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SUSCEPTIBILITY OF *ARCOBACTER BUTZLERI* TO HEAVY METALS

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ABSTRACT

The susceptibility of 50 strains of *Arcobacter butzleri* isolated from chicken liver [12], mussels [18], river water [6] and bovine [5], duck [2] and pelicans [7] feces to mercury (Hg), chromium (Cr), silver (Ag), nickel (Ni), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo) and lead (Pb) was determined. All the strains were resistant to Mo, Mn, Ni, Co, Pb and Fe and susceptible to Hg, Ag and Cr. MIC values showed high variability, indicating a non homogeneous behavior among the strains.

Key words: *Arcobacter butzleri*, heavy metals, resistance

Arcobacter butzleri is an emerging pathogen that has been associated with abortion and enteritis in animals and with diarrhea and bacteremia in adults and children. This *Arcobacter* species seems to be the most frequent in human beings. *A. butzleri* recognizes a wide reservoir range, being isolated from domestic and free living mammals and birds, from shellfish and environmental water bodies as well. It has been suggested that water may play an important role in the transmission of these organisms (6,12).

Thus, *A. butzleri* seems to be an ubiquitous microorganism that can be isolated from the environment, animals and also in association with infectious processes in human beings (5,6,7,9,12). However, many of the biological characteristics of this bacterium including the growth in the presence of heavy metals remain unknown. In fact, it seems that there are no references in the literature about the susceptibility or resistance of *A. butzleri* to heavy metals. Information is restricted to *Campylobacter*, (4,13), their formerly taxonomic description, and limited to cadmium chloride.

Since *A. butzleri* is an emerging pathogen frequently recovered from environmental waters that could be contaminated with heavy metals, the susceptibility of 50 *A. butzleri* strains to nine heavy metal compounds was studied. The strains were isolated from chicken liver for human consumption [12], mussels [18], river water [6] and feces obtained from bovines [5], ducks [2] and pelicans [7].

The susceptibility to heavy metals was assessed using an adaptation of the double dilution agar method for determining antibiotics minimal inhibition concentration (MIC). In brief, series of Mueller-Hinton agar plates containing dilutions of salts of the assayed heavy metals were prepared. The following heavy metals salts and concentration ranges were used $HgCl_2$, $AgNO_3$, K_2CrO_4 (0.01-0.16 mmol/L), $FeSO_4$, Pb ($C_2H_2O_2$), $NiSO_4$ (1-16 mmol/L), $MnSO_4$, $NaMoSO_4$ and CoO (5-80 mmol/L).

The plates were inoculated using a Steers replicator, depositing 0.001 mL of a suspension of each strain in *Arcobacter* broth. Inocula were prepared diluting a 48h broth culture to a relation 1:5. The plates were incubated at 26ºC, during 48 h under aerobic conditions. The lowest heavy metal concentration producing complete inhibition of macroscopic growth was considered as MIC. Following Mohammad's (8) recommendations, a strain was considered resistant when it was able to grow at a concentration of 1 mmol/L or more.

As shown in Table 1, all strains were susceptible to mercury, silver and chrome salts and resistant to the other heavy metals salts. $HgCl_2$ was the most active compound inhibiting 28% of the strains at a concentration ≤0.01 mmol/L whereas $NaMoSO_4$ and CoO exhibited the lowest inhibitory activity.

In other bacteria, like *Pseudomonas* sp., resistance to chrome and mercury is codified by plasmids (3). Since all the strains in the present study were susceptible to chrome and mercury salts, it is possible to infer that they do not carry this kind of plasmids.

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Table 1. Minimal inhibitory concentrations and resistance (%) of 50 strains of *arcobacter butzleri* to nine heavy metals salts

				M. I. mcg/mL C.							
Metal salt of	≤0.01 Nº/%	0.02 Nº/%	0.04 Nº/%	0.08 Nº/%	0.016 Nº/%	4 Nº/%	8 Nº/%	10 Nº/%	20 Nº/%	≥80 Nº/%	% of resistance
Mercury	14/28	22/44	13/26	1/2							0
Silver			13/26	37/74							0
Chrome			11/22	33/66	6/12						0
Nickel						49/98	1/2				100
Lead							50/100				100
Iron							50/100				100
Manganese								30/60	20/40		100
Molybdenum										50/100	100
Cobalt										50/100	100

On the other hand, chrome, mercury and silver compounds are used as disinfectants. The high susceptibility of *A. butzleri* strains to salts of these three metals leads to speculate that they could be highly susceptible to disinfectants having those metals in their formulation. Studies in order to determine the susceptibility of *A. butzleri* to different disinfectants are currently being carried out in our laboratory.

Bacteria can use some metals, like cobalt and nickel, as micronutrients, in redox processes, as components of various enzymes and in the osmotic pressure regulation, (2), which can explain in part the resistance observed in *A. butzleri* to those heavy metals. In the same way, the high resistance observed to $FeSO_4$ could be explained because iron is an essential nutrient for bacterial growth and plays a substantial role in cellular respiration as an important component of citochromes and electron transporting proteins (1). Resistance to cobalt, nickel and lead could be due to plasmids (3) but the resistance mechanisms to heavy metals remain unknown in *A. butzleri*.

An important number of the strains under study have been isolated from mussels (18/36%) and river water (6/12%). In earlier studies, Paredes (10) established that low concentrations of lead and nickel could be found in mussels of our region, whereas Quiroz *et al*. (11) demonstrated the presence of several heavy metals in Valdivia river, located at 73º11'W, 39º46'S.

Further studies are needed for a better understanding of the resistance of *A. butzleri* to heavy metals as well as to establish if the environmental presence of heavy metals compounds could be related to this resistance.

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RESUMO

Sensibilidade de *Arcobacter butzleri* a metais pesados

Arcobacter butzleri é um bacilo Gram negativo de caráter zoonótico, pertencente à Família *Campylobacteraceae*, que tem sido associado a diarréia e septicemia no ser humano. A susceptibilidade de 50 amostras de *A. butzleri* isoladas de fígados de frango [12], mariscos [18], água de rio [6] e fezes de bovinos [5], patos [2] e pelicanos [7] aos metais pesados mercúrio (Hg), cromo (Cr), prata (Ag), níquel (Ni), cobalto Co), ferro (Fe), manganês (Mn), molibdênio (Mo) e chumbo (Pb) foi determinada. Todas as amostras foram resistentes a Mo, Mn, Ni, Co, Pb e Fe, sendo susceptíveis a Hg, Ag e Cr. Os valores das CIM apresentaram alta variabilidade indicando um comportamento não homogêneo entre as amostras.

Palavras-chave: *Arcobacter butzleri*, metais pesados, resistência

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