Table 1. Minimal inhibitory concentrations for gentamicin and each plant extract against selected bacteria.

	E. aerogenes	*S. aureus*	*P. vulgaris*	*S. epidermidis*	*S. aureus* ATCC 29213
Gentamicin (μg/mL)	<50	1565	390	780	780
E. polystachya (μg/mL)	3125	3125	1565	390	3125
E. texana (μg/mL)	ND	ND	ND	1565	3125

ND: not determined

lactones in *E. polystachya* and carbohydrates and coumarins in *E. texana* were also detected.

The bacteria included in this study are the most frequent causal agents of urinary tract infection. According to the results of this study, methanolic extract of *E. polystachya* has broad-spectrum effect because the antibacterial activity observed included both Gram-positive and Gram-negative organisms.

Wächter *et al.*, using a methanol-dichloromethane extract obtained from the aerial parts of *E. texana*, isolated two new antibacterial and antifungal flavanones together with a known flavanone.[7] According to the results of the present study, methanolic extracts of *E. texana* have only limited antibacterial activity (only against *S. epidermidis*). No other antimicrobial activity for compounds isolated from *E. texana* has been reported.

The present results suggest that a methanolic extract of *E. polystachya* is a good alternative to other antibacterial compounds and underlines the importance of screening plant extracts in the search for new agents.

Finally, according to phytochemical screening, the *E. polystachya* extract contains flavonoids, terpenoids, carbon-carbon double bonds and phenolic compounds. Based on these results, the isolation and characterisation of active compounds from *E. polystachya* is particularly important in light of the multidrug resistance observed in certain Gram-positive and Gram-negative bacteria,[12,13] against which only a few therapeutic options are available.

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Absence of intervening sequences (IVSs) in helix 11 region within 16S rRNA genes among more than 240 isolates of the seven *Campylobacter* species

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Thermophilic *Campylobacter jejuni* and *C. coli* are curved Gram-negative bacteria that are the most recognised cause of acute bacterial diarrhoea in the Western world. Infrequently, human illness is associated with *C. lari*, *C. upsaliensis* and *C. fetus*.[1,2] The genus *Campylobacter* belongs to the ε-subdivision of the Proteobacteria.

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Ribosomal RNA genes are essential for the survival of all organisms and are therefore the most intensely studied genes in any bacteria. With regard to 16S rRNA genes of *Campylobacter* organisms, three *C. sputorum* biovars , namely bubulus, fecalis and sputorum, have been shown to carry longer 16S rRNA genes (approximately 250 base pairs [bp] in the helix 11 region) whose internal transcribed spacers are not present, and the 16S rRNA molecules were found to be fragmented in this organism.[3] In addition, in five of 12 *C. helveticus* isolates, the enlarged 16S rRNA gene was shown to contain an atypical intervening sequence (IVS).[4] Etoh *et al*. found and sequenced IVSs in the polymerase chain reaction (PCR) amplicons of 16S rRNA genes of three isolates of *C. rectus*, two of *C. curvus* and two of *C. sputorum*.[5]

Although some atypical *Campylobacter* isolates were subjected to clarify IVSs within 16S rRNA genes, studies on the identification of IVSs within 16S rRNA genes from major and typical *Campylobacter* species, namely *C. jejuni*, *C. coli* and *C. fetus*, have yet to appear.

The present study aims to clarify whether or not IVSs occur within 16S rRNA genes in more than 240 isolates of the seven *Campylobacter* species including the major and typical campylobacters, *C. jejuni*, *C. coli* and *C. fetus*, as well as atypical campylobacters, *C. lari*, *C. upsaliensis*, *C. concisus* and *C. hyointestinalis*.

A total of 241 *Campylobacter* isolates (*C. jejuni* [*n*=51], *C. coli* [*n*=11], *C. fetus* [*n*=33], *C. lari* [*n*=62], *C. upsaliensis* [*n*=44], *C. consisus* [*n*=10] and *C. hyointestinalis* [*n*=30]) were used (Table 1). Genomic DNA was prepared from the *Campylobacter* cells by sodium dodecyl sulphate (SDS) and proteinase K treatment, phenol-chloroform extraction and ethanol precipication.[6]

In the present study, a PCR primer pair of fD1 (5′-GAGTTTGATCCTGGCTCAG-3′)[7] and r-*Ca*16h11 (5′-TGGACCGTGTCTCAGTTCC-3′) was used to generate the helix 11 region within 16S rRNA gene sequences from the *Campylobacter* isolates. The r-*Ca*16h11 was constructed based on the sequence information from 14 isolates within the genus *Campylobacter* and eight isolates within the genus *Helicobacter* (data not shown). The PCR mixture contained 100 ng template DNA, 10 mmol/L Tris-HCl (pH 8.3); 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 100 mmol/L each dNTP, 0.64 µmol/L each primer, and 1 unit *Thermus aquaticus* [*Taq*] DNA polymerase [Takara Bio Inc, Shiga, Japan]).

The PCR was performed in 25-µL reaction volumes at 94°C for 5 min, 30 cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and finally 72°C for 5 min. The PCR products, separated by 1% (w/v) agarose gel electrophoresis in 0.5×TBE, were purified using a QIA quick PCR purification kit (Qiagen, Tokyo, Japan). The purified fractions were subjected to cycle sequencing with BigDye Terminator (version 3.1; Applied Biosystems, Tokyo, Japan) and with the sequencing primers. Sequence analysis was carried out using the Genetyx Windows software (version 9; Genetyx, Tokyo, Japan).

Nucleotide sequences of the helix 11 region within 16S rRNA gene sequences from the isolates of seven *Campylobacter* species analysed in the present study were compared to each other and with the accessible sequence data from other campylobacters using CLUSTAL W software (1.7 program),[8] which was incorporated in the DDBJ/EMBL/GenBank databases.

At present, the PCR primer pair (fD1 and r-*Ca*16h11) was designed to amplify the helix 11 region within 16S rRNA gene sequences in the 241 *Campylobacter* isolates examined. When PCR was first carried out with the isolates using the primer pair, amplicons were generated from all isolates (data not shown). Following sequencing and alignment analyses, all 241 *Campylobacter* isolates (51 *C. jejuni*, 11 *C. coli*, 33 *C. fetus*, 62 *C. lari*, 44 *C. upsaliensis*, 10 *C. concisus* and 30 *C. hyointestinalis*) were shown not to carry any IVSs in the helix 11 region within the 16S RNA genes (Figure 1a). Moreover, the nucleotide sequence of the helix 11 region from the *C. sputorum* biovar sputorum LMG7795^T strain, which has already been shown to carry IVS (240 nucleotides long) in the region,[3] was aligned together with those from *C. jejuni* 81–176, *C. coli* 23, *C. fetus* 8414c and *C. lari* JCM2530^T isolates for comparison (Fig. 1b). With regard to the sequences of the helix 11 region from three isolates, *C. lari* JCM2530^T UPTC NCTC12894 and UPTC NCTC12895, which were analysed in the present study, the authors have described their nearly full-length 16S rDNA and have employed these three DDBJ accession numbers in Table 1.[9]

Thus, no IVSs were identified in the helix 11 region within the 16S rRNA gene sequences among all 241 isolates from seven *Campylobacter* species (*C. jejuni*, *C. coli*, *C. fetus*, *C. lari*, *C. upsaliensis*, *C. concisus* and *C. hyointestinalis* [Figs. 1a and 1b]). With regard to the helix 11 region amplified and sequenced in the present study, UPTC NCTC12894 and 12895 isolates showed an identical nucleotide sequence of 257 bp (Fig. 1a, Table 1). In addition, an IVS of 233 bp (putative nucleotide position [np] 185–417; X67775) was shown for *C. suputorum* biovar sputorum LMG7795^T in the present alignment analysis, as indicated in Figure 1b. This IVS sequence has an extremely high A+T content (approximately 89%; Fig. 1b), as described by van Camp *et al*.[3]

In relation to the enlarged 16S rRNA genes containing IVSs in the genus *Campylobacter*, a total of 17 cases (three

Table 1. Summary of the identification of IVSs in the helix 11 region within 16S rRNA genes from *Campylobacter* organisms following sequencing and alignment analysis, and with an example of their accession number.

Campylobacter species	IVS in helix 11	Isolate	Accession number
C. jejuni (*n*=51)	0	*C. jejuni* 81-176	AB454519
		C. jejuni HP5110	AB453262
C. coli (*n*=11)	0	*C. coli* 23	AB453254
		C. coli 27	AB453255
C. fetus (*n*=33)	0	*C. fetus* 8414c	AB453258
		C. fetus 9813a	AB453259
C. lari (*n*=62)	0	*C. lari* 28	AB453263
		C. lari JCM2530^T	AB181368
		UPTC NCTC12894	AB181359
		UPTC NCTC12895	AB181360
C. upsaliensis (*n*=44)	0	*C. upsaliensis* neko104-1	AB453264
		C. upsaliensis neko 37-1	AB453265
C. concisus (*n*=10)	0	*C. concisus* LMG7961	AB453256
		C. concisus LMG7962	AB453257
C. hyointestinalis (*n*=30)	0	*C. hyointestinalis* 3014	AB453260
		C. hyointestinalis ATCC 35217	AB453261

IVS: intervening sequence.

isolates of *C. sputorum* biovars bubulus, fecalis and sputorum;[3] five of *C. helveticus*;[4] three *C. rectus*, two *C. curvus* and two *C. sputorum*;[5] and two *C. hyointestinalis* subsp. lawsonii[10]) have been described previously. In addition, these isolates are atypical members of the genus *Campylobacter*. Therefore, no examples have been described for the IVSs within 16S rRNA genes with the major and typical campylobacters *C. jejuni*, *C. coli* and *C. fetus*.

In the present study, seven *Campylobacter* species (both typical and atypical species) were shown not to carry any IVSs in the helix 11 regions. Moreover, no IVSs were identified in these seven *Campylobacter* species, suggesting that they may not have had an opportunity to interact with any other sources of IVS until now, or have not been able to integrate IVSs into their genomes.

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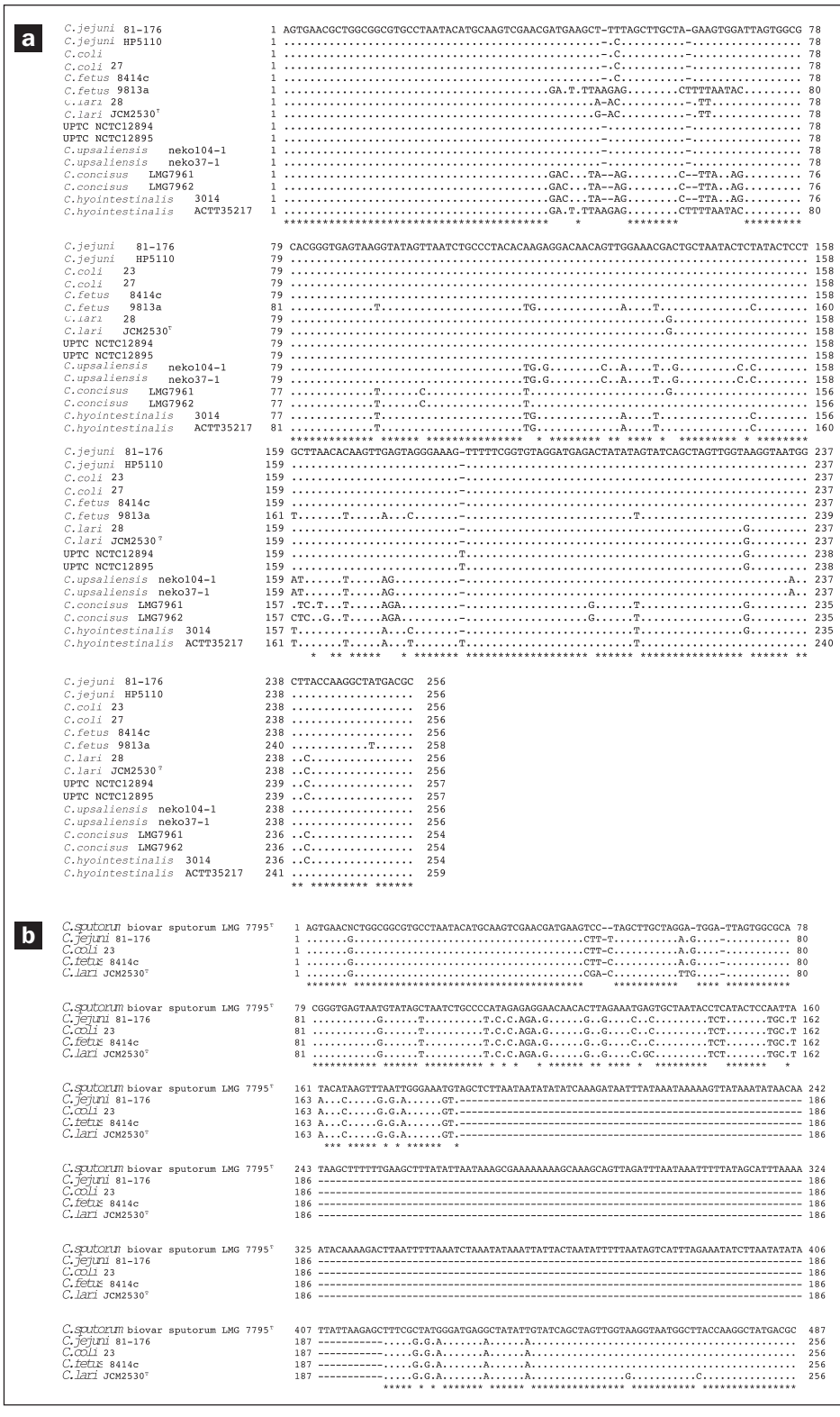

Fig. 1. Nucleotide sequence alignment analyses in the helix 11 region within 16S rRNA genes from seven *Campylobacter* species (A) examined. The accession numbers of the sequences analysed in the present study are shown in Table 1. Numbers on the left and right refer to the nucleotide positions determined in the present study. Nucleotide sequence from the *C. sputorum* biovar sputorum LMG7795^T strain (X67775)[3] was also aligned with those from the *C. jejuni* 81-176, *C. fetus* 8414c and *C. lari* JCM2530T isolates for comparison (B). Dots indicate identical bases; changes are explicitly indicated; dashes are deletions; identical positions in all cases are marked by asterisks.

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