Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.497

Survival of *Acinetobacter* spp. isolated from food to simulated gastrointestinal conditions

Ricardo Campos MONTEIRO[1], Michel Santos Gomes do NASCIMENTO[1], Janaína dos Santos NASCIMENTO[1]*

Abstract

In recent years, *Acinetobacter* isolates have frequently been obtained from foods of different origins, and the relevance of their presence and their role as a foodborne pathogen are rarely discussed. Surviving the passage through the gastrointestinal system is paramount to its intestinal colonization and, consequently, to triggering foodborne illnesses and other subsequent infections in the consumer. This work evaluated food isolates of *Acinetobacter* spp. (from ready-to-eat salads and goat milk) for their ability to survive in simulated gastrointestinal conditions. The isolates were exposed for 40 min at pH 2.0 in the presence of pepsin to simulate gastric conditions, followed by subsequent exposure to simulated intestinal fluid (with bile salts and trypsin) for 120 min. In general, reductions of 4.2–48.1% in viable counts of isolates were observed after exposure to the simulated gastrointestinal conditions. It is worth noting that six isolates showed a population reduction of less than 1 log CFU. mL^{-1}. This study points out that some isolates of *Acinetobacter* spp. found in food could reach the intestine after ingestion of contaminated food, remaining viable and at high counts, therefore posing a potential risk as a food pathogen, being able to develop infections in consumers, especially in the most vulnerable ones.

Keywords: gastrointestinal digestion, opportunistic foodborne pathogen, goat milk, ready-to-eat salad, *A. baumannii*.

Practical Application: *Acinetobacter* may be an opportunistic foodborne pathogen by surviving the gastrointestinal tract.

1 INTRODUCTION

Acinetobacter spp. has been isolated from different foods of plant and animal origin, including foods that do not undergo heat treatment before ingestion. Many of these isolates are resistant and multiresistant to antibiotics (Ababneh et al., 2022; Carvalheira, Casquete et al., 2017; Carvalheira, Silva, & Teixeira, 2017; Malta et al., 2020, 2021; Marí-Almirall et al., 2019). However, little is known about *Acinetobacter*'s ability to colonize the gastrointestinal system.

Some studies carried out in different countries with hospitalized patients report indications that this may occur. The studies highlighted very similar cases: Patients generally under 5 years of age or elderly were admitted to hospitals initially presenting with gastrointestinal infections. *Acinetobacter* strains were isolated from feces, and, in some cases, the patient's clinical condition progressed to bacteremia, also caused by *Acinetobacter* spp. Bloodstream infections are closely associated with previous infections from the respiratory tract and infections related to intravenous devices (Regalado et al., 2009; Thom et al., 2010; Yakut et al., 2016). This may be why the association between previously developed gastrointestinal tract infections caused by *Acinetobacter* spp. and bloodstream infections is underinvestigated.

One of the primary routes for *Acinetobacter* spp. to enter the gastrointestinal tract is through food. Recent global studies have demonstrated that various foods of plant and animal origin could serve as entry points for *Acinetobacter* spp. in the human body. This mode of transmission has also been observed in other bacterial species, such as *Escherichia coli*.

In recent decades, the number of individuals with weakened immune systems, such as in cases of HIV, cancer, tuberculosis, transplants, chemotherapy, and carriers of autoimmune diseases, among others, has increased thanks to advances in medicine and, therefore, greater life expectancy. These conditions further predispose affected individuals to infectious diseases (Cant & Cole 2010; Dropulic & Lederman 2016). Thus, the contact by the ingestion and subsequent colonization of these individuals with resistant and multiresistant bacteria, such as *Acinetobacter* spp., can be highly critical.

As the existing literature is limited, our study fills a crucial gap by providing evidence that *Acinetobacter* spp. could potentially bypass gastrointestinal defenses and emerge as an opportunistic foodborne pathogen. Our research is novel in that it investigates the survival capacity of *Acinetobacter* spp. isolated from food to simulated gastrointestinal conditions, thereby suggesting its potential as a food-associated pathogen.

1.1 Relevance of the work

Milk and ready-to-eat salads could serve as entry points for Acinetobacter spp. in the human body. Our research investigated the survival capacity of Acinetobacter spp. from

Received: April 12, 2025.
Accepted: May 11, 2025.
[1]*Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Laboratório de Microbiologia, Rio de Janeiro, Rio de Janeiro, Brazil.*
*Corresponding author: janaina.nascimento@ifrj.edu.br
Conflict of interest: nothing to declare.
Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

food to simulated gastrointestinal conditions. The results showed that this pathogen could reach the intestine after ingestion of contaminated food, remaining viable and at high counts, thus highlighting its potential as an opportunistic foodborne pathogen.

2 MATERIALS AND METHODS

2.1 Bacterial isolates

Acinetobacter spp. used in this work came from raw goat's milk and ready-to-eat salads, identified by MALDI-TOF mass spectrometry in previous studies (Beltrão, 2019; Ramos & Nascimento, 2019) in Rio de Janeiro, Brazil, and are shown in Table 1. The isolates were activated from stocks frozen at –20°C using inoculation onto plates containing Casoy agar (tripticase soy agar, HiMedia, São Paulo, Brazil). The plates were incubated at 37°C for 18–24 h.

2.2 Digestion fluid stock solution

The components and concentrations proposed by Minekus et al. (2014) were used to prepare the digestion fluid stock solution for simulated gastric juice (SGJ) and simulated intestinal fluid (SIF). The solution was autoclaved, aliquoted into Falcon tubes, and frozen at –20°C until use.

2.3 Assessment of tolerance to simulated gastric juice

To compose the SGJ, a pepsin solution (Sigma-Aldrich, USA) was added to the digestion fluid at the time of use. The solution was sterilized by filtration through a nitrocellulose membrane (0.22 µm) up to a final concentration of 2,000 U/mL and with the pH adjusted to 2.0.

Tolerance to SGJ was assessed as described by Pettersen et al. (2019), exposing 1 mL of saline solution containing a cell suspension of approximately 8.0 log CFU.mL^{-1} of *Acinetobacter* spp. to 9 mL of SGJ, previously warmed to 37°C. Exposure was performed for 40 min at 37°C and agitation at 100 rpm to simulate peristaltic movement.

Suspension aliquots were collected at time zero and after 40 min of exposure for quantification, as described by Mao et al. (2006). Serial dilutions were performed and inoculated, in duplicate, on plates containing Casoy agar. Plates were incubated at 37°C for 24 h. After the incubation time, the colony count was performed.

Table 1. *Acinetobacter* spp. used in this study.

Identification	Isolates	Source
Acinetobacter baumannii	F3R18/7	S
	F3R13/1	S
	F1R13/6	S
Acinetobacter gerneri	F5R14/3	S
Acinetobacter guillouiae	1708	M
	1715	M
Acinetobacter nosocomialis	F4R15/7	S
	F4R15/6	S
	F1R13/7	S
Acinetobacter ursingii	2008	M
	2017	M

M: samples from raw goat milk; S: amples from ready-to-eat salads.

2.4 Assessment of simulated intestinal fluid tolerance

In order to mimic what happens in the human body, the isolates were subjected to SIF action, as described by Pettersen et al. (2019), with minor changes. After the time of exposure to SGJ, the pH of the medium was adjusted to 7.0 using a sterile solution of 1.0 M NaOH, and, subsequently, the intestinal fluid was simulated from the addition of bovine pancreas trypsin (Sigma-Aldrich, USA) in order to reach a final concentration of 100 U/mL and bile salts (ox gall, Sigma-Aldrich, USA) at a final concentration of 0.3% (w/v), as adapted by Madureira et al. (2005). The SIF was maintained at 37°C for 120 min with 100 rpm stirring. The quantification of viable cells was performed after 60 and 120 min of bacteria exposure to SIF, following the same methodology used to quantify viable cells after exposure to SGJ.

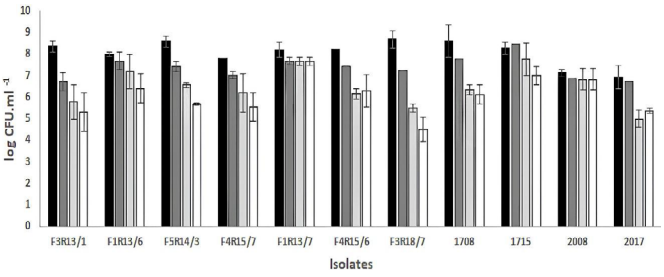
2.5 Statistical analysis

Differences in the counting values between the isolates obtained from salads and raw milk were compared using an unpaired t-test using GraphPad software. p-values < .05 were considered significant.

3 RESULTS AND DISCUSSION

Unlike classic foodborne pathogens, *Acinetobacter* is rarely described as associated with diarrheal disease. However, the main reason for this disparity lies in the fact that these bacteria usually cause much more severe infections, and even when they are detected in the gastrointestinal system, their presence is usually overshadowed by the co-occurrence of more common infectious agents, which are then collectively treated as opportunistic pathogens (Amorim & Nascimento, 2017).

Notably, seven isolates from salads and four from goat's milk were used in this study to investigate the survival capacity of food *Acinetobacter* spp. The initial counts of *Acinetobacter* spp. from ready-to-eat salads before treatments ranged from 7.99 to 8.67 log CFU.mL^{-1}. After 40 min of exposure to SGJ, the obtained counts varied between 6.70 and 7.68 log CFU.mL^{-1}. Except for the isolates F3R13/1, F5R14/3, and F3R18/7, which presented a reduction higher than 1.0 log CFU.mL^{-1}, reductions



SGJ: simulated gastric juice; SIF: simulated intestinal fluid; ■: initial inoculum; ■: exposure to SGJ for 40 min; ■: exposure to SIF for 1 h; □: exposure to SIF for 2 h.

Figure 1. Survival of *Acinetobacter* spp. in the simulated gastrointestinal system.

lower than this value were observed for the other isolates from salads (Figure 1).

This study breaks new ground by examining the tolerance of *Acinetobacter* spp. from raw and fresh food to simulated gastrointestinal fluids. This unique approach makes it difficult to directly compare our findings with other studies on the same microorganism. However, our results align with those of studies conducted on classic food pathogens, providing valuable insights into the survival mechanisms of these organisms.

Pienaar and his team conducted a comprehensive analysis of two groups of isolates of enteropathogenic and enterotoxigenic *E. coli*. These isolates were subjected to gastric conditions with varying pH levels for a duration of 180 min. The researchers discovered that both groups of *E. coli* demonstrated remarkable resilience, surviving even with membrane damage. They also observed that strains of enterotoxigenic *E. coli* remained viable and cultivable across all pH levels, a finding of significant interest (Pienaar et al., 2016).

In our studies, *Acinetobacter* isolates isolated from food showed good tolerance to SGJ at pH 2.0, reducing a maximum of 19.7% of the initial inoculum. Low pH also does not seem decisive for the survival of food pathogens such as *Shigella flexneri*, *S. dysenteriae*, *Salmonella* Typhimurium, and *Vibrio cholerae*. Even though they suffer some damage to their cells, these pathogens can tolerate exposure to gastric juice for 30–180 min (Singh & Barnard, 2016, 2017).

However, for a food pathogen to establish colonization in the human intestinal tract, it must be able to resist local stress, which includes changes in pH, concentration of bile salts (which play a detergent role in the emulsification of fats, facilitating the degradation of lipids present in the plasma membrane), and the presence of trypsin in pancreatic juice, which is crucial in this process, since this proteolytic enzyme plays the role of proteolysis, promoting the breakdown of proteins into amino acids (Joffre et al., 2019; Zhang et al., 2016).

Mimicking the passage from the stomach to the intestine, when the isolates were exposed to the action of bile salts and trypsin (SIF composition) for 60 min, the counting of the log CFU.mL^{-1} varied little for seven of the salads's isolates tested.

The isolates were kept in the same condition for another 60 min, obtaining, at the end of the incubation, reductions of about 0.53 and 4.17 log CFU.mL^{-1} compared with the initial inoculum. In general, the isolates showed a population reduction higher than 1.5 log CFU.mL^{-1} in the last hour, except for isolate F1R13/7, whose reduction was only 0.53 CFU.mL^{-1}. The *A. baumannii* F3R18/7 isolate showed a higher sensitivity to simulated gastrointestinal conditions, with an average final reduction of 4.17 log CFU.ml^{-1} (48.1% of the initial concentration), followed by the isolate F3R13/1, also *A. baumannii*, whose counts after treatment showed a reduction of 3.05 log CFU.mL^{-1} (36.6% of the initial concentration).

The other group of *Acinetobacter* spp. from raw goat milk was also subjected to simulated gastrointestinal conditions (Figure 1). At the beginning of the experiments, before exposure to SGJ, counts of 8.59, 8.27, 7.12, and 6.92 log CFU.mL^{-1} were obtained for isolates 1708, 1715, 2008, and 2017, respectively. When exposed to SGJ for 40 min, the population of isolates slightly dropped, reaching counts of 7.75, 8.45, 6.84, and 6.71 log CFU.mL^{-1}, respectively.

When exposed to SIF for 2 h, it was observed that the population of the isolates suffered a decrease, with reductions from 0.30 to 2.46 CFU.mL^{-1} compared to those obtained after exposure to SGJ. The *A. ursingii* 2008 isolate was the one that showed the highest tolerance to the simulated gastrointestinal conditions since, throughout the entire exposure, there was a reduction of only 0.30 log CFU.mL^{-1} (4.2 % of the initial concentration).

In general, reductions of 3.0–19.7% in viable counts of isolates from both sources were observed during exposure to SGJ. In comparison, 4.2–48.1% reductions were obtained after exposure to SIF for the subsequent 2 h. The analysis of the survival fractions in the simulated stomach and intestine revealed the difference between the two groups of *Acinetobacter* spp. isolates from goat's milk and salad were not considered statistically significant (p = .1621).

In our study, all isolates from raw goat's milk and six salad isolates showed a reduction of less than 1 log CFU.mL^{-1} when exposed to FIS, suggesting greater tolerance to these conditions. The total reduction in the population of these isolates after the total exposure to simulated gastrointestinal conditions corresponded to a survival of 51.9–95.6% of the initial inoculum. This high tolerance has also been observed with other foodborne pathogens. Mathipa and Thantsha (2015) described the behavior of strains of enterohemorrhagic *E. coli* in a simulated gastrointestinal model, where tolerance of these bacteria was verified after 120 min of exposure. The tolerance of *Salmonella* spp. and *Listeria monocytogenes* under the same conditions was investigated in another study, in which the authors highlighted the high tolerance of these pathogens to bile salts (Akritidou et al., 2022).

4 CONCLUSION

Our results demonstrate that some isolates of *Acinetobacter* spp. studied in this work, both isolated from raw goat's milk and ready-to-eat salads, could reach the intestine and remain viable under the action of bile salts and proteolytic enzymes in concentrations very similar to the initial inoculum (e.g., isolates F1R13/7 and 2008), being highly tolerable under these conditions. Our data also show that the total reduction in values from the initial inoculum of most of the isolates until the end of exposure to the simulated gastrointestinal conditions was not remarkable, reaching a median of 24%, highlighting that only one isolate proved to be more sensitive, with a reduction near to 50% (F3R18/7). This work alerts to the ability of *Acinetobacter* spp. to circumvent the primary defenses of the human gastrointestinal system, especially in the elderly, young children, and immunocompromised individuals, who could be more susceptible to gastrointestinal infections caused by *Acinetobacter*.

ACKNOWLEDGMENTS

Janaína dos Santos Nascimento thanks the research grants from Conselho Nacional de Desenvolvimento Científico e

Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

REFERENCES

Ababneh, Q., Al-Rousan, E., & Jaradat, Z. (2022). Fresh produce as a potential vehicle for transmission of Acinetobacter baumannii. *International Journal of Food Contamination*, *9*, Article 5. https://doi.org/10.1186/s40550-022-00092-7

Akritidou, T., Akkermans, S., Gaspari, S., Azraini, N. D., Smet, C., Van De Wiele, T., & Van Impe, J. F. M. (2022). Effect of gastric pH and bile acids on the survival of Listeria monocytogenes and Salmonella Typhimurium during simulated gastrointestinal digestion. *Innovative Food Science and Emerging Technologies*, *82*, Article 103161. https://doi.org/10.1016/j.ifset.2022.103161

Amorim, A. M. B., & Nascimento, J. S. (2017). Acinetobacter: an underrated foodborne pathogen? *Journal of Infections in Developing Countries*, *11*, 111–114. https://doi.org/10.3855/jidc.8418

Beltrão, J. C. C. (2019). *Avaliação da qualidade microbiológica de saladas de hortaliças cruas prontas ao consumo e identificação do perfil de resistência a antibióticos das enterobactérias isoladas* [Master's dissertation, Universidade Federal Fluminense]. Repositório Institucional da UFF. https://app.uff.br/riuff/handle/1/12702?show=full

Cant, A., & Cole, T. (2010). Infections in the immunocompromised. In A. Finn, N. Curtis, & A. J. Pollard (Eds.), *Hot Topics in Infection and Immunity in Children VI* (Vol. 659, pp. 1–18). Springer. https://doi.org/10.1007/978-1-4419-0981-7_1

Carvalheira, A., Casquete, R., Silva, J., & Teixeira, P. (2017). Prevalence and antimicrobial susceptibility of Acinetobacter spp. isolated from meat. *International Journal of Food Microbiology*, *243*, 58–63. https://doi.org/10.1016/j.ijfoodmicro.2016.12.001

Carvalheira, A., Silva, J., & Teixeira, P. (2017). Lettuce and fruits as a source of multidrug resistant *Acinetobacter* spp. *Food Microbiology*, *64*, 119–125. https://doi.org/10.1016/j.fm.2016.12.005

Dropulic, L. K., & Lederman, H. M. (2016). Overview of infections in the immunocompromised host. In R. T. Hayden, D. M. Wolk, K. C. Carroll, & Y.-W. Tang (Eds.), *Diagnostic Microbiology of the Immunocompromised Host* (2nd ed., pp. 1–50). American Society for Microbiology. https://doi.org/10.1128/9781555819040.ch1

Joffre, E., Nicklasson, M., Álvarez-Carretero, S., Xiao, X., Sun, L., Nookaew, I., Zhu, B., & Sjöling, Å. (2019). The bile salt glycocholate induces global changes in gene and protein expression and activates virulence in enterotoxigenic Escherichia coli. *Scientific Reports*, *9*, Article 108. https://doi.org/10.1038/s41598-018-36414-z

Madureira, A. R., Pereira, C. I., Truszkowska, K., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2005). Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. *International Dairy Journal*, *15*(6–9), 921–927. https://doi.org/10.1016/j.idairyj.2004.08.025

Malta, R. C. R., Cruz, C. H. S., Santos, J. B., Ramos, G. L. P. A., & Nascimento, J. S. (2021). *Acinetobacter em alimentos: Uma visão geral* (1st ed.). Atena Editora.

Malta, R. C. R., Ramos, G. L. P. A., Nascimento, J. S. (2020). From food to hospital: we need to talk about Acinetobacter spp. *Germs*, *10*(4), 210–217. https://doi.org/10.18683/germs.2020.1207

Mao, Y., Doyle, M. P., & Chen, J. (2006). Role of colanic acid exopolysaccharide in the survival of enterohaemorrhagic Escherichia coli O157:H7 in simulated gastrointestinal fluids. *Letters in Applied Microbiology*, *42*(6), 642–647. https://doi.org/10.1111/j.1472-765X.2006.01875.x

Marí-Almirall, M., Cosgaya, C., Pons, M. J., Nemec, A., Ochoa, T. J., Ruiz, J., Roca, I., & Vila, J. (2019). Pathogenic Acinetobacter species including the novel Acinetobacter dijkshoorniae recovered from market meat in Peru. *International Journal of Food Microbiology*, *305*, Article 108248. https://doi.org/10.1016/j.ijfoodmicro.2019.108248

Mathipa, M. G., & Thantsha, M. S. (2015). Cocktails of probiotics pre-adapted to multiple stress factors are more robust under simulated gastrointestinal conditions than their parental counterparts and exhibit enhanced antagonistic capabilities against Escherichia coli and Staphylococcus aureus. *Gut Pathogens*, *7*, Article 5. http://doi.org/10.1186/s13099-015-0053-5

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Feuteun, S. L., Lesmes, U., Macierzanka, A., Mackie, A., … Brodkorb, A. (2014). A Standardised static in vitro digestion method suitable for food – an international consensus. *Food and Function*, *5*, 1113–1124. https://doi.org/10.1039/c3fo60702j

Pettersen, K. S., Skjerdal, T., Wasteson, Y., Lindbäck, T., Vegarud, G., Comi, I., & Aspholm, M. (2019). Survival of Listeria monocytogenes during in vitro gastrointestinal digestion after exposure to 5 and 0.5 % sodium chloride. *Food Microbiology*, *77*, 78–84. https://doi.org/10.1016/j.fm.2018.08.010

Pienaar, J. A., Singh, A., & Barnard, T. G. (2016). Exploratory study into the culturability and viability of three strains of Escherichia coli after exposure to simulated gastric fluid of sub-lethal pHs. *Medical Technology SA*, *30*(1), 36–44. https://hdl.handle.net/10520/EJC193810

Ramos, G. L. P. A., & Nascimento, J. S. (2019). Characterization of Acinetobacter spp. from raw goat milk. *Ciência Rural*, *49*(10), Article e20190404. https://doi.org/10.1590/0103-8478cr20190404

Regalado, N. G., Martin, G., & Antony, S. J. (2009). Acinetobacter lwoffii: Bacteremia associated with acute gastroenteritis. *Travel Medicine and Infectious Diseases*, *7*(5), 316–317. https://doi.org/10.1016/j.tmaid.2009.06.001

Singh, A., & Barnard, T. G. (2016). Surviving the acid barrier: responses of pathogenic Vibrio cholerae to simulated gastric fluid. *Applied Microbiology and Biotechnology*, *100*, 815–824. https://doi.org/10.1007/s00253-015-7067-2

Singh, A., & Barnard, T. G. (2017). Adaptations in the physiological heterogeneity and viability of Shigella dysenteriae, Shigella flexneri and Salmonella typhimurium, after exposure to simulated gastric acid fluid. *Microbial Pathogenesis*, *113*, 378–384. https://doi.org/10.1016/j.micpath.2017.11.014

Thom, K. A., Hsiao, W. W. L., Harris, A. D., Stine, O. C., Rasko, D. A., & Johnson, J. K. (2010). Patients with Acinetobacter baumannii bloodstream infections are colonized in the gastrointestinal tract with identical strains. *American Journal of Infection Control*, *38*(9), 751–753. https://doi.org/10.1016/j.ajic.2010.03.005

Yakut, N., Kepenekli, E. K., Karaaslan, A., Atici, S., Akkoc, G., Demir, S. O., Soysal, A., & Bakir, M. (2016). Bacteremia due to Acinetobacter ursingii in infants: reports of two cases. *The Pan African Medical Journal*, *23*, Article 193. https://doi.org/10.11604/pamj.2016.23.193.8545

Zhang, X. H., Wang, Z., Yin, B., Wu, H., Tang, S., Wu, L., Su, Y. N., Lin, Y., Liu, X. Q., Pang, B., Kemper, N., Hartung, J., & Bao, E. D. (2016). A complex of trypsin and chymotrypsin effectively inhibited growth of pathogenic bacteria inducing cow mastitis and showed synergistic antibacterial activity with antibiotics. *Livestock Science*, *188*, 25–36. https://doi.org/10.1016/j.livsci.2016.03.017