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Communication

[*Comunicação*]

Susceptibility of *Arcobacter butzleri* to human blood serum

[*Susceptibilidade de Arcobacter butzleri ao soro humano*]

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Arcobacter butzleri, formerly known as *Campylobacter butzleri*, is a zoonotic bacterium isolated from mammals (dogs, cattle, monkeys, and swine) and domestic and wild birds (hens, turkeys, ducks, sparrows, and pelicans). It is recognized as an emerging enteric pathogen for human beings that could be also involved in extraintestinal infections such as bacteraemia and endocarditis (Kiehlbauch et al., 1991; Anderson et al., 1995; On et al., 1995; Jacob et al., 1998; Mansfield and Forsythe, 2000; Yan et al., 2000; Fernández et al., 2007). Some of the virulence mechanisms of this bacterium have been studied, but the protective effects of normal serum and the role of complement in host defence remain to be evaluated. The protective effects of normal serum and the role of complement in host defence against invasive disease due to other enteropathogenic Gram negative bacteria, like *C. jejuni*, are well established (Leddy and Steignigel, 1979; Fernández et al., 1995; Rother et al., 1998). In immunocompetent individuals, the bactericidal effect of normal human blood serum may act as a protective barrier against bloodstream invasion of *C. jejuni* (Lever, 1984), a species closely related to *A. butzleri*.
The aim of this study was to determine the susceptibility of *A. butzleri* to normal as well as to complement-deficient human sera.

To establish the susceptibility of *A. butzleri* to the bactericidal activity of human serum, ten strains isolated from different sources (chicken liver-two, bovine faeces-two, pelican faeces-three, shellfish-one, and river water-two), in Valdivia city (39° 46' Southern latitude), Chile, were studied. The strains were isolated using the passive membrane filter method proposed by Le Roux and Lastovica (1998). Briefly, a 0.45µm membrane filter (Millipore) was placed on a sheep blood agar plate without antimicrobials and 0.2mL of the fecal suspension was placed onto the membrane filter. After 30min, another 0.2mL was added onto the membrane and the filter was removed from the plate 30min later. The identification at species level was done using the multiplex-PCR technique proposed by Houf et al. (2000).

Susceptibility to the bactericidal activity of human serum was assessed using three types of sera: 1– a pool of normal sera (average values for: C_3 130mg/dL, C_4 72mg/dL, IgM 210mg/dL, IgA 325mg/dL, and IgG 1,210mg/dL); 2– a gammaglobulin-reduced serum (IgM <69mg/dL, IgA <70mg/dL, IgG <34mg/dL, C_3 124mg/dL, and C_4 64mg/dL); 3– a complement-deficient serum (C_3 <75.0mg/dL, C_4 <10.0mg/dL, IgM 190mg/dL, IgA 285mg/dL, and IgG 980mg/dL).

The pool of normal sera was used both undiluted and diluted in Hanks-gelatine solution at the following concentrations: 10, 30, 50, and 70%. Part of the undiluted pool was inactivated at 56ºC for 30 minutes. A portion of the latter was enriched with fresh pooled serum as complement source, enough to obtain an inactivated/fresh serum ratio of 80/20. Susceptibility tests were carried out by separately mixing 100µL of a suspension ($2x10^9$ CFU/mL) of each strain in

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Hanks-gelatine solution with 100µL of fresh pooled normal sera at each dilution in Eppendorf tubes using the sera fore mentioned. The experiments were carried out in triplicate.

Each individual mixture was incubated for 15, 30, and 60 minutes at 37ºC on a rotating platform at 20 cycles/minute. After incubation, a 50µL aliquot of each mixture was diluted in Hanks-gelatine solution for bacterial counts according to Miles and Misra (1938) method. The number of colony-forming units (CFU) thus obtained represented the actual bacterial survival and it was expressed as a percentage (mean of the percentages obtained in the three experiments) of the survival rates obtained in control experiments. The latter were carried out using brain-heart infusion broth (Oxoid - Basingstoke, Hants, U.K.) instead of serum, for the same incubation periods. Statistical evaluation was done by the Kruskal-Wallis test using EpiInfo 3.4 program.

All the strains under study showed similar behavior with regard to the bactericidal activity of human serum. The non parametric Kruskal-Wallis showed that the survival rates obtained in all the experimental groups were significantly different to those obtained with the control group. The results are summarized in Table 1.

Decreasing bacterial survival rates depending on the incubation time were observed when the activity of the undiluted pooled sera was tested. In this case, the mean bacterial survivals were $4.2x10^7$ (2.1%) for the first 15 minutes, $2.2x10^7$ CFU/mL (1.1%) for 30 minutes, and $6.6x10^6$ CFU/mL (0.3%) for 60 minutes. Survival averages were higher when the different dilutions of the sera were assayed. Increasing survival rates were observed along with dilutions of the sera.

The highest survival rates were observed with heat-inactivated sera ($\geq 1.88x10^9$; ≥94%) for the three incubation periods. When fresh serum was added to the inactivated serum pool, survival rates decreased to $1.506x10^9$ CFU/mL (75.3%), $1.496x10^9$ CFU/mL (74.8%), and $1.474x10^9$ CFU/mL (73.7%) at 15, 30, and 60 min of incubation, respectively.

The serum with decreased complement levels had weak bactericidal activity ($1.458x10^9$–$1.422x10^9$ CFU/mL; 72.9–71.1% survival rates), whereas gammaglobulin-reduced serum with normal levels of complement reduced the survival rate between $6.28x10^8$ CFU/mL (31.4%) at 15 min and $4.04x10^8$ CFU/mL (20.2%) at 30 min incubation.

Table 1. Susceptibility of *Arcobacter butzleri* to human serum

Experimental conditions	Survival					
	15 min		30 min		60 min	
	CFU/mL	%	CFU/mL	%	CFU/mL	%
Pool of normal sera	$4.2x10^7$	2.1	$2.2x10^7$	1.1	$6.6x10^6$	0.33
70% diluted normal sera	$6.82x10^8$	34.1	$6.48x10^8$	32.4	$6.16x10^8$	30.8
50% diluted normal sera	$1.228x10^9$	61.4	$1.096x10^9$	54.8	$1.018x10^9$	50.9
30% diluted normal sera	$1.394x10^9$	69.7	$1.128x10^9$	56.4	$9.88x10^8$	49.4
10% diluted normal sera	$1.492x10^9$	74.6	$1.39x10^9$	69.5	$1.34x10^9$	67.0
Inactivated sera	$1.88x10^9$	94.0	$1.892x10^9$	94.6	$1.894x10^9$	94.7
Inactivated sera + 20% normal sera	$1.506x10^9$	75.3	$1.496x10^9$	74.8	$1.474x10^9$	73.7
Complement-deficient sera	$1.458x10^9$	72.9	$1.438x10^9$	71.9	$1.422x10^9$	71.1
Gammaglobulin-reduced sera	$6.28x10^8$	31.4	$6.0x10^8$	30.0	$4.04x10^8$	20.2
Control (Brain Hearth Infusion Broth)	$2x10^9$	100	$2x10^9$	100	$2x10^9$	100

The highest bactericidal activity was observed with pooled fresh normal sera, and it decreased with incubation time. Similar results were reported by Blaser et al. (1985) for *C. jejuni* and Fernández et al. (1995) for *C. jejuni* and *C. coli* but their bacterial survival rates were higher than those reported by the present study, thus suggesting that *A. butzleri* could be more susceptible than these two *Campylobacter* species to the bactericidal effect of the human serum.

Heat-inactivated serum showed the lowest bactericidal activity. This was reversed when

complement was partially restored by adding normal serum. On the other hand, the gammaglobulin-reduced but normal complement-containing serum showed active bactericidal effect. These results are similar to those obtained by Fernández et al. (1995) for *C. jejuni* and *C. coli* and suggest that *A. butzleri* could also directly activate complement by an alternative pathway, without the presence of pre-existent immune complexes, as required by many Gram negative bacteria (Joiner et al., 1984).

The obtained results allowed to conclude that *A. butzleri* is highly susceptible to normal human serum. Serum bactericidal capacity may constitute a protective barrier against systemic invasion by *C. jejuni* which is highly susceptible to this non specific defensive mechanism. The opposite is observed in *C. fetus* subsp. *fetus* that, being resistant to serum bactericidal activity is strongly associated with systemic infections (Blaser et al., 1987).

The incidence of *A. butzleri* bacteraemia remains still unknown and from the few published reports it could be inferred that this clinical presentation is related to underlying diseases or impaired immunological conditions of the subject (On et al., 1995; Yan et al., 2000). The latter could be related to the high susceptibility of *A. butzleri* to serum bactericidal effect that might also explain the occasional association of this bacterial species with bacteraemia.

Keywords: *Arcobacter*, blood serum, complement, zoonosis

RESUMO

A susceptibilidade de 10 amostras de Arcobacter butzleri *ao soro humano foi estudada. A maior atividade bactericida foi encontrada no soro humano normal, com taxas de sobrevivência bacteriana inversamente proporcionais à diluição do soro. As maiores taxas de sobrevivência foram obtidas com o soro inativado pelo calor. As taxas de sobrevivência decresceram com a adição de soro fresco ao inativado. O soro com valores reduzidos de gamaglobulinas e valores normais de complemento mostrou ativo efeito bactericida. Os resultados demonstraram que* A. butzleri *é altamente susceptível ao efeito bactericida do soro humano, sugerindo que pode ser capaz de ativar diretamente o complemento pela via alternativa.*

Palavras-chave: Arcobacter, *soro sanguíneo, complemento, zoonose*

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