

RESISTANCE PROFILE OF *Aeromonas* spp. ISOLATED IN DAIRY PRODUCTS INDUSTRY

PERFIL DE RESISTÊNCIA DE *Aeromonas* spp. ISOLADA NO FLUXOGRAMA DE PRODUÇÃO DO QUEIJO MINAS FRESCAL INDUSTRIAL E ARTESANAL

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SUMMARY

Bacteria of the genus *Aeromonas* can be isolated from clinical, food and environmental samples. For dairy products contamination can be caused by the presence of the agent in the feedstock used for production or due to unsatisfactory hygienic conditions during processing. The present study aimed to assess *Aeromonas* isolates behavior in the presence of common antimicrobials, considering the importance of *Aeromonas* spp. as an emerging pathogen of foodborne diseases, the indiscriminate use of antimicrobials to treat human pathologies, in addition to the role that milk and its derivatives play in human feeding habits. The processing steps during dairy products manufacturing were studied looking for vulnerable points or products where *Aeromonas* could be present and, subsequently, either pass it to the final products or disseminate the micro-organism in different processing stages of Minas and Colonial cheeses. The results show high levels of resistance: 33.6% of 98 isolates of *Aeromonas* spp. were resistant to all 15 antibiotics tested, corresponding to 7.5% of the isolates from Colonial cheese and 51.7% from Minas cheese, thus showing greater resistance among the samples collected in the industry. Multiple antimicrobial resistance was verified in 100% of the isolates, which reveals a profile which is alarming for public health.

KEY-WORDS: Antimicrobial. Colonial Cheese. Minas Frescal Cheese.

RESUMO

Bactérias do gênero *Aeromonas* podem ser isoladas de amostras clínicas, alimentares e ambientais. Para os derivados lácteos a contaminação decorre da presença do agente na matéria prima utilizada para produção, ou devido a condições higiênicas insatisfatórias durante o processamento. Considerando a importância da *Aeromonas* spp. como patógeno emergente de doenças de origem alimentar, o uso indiscriminado de antimicrobianos nas patologias humanas, além do papel fundamental que o leite e seus derivados representam na alimentação humana, o presente estudo teve por objetivo verificar o comportamento dos isolados frente à ação de antimicrobianos de uso comum. Foram pesquisados produtos e pontos do fluxograma do processamento de derivados lácteos, que poderiam veicular o gênero *Aeromonas* e permitir sua ocorrência nos produtos finais, ou atuar como disseminadores do micro-organismo nas diferentes etapas do processamento do queijo Minas Frescal e do queijo Colonial. Os resultados demonstram altos níveis de resistência: 33,6% dos 98 isolados de *Aeromonas* spp. foram resistentes a todos os 15 antimicrobianos testados, correspondendo a 7,5% dos isolados do queijo Colonial e 51,7% do queijo Minas Frescal, ocorrendo, portanto, maior resistência entre as amostras coletadas na indústria. 100% dos isolados apresentaram resistência múltipla, resultado que revela um perfil preocupante para saúde pública.

PALAVRAS-CHAVE: Antimicrobiano. Queijo Colonial. Queijo Minas Frescal.

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INTRODUCTION

The genus *Aeromonas* belongs to the *Aeromonadaceae* family and consists of Gram-negative, facultative anaerobes, mostly mobile and able to multiply at refrigeration temperatures (IGBINOSE et al., 2012). It is distributed worldwide, and has been isolated in various niches such as aquatic, fish, food, pets, invertebrates, birds, insects, and soil (JANDA & ABBOTT, 2010).

Aeromonas spp., considered an emerging foodborne agent, can act either as infectious or enterotoxigenic organism, resulting in serious consequences for humans (ISONHOOD & DRAKE, 2002; MARTINELI et al., 2010; IGBINOSE et al., 2012). The genus has been identified as the agent of various infections, gastroenteritis being the most common. *A. hydrophila*, *A. caviae* and *A. veronii* biovar *sobria* are the most frequently isolated species (PARKER & SHAW, 2011). In addition to causing diseases in humans and animals, it is also an important deteriorating agent that contributes to decreasing shelf life of various foods (meat, milk, eggs, seafood and cooked food) even when kept under refrigeration (KIROV, 1993).

The appearance of strains with multiple antibacterial resistance (MAR) is a major public health issue. The antimicrobial resistance among pathogens routinely associated with gastroenteric diseases is highlighted (IGBINOSA et al., 2012). Resistance is particularly relevant in the pathogenic species of *Aeromonas* spp. in which multidrug resistance have been identified (ALCAIDE et al., 2010; MARTINELI et al., 2010; JANDA & ABBOTT, 2010; IGBINOSA et al., 2012). However, little is known about foodborne and environmental strains, especially in Brazil.

Given the importance of *Aeromonas* as an agent of foodborne diseases, the role that dairy products play in the population feeding habits and also the indiscriminate use of antimicrobials to treat humans, the present study aims to assess the behavior of *Aeromonas* spp. isolates from different processing stages of Minas and Colonial cheeses, to the action of commonly used antibiotics.

MATERIAL AND METHODS

We analyzed the resistance profiles of a total of 98 isolates of different species of *Aeromonas* (*A. hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, *A. schubertii* and *A. jandaei*) obtained from the final product and from the processing steps of the industrially produced Minas cheese and the handmade Colonial cheese. The sampling points included water supply, surface equipment, handlers' hands, raw and pasteurized milk, curd and cheese ready for consumption.

Samples were collected on normal working days following the methodology established by the American Public Health Association (2001). Samples of Minas cheese were obtained during 12 days from a large plant, located in the state of Rio Grande do Sul, subjected to

permanent sanitary-hygienic control. Samples of Colonial cheese were collected during five samplings performed directly on a dairy farm, also located in the state of Rio Grande do Sul, which allocates part of its daily milk production for making artisanal cheese.

The bacteria *Aeromonas* spp. was isolated by selective enrichment performed initially in tryptic soy broth (TSB) supplemented with ampicillin at a concentration of 30 milligrams per liter (ABEYTA JUNIOR et al., 1990). After incubation, the selective enrichment cultures were plated on phenol-amide-ampicillin red agar (PALUMBO et al., 1985) and ampicillin-dextrin agar (HAVELAAR & VONK, 1988), which were incubated again at 28°C for 24 hours and examined for the presence of large yellow colonies (3-5 mm) surrounded by a halo resulting from the hydrolysis of starch or dextrin, characteristics of *Aeromonas*. Up to five colonies were inoculated into tilted tubes of trypticase-soy agar (TSA) and, after confirming the presence of pure cultures; they were plated on triple-sugar-iron (TSI) agar and subjected to the tests of motility, oxidase, catalase and resistance to the vibriostatic agent O/129 (POPOFF, 1984). The characterization of the species was performed following the scheme of Popoff (1984), plus evidence recommended by Abeyta Jr. et al. (1990), with updates submitted by Furuwatari et al. (1994) and Abbott et al. (2003).

The antimicrobial susceptibility tests were performed according to the method described by Bauer et al. (1966) and NCCLS (1986). To carry out these tests, the strains were subcultured in tubes containing 5 mL of trypticase-soy broth (TSB) and incubated at 37°C for 18-20 hours. Then, the diluted cultures were plated, using sterile swabs on plates with Mueller-Hinton agar and after approximately three minutes, the time needed for drying the medium from the surface; polidisks⁶ containing antimicrobials were placed. Readings were performed using a millimeter ruler to measure the inhibition halos, after 18 to 24 hours of incubation at 37°C. The diameter measurements (mm) were compared with the table provided by the manufacturer of the disks used. We tested 15 active ingredients used routinely in human and veterinary medicine, as follows: amikacin, amoxicillin, ampicillin, aztreonam, cephalothin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, piperacillin/tazobactam, sulfazotrim and tetracycline.

The nonparametric chi-square test was used to compare the frequency of antimicrobial resistance profiles of *Aeromonas* bacteria between samples of industrial and colonial (STEEL & TORRIE, 1960).

RESULTS AND DISCUSSION

As shown in Tables 1 and 2, cultures of *Aeromonas* were resistant to multiple drugs, particularly those isolates from the Minas cheese production line. In this case, 98.3% of the samples

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Table 1 - Cultures of *A. caviae*, *A. sobria* and *A. schubertii* isolated in samples from the production line of Minas cheese, produced in northwestern Rio Grande do Sul, in relation to the number of drugs they were resistant to.

RESISTANCE (No. drugs) ¹	<i>A. caviae</i> (No. cultures)	<i>A. sobria</i> (No. cultures)	<i>A. schubertii</i> (No. cultures)	TOTAL
1				
2				
3				
4				
5				
6	01 (2.0) ²			01 (1.7) ³
7				
8				
9				
10	02 (4.0)	01 (16.7)		03 (5.2)
11	03 (6.0)			03 (5.2)
12	03 (6.0)	02 (33.3)		05 (8.6)
13	06 (12.0)			06 (10.3)
14	09 (18.0)	01 (16.7)		10 (17.2)
15	26 (52.0)	02 (33.3)	02 (100.0)	30 (51.8)
TOTAL	50 (100.0)	06 (100.0)	02 (100.0)	58 (100.0)

¹Antimicrobial agents: tetracyclines, chloramphenicol, gentamicin, amikacin, ampicillin, cephalothin, cefoxitin, sulfazotrim, amoxicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, and piperacillin.

²Percentage of isolates resistant to the total isolates of the species.

³Percentage of isolates resistant to the total isolates tested.

Table 2 - Distribution of cultures of *A. hydrophila*, *A. caviae*, *A. sobria*, *A. Veroni* and *A. jandaei* isolated from different points of the handmade Colonial cheese produced in the northwestern part of the state of Rio Grande do Sul, in relation to the number of drugs that they were resistant to.

RESISTANCE (No. drugs) ¹	<i>A. hydrophila</i> (No. cultures)	<i>A. caviae</i> (No. cultures)	<i>A. sobria</i> (No. cultures)	<i>A. Veroni</i> (No. cultures)	<i>A. jandaei</i> (No. cultures)	TOTAL
1						
2						
3	01 (5.9) ²	01 (10.0)				02 (5.0) ³
4		01 (10.0)				01 (2.5)
5		02 (20.0)				02 (5.0)
6	01 (5.9)	01 (10.0)				02 (5.0)
7			02 (28.5)			02 (5.0)
8			01 (14.3)			01 (2.5)
9						
10		02 (20.0)				02 (5.0)
11	01 (5.9)		02 (28.5)			03 (7.5)
12	06 (35.3)	01 (10.0)			02 (66.7)	09 (22.5)
13	03 (17.6)	01 (10.0)				04 (10.0)
14	05 (29.4)	01 (10.0)	01 (14.3)	01 (33.3)	01 (33.3)	09 (22.5)
15			01 (14.3)	02 (66.7)		03 (7.5)
TOTAL	17 (100.0)	10 (100.0)	07 (100.0)	03 (100.0)	03 (100.0)	40 (100.0)

¹Antimicrobial agents: tetracyclines, chloramphenicol, gentamicin, amikacin, ampicillin, cephalothin, cefoxitin, sulfazotrim, amoxicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, and piperacillin.

²Percentage of isolates resistant to the total isolates of the species.

³Percentage of isolates resistant to the total isolates tested.

showed resistance to ten or more antimicrobials. Note the fact that 51.8% of the isolates were resistant to all tested principles. For *A. caviae*, *A. sobria* and *A. schubertii*, 100.0% of the cultures were resistant to six or more drugs.

From the isolates obtained from Colonial cheese production, 75.0% were resistant to at least ten antimicrobials. It is noted that of the 17 cultures of *A. hydrophila* tested for sensitivity to antibiotics, 15 (88.2%) were resistant to at least 11. Multiple resistance was also identified for other isolated species. *A. caviae* was resistant to at least three antimicrobials while *A. sobria* isolates were resistant to seven or more. On the other hand, for *A. Veroni*, 100.0% of isolates were resistant to at least 14 of the 15 antimicrobials tested. *A. jandaei* showed resistance to 12 or more antimicrobial principles.

Research conducted with isolates of *Aeromonas* spp. from environmental and food sources and clinical specimens showed that these bacteria may exhibit multiple resistance to drugs such as ampicillin, penicillin G, carbecillin, tetracycline, cephalothin, rifampicin, methicillin, novobiocin, vancomycin, erythromycin (BORREGO et al., 1991; ROSSI JR et al., 2000; COSTA & ROSSI JR, 2002; MAYKOT, 2002; VIVEKANANDHAN et al., 2002; MARTINELI et al., 2010). However, for all *Aeromonas* species identified, the resistance profile to drugs commonly used in human and veterinary medicine observed in

this study was higher than those reported by the authors.

Figure 1 shows the best antimicrobials against the *Aeromonas* spp. strains isolated from the production process of Minas cheese. Higher sensitivity of *Aeromonas* has been observed to the antimicrobials chloramphenicol, colistin, kanamycin, aminoglycosides, nalidixic acid, neomycin, streptomycin and gentamicin (BIZANI et al., 2001; MAYKOT, 2002; VIVEKANANDHAN et al., 2002; RADU et al., 2003; CARNEIRO & ROSSI JR, 2006; AWAN et al., 2009; MARTINELI et al., 2010). This result has been confirmed in this study since gentamicin showed the best sensitivity profile to these bacteria; however, still 70.6% of the isolates were resistant to this antibiotic, revealing the public health risks in cases when it is necessary to treat human infections. Moreover, 100% of the isolates were resistant to ampicillin, amoxicillin and piperacillin/tazobactam while only one isolate was sensitive to amikacin and cephalothin. Because ampicillin was used as the selective agent during the isolation of *Aeromonas* spp. the 100.0% sample resistance result was expected. Rossi Junior et al. (2000), Ghenghesch et al.(2001), Falgas (2003), Sharma et al.(2005), Carneiro & Rossi Junior (2006), Pallu et al. (2006) and Martineli et al. (2010) have also reported the same profile.

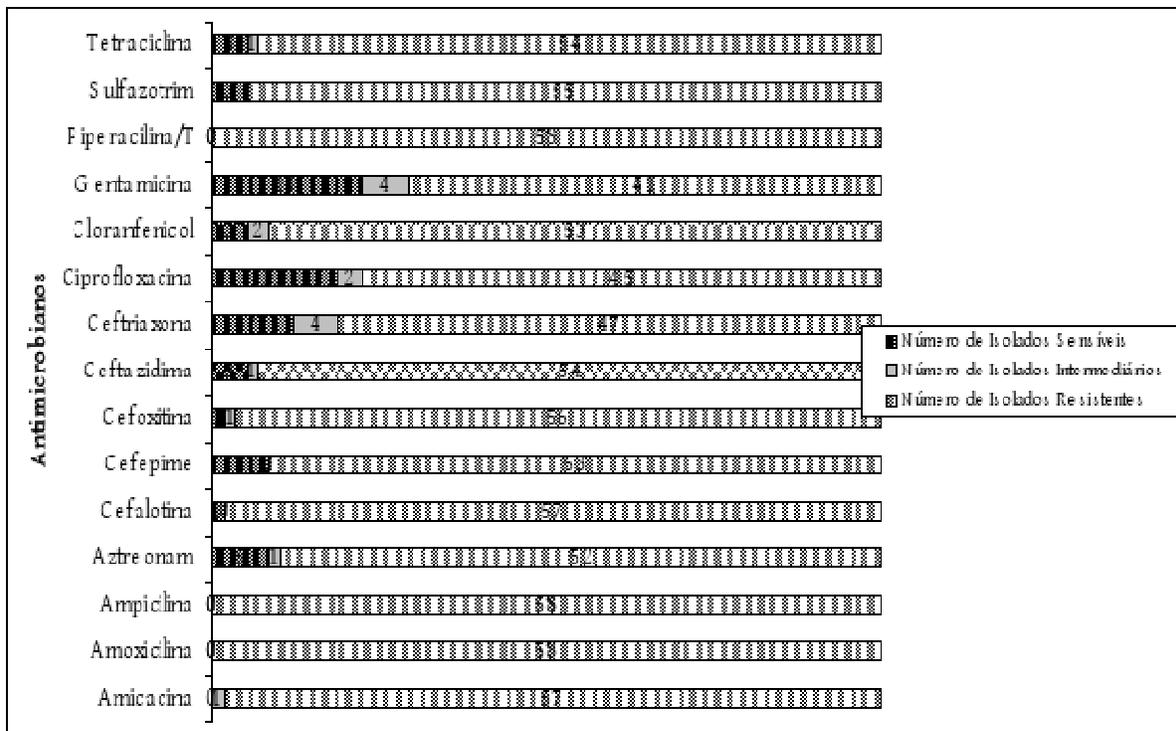


Figure 1 - Profile of resistance of *Aeromonas* spp. strain isolates of samples collected in the processing line of Minas cheese in the northwest region of Rio Grande do Sul.

Multiple antimicrobial resistance was also observed among the 40 isolates obtained from the Colonial cheese production, but there was a larger number of isolates sensitive to drugs compared to the isolates from Minas cheese (Figure 2). Gentamicin, cefepime and ciprofloxacin had the lowest levels of resistance (42.5%, 45.0% and 47.5%, respectively). The resistance rates were as follows: aztreonam, 55.0%

of the isolates were resistant; ceftriaxone, 65.0%; amikacin, 67.5%; ceftazidime, 70.0%; and, for chloramphenicol and cefoxitin, 75.0%. The resistance rates for tetracycline, piperacillin/tazobactam, sulfazotrim and cephalothin were, respectively, 82.5%, 85.0%, 87.5% and 97.5%. Furthermore, 100% of the isolates were resistant to amoxicillin and ampicillin.

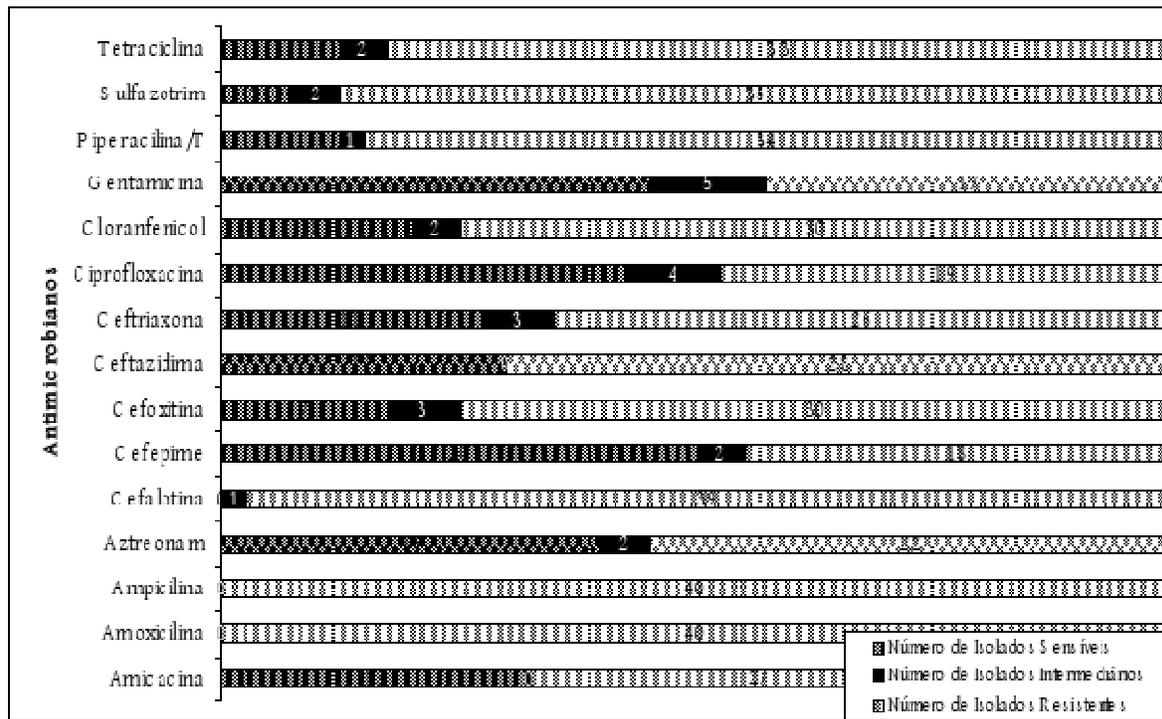


Figure 2 - Profile of resistance of *Aeromonas* spp. strain isolates of samples collected from the production line of the handmade Colonial cheese in the northwest region of Rio Grande do Sul.

For some groups of antibiotics, there was significant difference in the resistance profile of isolates obtained from the samples of different types of cheeses. For beta-lactams and the amino-glycosides ($p < 0.01$), as well as quinolon and chloramphenicol ($p < 0.05$), resistance was higher in isolates of *Aeromonas* spp. identified in the samples collected in the production line of Minas cheese. Tetracyclines and sulfamidic did not differ statistically between groups. It is possible that the higher resistance observed in isolates from industrial production is associated with the use of antimicrobials, representing the reality of many rural farms, while the handmade Colonial cheese used just its own raw material.

The concern for public health is cited by other authors; Guerra et al. (2007) isolated *Aeromonas* spp. in 6.6% of patients suffering from acute gastroenteritis in two hospitals of Rio Grande do Sul. *A. hydrophila*, *A. caviae* and *A. veronii* were isolated in 51.8%, 40.8% and 7.4% of patients, respectively. Likewise, the

antimicrobial susceptibility test also revealed resistance of all isolates to ampicillin and cephalothin, in addition to ticarcillin/clavulanic acid and ceftazidime. These authors reported that some multidrug-resistant isolates can be considered multi-resistant, as they were resistant to more than four antimicrobials.

These findings are worrisome since the cultures of these species were obtained from the processing lines of two different types of cheese widely consumed in Brazil, thus posing a health risk for the consumer. In addition, multiple resistance has been presented by *A. hydrophila*, *A. caviae* and *A. veronii*, species that account for 85.0% of human infections caused by the genus (JANDA & ABBOTT, 2010).

CONCLUSION

Isolates of *Aeromonas* spp. studied showed multiple resistance to antimicrobials used in human

and veterinary medicine, thus illustrating a public health threat. The agent showed the highest sensitivity to gentamicin, however, 33.6% of the strains were resistant to all antibiotics tested, with increased resistance in isolates of *Aeromonas* spp. obtained from the samples collected in the production line of Minas cheese.

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