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Short communication

Antimicrobial resistance in food and clinical *Aeromonas* isolates

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Abstract

This study highlights the incidence of resistance and the presence of plasmids in human and food isolates of *Aeromonas* in Brazil. A total of 83 *Aeromonas* spp. strains (28 isolated from human and 55 from fresh lettuce) were studied. Thirty-five were identified as *A. hydrophila* complex and 48 as *A. caviae* complex. All strains were shown to be susceptible to imipenem, amikacin, gentamicin, tobramycin and ciprofloxacin by the disk diffusion method. Resistance to antimicrobial agents was observed in strains of both food and clinical origin. The food strains were resistant to ampicillin/sulbactam, cefoxitin and tetracycline, while the clinical strains presented resistance to ampicillin/sulbactam, cefotaxime, ceftazidime, cefoxitin, sulfamethoxazole/trimethoprim, chloramphenicol and tetracycline. The minimal inhibitory concentrations of chloramphenicol, tetracycline and sulfamethoxazole/trimethoprim were tested by agar dilution. Thirteen strains isolated from vegetables were resistant to tetracycline (MIC 16 μg ml^{-1}). Two *A. hydrophila* strains and one *A. caviae* strain presented extracromosomal DNA (3 and 15 kb plasmids, respectively). The tetracycline resistance phenotype determinant was related to the 15 kb plasmid according to cure and transformation experiments.

Keywords: *Aeromonas*; Antibiotics; Plasmids

1. Introduction

Aeromonas spp. have been considered important pathogens for cold- and warm-blooded organisms (Montoya et al., 1992). Recent works have emphasized their emergence as human primary pathogens, since they have been related to a variety of local and systemic infections, even in immunologically competent hosts (Janda and Abbott, 1998; Joseph and Carnahan, 2000; Taneja et al., 2004). *Aeromonas* can be found in soil, fresh and saline water, drinking water and animal faeces (Fiorentini et al., 1998). It has also been isolated from different kinds of food, such as fish, milk, meat and meat products, lettuce, other salad leaves, ice-cream and cheese (Freitas et al., 1993; Rhodes et al., 2000 Villari et al., 2000). Water and food are important sources of transmission of *Aeromonas* spp. infections to humans (Kirov, 2001). Gastroenteritis is the most common infection caused by *Aeromonas* spp., which can be self-limited or severe. Diarrhea, cholera-like illness and colitis have also been reported (Champsaur et al., 1982; Freitas et al., 1998; Janda and Abbott, 1998).

Antibiotic susceptibility of clinical isolates of *Aeromonas* spp. have been studied (Janda and Abbott, 1998; Villari et al., 2000). However, there is little knowledge about the vegetable strains. Antimicrobial resistance and virulence factors have been correlated with plasmids in *Aeromonas* spp. (Adams et al., 1998; Casas et al., 2005).

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The presence of plasmids may be a risk since they can be transferred to other bacterial pathogens and may hinder antibiotic therapy of *Aeromonas* spp. infections.

In this work, we report the isolation and identification of *Aeromonas* spp. from lettuce, determine the antimicrobial resistance found in strains isolated from lettuce and humans, and investigate the association of resistance with the presence of plasmids, in order to evaluate the potential risks these findings represent.

2. Materials and methods

2.1. Strains

Eigthy-three strains were used in this work: 28 previously isolated from patients (faeces, with or without diarrhea) (Freitas et al., 1998), and 55 strains isolated from lettuce. Routine propagation of *Aeromonas* strains was performed on plates of LB (1% tryptone [Oxoid], 0.5% yeast extract [Oxoid], and 0.5% of NaCl, pH 7.0 ± 0.2) plus 2.0% of agar [Difco], incubated at 37 °C. All strains were maintained at −70 °C in LB medium containing 20% glycerol.

2.2. Isolation and phenotypic identification

Primary isolation of *Aeromonas* spp. from 25 samples of lettuce was done according to the Compendium of Methods for the Microbiological Examination of Food (Palumbo et al., 1992). Pre-enrichment in alkaline peptone water at 28 °C for 24 h streaked onto to surface of *Pseudomonas–Aeromonas* selective agar [Merck] and presumptive identification tests were done as described by Araújo et al. (2002). The identification of the phenospecies was based on biochemical and physiological tests, according to Bergey's manual (Holt et al., 1994) and Janda and Abbott (1998).

2.3. Antimicrobial susceptibility tests

LB broth cultures of the strains were diluted to a turbidity of 0.5 on the McFarland scale. The antimicrobial susceptibility was determined by the disk diffusion technique as described by The National Committee of Clinical Laboratory Standards (NCCLS, 2004) for the *Enterobacteriaceae*. Strains showing resistance in the disk diffusion test had the minimal inhibitory concentration (MIC) of some drugs determined by the agar dilution method (NCCLS, 2004). The strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were used as control for these experiments.

2.4. Plasmid DNA extraction and detection

Plasmid DNA was isolated by the methods of Birnboim and Doly (1979) and Kado and Liu (1981). The lysis in the Birnboin and Doly method was performed in the absence of lysozyme, and the precipitation of cellular debris was done in the presence of 3 M sodium acetate pH 4.8. The lysates were separated by horizontal electrophoresis at 80 V, 50 mA, for 5 and 8 h in 0.7% agarose (Life Technologies, Brazil) gels prepared with tris borate buffer Sambrook et al., 1989. The gels were stained with ethidium bromide solution (0.5 μg ml^{-1}) for 30 min and plasmid bands were viewed with an ultraviolet transilluminator. When needed, the supercoiled DNA Ladder (Life Technologies, Brazil) was included in the gels as a molecular weight standard marker.

2.5. Curing experiments

Strains carrying plasmids were grown at 37 °C for 18 h. A 1:4 dilution was prepared in a fresh LB medium, gently shaken at 37 °C for 1 h, and 40 μl of the culture was then transferred to 5 ml of LB. After 2 consecutive cultures at 40.5 °C for 5 h without shaking, the culture was appropriately diluted and seeded on LB agar plates. The susceptibility of the colonies isolates to tetracycline (4 μg ml^{-1}) was investigated by replica plating. Colonies sensitive to tetracycline and the wild type were analysed for the presence of plasmids.

2.6. Transfer of drug resistance

Plasmids were purified by using Wizard SV plus miniprep kits (Promega, USA), and 50 μl of the recovered DNA was used to transform the plasmid-free competent cells of *A. hydrophila* ATCC 7966 (a tetracycline-sensitive strain). The competent cells were prepared by heat shock treatment, as described for *E. coli* earlier (Sambrook et al., 1989). Transformants were selected on LB agar containing tetracycline (4 μg ml^{-1}), and the presence of plasmids in them was shown by DNA electrophoresis.

3. Results and discussion

There are some recent reports on the presence of *Aeromonas* in food and environmental samples, suggesting that they are important vehicles of dissemination of this pathogen (Neyts et al., 2000; Rhodes et al., 2000; McMahon and Wilson, 2001). In the present work, a total of 55 vegetable strains were analysed: 26 strains of *A. hydrophila* complex and 29 of *A. caviae* complex. *Aeromonas* spp. have been isolated from raw, ready-to-eat and organic vegetables (Villari et al., 2000;