ISSN 0103-8478

ISOLATION OF *Arcobacter spp* FROM POULTRY CARCASSES, IN BRAZIL

ISOLAMENTO DE *Arcobacter spp* DE CARCAÇAS DE FRANGO NO BRASIL

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SUMMARY

*Fourty eight isolates of **Arcobacter spp** were obtained from 37 poultry carcasses, from abattoir, among 80 carcasses examined. Attempts for culturing were made from the skin and muscle, resulting on 25 positive cultures from muscle and 23 from skin. Classification was achieved by phenotypic characterization and PCR and multiplex PCR, resulting 41 samples of **Arcobacter butzleri** and 07 **Arcobacter sp**. This is the first report on the occurrence of Arcobacter in animal carcasses in Brazil.*

Key words: *Arcobacter sp, Arcobacter butzleri, poultry carcasses.*

RESUMO

*Foram isoladas 48 amostras de **Arcobacter spp** de 37 carcaças de frangos colhidas em frigorífico, prontas para consumo, entre 80 carcaças examinadas. Foram feitas tentativas de cultivo a partir de pele e de músculo, sendo obtidas 25 cultivos positivos de músculo e 23 de pele. As bactérias foram classificadas pelas características fenotípicas e pelo teste de PCR e PCR múltiplo, obtendo-se 41 amostras classificadas como **Arcobacter butzleri** e 07 com classificação a nível de gênero **Arcobacter sp.** Estes são os primeiros relatos sobre a ocorrência das bactérias em carcaças de animais no Brasil.*

Palavras-chave: ***Arcobacter sp**, Arcobacter butzleri, carcaças de frango.*

INTRODUCTION

The genus ***Arcobacter*** includes bacteria that were formerly designated "aerotolerant ***Campylobacter***" because they grew in the presence of atmospheric oxygen. It was suggested the designation of ***Campylobacter cryaerophila*** (NEILL ***et al.***, 1985) and inclusion in the genus ***Campylobacter***.

In view of phenotypic and genotypic differences from ***Campylobacter spp***, the genus ***Arcobacter*** was proposed for the organisms (VANDAMME ***et al.***, 1991). The species of ***Arcobacter*** associated with human and animal disease are ***A. cryaerophilus*** (DNA groups 1A and 1B), ***A. butzleri*** and ***A. skirrowii***.

A. cryaerophilus has been isolated from aborted porcine fetuses, sows with reproductive problems and preputial fluid of male pigs (ELLIS ***et al.***,1978; NEILL ***et al.***,1979; SCHROEDER-TUCKER ***et al.***, 1996). ***A. butzleri*** has been cultured from human cases of enteritis (TAYLOR ***et al.***, 1991; KIEHLBAUCH ***et al.***, 1991; PUGINA ***et al.***, 1991; LERNER ***et al.***, 1994; LAUWERS ***et al.***, 1996), drinking water reservoirs (JACOB ***et al.***, 1993), canal waters (DAHMABUTRA ***et al.***, 1992; STAMPI ***et al.***, 1993), poultry and pork (FESTY ***et al.***,1993; COLLINS ***et al.***, 1994; LAMMERDING ***et al.***, 1994).

Although in very low porcentage, ***A. butzleri*** has been found also in oviduct of sows and preputial fluid of male pigs (OLIVEIRA ***et al.***, 1997; 1999).The association of ***A. butzleri*** with human

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Recebido para publicação em 27.07.00. Aprovado em 22.11.00

enteritis, and its recovery in hogs and poultry suggests a potential human foodborne pathogen (WESLEY, 1996). In recent surveys, ***Arcobacter spp*** has been found more frequently from poultry than from red meats (DE BOER ***et al.***, 1996; ATABAY & CORRY, 1997; HARRAB ***et al.***, 1998), porcine fetuses, uterus and oviducts of sows and preputial fluid of boars and fattening pigs (OLIVEIRA ***et al.***, 1995; 1997; 1999).

The purposes of this study were to isolate ***Arcobacter spp*** from poultry carcass from abattoir in Brazil, and to utilize PCR analysis to establish the identity of the isolates.

MATERIALS AND METHODS

Carcasses. A total of 80 poultry carcasses (10 weekly) were taken from the line in the processing plant of an abattoir in the State of Rio Grande do Sul, Brazil. They were separately wrapped and transported from the factory to the laboratory under refrigerated conditions and examined within 2h of slaughter.

Isolation Procedure. Samples were taken respectively from the skin and muscle of the neck of each carcass, for bacteriological examination. A piece of skin was cut and placed into liquid EMJH (Ellinghousen MacCulough Johnson and Harris – Difco) medium in tubes. Portions of muscle of the neck were grinded in a mortar with liquid EMJH. Tubes were incubated for 5 days at 30°C under aerobic conditions, followed by plating by the STEEL & MCDERMOTT (1984) membrane filter method onto sheep blood agar (brain heart infusion agar supplemented with 10% defibrinated sheep blood). One drop of the growth from EMJH was filtered onto blood agar through a 0.45µ pore size cellulose acetate filter (ATABAY & CORRY, 1997). The plates were left lids-up on the bench for about 30 min to dry and then inverted and incubated under aerobic conditions at 30°C for 2 days.

Phenotypic testing. Suspect colonies on each plate were picked and checked by gram stain and inoculated into semi-solid EMJH (0.15% agar). Growths from semi-solid EMJH were examined under dark-field microscopy (40x magnification) for motile gram negative curved rods. Cultures were purified by streaking on blood agar and checked for oxidase, catalase and growth on MacConkey Agar. Isolates were subcultured twice weekly into semi-solid BHI medium, waiting for genotypic testing.

Electron micrograph. To visualize the cell wall a plasmolisis was promoted using 0.75% saline solution. The material was placed over support membrane and metalized with palladium-gold, and examined by the electronic microscope EM 208 S with 70kv, 40,000 X final magnification.

Isolation of DNA. Total DNA from each sample was isolated by phenol-chloroform procedure (SAMBROOK ***et al.***, 1989). Briefly, bacteria were harvested (15,000 X g; 2 min.), washed in phosphate bufered saline and suspended in distilled water. Sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 0.5%. The samples were incubated for 10 min at 37°C with 1.0mg proteinase K/ml (Sigma) and extracted with an equal volume of phenol and then chloroform:isoamyl alcohol (24:1). The DNA was precipitated using ethanol and resuspended in 50µl of distilled water.

Genotypic testing. Sequences for PCR primers were derived from the sequences of ***Arcobacter***-specific and ***Arcobacter butzleri***-specific 16S rRNA-based DNA probes (WESLEY ***et al.,*** 1995). Primers Arco I (5'- AGA GAT TAG CCT GTA TTG TAT C – 3') and Arco II (5'- TAG CAT CCC CGC TTC GAA TGA – 3') are targeted to 1223bp region of the ***Arcobacter*** 16S rRNA gene. Primers Arco II e Butz (5'- CTT GAC ATA GTA AGA ATG ATT TAG – 3') amplify a 463bp fragment of the 16S rRNA gene.

Amplification was performed in a 50µl volume containing 5.0ng of template DNA using Arco I and Arco II primers (HARMON & WESLEY, 1996). PCR reaction products were visualized after separation by electrophoresis on 12.5% polyacrylamide gel and stained with a rapid silver nitrate staining method (SANGUINETTI ***et al.,*** 1994).

RESULTS AND DISCUSSION

Forty eight ***Arcobacter spp*** strains were isolated from 37 poultry carcasses (46.25% from a total of 80 examined), as shown in table 1. PCR tests identified 41 strains as ***A. butzleri*** and 07 as ***Arcobacter sp***. Figure 1 presents the PCR products of some isolates. All ***Arcobacter*** isolates yielded a characteristic 1,223bp product. Only ***A. butzleri*** strains yielded both the 1,223 and 463bp amplicons.

All the isolates were oxidase positive and presented variable growth on MacConkey agar. Catalase test differentiated ***A. butzleri*** (negative or weak reaction) from other isolates typed as ***Arcobacter sp*** (strong positive reaction for catalase). Characteristic motile curved rods were seen by darkfield microscopy. Electron micrograph showed helycoidal bacteria with one polar flagella (Figure 2).

Table 1 - Characterization of ***Arcobacter spp*** isolated from poultry carcasses.

Samples	Muscle	Skin
2	***A. butzleri***	-
6	***A. butzleri***	-
7	***A. butzleri***	-
8	-	***A. butzleri***
11	-	**Arcobacter sp**
12	***Arcobacter sp***	***A. butzleri***
13	***Arcobacter sp***	***A. butzleri***
15	-	***A. butzleri***
16	-	***A. butzleri***
18	-	***A. butzleri***
21	***A. butzleri***	-
24	***A. butzleri***	***A. butzleri***
27	***A. butzleri***	-
29	-	***Arcobacter sp***
31	***A. butzleri***	-
32	***A. butzleri***	-
33	***A. butzleri***	***A. butzleri***
34	***A. butzleri***	***A. butzleri***
35	***Arcobacter sp***	***A. butzleri***
36	-	***A. butzleri***
37	***A. butzleri***	***A. butzleri***
38	-	***A. butzleri***
39	***A. butzleri***	***A. butzleri***
40	***A. butzleri***	***A. butzleri***
42	-	***A. butzleri***
51	-	***A. butzleri***
52	***A. butzleri***	-
53	-	***A. butzleri***
54	***A. butzleri***	-
56	***A. butzleri***	-
58	***A. butzleri***	-
59	***A. butzleri***	***Arcobacter sp***
62	***A. butzleri***	-
65	***A. butzleri***	***A. butzleri***
67	***A. butzleri***	-
68	***Arcobacter sp***	-
69	-	***A. butzleri***

Arcobacter spp have been reported more frequently from poultry than from red meats (WESLEY, 1996). Accordingly, poultry may be a significant reservoir of ***A. butzleri***. In France, ***A. butzleri*** was recovered from 81% of poultry carcasses examined (LIOR & WOODWARD, 1994). In Germany, HARRAB ***et al.*** (1998), isolated 52.3% (89 of 170) of freshly slaughtered broilers from 15 different flocks.

In the present report it is shown a similar high prevalence of ***Arcobacter*** in poultry, in which ***A. butzleri*** was recovered from 42.5% (34 of 80) and other ***Arcobacter sp*** from 8.7% (7 of 80) of the poultry carcasses. The characterization of samples as ***Arcobacter sp*** in the present report means that they could be ***A. cryaerophilus*** or ***A. skirrowii.*** Differentiation between these two species was not done because it would require RFLP (restriction fragment length polymorphism). However, ***A. butzleri*** were precisely identified by multiplex PCR.

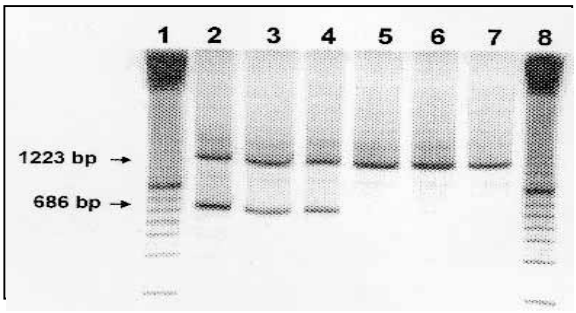

Figure 1 - Multiplex PCR on some ***Arcobacter*** samples isolated from poultry. Molecular weight marker lanes 1 and 8. ***A. butzleri*** lanes 2, 3 and 4. ***Arcobacter sp*** lanes 5, 6 and 7.

The use of enrichment medium followed by plating after filtration (STEELE & MC DERMOTT, 1984) was effective for isolation of ***Arcobacter*** from carcasses, as reported by LAMMERDING ***et al.*** (1994).

Tables 1 and 2 show the results of culturing skin and muscle samples from each carcass. It was not the objective of this research to establish a relation between porcentages of positive culture obtained from skin or muscle, nor relating the ***Arcobacter sp*** isolated. Nevertheless, it was shown that 52% of the strains were obtained from the muscle, while 48% from the skin. Data from table 2 also shows that ***A. butzleri*** were isolated from seven carcasses from both skin and muscle. RFLP patterns would define if they were the same strain. Different ***Arcobacter*** species were isolated respectively from skin and muscle of four poultry carcasses, as shown in table 2, meaning that there was infection or contamination by different microorganisms. ***A. butzleri*** were evenly distributed

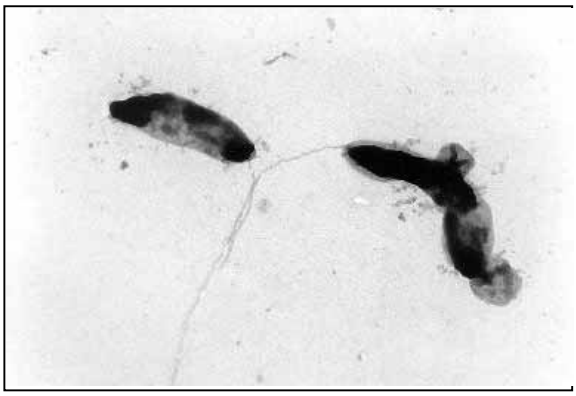

Figure 2 - Electron micrograph of two cells of ***Arcobacter spp*** showing their flagella.

Table 2 - Isolation of ***Arcobacter spp*** from skin or muscle or both, from poultry carcasses.

Samples	Muscle	Skin	Skin/muscle	Total	%
Arcobacter butzleri	13	10	7[1]	30	81.08
Arcobacter sp	1	2	-	3	8.11
A. butzleri and ***Arcobacter sp***	-	-	4[1]	4	10.81
Total	14	12	11	37[*]	100
%	37.84	32.43	29.73	100.00	

(1) = Two positive culture from each carcass.
(*) = 37 carcasses

in skin and/or muscle (21 from muscle and 20 from skin). There is no reference in the literature about reports on the distribution of the microorganisms in the skin and/or muscle from poultry carcasses.

CONCLUSIONS

Arcobacter spp, mainly ***A. butzleri*** were isolated from poultry carcasses in Brazil at a similar high prevalence as reported in some other countries. It is the first report on the occurrence of this microorganism in muscle and skin of poultry in South America.

ACKNOWLEDGEMENTS

We are indebted to José Gilvan Mancuso and Angelo Cesar Túlio for technical cooperation.

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