

Survival of thermophilic campylobacters on fingertips and their elimination by washing and disinfection

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SUMMARY

A simple impression-plate technique has been used to investigate the survival of four thermophilic campylobacter strains applied to fingertips. Campylobacters suspended in 0.1% peptone water and dried on the fingertips survived for different periods of time ranging from <1 to ≥ 4 min. However, campylobacters suspended in chicken liquor or blood survived for much longer periods. The most resilient organism was *Campylobacter jejuni* NCTC 11392 which, when suspended in 50% horse blood, survived for an hour. Suspensions containing 10^6 – 10^7 organisms prepared in 50% blood and dried on to fingertips were removed by thorough hand washing with either soap and water or water alone followed by drying on paper towels, but persisted on wet hands. The organisms were also eliminated by wiping the hands with a tissue saturated with 70% isopropyl alcohol for 15 sec.

INTRODUCTION

In the United Kingdom campylobacter enteritis has emerged as the most frequently reported form of acute bacterial diarrhoea (Skirrow, 1982). Whilst outbreaks have been shown to follow the consumption of contaminated meat (Brouwer *et al.* 1979), milk (Robinson *et al.* 1979) and water (Mentzing, 1981) the vehicle of most of the day-to-day sporadic cases has remained elusive.

Campylobacters are unlikely to survive for prolonged periods outside the body because of their microaerophilic nature and sensitivity to drying (Doyle & Roman, 1982), but because as few as 500 organisms swallowed in a glass of milk can produce infection (Robinson, 1981) transmission via contaminated hands is possibly a major route of infection in sporadic cases. This may take place directly by the hand-to-mouth route when food handlers process raw food, and through the cross-contamination of foods and utensils in kitchens when cooked food is handled immediately after uncooked. Infections transmitted from young children to siblings and parents (Butzler & Skirrow, 1979) and from sick dogs (Blaser *et al.* 1978) and cats (Svedhem & Norkrans, 1980) to their handlers are most likely to be acquired by the faecal-oral route from contaminated hands. Inadequate hand washing is probably the major contributory factor involved in transmission in all such instances.

The present study had two main aims. The first was to investigate the ability of campylobacters to survive on fingertips taking into account the variables: (1)

Campylobacter species and inoculum size; (2) the age and sex of volunteers; and (3) the suspending fluid – peptone water, blood or thawed chicken liquor. The second was to determine whether washing with water alone, or with unmedicated bar soap and water, or disinfection with a tissue saturated with 70% isopropyl alcohol eliminated campylobacters from the fingertips.

MATERIALS AND METHODS

Organisms

The test organisms were *Campylobacter jejuni* NCTC 11168 (biotype 1), (Skirrow & Benjamin, 1980), *C. jejuni* NCTC 11392 (biotype 2), *C. coli* NCTC 11353 and *C. laridis* NCTC 11352.

Preparation of standardized suspensions

The organisms were grown on Columbia agar (Oxoid CM 331) containing 5% horse blood, incubated microaerobically at 37 °C for 24 h. Bacteria were harvested into 0.1% peptone water (Oxoid L 37) and the suspensions standardized to 10⁸–10⁹ colony forming units (c.f.u.) per ml by reference to an absorbance versus viable count plot obtained using a Perkin–Elmer model 6/20 spectrophotometer at a wavelength of 450 nm. Tenfold dilutions in 0.1% peptone water were prepared as required. Suspensions in blood were prepared by mixing the dilutions with sterile, defibrinated horse blood (Tissue Culture Services). Suspensions in thawed chicken liquor were prepared by harvesting plate cultures directly into liquor and were standardized spectrophotometrically against a liquor blank.

Preparation of thawed chicken liquor

Twelve frozen boiling hens (old egg-laying birds) were allowed to thaw out overnight on an aluminium tray. About 500 ml of liquor were collected and filtered gravitationally through a coarse filter to remove debris. The filtrate was then distributed into universal bottles and frozen at –20 °C. When required the liquor was thawed and sterilized by sequential filtration through 1.2, 0.45 and sterile 0.22 µm membrane filters. Sterility was checked by culturing on to blood agar plates.

Impression plate medium

The medium used for impression plates was Modified CCDA-Preston (Bolton, Hutchinson & Coates, 1984; Hutchinson & Bolton, 1984) except that the agar concentration was increased to 2% to give discrete colonies.

Impression plate technique

Volunteers were instructed not to use any antibacterial preparations on their hands in the 72 h pre-test period.

Suspensions of organisms in 0.1% peptone water with or without added blood, or in chicken liquor, were inoculated on to the fingertips using a 50-dropper pipette. After application of one drop (0.02 ml) to each fingertip of either or both hands, the fingertips were gently rubbed together for 1 min to spread the inoculum evenly and then left for a further minute to dry. At this stage (taken as zero time)

the thumb (finger 1) was pressed on to the surface of the impression plate for 2 sec. Subsequently, at timed intervals, the index finger (finger 2) and remaining fingers (3, 4 and 5) were pressed on to the impression plate. One plate was used for the five fingertips of each hand. Plates were incubated microaerobically at 37 °C for 48 h. Colony counts were then made from each fingertip impression.

After each experiment volunteers were instructed to scrub their fingers and hands with non-medicated bar soap and water and, after drying thoroughly, disinfect them with a tissue saturated with 70% isopropyl alcohol (Alcowipe; Schering Prebbles Ltd).

Hand washing and disinfection studies

The fingertips of both hands of volunteer A were contaminated with 0.02 ml of suspension containing 10^6 – 10^7 organisms in 50% peptone water (0.1%):50% defibrinated horse blood. The fingertips were rubbed together gently for 1 min to spread the inoculum and then left to dry for a further 1 min. At this stage the fingertips of one hand (control) were pressed for 2 sec on to the surface of an impression plate. The hands were then decontaminated by one of five methods after which the fingers of the other hand (test) were pressed on to a second plate. Using soap and water (method 1) the hands were washed under warm running water using government issue, non-medicated bar soap. Twenty seconds were allowed for building up a lather, 20 sec for rubbing the finger and thumb tips against the palms of the opposite hand and against each other (right fingertips against left palm five times and vice versa; right thumb tip against left palm five times and vice versa; right fingertips against left fingertips five times; and finally right thumb tip against right fingertips and left thumb against left fingertips until time was completed) and a final 20 sec for rinsing. The hands were then dried on two clean paper towels for 30 sec. Method 2 was similar to method 1 except that the hands were shaken 'dry' for 10 sec before impression culture. When water alone (method 3) was used the hands were rubbed together under warm running water for 20 sec to simulate building up a lather and thereafter the procedure was similar to method 1. Method 4 was as for method 3 except that the hands were shaken 'dry' after washing. In method 5 fingertips were rubbed with a tissue saturated with 70% isopropyl alcohol (Alcowipe) for 15 sec and then air-dried for 30 sec. Plates were incubated microaerobically at 37 °C for 48 h and then examined for campylobacter colonies. All experiments were performed at least twice and the mean count/fingertip impression calculated.

RESULTS

Selectivity of impression plate medium

In preliminary experiments it was established that the Modified CCDA–Preston medium inhibited the growth of the normal bacterial flora present on fingertips (predominantly coagulase-negative cocci) and thus eliminated the difficulty of differentiating campylobacters from other bacteria. Moulds were occasionally isolated but were easily recognized.

Table 1. *Survival of C. jejuni* NCTC 11168 suspended in 0.1% peptone water on fingertips

Volunteer			Inoculum (organisms/ fingertip)	Hand	Recovery (c.f.u./fingertip) after intervals (min)				
Identity	Sex	Age			0*	1	2	3	4
A	M	37	1.8×10^3	L	1	0	0	0	0
			2.4×10^4	L	C†	0	0	0	0
			1.5×10^5	L	C	32	1	0	0
			2.5×10^6	L	C	16	6	6	0
			2.4×10^7	L	C	4	4	0	2
			8.0×10^3	R	0	0	0	0	0
			4.5×10^4	R	21	0	0	1	0
			1.2×10^5	R	C	9	7	3	0
			4.5×10^6	R	C	1	4	1	0
B	M	35	2.4×10^7	R	C	15	5	2	2
			1.8×10^3	L	0	0	0	0	0
			1.8×10^5	L	0	1	5	5	0
			1.3×10^7	L	C	8	0	0	0
			1.5×10^7	R	C	11	8	43	21
C	M	41	4.5×10^6	L	0	0	0	0	0
			4.5×10^6	R	1	0	0	0	1
			1.2×10^7	R	0	0	0	0	0
D	M	55	1.2×10^4	L	0	0	0	0	0
			5.6×10^5	L	22	22	1	1	0
			1.8×10^7	L	C	2	3	2	1
E	M	68	2.2×10^5	L	0	0	0	0	0
			1.8×10^6	L	0	1	0	0	0
			1.2×10^7	L	C	C	24	1	2
			1.2×10^7	R	C	C	C	1	0
F	F	23	1.2×10^4	L	0	0	0	0	0
			3.7×10^6	L	42	27	12	1	0
			2.4×10^7	L	C	C	58	35	10
G	F	24	7.0×10^6	L	C	0	1	0	0
			7.0×10^6	R	41	0	0	0	0
H	F	23	1.3×10^7	R	C	35	30	5	2

* Zero time was regarded as 2 min after inoculation of drops on to fingertips, this allowing time for spreading and drying to occur.

† C, confluent growth.

Survival of campylobacters suspended in 0.1% peptone water on fingertips

The survival of *C. jejuni* NCTC 11168 when suspended in 0.1% peptone water on the fingertips of eight volunteers is shown in Table 1. Survival was not apparently affected by the sex, age or hand (right or left) of volunteers. Within the preliminary 2 min spreading and drying period a 3–7 \log_{10} reduction of viable cells occurred, which was probably due primarily to the lethal effect of drying. Some variation in survival from volunteer to volunteer was apparent – whereas 10^4 – 10^5 cells dried on to the fingers of volunteer A produced confluent growth on impression plates at zero time, 10^7 cells dried on to the fingers of volunteer C produced no colonies at zero time. The survival time after drying increased with the inoculum size: the results obtained with volunteer A (right hand) show that whereas an inoculum of 10^3 cells failed to survive the 2 min drying period, an

Table 2. *Survival of different campylobacters suspended in 0.1% peptone water on fingertips of volunteer A*

Species	Inoculum (organisms/fingertip)	Recovery (c.f.u./fingertip) after intervals (min)				
		0	1	2	3	4
<i>C. jejuni</i> NCTC 11392	9.0×10^2	0	0	0	0	0
	9.0×10^4	0	0	0	0	0
	9.0×10^6	C	2	1	1	0
<i>C. coli</i> NCTC 11353	1.4×10^3	4	0	0	0	0
	1.4×10^5	C	0	5	0	0
	1.4×10^7	C	38	6	1	0
<i>C. laridis</i> NCTC 11352	2.2×10^3	9	0	0	0	0
	2.2×10^5	C	3	4	5	0
	2.2×10^7	C	4	47	0	2

Table 3. *Survival of different campylobacters suspended in 0.1% peptone water plus defibrinated horse blood on fingertips of volunteer A*

Species	Inoculum		Recovery (c.f.u./fingertip) after intervals (min)								
	(organisms/ fingertip)	Blood (%)	0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0
<i>C. jejuni</i> NCTC 11168	3.2×10^4	10	78	0	0	0	0	0	0	0	0
	6.0×10^6	10	C	16	0	5	0	1	0	0	0
	2.3×10^4	25	40	0	0	0	0	0	0	0	0
	3.6×10^6	25	C	92	53	19	0	8	0	0	0
	1.1×10^4	50	25	3	1	0	0	0	0	0	0
<i>C. jejuni</i> NCTC 11392	9.0×10^6	50	C	C	C	C	C	10	0	0	0
	7.5×10^2	50	10	0	0	0	0	0	0	0	0
	7.5×10^4	50	C	56	71	22	2	12	17	4	3
	7.5×10^6	50	C	C	C	C	C	C	C	C	C
<i>C. coli</i> NCTC 11353	6.7×10^2	50	6	0	0	0	0	0	0	0	0
	6.7×10^4	50	10	0	0	0	0	0	0	0	0
	6.7×10^6	50	C	C	92	78	9	20	11	3	0
<i>C. laridis</i> NCTC 11352	4.5×10^2	50	13	0	0	0	0	0	0	0	0
	4.5×10^4	50	11	2	0	0	0	3	1	2	0
	4.5×10^6	50	C	C	C	C	4	49	32	59	6

inoculum of 10^7 cells still contained survivors after 4 min. For all eight volunteers, impressions made after just 4 min produced few or no colonies, demonstrating that suspensions of *C. jejuni* NCTC 11168 in 0.1% peptone water deposited on the fingertips rapidly lose viability after drying.

The survival of *C. jejuni* NCTC 11392, *C. coli* NCTC 11353 and *C. laridis* NCTC 11352 when suspended in 0.1% peptone water on the fingertips of volunteer A is shown in Table 2. The results were similar to those obtained for *C. jejuni* NCTC 11168, with all three organisms rapidly losing viability after drying.

Survival of campylobacters suspended in peptone water plus blood on fingertips

In preliminary experiments it was established that the defibrinated horse blood used in tests had no anti-campylobacter activity.

The survival of the four test organisms when suspended in mixtures of 0.1%

Table 4. *Survival of different campylobacters suspended in undiluted chicken liquor on fingertips of volunteer A*

Species	Inoculum (organisms/ fingertip)	Recovery (c.f.u./fingertip) after intervals (min)								
		0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0
<i>C. jejuni</i> NCTC 11168	2.4×10^3	3	0	0	0	0	0	0	0	0
	2.4×10^5	C	15	14	4	0	0	0	0	0
	2.4×10^7	C	C	SC*	SC	55	37	31	3	9
<i>C. jejuni</i> NCTC 11392	5.1×10^2	0	0	0	0	0	0	0	0	0
	5.1×10^4	C	5	9	0	1	1	2	0	0
	5.1×10^6	C	SC	SC	59	5	5	0	3	4
<i>C. coli</i> NCTC 11353	1.9×10^3	1	0	0	0	0	0	0	0	0
	1.9×10^5	SC	6	3	0	0	0	0	0	2
	1.9×10^7	C	SC	SC	SC	37	58	32	10	5
<i>C. laridis</i> NCTC 11352	8.0×10^2	36	0	0	0	0	0	0	0	0
	8.0×10^4	C	17	22	7	2	0	1	0	0
	8.0×10^6	C	SC	SC	SC	7	23	38	17	33

* SC, Semi-confluent growth (>100 c.f.u.).

peptone water and blood on the fingertips of volunteer A is shown in Table 3. For tests on *C. jejuni* NCTC 11168 suspensions were prepared in either 10, 25 or 50% horse blood. It can be seen that as the concentration of blood increased the survival time was extended. Likewise the survival time was prolonged when suspensions of *C. jejuni* NCTC 11392, *C. coli* NCTC 11353 and *C. laridis* NCTC 11352 were prepared in 50% blood, and also when the inoculum size was increased. In extended studies with *C. jejuni* NCTC 11392 using an inoculum of 2×10^7 cells in 50% blood per fingertip 10 c.f.u. per fingertip were obtained on an impression plate after 45 min and one c.f.u. per fingertip after 60 min.

Survival of campylobacters suspended in chicken liquor on fingertips

In preliminary experiments it was established that the chicken liquor had no anti-campylobacter activity.

The survival of the four test organisms when suspended in undiluted chicken liquor on the fingertips of volunteer A is shown in Table 4. Similar results were obtained for all four organisms. Comparison with Table 3 shows that the protective effect of undiluted chicken liquor and 50% defibrinated horse blood were generally similar, although the chicken liquor did not match the degree of protection given by blood to *C. jejuni* NCTC 11392. The survival times of organisms dried on the fingers in chicken liquor increased with inoculum size.

Washing and disinfection of fingertips contaminated with campylobacters

It was found (Table 5) that washing with either soap and water or water alone, combined with drying on paper towels, physically removed all campylobacters from the fingertips. However, when hands were merely shaken 'dry' after washing some campylobacters remained, in which case washing with soap and water appeared to be more efficient than washing with water alone. Rubbing the fingertips with a tissue saturated with isopropyl alcohol was extremely effective,

Table 5. *Washing and disinfection of fingertips contaminated with campylobacters**

Method of decontamination	Time (s)	Mean c.f.u./fingertip impression	
		Control‡	Test
Soap and water	60	Confluent growth	0†
Paper towels	30		
Soap and water	60	Confluent growth	4 <i>C. jejuni</i> biotype 1
Shaking	10		3 <i>C. jejuni</i> biotype 2
			7 <i>C. coli</i>
			1 <i>C. laridis</i>
Water	60	Confluent growth	0
Paper towels	30		
Water	60	Confluent growth	1 <i>C. jejuni</i> biotype 1
Shaking	10		43 <i>C. jejuni</i> biotype 2
			> 50 <i>C. coli</i>
			14 <i>C. laridis</i>
Isopropyl alcohol	15	Confluent growth	0
Air dry	30		

* 10^6 – 10^7 cells of either *C. jejuni* NCTC 11168, *C. jejuni* NCTC 11392, *C. coli* NCTC 11353 or *C. laridis* NCTC 11352 suspended in 50% peptone water (0.1%):50% defibrinated horse blood and dried on for 2 min.

† 0, No growth of the four strains of campylobacter in each of two experiments.

and all of the campylobacters were killed within 15 sec, which was probably due to a combination of the bactericidal properties of the disinfectant and its drying effect on the skin.

DISCUSSION

Organisms present on the skin may be divided into three categories (Noble, 1981): (1) transient organisms which contaminate the skin but do not multiply on it; (2) temporary residents which contaminate the skin, multiply on it, and persist for short periods; and (3) resident organisms which are permanent inhabitants of the skin. Campylobacters picked up on the hands are likely to be transient organisms, which are situated superficially and hence should be easily removed by washing or killed by application of skin disinfectants. Hence it was considered that the fingertip impression-plate technique was suitable for studying the survival of campylobacters deposited superficially on the fingertips. The technique used has the disadvantage that both single cells and clumps of cells (colony-forming units) transferred from the fingertips to the impression plate produce single colonies. Hence the counts obtained underestimate the number of viable cells transferred. As the infective dose of campylobacters can be as low as 500 organisms (Robinson, 1981) it is possible that just one c.f.u. detected by the impression plate technique might have developed from a cluster of cells numerically sufficient to cause infection. For the purposes of this study the survival time of campylobacters on fingers, after drying, was investigated. However, in practice most cross-infection or auto-infection is likely to occur before the fingers have dried. In many

situations such as in poultry processing or in kitchens where large numbers of chickens are cooked it is likely that the hands will be repeatedly contaminated with campylobacters, and this must increase the opportunity for transmitting infection. The effect of this repeated contamination on survival of campylobacters was not investigated in the present study.

Healthy skin constitutes an unfavourable environment for the survival of most bacteria. The superficial layers of the skin are normally dry, but the majority of bacteria only survive in a moist environment. *C. jejuni* is not exceptional and is generally quite sensitive to drying at room temperature (Doyle & Roman, 1982). Apart from drying, the most important factor controlling the survival of campylobacters on skin surfaces exposed to air is their microaerophilic nature. Other factors affecting their survival are the presence in skin of bactericidal unsaturated fatty acids such as oleic acid (Ricketts *et al.* 1951) and competition with the adapted resident flora, which may account for the variation in survival time on the hands of different volunteers. Gram-negative bacilli picked up on the hands and fingers can sometimes become temporary residents especially in the presence of hand dermatitis (Adams & Marrie, 1982). Usually, however, the survival times are limited. The ability of *Escherichia coli* and several salmonella serotypes to survive on fingertips was investigated by Pether & Gilbert (1971). These workers found that the percentage recoveries of *E. coli* and *Salmonella anatum* fell sharply in the 0–15 min period after contamination of the fingers, though it took much longer for all the cells to die. When the fingertips of 19 volunteers were contaminated with 10^5 *E. coli* cells, in 10 cases the inoculum was not reduced to 1% until 60 min had elapsed. Furthermore, when just 500 *S. anatum* cells were used the organism could be recovered for at least 3 h. The survival of klebsiella serotypes on hands was investigated by Casewell & Phillips (1977), who contaminated the hands of volunteers with suspensions containing 10^3 organisms per ml, which survived for up to 150 min. In the present studies it was found that campylobacters survived on the fingertips for much shorter periods, although there appears to be strain difference. With 0.1% peptone water suspensions a 3–7 \log_{10} reduction of viable cells occurred during the 2 min drying stage, and the remaining cells survived for only a few minutes. However, both chicken liquor and blood had a protective effect which markedly increased the survival times. Blood was found to be particularly protective towards *C. jejuni* NCTC 11392, and in one experiment using an inoculum of 2×10^7 cells in 50% blood per fingertip the survival time was 1 h. Hence there is ample time for anyone with contaminated hands to spread the organisms or possibly to infect themselves directly by the hand-to-mouth route.

Various groups of workers have an occupational risk of contracting campylobacter infection such as farm workers, abattoir workers, kitchen staff, laboratory technicians and nurses. Poultry is an important source of campylobacters, and a strong association exists between sporadic campylobacter infections and the handling and preparation of raw chickens (Hopkins & Scott, 1983). Inexperience in those preparing chicken meals is known to be a major risk factor through cross-contamination of other foods or consumption of raw or under-cooked meats (Editorial, 1985). In the present study it was shown that the juices which drain out of frozen chickens on thawing (chicken liquor) have a

protective effect on campylobacters. These fluids are frequently contaminated with campylobacters, and therefore this is an ideal medium for transferring organisms from chickens to the hands of operatives, and emphasizes the need for good catering and personal hygiene practices (Dawkins, Bolton & Hutchinson, 1984). Recently, an outbreak of *C. jejuni* infection in a neonatal intensive care unit was reported (Hershkowici *et al.* 1987), which was attributed to person-to-person spread, presumably via contaminated hands.

These investigations (Table 5) have shown that washing the hands with either soap and water, or water alone, combined with drying on paper towels can remove a heavy inoculum of campylobacters from the fingertips. However, if hands are merely shaken 'dry' after washing some campylobacters are likely to remain; with this practice washing with soap and water is more efficient at removing campylobacters than washing with water alone. In kitchens we have observed that staff preparing food commonly rinse their hands with water when moving from one task to another. Unfortunately, the hands are usually given only a quick wipe on a cloth or overall and rarely are they properly dried. Our studies have demonstrated the importance of thorough hand drying, and we believe that greater attention to completely drying the hands after washing would help reduce the incidence of sporadic campylobacter infections.

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