 35 and 39	chicken	*C. lari*	0031	NT
CD 37	chicken	*C. lari*	0071	NT
CD 40 and 41	chicken	*C. jejuni*	6021	NT and 53
CD 43	chicken	*C. jejuni*	6033	53
CD 44 and 45	chicken	*C. jejuni*	6011	53
CD 42, 46 and 48	chicken	*C. jejuni*	6031	53
CD 63	human	*C. jejuni*	6000	13, 16,50
CD 64–68	human	*C. jejuni*	6016	27
CD 69–72	human	*C. coli*	2600	NT

NT, non – typable.
6––– *C. jejuni*; 2––– *C. coli*; 00–– *C. lari*; ––– are variables which differ depending on the biotype.

The technique was able to sub-group eleven Penner 53 serogroup into eight biotypes, with Preston biotype codes, 6021, 6031, 6000, 6002, 6410, 6050, 6033 and 6011 (Table II). All Penner serogroup 27 had the same Preston biotype code 6016, while four of the non- typable *C. coli* strains by the Penner scheme had the same Preston biotype 2600 (Table II) . All the Penner 24 serogroup *C. coli* strains had the same Preston biotype 2440 (Table II). The Preston biotype was able to sub – type 8 Penner 4, 13, 16, 50 antigens into 4 biotypes; 6304, 6314, 6704 and 6706. All the non – typable strains by the Penner serogroup were all biotyped (Table II). However, strains with the same Preston biotype had different Penner serotypes (Table II). Three of the chicken isolates with Preston biotype 6002, was serotyped by the Penner scheme as be-

longing to three different strains of *C. jejuni* (21, 53 and 38).

Discussion

In this present study, the modified Preston biotype was able to type all human and chicken isolates. This highlights the potential value of the scheme for studying the epidemiology of *Campylobacter* infections. All strains were biotyped into 10 (17%) *C. coli*, 3 (5%) *C. lari* and 45 (76%) *C. jejuni*. This shows the preponderance of *C. jejuni* strains amongst all other strains isolated during sporadic cases of diarrhoea and from healthy chicken in our environment. Reliance on the results of serotyping alone can be misleading and confusing. Strains of Penner serogroup complex 4, 13, 16, 50 can express these antigens to different degrees and this may lead to confusion about the similarity of such isolates. In this study, 8 strains of this Penner complex were sub – typed into 4 biotypes, while 11 Penner 53 were sub – typed into 8 biotypes. This shows that the Preston biotype can sub – type strains of the same serogroup. Conversely, it can be seen that Penner serotyping scheme will distinguish between strains of the same Preston biotype. For example, strains of Preston biotype 6002, isolated from three different chicken, had three different Penner serotypes (21, 53 and 38). A similar report was given by Hutchinson *et al.* (1987). The Preston biotyping scheme was also compared with the phage typing, Penner serotyping and Lior serotyping scheme and was also found to be suitable in combination with one of the typing schemes (Salama *et al.*, 1990). The Preston biotyping scheme is therefore complementary to the Penner serotyping scheme. However, strains of Penner serogroup 24 and 27 all had the same Preston biotype respectively. This confirms that all the isolates from Penner 24 and 27 are the same species, since they also share the same Preston biotype. The Preston biotype has an edge over the Penner serotyping scheme in that all the non – typable strains by the Penner scheme were biotyped. The Preston biotype was able to identify twenty – eight *Campylobacter* species including 3 *C. lari*, a feature which is uncommon with the Lior biotype as the Lior biotype does not speciate campylobacters. The modified Preston biotype is however not commonly used world – wide, because it requires a lot of tests. In conclusion, the modified Preston biotype clearly differentiated between the campylobacters and was able to sub – type strains, which is a useful tool for distinguishing epidemiologically related strains, most especially as common biotyping schemes do not speciate campylobacters. We would, however, recommend that the Preston biotype scheme be used in conjunction with the Penner serotyping method for excellent results.

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