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Evaluation of 11 PCR Assays for Species-Level Identification of *Campylobacter jejuni* and *Campylobacter coli*

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We examined the sensitivity and specificity of 11 PCR assays described for the species identification of *Campylobacter jejuni* and *Campylobacter coli* by using 111 type, reference, and field strains of *C. jejuni*, *C. coli*, and *Campylobacter lari*. For six assays, an additional 21 type strains representing related *Campylobacter*, *Arcobacter*, and *Helicobacter* species were also included. PCR tests were initially established in the laboratory by optimizing conditions with respect to five type and reference strains of *C. jejuni*, *C. coli*, and *C. lari*. One PCR test for *C. coli* failed to give appropriate results during this initial setup phase and was not evaluated further. The remaining 10 assays were used to examine heated lysate and purified DNA templates as appropriate of well-characterized type, reference, and field strains of *C. jejuni* ($n = 62$), *C. coli* ($n = 34$), and *C. lari* ($n = 15$). The tests varied considerably in their sensitivity and specificity for their respective target species. No assay was found to be 100% sensitive and/or specific for all *C. jejuni* strains tested, but four assays for *C. coli* gave appropriate responses for all strains examined. Between one and six strains of *C. jejuni* gave amplicons in four of seven *C. jejuni* PCR tests only where purified DNA was used as the template; corresponding results were seen with one strain of *C. coli* in each of three assays for the latter species. Our findings indicate that a polyphasic strategy for PCR-based identification should be used to identify *C. jejuni* and *C. coli* strains. The data may assist laboratories in selecting assays suited for their needs and in designing evaluations of future PCR tests aimed to identify these species.

Campylobacter spp. are gram-negative, microaerophilic and/or anaerobic, mainly spiral-shaped bacteria, most of which are established or suspected human gastrointestinal pathogens (28). Of the 16 species and six subspecies currently known (21), *Campylobacter jejuni* and *Campylobacter coli* are those most often isolated from human diarrhea (28). Most developed countries report *C. jejuni* as predominant, but in other areas, *C. coli* accounts for up to 50% of human cases (28). The main source of campylobacter infections is considered contaminated foods, since the bacteria are normal flora in animals such as poultry, pigs, and cattle.

The accurate identification of *C. jejuni* and *C. coli* provides important data for surveillance and risk assessment studies on which intervention strategies can be based. The principal hosts of *C. jejuni* and *C. coli* are widely regarded as poultry and pigs, respectively, but significant proportions of *C. coli* have been found in poultry (5), and genetically identical clones of *C. jejuni* have been observed in humans, cattle, and various animals living in the wild, as well as poultry (25, 26). In addition, rapid identification of *C. coli* may be useful clinically, since up to 68.4% of strains are resistant to erythromycin, the antibiotic of first choice for treatment of severe campylobacter infections (18).

Identification of campylobacters and related bacteria is well known to be problematic, principally because of their complex taxonomy, biochemical inertness, and fastidious growth requirements (20, 21). *C. jejuni* and *C. coli* are traditionally differentiated by the hippurate hydrolysis test, for which only *C. jejuni* gives a positive reaction. However, hippurate-negative strains of this species are well recognized (17, 36), and problems with false positive test results for non-*C. jejuni* species have also been described (5). Additional phenotypic characters, such as growth on a minimal medium and alpha-hemolytic activity, are useful but do not provide unequivocal discrimination, require stringent standardization, and are seldom used in routine laboratories (20). As a consequence, there has been considerable interest in the development of molecular identification methods for *C. jejuni* and *C. coli*.

PCR tests are considered especially attractive due to their relative ease of use, low cost, and potential application in large-scale screening programs by means of automated technologies (12). A wide range of PCR assays for *C. jejuni* and *C. coli* have been described, several of which are based on a variety of genes (e.g., 23S rRNA, *ceuE*, and *mapA*) (8, 9, 34) and others of which are derived from different randomly generated fragments (4, 32–33, 37) (see Table 1). The sensitivity and specificity of each PCR test have been examined, but the bases of the evaluations differed significantly, particularly with respect to the selection of strains of *C. jejuni*, *C. coli*, and *Campylobacter lari*, a group of species that are closely related by phylogenetic and genetic criteria (21). For example, one study included the reference strains used to establish an international serotyping scheme for *C. jejuni* and *C. coli* (13), another tested six *C. jejuni* strains and one *C. coli* strain but no *C. lari* isolates (4), and one was evaluated solely in the context of a specific taxonomic investigation (38). In addition, only one (13) appears to have been applied to hippurate-negative *C. jejuni* strains. The differences in the numbers and choices of strains used to evaluate the PCR tests make an objective comparison of their efficacy difficult.

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In this study, we investigated the sensitivity and specificity of 11 PCR assays for identifying *C. jejuni* and *C. coli*, mainly by use of 111 well-characterized strains of the most closely related species *C. jejuni*, *C. coli*, and *C. lari*.

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MATERIALS AND METHODS

Study design. Six PCR tests for *C. jejuni* (4, 8, 9, 13, 32, 34), four for *C. coli* (8, 9, 13, 33), and a multiplex assay designed for concurrent identification and differentiation of both species (38) were examined. For initial PCR setup, all primers and PCR cycling conditions as specified by the respective authors were first examined for their ability to obtain appropriate results with purified DNA template from *C. jejuni* CCUG 11284^T and CCUG 10958, *C. coli* CCUG 11283^T and CCUG 24865, and *C. lari* CCUG 23947^T. The specified reaction conditions were modified by altering key variables (primer annealing temperature, concentration of $MgCl_2$, concentration of *Taq* polymerase) only where appropriate results were not obtained. Details of the PCR assay variables finally used are given in Table 1. Optimized PCR assays were then applied to heated lysates of 111 strains of *C. jejuni*, *C. coli*, and *C. lari* (see Tables 2 and 3). Strains giving inappropriate results were reexamined by use of the original heated lysate and also purified DNA. For selected *C. jejuni* (13, 38) and *C. coli* (9, 13, 33, 38) assays, specificity was reexamined by use of purified DNA extracts from the type strains of related taxa; *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter fetus* subsp. *fetus*, *Campylobacter fetus* subsp. *venerealis*, *Campylobacter gracilis*, *Campylobacter helveticus*, *Campylobacter hominis*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter hyointestinalis* subsp. *lawsonii*, *Campylobacter lanienae*, *Campylobacter mucosalis*, *Campylobacter rectus*, *Campylobacter showae*, *Campylobacter sputorum*, *Campylobacter upsaliensis*, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter nitrofigilis*, *Arcobacter skirrowii*, *Helicobacter pametensis*, and *Helicobacter pullorum*.

Bacterial strains. Tables 2 and 3 list the 57 *C. jejuni* subsp. *jejuni*, 5 *C. jejuni* subsp. *doylei*, 34 *C. coli*, and 15 *C. lari* strains used. Of these, 81 are reference strains from international culture collections. The identities of the remaining strains (including hippurate-negative or variable *C. jejuni* subsp. *jejuni*) were verified in previous studies, mainly involving DNA-DNA hybridization and/or amplified fragment length polymorphism (AFLP) technologies (17, 22–23, 31, 36–38).

Preparation of heated lysate and purified DNA templates. Diluted (1:10 in purified distilled water) heated lysates (24) and purified DNA samples (11) from the bacterial strains used here were prepared as described previously. Where purified DNA was employed as the template for the PCR assays described by Gonzalez et al. (9), 100 ng was used per the authors' instructions.

PCR assay conditions. All PCRs were performed in 50-μl volumes containing 5 μl of 10× PCR buffer (N808-0171; Applied Biosystems, Foster City, Calif.), 0.5 μl of each PCR primer (final concentration, 130 μg/ml; DNA Technology A/S, Aarhus, Denmark), 0.5 μl of deoxynucleoside triphosphate mix (final concentration, 1 mM; Amersham Pharmacia Biotech, Hørsholm, Denmark), and 2.0 μl of template. Volumes of DNA polymerase (AmpliTaq; Applied Biosystems), $MgCl_2$ (Applied Biosystems), and DNA-free purified water were used as appropriate to each assay (Table 1). Reaction mixtures were then overlaid with 1 μl of sterile mineral oil and heated for 5 min at 94°C as an initial denaturation step. PCR was then performed using the cycling conditions specified by the original authors of the respective works, with amendments to the annealing temperature as specified in Table 1. All assays were terminated with a 5-min extension period of 72°C and were performed with Trio Biometra 20 thermocyclers (Biometra, Göttingen, Germany), the efficacy of which was regularly tested by in-house quality assurance procedures (10). Amplicons were detected by ethidium bromide staining of electrophoresed samples as described previously (24).

RESULTS

Initial PCR setup. Appropriate results in terms of species specificity and amplicon size for 10 of the 11 PCR assays examined for the five type and reference strains used were successfully obtained. Most assays required some alteration to one or more of the specified reaction variables to obtain suitable results (Table 1). The 23S rRNA-based PCR test for *C. coli* (8) consistently gave amplicons with the strains of *C. jejuni* and *C. lari* during the initial setup phase, despite extensive testing (Fig. 1). Table 1 shows the range of different variable parameters used in various combinations in attempts to establish the assay. Consequently, this PCR was not evaluated further. No amplicon was obtained with any of the 21 related *Campylobacter*, *Arcobacter*, or *Helicobacter* taxa in five assays reexamined for species specificity (9, 13, 33, 38).

Sensitivity and specificity of PCR tests by evaluation with 111 *C. jejuni*, *C. coli*, and *C. lari* strains. Table 2 lists the strains for which accurate results in all PCR tests evaluated were obtained. Table 3 gives the results for strains for which PCR tests failed in their accuracy and summarizes the sensitivity (percentage of strains of the correct species identified) and specificity (100 − percentage of strains of the nontarget species giving a reaction) of each assay. For the latter, only consistent results obtained from analyses performed on heated lysates and subsequently on purified DNA were considered. All assays tested for identification of *C. coli* proved both 100% specific and sensitive, although three tests gave amplicons of strains of CCUG 11283 or CDC D145 only when purified DNA was used as the template (Table 2).

The performance of *C. jejuni* PCR assays varied considerably. Test specificity varied from 84 to 100%, and test sensitivity ranged from 88 to 100%. No test proved 100% accurate; tests that yielded amplicons from all *C. jejuni* strains proved to be the least specific (Table 3). Conversely, the two assays found to be 100% specific for *C. jejuni* (13, 38), respectively, detected 91 and 93% of strains of this species. Strains of *C. jejuni* subsp. *doylei* contributed significantly to the failure rate of these and two other PCR tests intended to identify *C. jejuni*; one test derived from an arbitrarily primed PCR product (4) failed to react with a single strain of this taxon (Table 3). In addition, there was an increased proportion of *C. jejuni* identification assays for which heated lysates did not serve as a suitable reaction template (Tables 2 and 3). Moreover, the use of heated lysate frequently (46% of *C. jejuni* strains examined) gave rise to additional PCR products of a lower molecular weight than the intended amplicon in one assay (9). These results were obtained with only four strains (7%) when purified DNA of the concentration recommended by these authors was employed as the reaction template. Results obtained with the PCR assay targeting the 23S rRNA gene (8) were noteworthy in that the molecular size of the amplicon in 14 strains of *C. jejuni* varied between the limits originally described (8); in two strains, two distinct products were observed. Representative results are shown in Fig. 1.

DISCUSSION

We observed considerable variation in the performance of 11 previously described PCR assays for identifying *C. jejuni* and *C. coli*. Both physicochemical (i.e., relating to the PCR) and biological (i.e., relating to the diversity of *Campylobacter*) factors account for this variability.

The quality of the template used for PCR is crucial to the reaction (10). Purified DNA can be regarded as the "gold standard" template for bacterial identification, but for busy

TABLE 1. PCR tests and assay variables

Target species	Reference	Target gene (amplicon size [bp])	Primers[b]	Variable PCR parameters used[a]			
				$MgCl_2$ concn (mM)	Amt (U) of *Taq*	Primer annealing temp (°C)	No. of cycles
C. jejuni	8	23S rRNA (710 or 810)	F 5′-TAAAGTAAGTACCGAAGCTG-3′ R1 5′-GTAAATCCTAATACAAAGCT-3′ R2 5′-TAAATCCTAGTACGAAGCT-3′	2.5[c]	0.5[c]	58	27 (Pub)
	4	Random (265)	F 5′-ATC GGG CTG TTA TGA TGA TA-3′ R 5′-CAT ATC CAG AGC CTC TGG AT-3′	3.0	0.5	57	35 (Pub)
	34	*mapA* (604)	F 5′-ATG TTT AAA AAA TTT TTG-3′ R 5′-AAG TTC AGA GAT TAA ACT AG-3′	1.5 (Pub)	2.5 (Pub)	55 (Pub)	35 (Pub)
	9	*ceuE* (793)	F 5′-CCT GCT ACG GTG AAA GTT TTG C-3′ R 5′-GAT CTT TTT GTT TTG TGC TGC-3′	2.5	0.5 (Pub)	65	30 (Pub)
	32	Random (358)	F 5′-GAA TGA AAT TTT AGA ATG GGG-3′ R 5′-GAT ATG TAT GAT TTT ATC CT GC-3′	2.5	0.5 (Pub)	57	35
	13	*hipO* (735)	F 5′-GAA GAG GGT TTG GGT GGT-3′ R 5′-AGC TAG CTT CGC ATA ATA ACT TG-3′	2.5 (Pub)	0.5	66 (Pub)	25 (Pub)
	38	Random[d] (773)	F 5′-CA TCT TCC CTA GTC AAG CCT-3′ R 5′-AAG ATA TGG CTC TAG CAA GAC-3′	2.0	0.5	61	30
C. coli	8	23S rRNA (390)	F 5′-TAT TCC AAT ACC AAC ATT AGT-3′ R 5′-TAA ATC CTA ATA CGA AGC G-3′	1.0–2.5	0.5–3.0	54–61	27–30
	32	Random (258)	F 5′-ATA TTT CCA AGC GCT ACT CCC C-3′ R 5′-CAG GCA GTG TGA TAG TCA TGG G-3′	2.5	0.5 (Pub)	57	35
	9	*ceuE* (894)	F 5′-ATG AAA AAA TAT TTA GTT TTT GCA-3′ R 5′-ATT TTA TTA TTT GTA GCA GCG-3′	3.0	0.5 (Pub)	57 (Pub)	30 (Pub)
	13	Putative aspartokinase (500)	F 5′-GGT ATG ATT TCT ACA AAG CGA G-3′ R 5′-ATA AAA GAC TAT CGT CGC GTG-3′	2.5 (Pub)	0.5	60 (Pub)	25 (Pub)
	38	Random[d] (364)	F 5′-AG GCA AGG GAG CCT TTA ATC-3′ R 5′-TAT CCC TAT CTA CAA ATT CGC-3′	2.0	0.5	61	30

[a] Pub, parameters as described in original publication.
[b] F, forward; R, reverse.
[c] Not stated by original authors; values shown are in-house parameters.
[d] Assay performed as a multiplex reaction for detection of both *C. jejuni* and *C. coli,* as originally described (38).

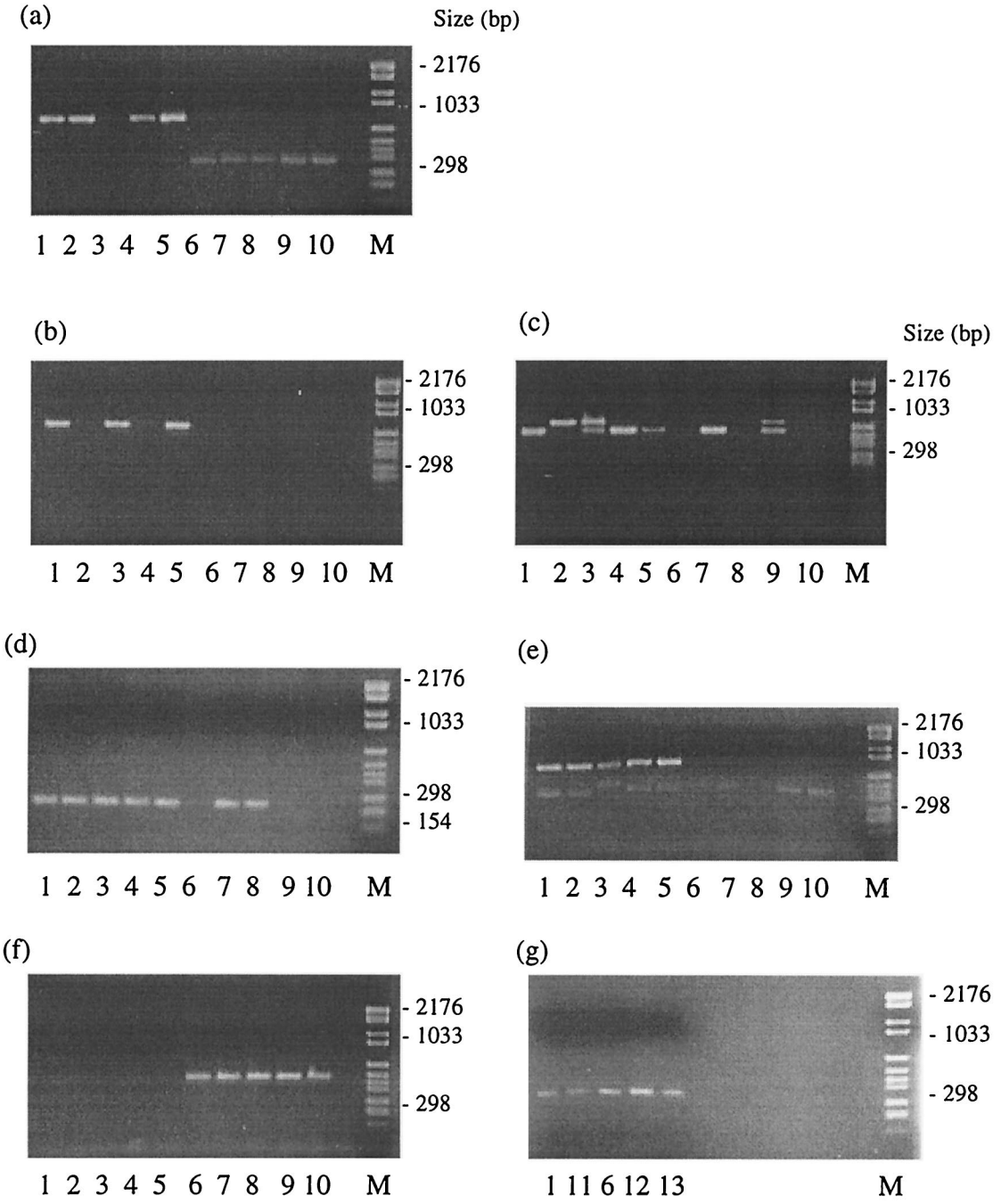

FIG. 1. Selected results from PCR tests. (a) Multiplex PCR for concurrent identification of *C. jejuni* and *C. coli* (38); (b to e) tests for *C. jejuni* (references 13, 8, 4, and 9, respectively); (f and g) tests for *C. coli* (references 13 and 8, respectively). Lanes: 1, CCUG 11284; 2, CCUG 10966; 3, CCUG 10970; 4, CCUG 10967; 5, CDC D133; 6, CCUG 11283; 7, CCUG 17715; 8, CCUG 10959; 9, LMG 9799; 10, CDC D145; 11, CDC D603; 12, CCUG 10951; 13, CCUG 23947; M, molecular size marker VI (Boehringer-Mannheim, Mannheim, Germany). Lanes 1 to 5 and 11, *C. jejuni*; lanes 6 to 10 and 12, *C. coli*; lane 13, *C. lari*. Results in panels a and e were obtained with heated lysate template; all other results were obtained with purified DNA.

routine laboratories the use of simple heated lysates is clearly advantageous. In general, the PCR tests examined here performed well when heated lysates were used, but in several cases amplicons were obtained only with purified DNA. Several factors may explain our results. Some *Campylobacter* strains do not release PCR-detectable DNA when boiled (16); the fact that PCR tests were not affected equally may reflect differences in the copy number and/or stability of each genetic marker. In addition, some PCR primers are affected more markedly than others by impurities present in crude DNA preparations (7, 27). The fact that nonspecific PCR products were frequently seen in one assay (9) when heated lysate was used as the

TABLE 2. *C. jejuni, C. coli,* and *C. lari* strains giving appropriate (species-specific) results in all 10 PCR tests subjected to full evaluation

Organism	Strain[a]
C. jejuni subsp. *jejuni*	CCUG 10935, CCUG 10936,[b] CCUG 10937, CCUG 10938, CCUG 12778, CCUG 10940, CCUG 16436, CCUG 10942, CCUG 10943, CCUG 10944, CCUG 17625, CCUG 10945,[b] CCUG 10946,[b] CCUG 10947,[b] CCUG 10948, CCUG 10949, CCUG 10950, CCUG 10952, CCUG 10954, CCUG 10958, CCUG 15361, CCUG 10961, CCUG 10962, CCUG 10963, CCUG 10968, CCUG 10971, CCUG 12782, CCUG 12783, CCUG 14567, CCUG 17755, CCUG 12790, CCUG12792, CCUG 15013,[c] CCUG 12795, CCUG 14538, CCUG 14541, CCUG 24866,[d] CCUG 24868,[b] CCUG 24869, CDC D114, CDC D123, CDC D133, CDC D 142, CDC D128[e] CCUG 11284[T], CDC D603,[f] CDC D634,[f] CDC D712,[b,f,g] CDC D835,[f] CDC D941,[f] CDC D977,[f] CDC D983[f]
C. coli	CCUG 11283[T],[h] CCUG 10957, CCUG 10960, CCUG 10964, CCUG 10969, CCUG 17754, CCUG 12791, CCUG 12794, CCUG 14537, CCUG 24865, CDC D134, CDC D145,[i] Ost-96-0096, LMG 9799, CCUG 33450, LMG 15884, LMG 15883, Lab 27, Lab 33, Lab 13, Lab 332
C. lari	CCUG 24567[T], CCUG 12774, CCUG 19528, CCUG 23948, LU 16/BTG4,[j] LU 21/12 OC3,[j] LU 29/4 OC5[j]

[a] T, type strain; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; Lab, our laboratory strain; LMG, Laboratorie voor Mikrobiologie, Ghent, Belgium; LU, Lancaster University, Lancaster, United Kingdom; Ost, ostrich isolate.

[b] Amplicon for *C. jejuni* test described in reference 34 obtained only with purified DNA template.

[c] Amplicon for *C. jejuni* test described in reference 38 obtained only with purified DNA template.

[d] Amplicon for *C. jejuni* test described in reference 4 obtained only with purified DNA template.

[e] Hippurate-variable strain (17).

[f] Hippurate-negative strain (36).

[g] Amplicon for *C. jejuni* test described in reference 13 obtained only with purified DNA template.

[h] Amplicon for *C. coli* tests described in reference 13 and 33 obtained only with purified DNA template.

[i] Amplicon for *C. coli* test described in reference 9 obtained only with purified DNA template.

[j] Urease-positive strain.

template but rarely when purified DNA was used may exemplify the influence of high DNA concentrations in heated lysates, which cause some PCR primers to misprime (2). Under such conditions, total inhibition of the PCR can also occur (2, 10).

Many factors apart from template quality can affect the efficacy of PCR, including source and type of DNA polymerase used, thermal cycler specification, and reaction buffer composition (1, 7, 15). Thus, stringent optimization procedures are recommended for implementation of a given PCR test (7, 10). We optimized 10 of 11 PCR tests under conditions concordant with recommended quality control procedures (10). Nonetheless, given the variables that can influence PCR test efficacy, in the hands of the original developers each PCR assay may perform satisfactorily. However, if such conditions cannot be easily reproduced, portability quickly becomes crucial.

In addition to technical factors contributing to the compromised sensitivity and specificity of certain PCR tests, a biological element is highly likely. *Campylobacter* is a taxonomically complex genus (21), and both *C. jejuni* and *C. lari* are genetically diverse species (6, 19, 21, 23). The finding that four of five PCR tests for identification of *C. coli* are effective may reflect the implication from AFLP analyses that this species appears to be comparatively homogeneous at the DNA level (6, 23). Our failure to obtain specific results for the *C. coli* PCR targeting the 23S rRNA gene resembles problems experienced by others (14) and may reflect the high level of conservation in the rRNA operon among closely related species (21).

C. jejuni comprises two genetically distinct but highly related subspecies, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*. Since the latter has no known animal reservoir and is infrequently observed in human disease, most attention is focused on *C. jejuni* subsp. *jejuni*, and most tests evaluated here were not originally used to examine *C. jejuni* subsp. *doylei* strains. The inability of many *C. jejuni* PCR tests to recognize strains of the latter taxon suggests that the genetic difference between the two subspecies (23, 30) contributes to PCR failure. Furthermore, genetic heterogeneity in *C. jejuni* subsp. *jejuni* can arise from various phenomena (19, 22, 35) that can affect the efficacy of a PCR if such change occurs within one or both of the binding sites (20). Mutation in *hipO* (29) has previously been identified as a source of failure for the PCR assay targeting that gene (13) and for a PCR based on *ureC* to identify the related organism *Helicobacter pylori* (3). Some of the negative PCR results we observed probably arose from the natural genetic diversity of *C. jejuni*. Such population variance is also demonstrated in our results for the *C. jejuni* subsp. *jejuni* PCR targeting the 23S rRNA gene (8). Amplicon size variation was also observed among the few strains of *C. coli* that gave a positive result with this assay (Fig. 1 and Table 3). The wide variation in distribution and sequence of intervening sequences in rRNA genes of *C. jejuni* and *C. coli* (37) and related bacteria, including interoperon differences, is now well established, and the effects and implications of intervening sequences for PCR tests have been discussed (21).

Our reexamination of the specificity of the five most accurate PCR tests by use of type strains of 21 related *Campylobacter*, *Arcobacter*, and *Helicobacter* species concurred with results obtained by the original authors (9, 13, 33, 38), emphasizing that more problems are encountered with accurate discrimination of closely related taxa. Our results endorse the use of a strain collection that adequately reflects the diversity and taxonomy of the target species to validate PCR assays. It is noteworthy that two of the PCR assays examined here that were readily established in our laboratory and yielded accurate results were first developed by testing with an extensive refer-

ence strain collection (13). Our data also support a polyphasic approach for identification of campylobacters (20), since no single PCR test identified all *C. jejuni* strains. Suspect *C. jejuni* subsp. *doylei* isolates should be confirmed by examining for their inability to reduce nitrate (30) or by alternative genetic methods such as AFLP fingerprinting (23). We now routinely use the multiplex PCR for concurrent identification and discrimination of *C. jejuni* and *C. coli* (38) as a first-line identification method and specific assays for these two species (13) as required. We hope the results of this study will assist others in selecting *C. jejuni* and *C. coli* PCR assays for their use.

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TABLE 3. Strains for which PCR tests failed to give appropriate results and summary of the sensitivity and specificity of each PCR

PCR assay target species and reference	Result for organism[a]																																Sensitivity (%)	Specificity
	C. jejuni subsp. *jejuni*					*C. jejuni* subsp. *doylei*					*C. coli*														*C. lari*									
	CCUG 10966	CCUG 10967	CCUG 10970	CCUG 14539	CCUG 24867	CCUG 24567[T]	CCUG 18265	CCUG 18266	CCUG 26152	CCUG 26155	CCUG 15360	CCUG 10951	CCUG 10955	CCUG 10956	CCUG 10959	CCUG 15362	CCUG 17715	CCUG 17755	CDC D112	CDC D126	Ost-95-3434	LMG 9799	Lab 215	Lab 421	CCUG 15035	CCUG 23949	CCUG 22396[b]	CCUG 22394[b]	CCUG 20707[b]	LU 18/3 BTG8[b]	LU 16/1 OC3[b]	LU 21/12 DN 18[b]		
C. jejuni																																		
8	L	S	S,L	+	I	L	L	L	L	L	–	–	–	S	–	–	S	–	S	S	L	L,I	L	S	–	–	–	–	–	–	–	–	100	84
4	+	+	+	+	+	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	92	92
34	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	W	W	U	–	–	–	+	+	100	90
9	+	+	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	88	98
31	+	+	+	+	+	+	+	+	+	+	W	+	+	+	–	–	–	+	–	–	–	–	–	–	+	+	W	W	W	W	W	–	100	86
38	+	+	+*	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	93	100
13	–	–	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	91	100
C. coli																																		
33	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	100	100
38	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	100	100
13	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	100	100
9	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	100	100

[a] T, type strain; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; Lab, our laboratory strain; LMG, Laboratorie voor Mikrobiologie, Ghent, Belgium; LU, Lancaster University, Lancaster, United Kingdom; Ost, ostrich isolate. +, amplicon of expected size obtained with heated lysate or purified DNA only (*; target species) or with heated lysate and purified DNA template (nontarget species); −, amplicon not obtained; S, small amplicon (710 bp); L, large amplicon (810 bp); I, intermediate amplicon (710 to 810 bp); W, weak amplicon; U, amplicon of unexpected size.

[b] Urease-positive strain.

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