Isolation and Characterization of *Campylobacter* spp. from Poultry Products and Detection of *cdt*A, *cdt*B and *cdt*C genes in *Campylobacter* spp.

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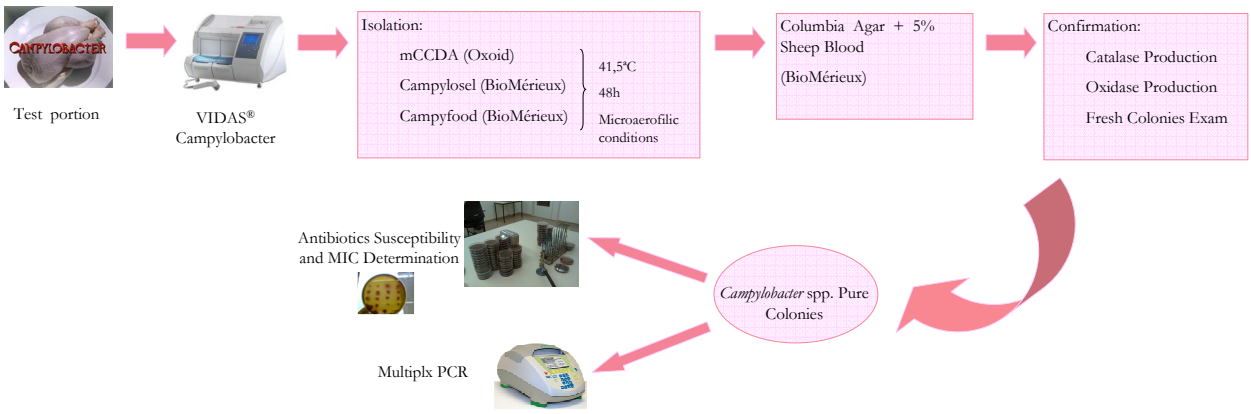
INTRODUCTION

Campylobacters are one of the most frequent causes of foodborne gastroenteritis in developing as well as developed countries. These species asymptomatically colonize the intestinal tracts of most mammals and birds, and one major route of human campylobacteriosis is assumed to be the consumption of contaminated poultry meat products. In poultry, *Campylobacter* spp. are considered to be commensals because the infection does not cause any clinical symptoms. However, the specific virulence mechanisms of *Campylabacter* spp. have not yet been adequately elucidated in humans diseases, although several virulence determinants have been proposed. The best characterized *Campylobacter* toxin is the cytolethal distending toxin (CDT) that enters into eukaryotic cells and breaks double-stranded DNA. *Campylobacter* diagnostics and determination of antibiotic resistence are also important for the treatment of infected individuals, and the distinction between the two most prevalent species in humans, namely *Campylobacter coli* and *Campylobacter jejuni*, is important for epidemological surveillance.

MATERIALS & METHODS

Experimental design



RESULTS & DISCUSSION

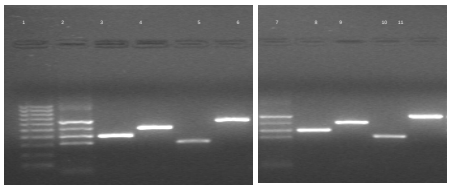


Fig. 1. Multiplex PCR detection and identification of the cdtA (422 bp), cdtB (531 bp), cdtC (339 bp) and 23S rRNA (650 bp) during the optimization process for randomly selected strains. Lane 1: 1000-bp DNA ladder (BioRad); lane 2: strain 6 (mixture of cdtA, cdtB, cdtC and 23S rRNA); lane 3: strain 6 (cdtA); lane 4: strain 6 (cdtB); lane 5: strain 6 (cdtC); lane 6: strain 6 (23S rRNA);lane 7: strain 81 (mixture of cdtA, cdtB, cdtC and 23S rRNA); lane 8: strain 81 (cdtA); lane 9: strain 81 (cdtB); lane 10: strain 81 (cdtC); lane 11: (23S rRNA)

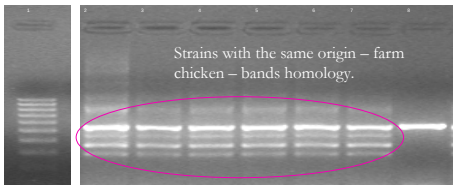


Fig. 3 . Multiplex PCR detection and identification of the *cdt*A (422 bp), *cdt*B (531 bp), *cdt*C (339 bp) and *23S* rRNA (650 bp) from food isolates . Lane 1: 1000-bp DNA ladder (BioRad); lane 2: strain 80; lane 3: strain 81; lane 4: strain 82; lane 5: strain 83; lane 6: strain 84/1; lane 7: strain 84/2; lane 8: strain 85

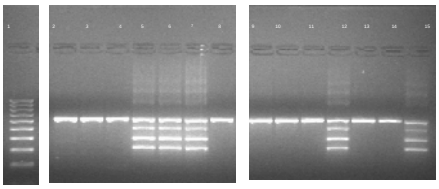


Fig. 2. Multiplex PCR detection and identification of the *cdt*A, *cdt*B, *cdt*C and *23S* rRNA from some of the studied isolates. Lane 1: 1000-bp DNA ladder (BioRad); lane 2: strain 1; lane 3: strain 3; lane 4: strain 4; lane 5: strain 6; lane 6: strain 7; lane 7: strain 8; lane 8: strain 9; lane 9: strain 16; lane 10: strain 17; lane 11: strain 18; lane 12: strain 19 ; lane 13: strain 20; lane 14: strain 12; lane 15: strain *Campylobacter jejuni* spp. *jejuni* ATCC® 33291

Table 1. Results of multiplex PCR for *Campylobacter* spp. strains

Strain	No. of strains tested	No. of strains that were PCR positive for:			
		*cdt*A	*cdt*B	*cdt*C	*23S* rRNA
Food	71	10	10	10	71
Human	20	11	11	11	20
HPA	3	3	3	3	3
Total	94	24	24	24	94

Table 2. MIC results for 18 *Campylobacter* spp. isolates randomly selected, as determined by agar dilution

Isolate	MIC (µg/ml)				
	Vancomycin	Ciprofloxacin	Rifampicin	Erythromycin	Nalidixic Acid
67	128	32	320	0,25	32
68	256	64	320	64	32
69/1	256	64	320	128	32
69/2	256	32	320	128	32
69/3	256	32	320	128	32
70/1	256	32	320	128	32
70/2	256	32	320	128	32
71	256	64	320	1	32
72	256	32	320	0,5	32
73	512	32	320	1	32
74	512	32	320	1	32
75	512	32	320	1	32
76	512	32	320	0,5	32
77	512	64	320	0,5	32
78	512	32	320	0,25	32
79	>512	32	320	0,5	32
80	256	32	320	<0,125	32
81	512	32	320	8	32

- Of the 32 poultry products analysed, 14 (43,8 %) were confirmed *Campylobacter* spp. positive
- From de antibiotics tested it was observed a high resistance to Vancomycin, Ciprofloxacin, Rifampicin and Nalidixic Acid (Table 2)
- There was no interaction between the primers *cdt*A, *cdt*B and *cdt*C (Fig.1)
- The *cdt*A, *cdt*B and *cdt*C genes were found in 14, 1 % of the food strains and 55,0 % of the human strains
- The *cdt*B genes were found in less number, for the generality of strains, than it was previously suggested (Bang D. D. et al., 2003)
- The multiplex PCR based approach developed here allows rapid and simultaneous detection of the *cdt*A, *cdt*B and *cdt*C genes in *Campylobacter* spp. isolates.

REFERENCES

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