

## Original Article

Tolerance of foodborne *Acinetobacter* spp. to sanitizer agents

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**Abstract**

**Introduction:** The bacteria *Acinetobacter* spp. are extremely relevant in clinical settings. Recently, they have emerged as potential food-borne opportunistic pathogens. Their ability to form biofilms contributes to antibiotic resistance by generating an environment that facilitates the acquisition and transfer of resistance genes. Studies on the tolerance of *Acinetobacter* spp. from food sources to sanitizers used in the food industry and homes are necessary to help mitigate food contamination by these microorganisms.

**Methodology:** Isolates from ready-to-eat salads (n = 11) and raw goat milk (n = 4) were evaluated for their tolerance to sodium hypochlorite (NaClO), quaternary ammonium compound/biguanide (QAC/BG), and peracetic acid (PAA). The Food and Drug Administration (FDA) recommends that the concentration of these sanitizers in food-processing equipment and utensils and other food-contact articles should not exceed 200 parts per million (ppm).

**Results:** The minimum inhibitory (MIC) and bactericidal (MBC) concentrations of NaClO were above 312.5 ppm for all isolates tested and  $\geq 2,500$  ppm for four isolates from salads. Only three isolates from salads and four isolates from goat milk were inhibited by an MIC lower than 200 ppm of PAA. QAC/BG presented the lowest MIC and MCB values (9.37/6.25 ppm for all isolates tested), suggesting that it is the most effective agent against the isolates used in this study.

**Conclusions:** Our results demonstrate that *Acinetobacter* spp. isolates from food can be tolerant to the recommended concentrations of NaClO and PAA, which highlights the health risks to consumers.

**Key words:** *Acinetobacter* spp.; sanitizers; salads; raw milk.

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**Introduction**

Lifestyle changes have resulted in some foods becoming very popular because they are fresh, healthy, practical, and without additives. Among these are ready-to-eat, fresh, raw, and minimally processed foods. However, these healthy products may have some hidden dangers.

Fresh foods can be considered high-risk from a microbiological point of view, since they can be consumed directly without undergoing any microbial inactivation processes. This has already been observed with the increase in disease outbreaks associated with the consumption of these foods [1,2]. Fresh and raw products, such as milk, or minimally processed products, such as ready-to-eat salads, can be sources of *Acinetobacter* spp.

*Acinetobacter baumannii* is a pathogen classified as an urgent threat by the Centers for Disease Control and Prevention (CDC), mainly because of its resistance to several antibiotics [3]. Most *A. baumannii* infections

are caused by strains resistant to multiple antibiotics (MDR, "multidrug-resistant"); this makes them difficult to treat and often results in hospital outbreaks. More importantly, *A. baumannii* is able to survive in different environments and adverse conditions, including long periods on surfaces [4]. Therefore, it is important to adequately decontaminate surfaces to prevent the spread of these bacteria.

*Acinetobacter* spp. are not only a concern in clinical settings. The presence of these bacteria in industries and other environments that produce or process food are a concern because pathogenic strains resistant to multiple antibiotics have been isolated from different food samples. Some studies have shown that food can act as a vector for the transmission of these bacteria to consumers [5-10].

Sanitizing agents are used in hospitals and industries involved with food production, preparation, and distribution [11]. However, some *A. baumannii* isolates are resistant to some of these agents, further

contributing to their ability to survive on surfaces and spread via food [4]. It is noteworthy that the capacity of bacteria to form a biofilm further increases their resistance to sanitizing agents; therefore, *Acinetobacter* spp. strains that form biofilms are often multidrug-resistant [12].

The efficiency of sanitizers used for the elimination of these microorganisms in the food industry and domestically, needs to be validated to ensure that these agents, at their recommended concentrations, are effective against *Acinetobacter* spp. strains. Several studies have evaluated the tolerance of *Acinetobacter* spp. isolates of clinical origin to sanitizers [13-16]. These studies revealed an association between clinical isolates with reduced susceptibility to sanitizers, such as sodium hypochlorite (NaClO), chlorhexidine, and peracetic acid (PAA), and the formation of biofilms.

Clinical isolates are typically more tolerant to sanitizers than environmental isolates; however, there are no specific studies on foodborne *Acinetobacter* spp., especially those isolated from fresh foods such as raw milk and salads.

In this study, the tolerance of *Acinetobacter* spp. isolates from raw goat milk and ready-to-eat salads was evaluated against NaClO, PAA, and quaternary ammonium compounds (QACs), which are generally used for cleaning food preparation areas.

## Methodology

### *Acinetobacter* spp. isolates

*Acinetobacter* spp. were isolated from raw goat milk products and ready-to-eat salads and identified by matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry in previous studies

performed at the Instituto Federal do Rio de Janeiro (IFRJ) [17] and Universidade Federal Fluminense (UFF) [18] (Table 1). The isolates were activated from stocks stored at -20 °C by inoculation onto Casoy agar (Soybean-Casein digest agar, Himedia, São Paulo, Brazil) plates. The cultures were incubated at 37 °C for 18–24 h.

### Characterization of the isolates

The initial characterization of the isolates is presented in Table 1. A qualitative assessment of biofilm production and multidrug resistance (MDR) of the isolates was performed.

Biofilm production was evaluated by inoculating the isolates into Congo Red agar (nutrient agar plus 0.8 g/L Congo red and 36 g/L sucrose) plates as described by Freeman *et al.* [19]. The cultures were incubated at 37 °C for 24 h. Biofilm-producing isolates appeared as black colonies, whereas non-producing isolates were depigmented or reddish. *Salmonella enterica* (ATCC14028) was used as a positive control.

Isolates were cultured on CHROMagar™ *Acinetobacter* MDR culture medium, a selective and differential chromogenic medium, to detect MDR [20]. The cultures were incubated at 37 °C for 18–24 h. MDR *Acinetobacter* spp. colonies were colored red. Other bacteria (including non-MDR *Acinetobacter* spp. isolates) were either inhibited or presented as light blue colonies.

### Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of NaClO, PAA, and QAC/biguanide (QAC/BG) were

**Table 1.** Isolates of *Acinetobacter* spp. used in this work.

Origin	Isolates	Identification	Characteristics previously studied *	Biofilm production	MDR phenotype expression
Ready-to-eat salads	F4R15/7	<i>Acinetobacter nosocomialis</i>	ND	-	+
	F3R18/7	<i>Acinetobacter baumannii</i>	ND	-	+
	F4R15/6	<i>Acinetobacter nosocomialis</i>	CAZ <sup>R</sup> , SUT <sup>R</sup>	-	+
	F5R14/3	<i>Acinetobacter gernerii</i>	ND	+	+
	F3R12/7	<i>Acinetobacter nosocomialis</i>	ND	-	+
	F1R13/7	<i>Acinetobacter nosocomialis</i>	ND	-	+
	F3R13/1	<i>Acinetobacter baumannii</i>	ND	-	+
	F1R13/6	<i>Acinetobacter baumannii</i>	ND	-	+
	F2R21	<i>Acinetobacter baumannii</i>	ND	+	+
	F2R13/7	<i>Acinetobacter baumannii</i>	ND	-	+
	F4R15/3	<i>Acinetobacter nosocomialis</i>	CAZ <sup>R</sup> , MIN <sup>R</sup> , SUT <sup>R</sup> (MDR)	-	+
	Raw goat's milk	1708	<i>Acinetobacter guillauiae</i>	KPC <sup>+</sup> , ESBL <sup>+</sup>	-
2017		<i>Acinetobacter ursingii</i>	KPC <sup>+</sup> , ESBL <sup>+</sup>	-	+
2008		<i>Acinetobacter ursingii</i>	ESBL <sup>+</sup>	-	+
1715		<i>Acinetobacter guillauiae</i>	KPC <sup>+</sup> , ESBL <sup>+</sup>	-	+

\* Characteristics previously studied by Beltrão [18] (isolates from ready-to-eat salads) and by Ramos, Nascimento [17] (isolates from raw goat's milk); R: resistance; MDR: multidrug resistance; CAZ: ceftazidime; MIN: minocycline; SUT: sulfamethoxazole/trimethoprim; ESBL<sup>+</sup>: extended-spectrum beta-lactamase producer; KPC<sup>+</sup>: *Klebsiella pneumoniae* carbapenemase producer; ND: not determined.

determined using the broth microdilution method in 96-well polystyrene microtiter plates, as described by Obe et al. [21], with minor alterations. Colonies of each *Acinetobacter* spp. isolate were inoculated in 10 mL of 0.85% (w/v) saline solution until the turbidity was equivalent to McFarland's 0.5 scale (approximately  $1.5 \times 10^8$  CFU/mL). Sanitizer (200  $\mu$ L) was added to the first well of the microtiter plates, and 100  $\mu$ L of tryptic soy broth (TSB, Himedia, São Paulo, Brazil) was added to the remaining wells in the same row. The sanitizer was diluted by transferring 100  $\mu$ L from the first to the last well. Then, 100  $\mu$ L of the inoculum prepared with the bacterial isolates was added to each well, resulting in a final volume of 200  $\mu$ L and a concentration of approximately  $10^6$  CFU in each well (confirmed by plating). The final concentration of sanitizers in the wells was 19.5–10,000 ppm for NaClO (CLORO RIO, Rioquímica, São Paulo Brazil), 1.9–1,000 ppm for PAA (PERAX RIO, Rioquímica, São Paulo, Brazil), and 1.17/0.78–150/100 ppm for QAC/BG (SEPTPRO, Prolink. São Paulo, Brazil). The QAC/BG was composed of a mixture of cationic surfactants (dimethyl ammonium didecyl chloride and benzalkonium chloride) and polyhexamethylene biguanide chloride. Positive control wells included 100  $\mu$ L of broth and 100  $\mu$ L of bacterial inoculum, and negative control wells included 200  $\mu$ L of TSB broth added before incubation of the plate at 37 °C for 24 h. Bacterial growth was visually determined based on turbidity. The MIC was determined as the lowest concentration of the sanitizer that inhibited the growth of each *Acinetobacter* spp. isolate. The experiment was repeated thrice for each isolate.

### Determination of the minimum bactericidal concentration (MBC)

The MBC was determined as described by Haubert et al. [22]. MBC was evaluated from wells without visible bacterial growth, as obtained in the previous experiment. Aliquots of 100  $\mu$ L were cultured on tryptic soy agar (TSA, Himedia, São Paulo, Brazil) plates and incubated at 37 °C for 24 h. After incubation, colonies were counted. MBC was defined as the lowest concentration of sanitizer at which 99.9% of the cells were killed. Three independent experiments were conducted for each isolate.

### Results

In this study, the tolerance of *Acinetobacter* spp. isolated from raw goat milk and ready-to-eat salads to NaClO, PAA, and QAC/BG was evaluated. Isolates used in this study were characterized before use by evaluating the production of biofilms and expression of the MDR phenotype.

Two (13.3%) of the 15 isolates, F5R14/3 and F2R21, included in this study were identified as *A. gernerii* and *A. baumannii*. Both of these isolates were obtained from ready-to-eat salads and produced biofilms on Congo red agar (Table 1).

The characterization of the isolate antibiotic resistance in previous studies was performed using an antibiogram, which may not reveal an MDR phenotype. All 15 isolates used in this study exhibited an MDR phenotype on CHROMagar *Acinetobacter* MDR-chromogenic agar (Plastlabor, Rio de Janeiro, Brazil) (Table 1).

**Table 2.** Minimum inhibitory and bactericidal concentration of sanitizers against *Acinetobacter* spp. studied in this work.

Identification	Origin	Isolate	MIC and MBC of the sanitizer agents in ppm					
			NaClO		PAA		QAC/BG	
			MIC	MBC	MIC	MBC	MIC	MBC
<i>Acinetobacter baumannii</i>	S	F3R18/7	1,250	1,250	500	500	9.37/6.25	9.37/6.25
	S	F3R13/1	$\geq 2,500$	$\geq 2,500$	125	125	9.37/6.25	9.37/6.25
	S	F1R13/6	1,250	$\geq 2,500$	250	250	9.37/6.25	9.37/6.25
	S	F2R21	$\geq 2,500$	$\geq 2,500$	125	250	9.37/6.25	9.37/6.25
	S	F2R13/7	625	1,250	250	250	9.37/6.25	9.37/6.25
<i>Acinetobacter nosocomialis</i>	S	F4R15/7	1,250	1,250	500	500	9.37/6.25	9.37/6.25
	S	F4R15/6	1,250	1,250	250	250	9.37/6.25	9.37/6.25
	S	F3R12/7	1,250	1,250	250	250	9.37/6.25	9.37/6.25
	S	F4R15/3	$\geq 2,500$	$\geq 2,500$	500	500	9.37/6.25	9.37/6.25
	S	F1R13/7	$\geq 2,500$	$\geq 2,500$	125	125	9.37/6.25	9.37/6.25
<i>Acinetobacter gernerii</i>	S	F5R14/3	312.5	312.5	500	500	9.37/6.25	9.37/6.25
<i>Acinetobacter guillouiae</i>	M	1708	625	625	31.2	31.2	9.37/6.25	9.37/6.25
	M	1715	312.5	312.5	62.5	62.5	9.37/6.25	9.37/6.25
<i>Acinetobacter ursingii</i>	M	2017	625	625	62.5	62.5	9.37/6.25	9.37/6.25
	M	2008	312.5	312.5	31.2	31.2	9.37/6.25	9.37/6.25

NaClO: sodium hypochlorite; PAA: peracetic acid; QAC/BG: quaternary ammonium compound and biguanide; S: isolates from salad samples; M: isolates from goat milk samples; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; ppm: parts per million.

The MIC and MBC values of NaClO against *Acinetobacter* spp. were 312.5 and  $\geq 2,500$  ppm, respectively. Isolates identified as *A. baumannii* and *A. nosocomialis* exhibited values higher than 1,250 ppm (Table 2). Only one isolate (F5R14/3, a biofilm producer) had a slightly lower NaClO MIC (312.5 ppm). The isolates F1R13/7, F3R13/1, F2R21, and F4R15/3 had high NaClO tolerance and were recovered from ready-to-eat salads.

The MIC of eight (71.7%) of the 11 isolates from ready-to-eat salads to PAA was  $\geq 250$  ppm (Table 2). The MIC and MBC of the raw milk isolates were between 31.2–62.5 ppm. The MIC and MBC values to QAC/BG were the same for all isolates tested (9.37/6.25 ppm) (Table 2).

## Discussion

The isolates studied in this work were characterized for the production of biofilm and for the expression of the MDR phenotype prior to evaluation of tolerance to sanitizers. Biofilms are communities of microorganisms attached to a biotic or abiotic surface surrounded by an extracellular polymeric matrix. The production of biofilms contributes to the adhesion, colonization, and infection capacity of *Acinetobacter* spp. [23,24].

The four isolates from raw goat milk had already been tested for biofilm production in a previous study [17], and these results were confirmed in this study. Only two isolates from ready-to-eat salads were biofilm producers, despite this being previously described for *Acinetobacter* spp. from food [25]. Recently, one study reported that all isolates ( $n = 17$ ) of *Acinetobacter* spp. derived from fresh vegetables and fruits were able to produce biofilms [26]. However, our results differ from the above findings, as a low frequency of isolates produced biofilms (13.3%). The ability of isolates to produce biofilms was further confirmed by amplifying genes associated with biofilm production by polymerase chain reaction (PCR).

Some studies have reported that the prevalence of antibiotic resistance in biofilms is higher than that in planktonic (free) cells, and that biofilm formation is more strongly associated with MDR isolates than susceptible isolates [27-29]. Biofilm-forming isolates can spread in food preparation environments and form biofilms on the surfaces of these facilities, making them a persistent source of food contamination. Additionally, biofilm production contributes to antibiotic resistance in *Acinetobacter* spp. by forming an environment that facilitates the acquisition and transfer of resistance genes [9].

All food isolates used in this study presented an MDR phenotype on CHROMagar *Acinetobacter* MDR chromogenic agar. This phenotype has been observed in *Acinetobacter* spp. isolated from different foods. In a previous study, 17 strains of the *Acinetobacter baumannii*-calcoaceticus complex (ABC complex) isolated from infant formula were characterized, 14 (82.3%) of which displayed MDR [5]. Another study identified 166 *Acinetobacter* spp. isolates by genetic sequencing of meat products [30]. A recent study detected four *A. baumannii* isolates from fresh fruits and vegetables (red apple, green grape, guava, and red radish) that were resistant to 16 antibiotics, including carbapenems, and were classified as extensively drug-resistant (XDR) [26].

The threat of MDR bacteria does not consist only of the difficulty of treating patients, but also of the risk of transfer of resistance genes to other bacteria [31]. The authors verified that the co-cultivation of *Acinetobacter baylyi* and *Escherichia coli* in lettuce enabled the transfer of resistance genes from *A. baylyi* to *E. coli*. These bacteria were then able to colonize the intestines of mice and promote the in vivo transfer of resistance genes to *Klebsiella pneumoniae*. Based on this, the results of our study are concerning, since the 15 isolates studied showed an MDR phenotype, increasing the risk of transferring resistance genes to other bacteria present in foods.

Bacterial growth in food production environments can be controlled using sanitizers. However, the tolerance of *Acinetobacter* spp. against these substances has been studied over the years, with greater emphasis on clinical isolates. Studies have shown that these bacteria of hospital origin generally tolerate different concentrations of NaClO and other sanitizers commonly used in hospital environments, such as antiseptic soaps [32-34]. However, few studies have reported the tolerance of *Acinetobacter* spp. isolates from food and food preparation surfaces to sanitizers.

In our study, the MIC and MBC values of NaClO against *Acinetobacter* spp. were between 312.5 and  $\geq 2,500$  ppm. Isolate F5R14/3 was the only isolate that presented a slightly lower MIC for NaClO, which is still above the recommended concentration by the FDA (up to 200 ppm) [35] for use in equipment and food processing utensils and other food contact articles for human consumption.

Some studies have suggested that the presence of organic matter in food-processing environments can reduce the effectiveness of antimicrobial agents, resulting in pathogens being exposed to sublethal concentrations of these agents [21,36,37]. In our study,

all the isolates with high chlorine tolerance (F1R13/7, F3R13/1, F2R21, and F4R15/3) were recovered from ready-to-eat salads in which chlorine is often used to disinfect vegetables.

If isolates are exposed to subinhibitory levels of chlorine due to the presence of organic matter or its inadequate application, this may contribute to a greater tolerance developing in these isolates against the agent. This has been observed in isolates of *Salmonella enterica* serotypes Typhimurium and Heidelberg, which developed chlorine tolerance after repeated exposure to sublethal concentrations of this agent [38,39]. Furthermore, Gadea *et al.* reported that exposure of Gram-negative and Gram-positive strains isolated from food to biocides results in a transient decrease in susceptibility to antimicrobial agents, such as sanitizers and antibiotics [40,41].

PAA is commonly used as a substitute for chlorine-based compounds in disinfection processes and has been approved by the FDA for use in the food industry [35,42]. The risk of developing tolerance or resistance to this agent is considered to be very low because of the low specificity of PAA reactions. This agent works by denaturing proteins, breaking the permeability of the cell wall, and oxidizing sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites. It also induces the formation and accumulation of hydroxyl radicals in the bacterial cell, which are lethal [43,44].

Our results showed that in the case of PAA, the MIC and MBC values of the raw goat milk isolates were lower than those of isolates from salads, and were below the concentration recommended by the FDA (> 100 ppm and < 200 ppm). However, the MIC for eight (71.7%) of the 11 isolates from ready-to-eat salads was greater than or equal to 250 ppm.

QACs are disinfectants and antiseptics that are widely used in agricultural facilities, healthcare, food industry, and homes [45]. The QACs used in this work were composed of a mixture of didecyldimethylammonium chloride, alkyl dimethyl benzyl ammonium chloride (benzalkonium chloride), and biguanide. The isolates' tolerance to QAC/BG was the same for all isolates, with MIC and MBC values of 9.37/6.25 ppm (Table 2). Among the three sanitizers tested, QAC/BG had the lowest MIC and MCB values, suggesting that it was the most effective agent against the isolates used in this study. Additionally, according to the FDA [35], the maximum concentration of these agents on food-processing surfaces and utensils should not exceed 200 ppm.

Despite their effectiveness in this and other studies, Kampf [46] suggested that QACs, such as

benzalkonium chloride, are not the most suitable biocidal agent for disinfection procedures because of the strong adaptive response developed by some bacteria, causing cross-resistance to other sanitizers and antibiotics.

Understanding the effective concentration of a sanitizing agent can prevent the spread of bacteria through food and their permanence on surfaces through biofilms. Thus, outbreaks such as the one described in a hospital environment, where inadequate concentrations of NaClO (800 ppm) used for disinfection resulted in the spread of imipenem-resistant *A. baumannii* (IRAB) in the intensive care unit can be avoided. Only 5,000 ppm NaClO was able to control and eradicate the IRAB outbreak [33].

## Conclusions

Efficient disinfection procedures are key to the prevention and elimination of pathogens in agricultural facilities, health care, food industry, and homes. Our results demonstrated that even *Acinetobacter* spp. isolates from food can be tolerant to the recommended concentrations of NaClO and PAA, which are widely used in the food industry and homes. Fresh food farmers, distributors, and retailers must ensure that these foods meet food safety requirements to prevent the transmission of *Acinetobacter* spp. and classic food pathogens to consumers. Ababneh *et al.* [26] also point out that consumers must also do their part to protect themselves from this type of contamination by ensuring that their fresh food is washed and cooked properly before being consumed. As pointed out by Lee *et al.*, the correct application of tools such as the standard sanitation operating procedure and hazard analysis of critical control points is necessary [47]. These tools, together with the complete and periodic inspection of equipment and utensils, are fundamental to the prevention of biofilm formation in food processing environments.

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## References

1. Callejón RM, Rodríguez-Naranjo MI, Ubeda C, Hornedo-Ortega R, Garcia-Parrilla MC, Troncoso AM (2015) Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog Dis* 12: 32-38.

2. Castro-Ibáñez I, Gil MI, Allende A (2017) Ready-to-eat vegetables: current problems and potential solutions to reduce microbial risk in the production chain. *LWT-Food Sci Technol* 85: 284-292.
3. CDC - Centers for Disease Control and Prevention (2019) AR threats report in: antibiotic/antimicrobial resistance (AR/AMR). Available: <https://www.cdc.gov/drugresistance/biggest-threats.html>. Accessed: 10 July 2022.
4. Betchen M, Giovinco HM, Curry M, Luu J, Frainow H, Carabetta VJ, Nahra R (2022) Evaluating the effectiveness of hospital antiseptics on multidrug-resistant *Acinetobacter baumannii*: understanding the relationship between microbicide and antibiotic resistance. *Antibiotics* 11: 614.
5. Araújo BC, Moraes MS, Costa LEO, Nascimento JS (2015) Multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex isolated from infant milk formula and utensils in a nursery in Rio de Janeiro, Brazil. *J Dairy Sci* 98: 2303–2306.
6. Amorim AMB, Nascimento JS (2017) *Acinetobacter*: an underrated foodborne pathogen? *J Infect Dev Ctries* 11: 111-114. doi: 10.3855/jidc.8418.
7. Zekar FM, Granier SA, Marault M, Yaici L, Gassilloud B, Manceau C, Touati A, Millemann Y (2017) From farms to markets: Gram-negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of North Africa. *Front Microbiol* 8: 1569.
8. Carvalheira A, Silva J, Teixeira P (2017) Lettuce and fruits as a source of multidrug resistant *Acinetobacter* spp. *Food Microbiol* 64: 119–125.
9. Malta RCR, Ramos GLPA, Nascimento JS (2020) From food to hospital: we need to talk about *Acinetobacter* spp. *Germs* 10: 210-217.
10. Malta RCR, Cruz CHS, Santos JB, Ramos GLPA, Nascimento JS (2021) *Acinetobacter* in food: an overview. 1st edition. Paraná: Atena Editora. 66 p. [Book in Portuguese].
11. Butucel E, Balta I, Ahmadi M, Dumitrescu G, Morariu F, Pet I, Stef L, Corcionivoschi N (2022) Biocides as biomedicines against foodborne pathogenic bacteria. *Biomedicines* 10: 379.
12. Yang CH, Su PW, Moi SH, Chuang LY (2019) Biofilm formation in *Acinetobacter baumannii*: genotype-phenotype correlation. *Molecules* 24: 1849.
13. Kampf G (2016). Acquired resistance to chlorhexidine—is it time to establish an ‘antiseptic stewardship’ initiative? *J Hosp Infect* 94: 213-227.
14. Kampf G (2018) Sodium hypochlorite. In *Antiseptic Stewardship*. Springer, Cham. 61-210.
15. Liu WJ, Fu L, Huang M, Zhang JP, Wu Y, Zhou YS, Zeng J, Wang GX (2017) Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant *Acinetobacter baumannii*. *J Med Microbiol* 66: 13-17.
16. Fernández-Cuenca F, Tomás M, Caballero-Moyano FJ, Bou G, Martínez-Martínez L, Vila J, Pachón J, Cisneros JM, Rodríguez-Baño J, Pascual A (2015) Reduced susceptibility to biocides in *Acinetobacter baumannii*: association with resistance to antimicrobials, epidemiological behaviour, biological cost and effect on the expression of genes encoding porins and efflux pumps. *J Antimicrob Chemother* 70: 3222-3229.
17. Ramos GLPA, Nascimento JS (2019) Characterization of *Acinetobacter* spp. from raw goat Milk. *Ciênc Rural* 49: e20190404.
18. Beltrão JC (2019) Evaluation of the microbiological quality of ready-to-eat raw vegetable salads and identification of the antibiotic resistance profile of isolated enterobacteria. Masters dissertation. Science Program Applied to Health Products, Universidade Federal Fluminense, Brazil. [Dissertation in Portuguese].
19. Freeman DJ, Falkiner FR, Keane CT (1989) New method for detecting slime production by coagulase negative staphylococci. *J Clin Pathol* 42: 872-847.
20. Chromagar™ (2022) CHROMagar™ *Acinetobacter* for detection of *Acinetobacter* spp. and MDR *Acinetobacter*. Available: <https://www.chromagar.com/en/product/chromagar-acinetobacter/>. Accessed: 10 July 2022.
21. Obe T, Nannapaneni R, Schilling W, Zhang L, Kiess A (2021) Antimicrobial tolerance biofilm formation and molecular characterization of *Salmonella* isolates from poultry processing equipment. *J Appl Poultry Res* 30: 100195.
22. Haubert L, Maia DSV, Rauber-Würfel SDF, Vaniel C, Silva WP (2022) Virulence genes and sanitizers resistance in *Salmonella* isolates from eggs in southern Brazil. *J Food Sci Technol* 59: 1097–1103.
23. Eze EC, Chenia HY, El-Zowalaty ME (2018) *Acinetobacter baumannii* biofilms: effects of physicochemical factors virulence antibiotic resistance determinants gene regulation and future antimicrobial treatments. *Infect Drug Resist* 11: 2277–2299.
24. Gedefie A, Demsis W, Ashagrie M, Kassa Y, Tesfaye M, Tilahun M, Sahle Z (2021) *Acinetobacter baumannii* biofilm formation and its role in disease pathogenesis: a review. *Infect Drug Resist* 14: 3711- 3719.
25. Cherif-Antar A, Moussa-Boudjemâa B, Didouh N, Medjahdi K, Mayo B, Flórez AB (2016) Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant. *Dairy Sci Technol* 96: 27-38.
26. Ababneh Q, Al-Rousan E, Jaradat, Z (2022) Fresh produce as a potential vehicle for transmission of *Acinetobacter baumannii*. *Int J Food Contam* 9: 5.
27. Babapour E, Haddadi A, Mirnejad R, Angaji SA, Amirmozafari N (2016) Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. *Asian Pac J Trop Biomed* 6: 528–533.
28. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, Du X, Liu X, Qiu S, Song H (2016) relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Acinetobacter baumannii*. *Front Microbiol* 7: 483.
29. Bardbari AM, Arabestani MR, Karami M, Keramat F, Alikhani MY, Bagheri KP (2017) Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental *Acinetobacter baumannii* isolates. *Microb Pathog* 108: 122–128.
30. Carvalheira A, Casquete R, Silva J, Teixeira P (2017) Prevalence and antimicrobial susceptibility of *Acinetobacter* spp. isolated from meat. *Int J Food Microbiol* 243: 58-63.
31. Maeusli M, Lee B, Miller S, Reyna Z, Lu P, Yan J, Ulhaq A, Skandalis N, Spellberg B, Luna B (2020) Horizontal gene transfer of antibiotic resistance from *Acinetobacter baylyi* to *Escherichia coli* on lettuce and subsequent antibiotic resistance transmission to the gut microbiome. *mSphere* 5: e00329-20.
32. Campana R, Baffone W (2017). Assessment of antimicrobial activity in different sanitizer products commonly used in food processing environment and home setting. *EC Microbiol* 12: 260-268.

33. Liu WL, Liang HW, Lee MF, Lin HL, Lin YH, Chen CC, Chang PC, Lai CC, Chuang YC, Tang HJ (2014) The impact of inadequate terminal disinfection on an outbreak of imipenem-resistant *Acinetobacter baumannii* in an intensive care unit. *PLoS One* 9: e107975.
34. Malta RCR (2021) *Acinetobacter* spp. isolates from food: study of tolerance to sodium hypochlorite and creation of a digital book. Masters dissertation. Postgraduate Program in Food Science and Technology. Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rio de Janeiro, Brazil. [Dissertation in Portuguese].
35. Food and Drug Administration (FDA) (2019) Code of Federal Regulations. Title 21, v. 3, 21CFR178.1010, Part 178: Indirect food additives: adjuvants, production aids, and sanitizers. Subpart B: Substances utilized to control the growth of microorganisms. Sec. 178.1010, sanitizing solutions. Available: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fi=178.1010>. Accessed: 04 August 2022.
36. Araujo PA, Lemos M, Mergulhão F, Melo L, Simões M (2013) The influence of interfering substances on the antimicrobial activity of selected quaternary ammonium compounds. *Int J Food Sci* 2013: 237581.
37. Parra A, Toro M, Jacob R, Navarrete P, Troncoso M, Figueroa G, Reyes-Jara A (2018) Antimicrobial effect of copper surfaces on bacteria isolated from poultry meat. *Braz J Microbiol* 49: 113–118.
38. Obe T, Nannapaneni R, Sharma CS, Kiess A (2018) Homologous stress adaptation antibiotics resistance and biofilm forming ability of *Salmonella enterica* serovar Heidelberg ATCC8326 on different food-contact surfaces following exposure to sublethal chlorine concentrations. *Poult Sci* 97: 951-961.
39. Obe T, Nannapaneni R, Sharma CS, Kiess A (2018) Rugose morphotype of *Salmonella enterica* serovar Typhimurium ATCC 14028 exhibits chlorine resistance and strong biofilm forming ability. *Int J Poult Sci* 17: 295–305.
40. Gadea R, Fuentes MAF, Pulido RP, Gálvez A, Ortega E (2016) Adaptive tolerance to phenolic biocides in bacteria from organic foods: effects on antimicrobial susceptibility and tolerance to physical stresses. *Food Res Int* 85: 131–143.
41. Gadea R, Glibota N, Pulido RP, Gálvez A, Ortega E (2017) Adaptation to biocides cetrимide and chlorhexidine in bacteria from organic foods: association with tolerance to other antimicrobials and physical stresses. *J Agr Food Chem* 65: 1758-1770.
42. Pironti C, Dell'Annunziata F, Giugliano R, Folliero V, Galdiero M, Ricciardi M, Mott O, Proto A, Franci G (2021) Comparative analysis of peracetic acid (PAA) and permaleic acid (PMA) in disinfection processes. *Sci Total Environ* 797: 149206.
43. European Chemicals Agency (ECHA) (2015) Opinion on the application for approval of the active substance: peracetic acid. Product-type: 2. ECHA/BPC/068/2015. Available: <https://echa.europa.eu/documents/10162/e10165ca10148f10165-10168c10158-10164baf-10168ced-10162ece65470ffa>. Accessed: 12 July 2022.
44. Kampf G (2018) Peracetic acid. *In* Antiseptic stewardship. Springer Nature Switzerland, Gewerbestrasse, Cham, Switzerland. 63-98.
45. Vereshchagin A, Frolov N, Egorova K, Seitkalieva M, Ananikov V (2021) Quaternary Ammonium Compounds (QACs) and Ionic Liquids (ILs) as biocides: from simple antiseptics to tunable antimicrobials. *Int J Mol Sci* 22: 6793.
46. Kampf G (2018) Adaptive microbial response to low-level benzalkonium chloride exposure. *J Hosp Infect* 100: e1-e22.
47. Lee SHI, Portinari MRP, Corassin CH, Oliveira CAF (2022). Biofilms from pathogenic bacteria in food processing environments: formation and preventive disinfection procedures. *Food Sci Eng* 3: 12-19.

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