PREVALENCE AND CONTROL OF THERMO-TOLERANT *CAMPYLOBACTER* SPECIES IN RAW POULTRY MEAT IN MOROCCO

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SAŽETAK

The prevalence of thermo-tolerant Campylobacter spp. was investigated in the raw poultry meat freshly slaughtered and retailed in Oujda area (Morocco). The results showed a high level of contamination of 62% of the meat samples analysed. The identification of the isolates showed the dominance of Campylobacter jejuni over Campylobacter coli, with frequencies of 90% and 10%, respectively. Some natural condiments, traditionally used in food preparations in Morocco, were tested for their antimicrobial activity on some isolates of C. jejuni and C. coli. Some of these condiments, including cinnamon, lemon juice, vinegar and sodium chloride showed a high inhibitory effect. While the others, including onion, lemon pH7, ginger, red and black peppers, cumin, garlic, parsley, coriander and saffron showed a slight or no inhibitory effect. The MICs of the most effective condiments were measured, and their in-vivo activity on fresh poultry meat, previously inoculated with Campylobacter cells, was evaluated. The results showed a high effectiveness of 1% (v/v) of lemon juice and vinegar and 2% (w/v) of cinnamon and sodium chloride in decontaminating the inoculated poultry meat, indicated by the elimination of Campylobacter cells to the undetectable level after 2 hours of inoculation.

Keywords: *Poultry meat, Campylobacter, control, condiments*

INTRODUCTION

Thermo-tolerant campylobacters are the most common causes of human bacterial gastro-enteritis in industrialised and developing countries (Friedman et al., 2000; Keener et al., 2004; Lastovica, 2006). They colonize the gastrointestinal tract of a wide range of domestic and livestock animals, such as birds, cows, chickens and pigs (Keener et al., 2004). The consumption and/or handling of their raw, undercooked and/or post-contaminated meat may be considered as a significant risk factor of Campylobacter infection for the health of consumer (Keener et al., 2004).

In Morocco, poultry slaughtering is still and mostly done by traditional procedures in shops, where the hygienic conditions are poor. The broilers are slaughtered, scalded in hot water, in order to facilitate the plucking, which is done by a machine, and carcasses are then manually eviscerated. The carcasses washed with tap water in another vessel are then sold or maintained at ambient temperature (20-30°C) during the day. The two water vessels, used for scalding and washing carcasses, are changed one to two times during the day. The poultry meat produced in these conditions is highly contaminated with coliform bacteria, *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp. and *Bacillus* spp. (*Amara* et al., 1994). However, campylobacters have never been checked in fresh poultry meat in Morocco. Many cases of acute diarrhoea associated with collective poultry meat

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consumption have been recorded in Morocco. The implication of *Campylobacter* species in these infections is highly suspected.

No antimicrobial drug therapy is usually required for *Campylobacter* infections, since they are of short duration and clinically mild. But, antimicrobial drug treatment is indicated for severe infections or persons at risk, such as children or immuno-compromised patients (Saenz et al., 2000; Engberg et al., 2001). However, the unrestricted use of antibiotics in poultry farming may lead to the emergence of antibiotic resistant *Campylobacter* strains (Pederson et al., 2003; Snelling et al., 2005) and compromise the health of infected consumers. The development of natural and more effective inhibitors may constitute an efficient and safe measure to avoid *Campylobacter* infections.

The main objectives of this work are to study (i) the prevalence of thermo-tolerant *Campylobacter* species in raw poultry meat produced in traditional shops in Morocco, and (ii) their control with natural condiments commonly used in food preparations.

MATERIAL AND METHODS

1. Isolation and identification of thermo-tolerant *Campylobacter spp.*

Fifty samples of raw poultry meat freshly slaughtered in traditional shops were collected from retail market in Oujda area (Morocco) and rapidly transported at 4°C to the laboratory. The pre-enrichment of *Campylobacter* was done by introducing 25 g of the skin of each sample in 225 ml of Brucella Broth (Biolife, Italy) supplemented with 5% (v/v) defibrinated horse blood. The incubation was done during 24 hours at 42°C under micro-aerophilic conditions using anaerobic jars with gas-generating Campy GasPak (BBL CampyPak, USA). The isolation was done by streaking a drop of the enriched Brucella Broth samples on modified Charcoal Cefoperazone Desoxycholate agar (mCCDA, Biolife Italy) and Karmali agar (Biolife, Italy), and then incubated under micro-aerophilic conditions as described above.

The suspected colonies were tested for their typical corkscrew-like motion, Gram stain, and catalase and oxidase reactions. The strains maintained were then studied for H_2S production and sugar utilisation on TSI agar (Biolife, Italy), Sodium hippurate hydrolysis, resistance/sensibility to the cephalotin and growth at 25°C. All the tests using incubation were done under micro-aerophilic conditions.

2. Antimicrobial activity of natural condiments

Preparation of condiments

The natural condiments tested for their antimicrobial activity on campylobacters were: onion, lemon, red pepper, black pepper, vinegar, cumin, cinnamon, garlic, parsley, coriander, saffron and salt.

The onion, garlic, parsley, lemon and coriander were ground and then the juices obtained were sterilised with 0.45 µm sterile membranes. The lemon pH 7 was obtained by neutralising lemon juice with NaOH 0.1 N to pH 7. The other compounds including red pepper, black pepper, vinegar, cumin, cinnamon, saffron and salt, were sterilised by autoclaving at 121°C/15 minutes.

Screening tests

One millilitre of a fresh culture of *Campylobacter* was diluted to 1/10 in Brucella Broth, and then inoculated by spreading on Karmali agar supplemented with 5% (v/v) of defibrinated horse blood. The strains selected for this test were *C. jejuni* (strains S1 and S4) and *C. coli* (strains S13 and S16). The sterilised compounds, by filtration or autoclaving, were impregnated in sterile Whatman paper discs, and then put on the medium. The incubation of the assays, made in duplicate, was done at 42°C for 3 days under micro-aerophilic conditions. The inhibition zones obtained around the paper discs were then measured.

Minimal Inhibitory Concentration measurement

The minimal inhibitory concentrations (MICs) of the compounds showing a high inhibitory effect, during the screening test, were measured on Karmali agar supplemented with 5% (v/v) of defibrinated horse blood. The condiments selected were sodium chloride, cinnamon, vinegar and lemon. The concentrations tested by incorporation in the medium were: 0%, 0.01%, 0.1%, 0.5%, 1%, 2% and 4% (w/v or v/v). Streaking an overnight culture of

the strain studied on the medium did the inoculation. The trials, made in duplicate, for the four studied strains of *C. jejuni* (strains S1 and S4) and *C. coli* (strains S13 and S16), were incubated at 42°C for 3 days under micro-aerophilic conditions. The lower concentration showing a total growth inhibition corresponds to the MIC of the condiment.

APPLICATION OF CONDIMENTS ON MEAT

The same condiments studied for the MICs measurement were applied on fresh poultry meat, previously contaminated with *Campylobacter* strains. For this, 10 g of fresh skin of poultry meat were introduced in 100 ml of sterile *Brucella broth* supplemented with the MIC of the condiment. The medium was then inoculated with an overnight culture of the strains of *C. jejuni* (S1 and S4) and *C. coli* (S14 and S16) of initially about 2.7 106 CFU/ml. The cultures, made in duplicate, were then incubated under micro-aerophilic conditions at 42°C during 5 days.

The viability of *Campylobacter* cells was followed by spreading 0.1 ml of the cultures, of each trial, on Karmali agar supplemented with 5% (v/v) of defibrinated horse blood. The inhibitory effect was evaluated by the presence or absence of colonies of *Campylobacter* on Karmali agar after incubation under micro-aerophilic conditions.

RESULTS AND DISCUSSION

1. Prevalence of *Campylobacter* species in fresh poultry meat

The analyses of the fresh poultry meat showed that 31 of 50 analysed samples are contaminated with thermo-tolerant *Campylobacter* strains (Table 1). The identification of the 42 strains isolated from samples showed that they are mainly represented by *C. jejuni* and *C. coli*, with frequencies of 90% and 10%, respectively (Table 1).

The high level of contamination with *Campylobacter* species obtained (62%) is comparable to the results found in other developed and developing countries (Friedman et al., 2000; Atanassova et al., 2007). The two species we isolated are the most common causes of human gastrointestinal diseases (Keener et al., 2004).

This finding may explain the implication of poultry meat in many cases of collective and non-identified origins of food poisonings in Morocco, and to consider poultry meat as a potential source of gastro-intestinal diseases caused by *C. jejuni* and/or *C. coli.* Furthermore, poultry meat is widely consumed in Morocco, particularly in rural areas, due to its low price compared to red meats. Therefore, the improvement of control strategies of these enteropathogens in poultry farming, slaughtering and during manipulation may reduce considerably this potential risk to public health.

The high level of contamination recorded in this study may be due to the poultry itself and mainly to the cross contamination, since the poultry carcasses preparation is mostly done in traditional shops, where the hygienic conditions are poor. In fact, the slaughtering and dressing of carcasses are done

▼ **Table 1.** Characteristics of *Campylobacter* strains isolated from raw poultry meat
▼ **Tablica 1.** Karakteristike sojeva *Campylobacter* spp. izoliranih iz sirovog mesa peradi

Strains / Sojevi		Gram	Cat	Ox	25°C	42°C	Sug	H_2S	Ceph	Hipp	Species / Vrste
Nb	%										
37	90.24	-	+	+	-	+	-	-	R	+	*C. jejuni* ssp *jejuni*
4	9.76	-	+	+	-	+	-	±	R	-	*C. coli*

Nb = number of strains, Cat = catalase, Ox= oxidase, Sug = sugars utilisation (glucose and lactose), H_2S = H2S production, Ceph = resistance to cephalotin, Hipp = Hippurate hydrolysis, R = Resistance, + = positive reaction, - = negative reaction, ± = slightly positive reaction.
Nb =broj sojeva, Cat = katalaza, Ox= oksidaza, Sug = Iskorištenje šećera (glukoza i laktoza), H_2S = H_2S proizvodnja, Ceph = otpornost na cefalotin, Hipp = Hidroliza hipurata, R = Otpornost, + = pozitivna reakcija, - = negativna reakcija, ± = slaba pozitivna reakcija.

manually, using two potable water vessels, changed one to two times a day for plucking and washing carcasses. In these conditions, *Campylobacter* species may contaminate easily the carcass, the environment and the operator, causing thereby pathogenesis for the consumer and/or the operator. The development of more efficient and safe antimicrobial agents is necessary for reduction of *Campylobacter* outbreaks.

2. Antimicrobial activity of condiments

The results of the screening test of the antimicrobial activity of the condiments studied are presented in table 2. The results show that lemon, vinegar, cinnamon and sodium chloride present a high inhibitory effect on the four studied strains of *C. jejuni* (S1 and S2) and *C. coli* (S14 and S16), with inhibition zones varying between 0.95 and 1.6 cm. The other compounds showed a slight or no inhibitory effect.

The MICs of the selected condiments on the four strains of two *Campylobacter* species were 2% for cinnamon and sodium chloride and 1% for vinegar and lemon (Table 3). The application of these MICs on meat previously inoculated with *C. jejuni* and *C. coli* showed a rapid elimination to the undetectable level, in one hour, of these pathogenic microorganisms (Table 4), leading to the improvement of the hygienic quality of the product.

The antimicrobial activity of vinegar and lemon may be attributed to their acidity, since the neutralised lemon juice showed no inhibitory effect. The treatment of poultry meat with organic acids and/or acidified sodium chloride was known for its effectiveness in reduction of *Campylobacter* charges (SCVMRPH, 1998; Kemp et al., 2001).

The most important finding in this work is the highest sensibility of *Campylobacter* strains to the cinnamon. This result is of great interest in food industry, not only in the prevention of campylobacteriosis, but in the improvement of sensor properties of foods as well, particularly in non-heat-treated foods, such as milk products (ice creams) and pastry making. Cinnamon was demonstrated for its improvement of the shelf life and sensor properties of almond pastes (Faid et al., 1995).

It should be emphasised that there are two culinary habits widely practiced in poultry cooking in Morocco, which may be considered as great barriers in preventing human *Campylobacter* infections. The first one consists of a pre-treatment of carcasses with vinegar and sodium chloride to take of the characteristic odour of poultry meat. And the second consists of overcooking meat with natural condiments to develop good colour and taste of meat. These traditional habits, used for a culinary

Table 2. Antimicrobial activity of natural condiments on *campylobacter* strains isolated from raw poultry meat
Tablica 2. Antimikrobijsko djelovanje prirodnih začina na sojeve *Campylobacter* spp. izolirane iz sirovog mesa peradi

	Onion Luk	Lemon Limun	Lemon Limun (pH 7)	Ginger Đumbir	Red pepper Crveni papar	Black pepper Crni papar	Vinegar Ocat	Cumin Kumin	Cinnamon Cimet	Garlic Češnjak	Parsley Peršin	Coriander Korijander	NaCl	Saffron Šafran
C. jejuni S1	0.6	0.9	0.7	0.8	0.8	0.7	1.1	0.8	1.4	0.7	0.7	0.8	1.0	0.7
C. jejuni S2	0.6	0.8	0.7	0.8	0.7	0.8	1.1	0.7	1.6	0.7	0.8	0.7	1.2	0.8
C. coli S13	0.6	1.1	0.7	0.7	0.8	0.7	1.1	0.9	1.2	0.8	0.7	0.7	1.1	0.8
C. coli S16	0.6	1.1	0.6	0.6	0.8	0.8	0.9	0.9	1.2	0.8	0.8	0.8	1.1	0.7

Legends= onion, lemon, lemon (pH = 7), red pepper, black pepper, vinegar, cumin, cinnamon, garlic, parsley, coriander, sodium chloride, saffron.
Values are given in cm, the paper disc diameter is 0.6 cm
Legenda = luk, limun, limun (pH = 7), crveni papar, crni papar, ocat, kumin, cimet, češnjak, peršin, korijander, natrijev klorid, šafran.
Vrijednosti su prikazane u cm, a promjer papirnatog diska iznosio je 0.6 cm

▼ **Table 3.** Minimal Inhibitory concentrations (MICs) of some condiments on *Campylobacter* strains isolated from raw poultry meat (these results are obtained with *C. jejuni* S1 and *C. coli* S16)

▼ **Tablica 3.** Minimalne inhibitorne koncentracije (MICs) nekih začina na sojeve *Campylobacter* spp. izolirane iz sirovog mesa peradi (ovi su rezultati dobiveni s *C. jejuni* S1 i *C. coli* S16)

	Concentrations of the condiments / Koncentracije začina (%)						
	0	0.01	0.1	0.5	1	2	4
Sodium chloride / Natrijev klorid (w/v)	+	+	+	+	+	-	-
Cinnamon / Cimet (w/v)	+	+	+	+	+	-	-
Lemon / Limun (v/v)	+	+	+	+	-	-	-
Vinegar / Ocat (v/v)	+	+	+	+	-	-	-

Legends: + growth, - no growth
Legenda: +rast, - bez rasta

objective, lead to the reduction of the incidence of microbial infections in population, because of their antimicrobial activity.

CONCLUSION

This work leads us to the conclusion that poultry carcasses should be treated at least during one hour with 2% of cinnamon and sodium chloride and 1% of vinegar and lemon before cooking and/or handling by consumers. This treatment may constitute a practical and more effective measure to improve the hygienic quality of the product, and prevent or reduce therefore the incidence of the acute *Campylobacter* gastrointestinal diarrhoeas in population.

SUMMARY

PREVALENCIJA I KONTROLA TERMO-TOLERANTNIH CAMPYLOBACTER SPP. U SVJEŽEM MESU PERADI U MAROKU

Prevalencija termo-tolerantnih Campylobacter spp ispitana je u sirovom mesu peradi neposredno nakon klanja i stavljanja u promet u području Oujda (Maroko). Rezultati su pokazali visok stupanj kontaminiranosti u 62% analiziranih uzoraka mesa. Identifikacija izolata pokazala je dominaciju Campylobacter jejuni nad Campylobacter coli, uz učestalost od 90% odnosno 10%. Ispitano je antimikrobijsko djelovanje nekih prirodnih začina, koji se tradicionalno koriste u pripremi hrane u Maroku, na neke izolirane sojeve vrste C. jejuni i C.coli. Pokazalo se da neki od tih začina, uključivši cimet, sok limuna, ocat i natrijev klorid, imaju snažan inhibitorni učinak. Nasuprot tome, neki drugi začini kao što su luk, limun s pH7, đumbir, crveni i crni papar, kumin, češnjak, peršin, korijander i šafran imaju slab ili nikakav inhibitorni učinak. Određene su minimalne inhibitorne koncentracije najdjelotvornijih začina, te je procijenjeno njihovo in-vivo djelovanje na svježe meso peradi, koje je prethodno inokulirano bakterijom. Dobiveni rezultati su pokazali značajnu djelotvornost od 1% (v/v) limunovog soka i octa, odnosno 2% (t/v) cimeta i natrijevog klorida na dekontaminaciju inokuliranog mesa peradi, a što je bilo vidljivo iz eliminacije stanica bakterija do nemjerljive razine 2 sata nakon inokulacije.

Ključne riječi: *Meso peradi, Campylobacter spp., kontrola, začini*

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▼ **Table 4.** Inhibition of campylobacter strains in raw poultry meat (these results are obtained with *C. jejuni* S1 and *C. coli* S16)

▼ **Tablica 4.** Inhibicija sojeva Campylobacter spp.u sirovom mesu peradi (ovi su rezultati dobiveni s *C. jejuni* S1 i *C. coli* S16)

	Duration of incubation (hours) / Trajanje inkubacije (sati)				
	0	1	2	24	120
Sodium chloride / Natrijev klorid (2% w/v)	+	+	-	-	-
Cinnamon / Cimet (2% w/v)	+	+	-	-	-
Lemon / Limun (1% v/v)	+	+	-	-	-
Vinegar / Ocat(1% v/v)	+	+	-	-	-

Legends: + growth, - no growth
Legenda: +rast, - bez rasta

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RAZGRADNJA LIPIDA MIŠIĆNOG I MASNOG TKIVA TIJEKOM ZRENJA PRŠUTA

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SAŽETAK

Lipoliza je složeni biokemijski proces u tkivima buta tijekom prerade pršuta u kojem pod utjecajem endogenih, a manjim dijelom i egzogenih enzima (enzimi mikroorganizama) dolazi do razgradnje lipida intramuskularnog i adipoznog tkiva do slobodnih masnih kiselina. Tijek lipolize intramuskularnih i lipida adipoznog tkiva bitno se razlikuju, što je uvjetovano razlikama u lipidnom i enzimskom sastavu. Intramuskularni lipidi sastavljeni su od triglicerida i fosfolipida te lipoliza ovdje započinje njihovom hidrolizom, pri čemu tijek hidrolize triglicerida ide preko di- i monoacilglicerola do slobodnih masnih kiselina, a hidroliza fosfolipida izravno do slobodnih masnih kiselina. Razgradnja lipida adipoznog tkiva (intermuskularna i potkožna masnoća) započinje hidrolizom triglicerida od kojih su uglavnom sastavljeni, preko di- i monoacilglicerola i glicerola do slobodnih masnih kiselina. Proces lipolize ima veliki utjecaj na kvalitetu pršuta zbog izravnog učinka na aromu i okus. Tijek lipolize uvelike ovisi o tipu pršuta, tipu masnog tkiva (adipozno i intramuskularno) te količini endogenih lipolitičkih enzima i specifičnim uvjetima u preradbenom procesu. Slobodne masne kiseline nastale u procesu lipolize, osobito one polinezasićene, stvaranjem prekursora okusa i arome koji služe kao supstrat za budu-

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