

Juliana Oliveira de Miranda,<sup>1</sup> Samily Aquino de Sá Oliveira,<sup>2</sup>  
 Sinara Laís Ramalho de Sales,<sup>3</sup> Danilo Sales Rosa,<sup>2</sup>  
 Jéssica Xavier Coelho,<sup>1</sup> Mateus Matiuzzi da Costa,<sup>4</sup>  
 Glayciane Costa Gois,<sup>4</sup> and Rafael Torres de Souza Rodrigues<sup>1\*</sup>

<sup>1</sup>Dept. of Veterinary Sciences in Semiárid, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, 56300-990, Brazil

<sup>2</sup>Dept. of Biosciences, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, 56304-205, Brazil

<sup>3</sup>Specialization Course in Hospital Practices in Dogs and Cats, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, 56300-000, Brazil

<sup>4</sup>Dept. of Animal Science, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, 56300-990, Brazil



## Prevalence, Biofilm Formation, and Antimicrobial Resistance of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* Isolates from Goat Meat Marketed in Petrolina, Brazil

### ABSTRACT

The aim of this study was to evaluate the prevalence and antibacterial resistance and biofilm formation by bacterial strains isolated from raw goat meat sold in street fairs (SF) and commercial establishments (CE) in Petrolina, Pernambuco, Brazil. SF samples had mesophilic aerobic bacteria counts of 3.71 to 7.57 log CFU/g, *S. aureus* counts of 1.78 to 5.38 log CFU/g, total coliform counts of  $2.3 \times 10^1$  to  $>1.1 \times 10^3$  most probable number (MPN)/g, thermotolerant coliform counts of  $<3.0$  to  $>1.1 \times 10^3$  MPN/g, and *Escherichia coli* counts of  $<3.0$  to  $>1.1 \times 10^3$  MPN/g. CE samples had mesophilic aerobic bacteria counts of 2.90 to 6.00 CFU/g, *S. aureus* counts of 2.00 to 4.49 log CFU/g, total coliform counts of  $2.3 \times 10^1$  to  $>1.1 \times 10^3$  MPN/g, thermotolerant coliform counts of  $3.0$  to  $>1.1 \times 10^3$  MPN/g, and *E. coli* counts of  $<3.0$  to  $>1.1 \times 10^3$  MPN/g. *Salmonella* was detected in 25% of SF and CE samples. All isolates of *S. aureus* and *Salmonella* and 95.6% of *E. coli* isolates were biofilm producers. Resistance to multiple drugs was found in

isolates of *Salmonella*, *E. coli*, and *S. aureus* from SF and CE samples. Goat meat marketed in Petrolina is heavily contaminated with pathogenic bacteria resistant to multiple drugs and capable of biofilm formation.

### INTRODUCTION

Petrolina is an inland city in the São Francisco River Valley (state of Pernambuco) in the Brazilian Semiárid Region and is currently known as an important economic hub in the region due to large-scale production of irrigated fruit (31). The municipality also has the second largest goat herd in Brazil, with 252,000 animals, and is adjacent to Casa Nova and Juazeiro (state of Bahia), which have the first (510,194 heads) and third (246,813 heads) largest herds in the country, respectively (21).

Petrolina is one of the main Brazilian hubs for the sale and consumption of goat meat, with an average consumption per capita (11.7 kg) higher than the national average (0.6 kg) (32). Petrolina also has the largest open-air food complex in Latin America, specialized in goat-based dishes typical in the region.

\*Author for correspondence: Phone: +55.87.2101.4861; Fax +55.87.2101.6830; Email: rafael.rodrigues@univasf.edu.br

The Bodódromo is currently one of the main tourist attractions in the municipality, receiving thousands of visitors every year (31).

However, despite the undeniable importance of goat meat for the municipality of Petrolina, no surveys have been conducted on the microbiological quality and risks related to the consumption of this product in the region. This monitoring is of paramount importance because meat is highly perishable (51) and is an extremely rich medium for the growth of harmful microorganisms (5), including pathogens that cause foodborne diseases.

In addition to the possible threat to public health due to the risk of foodborne diseases, meat can be a vehicle for antibiotic-resistant bacteria (8, 22, 33, 52, 53). These bacteria can be transferred to humans, causing clinical diseases with limited treatment options (47). The use of antibiotics in veterinary medicine has gained much attention in recent years because of the increasing amount of evidence linking the use of antimicrobials in animal production and selection for important multidrug-resistant pathogens (29). The main mechanism for accumulation of antimicrobial resistance genes is selection pressure exerted by the indiscriminate use of antimicrobials (2).

In addition to resistance to antimicrobials, another strategy that promotes bacterial survival is the ability to form biofilms (24). Biofilm formation is extremely important in the food industry because biofilms can compromise food safety and put consumer health at risk (3). Biofilms on equipment and contact surfaces provide a persistent source of contamination (34), which can contribute to the occurrence of foodborne outbreaks (46).

This study was conducted to determine the prevalence, antimicrobial resistance profiles, and biofilm formation capacity of pathogenic isolates of *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* obtained from goat meat samples sold in street fairs and commercial establishments in the municipality of Petrolina, Brazil.

## MATERIALS AND METHODS

### Sampling and microbiological analysis

Raw goat meat samples were collected from 40 points of sale in the municipality of Petrolina. The samples were collected from commercial establishments (butchers or markets) authorized by the municipal health authority and from street fair stalls, which did not have this authorization. Meat sold in commercial establishments was kept under refrigeration, whereas meat sold in street market stalls was stored and sold without refrigeration. The meat samples were removed from the breast cut because it has low commercial value and removal of a small sample did not affect its subsequent sale.

Goat meat samples were collected from 20 street market stalls, one sample per stall. The city of Petrolina hosted eight street fairs, and goat meat samples were collected from the four largest fairs, which were located in four neighborhoods in different parts of the city. The four fairs

were chosen because they had a large number of stalls selling meat and the neighborhoods in which they were located had enough commercial establishments to collect the same number of samples. Meat samples were collected from five stalls at each street fair. Because the street fair stalls were arranged side by side in a linear formation, the sampling plan was designed to cover the stalls located at the ends and center of the formation. Thus, samples were collected from points across the entire street market.

Goat meat samples also were collected from 20 commercial establishments, one sample per establishment. The 20 samples were collected from five establishments in each of the four neighborhoods in which the samples from street fairs were also collected. The five largest commercial establishments in each neighborhood were chosen, and they had similar structures and procedures for handling meat.

The research team identified itself to sellers at both commercial establishments and street fair stalls. All samples used in this study were purchased. However, some vendors at the fairs refused to sell the samples to us, probably due to their fear of the study results and because they were aware of the illegality of their situation. Regardless of the place of collection, all samples were excised from the meat cuts by the vendors.

The temperature and pH of the samples were measured immediately after collection at commercial establishments and street fair stalls. All samples were packaged in sterile polyethylene bags and transported in coolers with ice to the laboratory, which was ca. 10 to 15 km from the neighborhoods where the street fairs and commercial establishments were located. The samples were immediately analyzed upon arrival at the laboratory. Culture, isolation, and identification of microorganisms were conducted according to the methods of the American Public Health Association (7).

Twenty-five grams of each sample was added to 225 mL of 0.1% peptone water (Difco, BD, Sparks, MD), homogenized, then serially diluted up to a 10<sup>-3</sup> dilution. For determination of total and thermotolerant coliforms and *E. coli*, aliquots of each dilution were inoculated into tubes with lauryl sulfate tryptose broth (Kasvi, Roseto degli Abruzzi, Italy) and incubated at 37 ± 0.5°C for 24 ± 2 h. Each tube with growth and gas production was transferred to 2% brilliant green bile (GB; HiMedia, Mumbai, India) and *E. coli* broth (EC; HiMedia) tubes. The presence of coliforms was confirmed by observing growth with gas production in the GB and EC tubes after 24 h incubation at 37 ± 0.5 and 45.5 ± 0.2°C, respectively. The presence of *E. coli* was confirmed from the positive EC tubes. One loop of culture from each tube was streaked onto plates containing Levine eosin methylene blue agar (Kasvi, Laboratorios Conda S.A., Torrejon de Ardoz, Spain). After incubation at 37 ± 1°C for 24 ± 2 h, typical *E. coli* colonies were selected and inoculated onto standard plate count agar (PCA; GranuCult, Merck, Darmstadt, Germany) and incubated at

37 ± 1°C for 24 ± 2 h. Gram staining and biochemical tests were performed for confirmation (35).

Total aerobic mesophiles were counted using the standard surface plating method, in which the samples were inoculated onto PCA and incubated at 37 ± 1°C for 48 ± 2 h. Colony counts were made to determine the number of CFU per gram of sample.

For *S. aureus*, samples were inoculated onto Baird-Parker agar plates (Kasvi, Italy) enriched with egg yolk and tellurite and incubated at 37 ± 1°C for 45 to 48 h. Colonies typical of *S. aureus* were selected, transferred to brain heart infusion agar plates (Kasvi, Spain), and incubated at 37 ± 1°C for 18 to 24 h. Gram staining and biochemical tests were performed for confirmation (35).

For *Salmonella* detection, samples cultures were preenriched by adding 25 g of each sample to 225 mL of buffered peptone water (Difco, BD) and incubating at 37 ± 1°C for 18 ± 2 h. Selective enrichment was conducted in bottles of Muller-Kauffmann tetrathionate broth (HiMedia) and Rappaport-Vassiliadis soybean broth (Micromed, Brasilia, Brazil). After incubation, cultures were plated on xylose lysine agar (Kasvi, Spain) and bright green agar (Kasvi, Italy) and incubated at 37 ± 1°C for 24 ± 3 h. Colonies with characteristics typical of *Salmonella* were transferred to nutrient agar plates (Kasvi, Italy) and incubated at 37 ± 1°C for 24 ± 3 h. Gram staining and biochemical tests were performed for confirmation (35).

#### Antibiotic susceptibility test

Antimicrobial sensitivity tests were performed with the disk diffusion method according to the methods of the Clinical and Laboratory Standards Institute (11). Commercial antibiotic disks impregnated with clavulanic acid + amoxicillin (AMC; 30 µg), ampicillin (AMP; 10 µg), azithromycin (AZI; 15 µg), cephalexin (CFE; 30 µg), ceftriaxone (CRO; 30 µg), ciprofloxacin (CIP; 5 µg), chloramphenicol (CLO; 30 µg), gentamicin (GEN; 10 µg), imipenem (IPM; 10 µg), norfloxacin (NOR; 10 µg), oxacillin (OXA; 1 µg), tetracycline (TET; 30 µg) and vancomycin (VAN; 30 µg) were tested with all isolates of *Salmonella*, *E. coli*, and *S. aureus*. Erythromycin (15 µg) and penicillin G (PEN; 10 IU) were tested only with *S. aureus* isolates. Polymyxin B (POL; 300 IU) was tested only against *Salmonella* and *E. coli*.

The disk diffusion test was performed on Mueller-Hinton agar (Kasvi, Italy) on which a suspension of each isolate on a 0.5 McFarland optical density (OD) scale was spread with a swab. After absorption of the inoculum, the antibiotic disks were placed, and the plates were incubated at 37 ± 1°C for 24 ± 2 h. Based on the diameter of the halo formed around each disk, isolates were classified as sensitive, intermediate, or resistant (11).

#### Biofilm production

Isolates were inserted with a bacteriological loop into test tubes with 0.85% saline to reach ca. 1.5 × 10<sup>8</sup> CFU/mL (0.5 on the McFarland scale). Inoculum levels were then adjusted in 0.85% saline to 6 × 10<sup>6</sup> CFU/mL. A 96-well microplate was loaded with specific media for each bacterium: tryptone soybean broth (TSB; Kasvi, Italy) plus glucose (Isifar, Duque de Caxias, Brazil) for *S. aureus*, TSB for *E. coli*, and Luria-Bertani broth (Sigma-Aldrich, St. Louis, MO) for *Salmonella*. Each inoculum was added, and the plates were incubated at 37 ± 1°C for 24 ± 2 h. Plate wells were washed with distilled water, and primary aliphatic methanol was added for 20 min to fix the biofilm. After discarding this material, the microplate was dried at room temperature overnight and stained with 0.25% crystal violet for 5 min. After washing with distilled water to remove excess dye, the biofilm was resuspended in ethanol:acetone (80:20), and the OD was measured on an Easys ELISA plate reader at 620 nm (30, 44). Isolates were classified according to Stepanović et al. (43) as non-biofilm producers (OD ≤ negative control OD [ncOD]), weak biofilm producers (ncOD < OD ≤ [2 × ncOD]), moderate biofilm producers ([2 × ncOD] < OD ≤ [4 × ncOD]), or strong biofilm producers ([4 × ncOD] ≤ ncOD). Tests were performed in technical triplicate.

#### Data presentation

Aerobic mesophilic bacteria and *S. aureus* counts were log transformed. The most probable number (MPN) method was used for quantification of total and thermotolerant coliforms and *E. coli*. *Salmonella* data were recorded as present or absent. Antibiotic resistance and biofilm formation data were reported relative to the total number of isolates tested in each analysis.

## RESULTS AND DISCUSSION

#### Microbiological contamination of goat meat

The mesophilic aerobic bacteria count represents the total number of microorganisms that multiply in a food in the presence of oxygen (aerobic) and at moderate temperatures (mesophilic) and is often used as a quality indicator (16). Many countries have regulations requiring a mesophilic aerobic bacteria level of <10<sup>5</sup> to 10<sup>7</sup> CFU/g or cm<sup>2</sup> (23). European Commission Decision 2001/471/EC (15) established that average mesophilic aerobic bacteria counts of >5 log CFU/g are unacceptable in meat, whereas Brazilian legislation (9) established a maximum limit for mesophilic aerobes of 10<sup>6</sup> CFU/g (or 6 log CFU/g) in raw meats. None of the samples acquired from commercial establishments had counts above the maximum limit (6 log CFU/g) in Brazil (9) (Table 1). However, the counts in most of the analyzed samples were close to this limit. These findings are similar to those described by Kim et al. (23), who found that 94.7% of fresh beef samples sold in butcher shops in South Korea between 2010 and 2014 had mesophilic aerobic bacteria counts <6 log CFU/g.

**TABLE 1. Microbiological analysis of goat meat sold in street fairs and commercial establishments in the city of Petrolina, state of Pernambuco, Brazil**

Sample	Mesophilic aerobes (log <sub>10</sub> CFU/g)	<i>S. aureus</i> (log <sub>10</sub> CFU/g)	Total coliforms (MPN/g)	Thermotolerant coliforms (MPN/g)	<i>E. coli</i> (MPN/g)	<i>Salmonella</i> spp.
<b>Street fairs</b>						
1	6.51	2.56	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	<3.0	Absent
2	6.23	2.94	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	3.6	Absent
3	4.41	3.11	9.3 × 10 <sup>1</sup>	7.4	3.6	Absent
4	5.08	3.94	>1.1 × 10 <sup>3</sup>	4.6 × 10 <sup>2</sup>	<3.0	Absent
5	3.93	2.88	4.6 × 10 <sup>2</sup>	4.6 × 10 <sup>2</sup>	7.2	Absent
6	4.93	0	2.4 × 10 <sup>2</sup>	2.4 × 10 <sup>2</sup>	2.4 × 10 <sup>2</sup>	Absent
7	4.52	2.75	>1.1 × 10 <sup>3</sup>	2.3 × 10 <sup>1</sup>	2.3 × 10 <sup>1</sup>	Absent
8	4.88	0	1.1 × 10 <sup>3</sup>	<3.0	<3.0	<b>Present</b>
9	6.65	1.78	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	Absent
10	3.71	2.00	1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	Absent
11	7.57	0	>1.1 × 10 <sup>3</sup>	2.7 × 10 <sup>1</sup>	3.5 × 10 <sup>1</sup>	Absent
12	7.38	5.38	>1.1 × 10 <sup>3</sup>	1.5 × 10 <sup>2</sup>	3.5 × 10 <sup>1</sup>	Absent
13	7.34	0	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	1.5 × 10 <sup>1</sup>	<b>Present</b>
14	7.54	4.88	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	9.4	<b>Present</b>
15	5.83	3.53	>1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	9.3 × 10 <sup>1</sup>	Absent
16	5.30	0	2.3 × 10 <sup>1</sup>	<3.0	<3.0	<b>Present</b>
17	5.60	2.98	2.3 × 10 <sup>1</sup>	<3.0	<3.0	Absent
18	3.92	0	2.4 × 10 <sup>2</sup>	9.3 × 10 <sup>1</sup>	2.1 × 10 <sup>1</sup>	<b>Present</b>
19	5.56	2.78	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	Absent
20	4.11	0	4.6 × 10 <sup>2</sup>	4.6 × 10 <sup>2</sup>	4.6 × 10 <sup>2</sup>	Absent
<b>Commercial establishments</b>						
1	5.08	3.46	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	Absent
2	5.26	3.59	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	Absent
3	5.18	2.62	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	Absent
4	4.53	2.48	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	2.3 × 10 <sup>1</sup>	Absent
5	2.90	0	2.4 × 10 <sup>2</sup>	2.4 × 10 <sup>2</sup>	2.3 × 10 <sup>1</sup>	Absent
6	5.18	2.38	>1.1 × 10 <sup>3</sup>	3.0	<3.0	Absent
7	4.04	2.30	2.3 × 10 <sup>1</sup>	9.2	3.6	Absent
8	5.20	2.78	9.3 × 10 <sup>1</sup>	9.3 × 10 <sup>1</sup>	<3.0	<b>Present</b>
9	4.85	3.04	2.4 × 10 <sup>2</sup>	2.4 × 10 <sup>2</sup>	1.5 × 10 <sup>1</sup>	Absent
10	4.00	2.00	9.3 × 10 <sup>1</sup>	9.3 × 10 <sup>1</sup>	9.3 × 10 <sup>1</sup>	Absent
11	6.00	2.48	4.6 × 10 <sup>2</sup>	2.4 × 10 <sup>2</sup>	3.6	Absent
12	5.95	2.30	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	<3.0	Absent
13	4.53	2.72	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	1.1 × 10 <sup>3</sup>	<b>Present</b>
14	5.75	2.60	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	4.6 × 10 <sup>2</sup>	Absent
15	4.46	0	2.3 × 10 <sup>1</sup>	2.3 × 10 <sup>1</sup>	<3.0	Absent

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**TABLE 1. Microbiological analysis of goat meat sold in street fairs and commercial establishments in the city of Petrolina, state of Pernambuco, Brazil (cont.)**

Sample	Mesophilic aerobes (log <sub>10</sub> CFU/g)	<i>S. aureus</i> (log <sub>10</sub> CFU/g)	Total coliforms (MPN/g)	Thermotolerant coliforms (MPN/g)	<i>E. coli</i> (MPN/g)	<i>Salmonella</i> spp.
<b>Commercial establishments</b>						
16	5.32	0	9.3 × 10 <sup>1</sup>	9.3 × 10 <sup>1</sup>	4.3 × 10 <sup>1</sup>	Absent
17	5.97	4.49	>1.1 × 10 <sup>3</sup>	4.6 × 10 <sup>2</sup>	3.6 × 10 <sup>1</sup>	<b>Present</b>
18	5.72	0	1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	2.8 × 10 <sup>1</sup>	Absent
19	5.83	0	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	9.2	<b>Present</b>
20	5.51	4.30	>1.1 × 10 <sup>3</sup>	>1.1 × 10 <sup>3</sup>	7.2	<b>Present</b>

<sup>a</sup>MPN, most probable number. Results in bold are above the limit established by Brazilian legislation for raw goat meat (9) (6 log<sub>10</sub> CFU/g for mesophilic aerobes and absence of sample for *Salmonella* spp.).

In contrast, 35% of the samples from street fairs in the present study had mesophilic aerobic bacteria counts >6 log CFU/g, with values ranging from 6.23 to 7.57 log CFU/g (Table 1). However, average counts were 5.55 log CFU/g for street fair samples and 6.06 log CFU/g for commercial establishment samples, which were not significantly different (unpaired two-tailed Student's *t*-test; results not shown). The high mesophilic aerobic bacteria count in most of the goat meat samples evaluated in our study, including those obtained in commercial establishments, could be due to inadequate meat storage temperatures. Meat samples obtained from street fairs had internal temperatures of 23.5 to 29.1°C, whereas samples from commercial establishments had temperatures of 6.7 to 17.9°C. The high temperatures of meat sold at street fairs were expected because the meat was stored without refrigeration. However, the high temperatures of meat sold in commercial establishments were not expected because the meat was stored under refrigeration. According to Brazilian legislation, refrigerated fresh meat must be sold with a maximum internal temperature of 7°C (10). The findings of this study indicate that the public health authorities in Petrolina must intensify inspection in these commercial establishments to enforce the law.

Kumar et al. (25) evaluated samples of fresh beef obtained from slaughterhouses and local markets in Ethiopia and found levels higher than those in the present study for mesophilic aerobic bacteria; 75.9% of samples had >6 log CFU/g and were classified as poor quality. An even higher prevalence of poor quality meat was reported by Tafesse et al. (45), who found that 100% of goat samples sold on the street in Jijiga, Somalia had mesophilic aerobic bacteria counts >6 log CFU/g. Although the meat storage conditions were not described by Kumar et al. (25), in the

study conducted by Tafesse et al. (45) the meat samples were stored and sold at ambient temperature, similar to the conditions found at street fairs in Petrolina.

For *S. aureus*, 65 and 75% of the samples from street fairs and commercial establishments, respectively, were contaminated (Table 1). A quick *t* test of the *S. aureus* data also revealed no significant difference between samples from commercial establishments (average, 2.18 log CFU/g) and those from street fairs (average, 2.08 log CFU/g). A lower prevalence than that found in the present study was reported by Adesiji et al. (4) in goat meat samples collected from slaughterhouses and local markets in Osogbo, Nigeria (12%). This difference could be due to different slaughter conditions. According to Adesiji et al. (4), the goat meat samples were obtained from a government slaughterhouse with hygienic processing and a clean environment. However, the high level of bacterial contamination of the goat meat samples analyzed in our study could be due to the large number of clandestine slaughterhouses in Petrolina, which had only one authorized slaughterhouse that did not meet the regional demand for goat meat. Thus, most of the goat meat sold in Petrolina comes from clandestine slaughterhouses, which operate in inappropriate places and do not follow good manufacturing practices (19).

Only 5% of samples from street fairs had *S. aureus* counts >5 log CFU/g (Table 1). However, none of the samples collected from commercial establishments had *S. aureus* counts >5 log CFU/g. This result could be due to the ambient temperature storage of goat meat sold in street fairs. According to the *Compendium of Microbiological Criteria for Food* (16), staphylococcal enterotoxins are produced in food during the exponential growth phase of *S. aureus*, and disease-causing concentrations of these toxins are reached when this pathogen grows to 5.00 to

8.00 log CFU/g. Tafesse et al. (45) found that 100% of the meat samples sold at a street fair (where the meat was stored without refrigeration) had *S. aureus* counts >5.00 log CFU/g.

Even though the prevalence of *S. aureus* contamination was low, under favorable conditions, particularly storage at >6°C, the bacteria reproduce quickly and begin to synthesize enterotoxins (14). These favorable conditions were found in the present study; 100% of samples sold at street fairs and in commercial establishments were >6°C at the time of collection. Sharma and Chattopadhyay (40) evaluated sheep meat samples collected in Calcutta, India and described conditions similar to those in our study in which samples were sold at street fairs without an adequate cold storage system. These favorable conditions for the growth of *S. aureus* made the meat a possible vehicle for food poisoning due to the production of enterotoxins (14).

In the present study, total coliforms, thermotolerant coliforms, and *E. coli* were present in 100% of evaluated goat meat samples (Table 1), similar to the prevalence found by Sharma and Chattopadhyay (40), who isolated *E. coli* from 98% of sheep meat samples collected randomly in street fairs, and by Vieira et al. (49), who found that 100% of beef samples sold in supermarkets in Sinop, Mato Grosso, Brazil were contaminated with coliforms.

Twenty-five percent of goat meat samples from street fairs and 25% of samples from commercial establishments were of unacceptable quality (Table 1) because they had *E. coli* levels above those stipulated by Brazilian legislation, which established a maximum of 10<sup>2</sup> MPN/g in samples of raw meat (9). In addition to being an important pathogen, *E. coli* is the only bacterium in the coliform group whose primary habitat is the intestine of humans and warm-blooded animals and is found in feces of all healthy individuals (48). Thus, screening for this microorganism in food provides information on the hygienic conditions of the product, which may indicate poor handling practices and/or improper storage (18).

*Salmonella* was found in 25% of samples acquired from both street fairs and commercial establishments (Table 1). Because this pathogen can have serious health consequences, Brazilian legislation has established that *Salmonella* should be absent in all samples (9). Compared with the *Salmonella* prevalence found in the present study, Ahmad et al. (6) found a lower prevalence in sheep (10%) and goat (10%) meat samples sold in various areas of Lahore, Pakistan. Sharma and Chattopadhyay (40) reported a *Salmonella* prevalence of only 2% in sheep meat samples, and Adesiji et al. (4) did not detect *Salmonella* in any of the samples of goat meat and beef evaluated. However, Adesiji et al. (4) noted that the lack of detection of *Salmonella* in goat meat samples could be due to the lack of a preenrichment step and selective media to optimize

*Salmonella* isolation. Most of the goat meat sold in Petrolina comes from clandestine slaughterhouses without adequate hygiene conditions (19). According to Silva et al. (41), the presence of *Salmonella* in food may indicate inadequate hygienic conditions associated with obtaining, processing, and sale of meat.

Lack of basic infrastructure and poor sanitary conditions in the sales area, lack of potable water and clean equipment, and cross-contamination due to improper handling of meat can also contribute to the presence of high microbial levels in food (45). In the Petrolina street fairs, the marketing environment was substandard, with animals and garbage in the vicinity of the stalls and unhygienic sanitary habits of the handlers not in compliance with good handling practices (Fig. 1). These inadequate conditions and the lack of refrigeration for storage of the meat could contribute to the slightly higher contamination levels observed in meat sold at street fairs compared with that in commercial establishments. Although the sale of meat at street fairs is a cultural activity in Petrolina, municipal authorities should provide better working conditions for local sellers, such as refrigeration equipment and adequate places for handling meat. Sellers also must be trained in good handling practices.

The insignificant difference in levels of bacteria between goat meat samples collected from commercial establishments and those from street fairs is worrying. Many consumers prefer to buy meat from legal commercial establishments precisely because these consumers believe they are buying safer products. However, as found in this study, the meat sold in these establishments poses risks to human health similar to those of meat sold in street fairs probably because of the failure of commercial establishments to maintain good practices for food handling and hygiene. During sample collection at these establishments, we observed substandard practices, such as simultaneous handling of meat and money, use of inappropriate clothing for handling food, lack of regular cleaning of facilities and utensils used for handling meat, and inadequate temperature for storage and handling. Many commercial establishments sell meat obtained from clandestine slaughterhouses because the only legal slaughterhouse that was operating in Petrolina at the time of this study was not able to meet the local demand for goat meat (19). Thus, the municipal health authorities should intensify inspection actions in commercial establishments.

Although most consumers in Petrolina cook goat meat, the high level of contamination observed in raw meat in the present study is of concern, because undercooked meat can still harbor some pathogenic microorganisms. The utensils used to prepare the meat also may be used to prepare other foods that will be eaten without a heat treatment, increasing the risk of illness from cross-contamination.



Figure 1. Stalls for sale of goat meat in a street fair in the municipality of Petrolina, state of Pernambuco, Brazil.

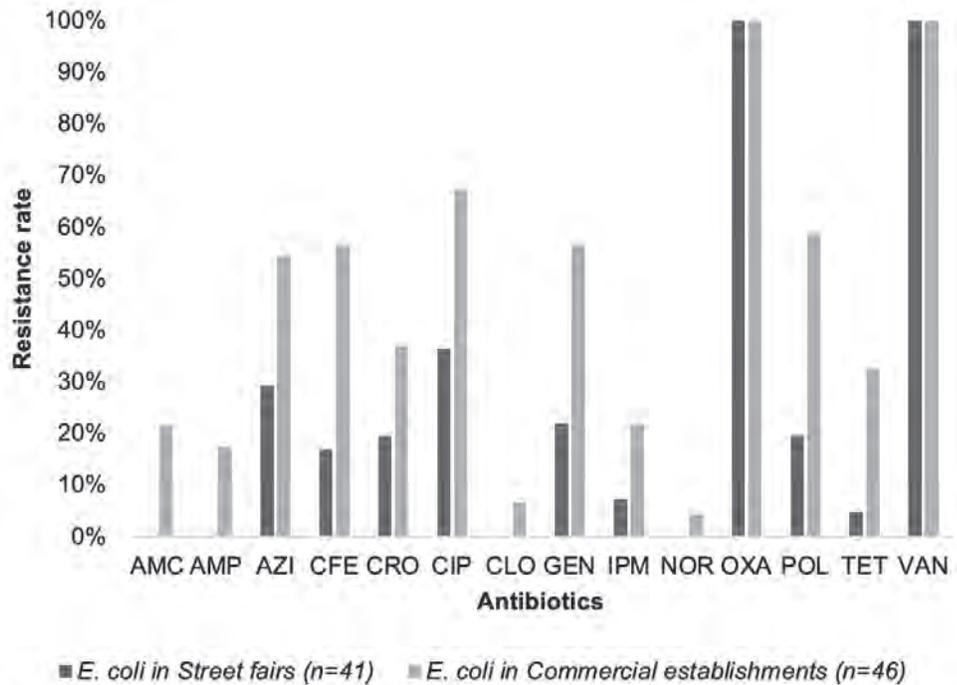


Figure 2. Antibiotic resistance in *Escherichia coli* isolates obtained from goat meat sold in street fairs and commercial establishments in Petrolina, Pernambuco, Brazil. AMC, clavulanic acid + amoxicillin (30 µg); AMP, ampicillin (10 µg); AZI, azithromycin (15 µg); CFE, cephalexin (30 µg); CRO, ceftriaxone (30 µg); CIP, ciprofloxacin (5 µg); CLO, chloramphenicol (30 µg); GEN, gentamicin (10 µg); IPM, imipenem (10 µg); NOR, norfloxacin (10 µg); OXA, oxacillin (1 µg); POL, polymyxin B (300 IU); TET, tetracycline (30 µg); VAN, vancomycin (30 µg).

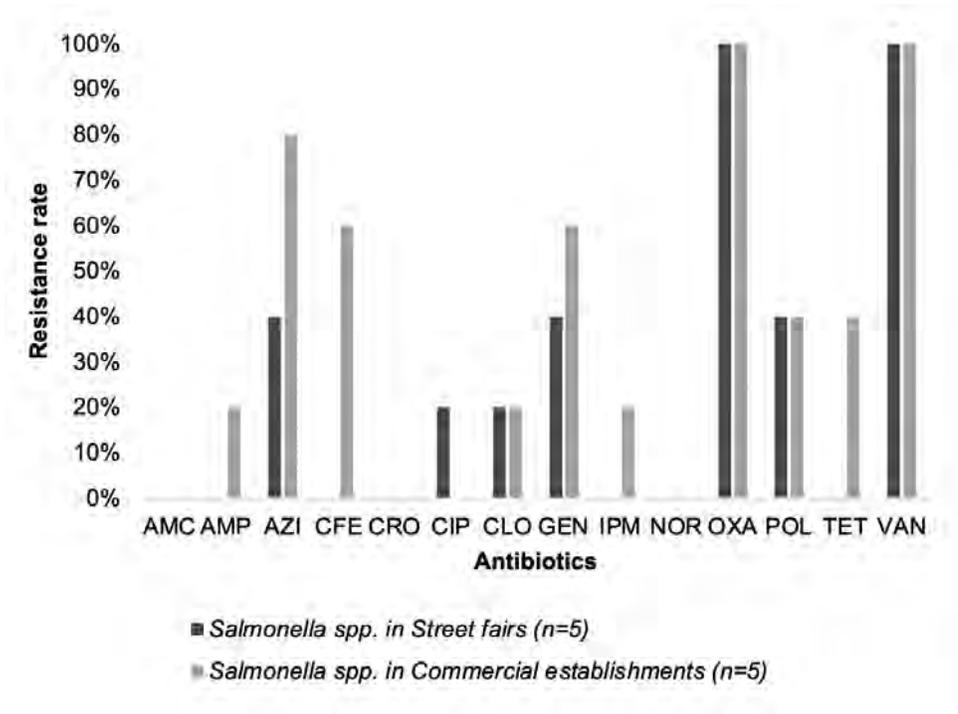


Figure 3. Antibiotic resistance in *Salmonella* isolates obtained from goat meat sold in street fairs and commercial establishments in Petrolina, Pernambuco, Brazil. AMC, clavulanic acid + amoxicillin (30 µg); AMP, ampicillin (10 µg); AZI, azithromycin (15 µg); CFE, cephalexin (30 µg); CRO, ceftriaxone (30 µg); CIP, ciprofloxacin (5 µg); CLO, chloramphenicol (30 µg); GEN, gentamicin (10 µg); IPM, imipenem (10 µg); NOR, norfloxacin (10 µg); OXA, oxacillin (1 µg); POL, polymyxin B (300 IU); TET, tetracycline (30 µg); VAN, vancomycin (30 µg).

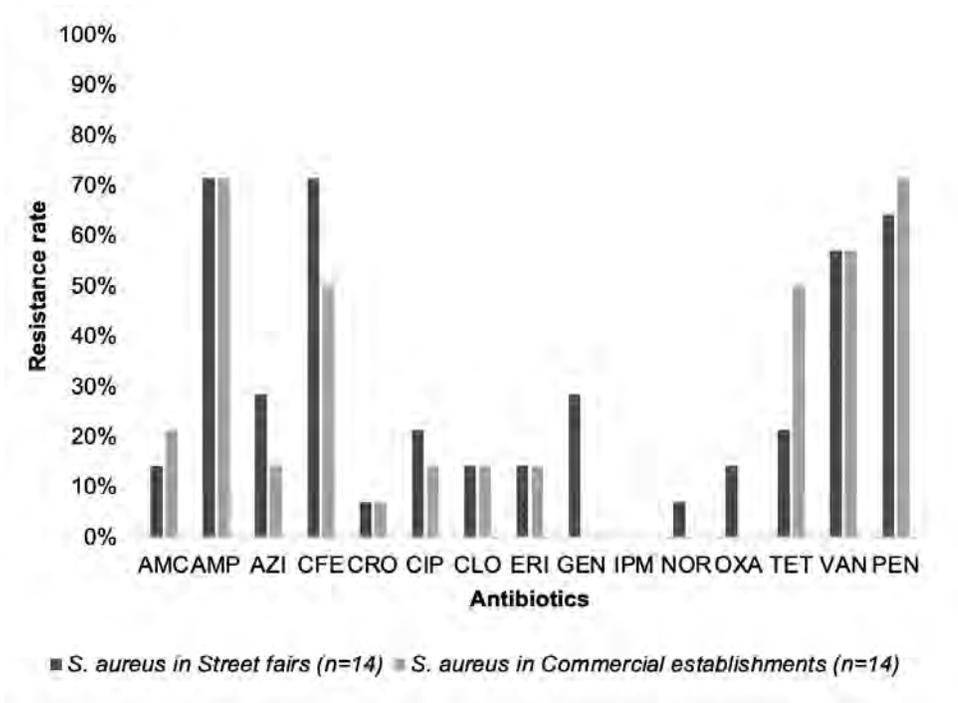


Figure 4. Antibiotic resistance in *Staphylococcus aureus* isolates obtained from goat meat sold in street fairs and commercial establishments in Petrolina, Pernambuco, Brazil. AMC, clavulanic acid + amoxicillin (30 µg); AMP, ampicillin (10 µg); AZI, azithromycin (15 µg); CFE, cephalexin (30 µg); CRO, ceftriaxone (30 µg); CIP, ciprofloxacin (5 µg); CLO, chloramphenicol (30 µg); ERI, erythromycin (15 µg); GEN, gentamicin (10 µg); IPM, imipenem (10 µg); NOR, norfloxacin (10 µg); OXA, oxacillin (1 µg); TET, tetracycline (30 µg); VAN, vancomycin (30 µg); PEN, penicillin G (10 IU).

## Antimicrobial resistance

A high prevalence of antimicrobial resistance was found in *E. coli* isolates (resistance to all antibiotics tested) (Fig. 2) and *Salmonella* (resistance to 10 of the 14 antibiotics tested) (Fig. 3) obtained from goat meat samples from commercial establishments and in *S. aureus* isolates obtained from samples acquired at street fairs (resistance to 14 of the 15 antibiotics tested) (Fig. 4).

High percentages of *E. coli* isolates were resistant to VAN (100%) and OXA (100%), followed by CIP (67.3%), POL (58.6%), CFE (56.5%), GEN (56.5%), and AZI (54.3%). No *E. coli* isolates were resistant to AMP (Fig. 2). A similar result was reported by Kaushik et al. (22), who evaluated chicken meat samples in India and verified resistance to VAN in 74.1% of *E. coli* isolates. In contrast, Parvin et al. (33) reported that only 7 and 8.1% of *E. coli* isolates from chicken meat in Bangladesh were resistant to POL and GEN, respectively, and 89.5% of the isolates were resistant to AMP. The differences could be related to the types of antibiotics most often used in these countries. In a previous study on commercial poultry farms in Bangladesh, the majority of the farms regularly used antibiotics without a prescription, and AMP was among the most commonly used antibiotics (20). To the best of our knowledge, no studies have been conducted to determine the most commonly used antibiotics on goat farms in Brazil.

High percentages of *Salmonella* isolates were resistant to VAN (100%) and OXA (100%), followed by AZI (80%), CFE (60%), GEN (60%), POL (40%), and TET (40%). No isolates were resistant to AMC, CRO, or NOR (Fig. 3). In contrast to the findings in the present study, Zhang et al. (53) found a low prevalence of POL-resistant *Salmonella* isolates in chicken (2.3%) and pork (1.6%) samples sold in retail markets in the People's Republic of China. These authors also verified a higher prevalence of resistance to TET (78.9%) and AMP (63.6%) in isolates obtained from pork samples and to TET (71.5%) and CLO (48.3%) in isolates obtained from chicken meat samples. Tetracyclines one of the most commonly used classes of antibiotics in Chinese animal production (12). Studies on the use of antibiotics in the Brazilian production of ruminants are scarce and do not precisely quantify those most used (36). Research carried out in Brazil has focused mainly on describing the antibiotic resistance of disease-causing bacteria in animals (17, 37).

Among *S. aureus* isolates, 71.4% were resistant to AMP, CFE, and PEN, 57.1% to VAN, 50% to TET, and 28.5% to AZI and GEN. No isolates were resistant to IPM (Fig. 4). A similar result was reported by Wu et al. (52), who found that 86 and 87% of *S. aureus* isolates from retail meat and meat products in China were resistant to PEN and AMP, respectively. A higher prevalence of resistance to GEN and TET was found in *S. aureus* isolates from samples of goat meat (50 and 66.6%, respectively), sheep

meat (55 and 77.7%, respectively), and beef (61.5 and 84.6%, respectively) marketed in retail centers in Iran (8). In a previous study carried out in Iran, tetracycline class antibiotics were the most commonly used antibiotics in local animal production (1).

For samples acquired at street fairs and commercial establishments, resistance to multiple drugs was found in *Salmonella* isolates (100 and 100%, respectively), *E. coli* isolates (63.4 and 91.3%, respectively), and *S. aureus* isolates (64.2 and 50%, respectively) (Fig. 5). Afolabi et al. (5), Zhang et al. (53), and Parvin et al. (33) also reported the presence of some pathogenic isolates resistant to multiple drugs in samples of pork, goat meat, and chicken meat, respectively.

The high prevalence of multidrug-resistant bacterial pathogens (isolates resistant to three or more antibiotic classes) found in this study can be explained by the indiscriminate and extensive use of antibiotics at subtherapeutic doses in animal production to prevent or control infections (28) and by plasmid-mediated resistance and mutational changes in resistance genes, which are crucial for the development of bacterial resistance (22).

Although supervision of the use of antibiotics in the beef, pork, and poultry industries has increased in some countries, the goat meat industry has not received similar attention, despite its growing market share (27). Current conditions present a serious threat to public health and reaffirm the role of meat as an important vehicle for antibiotic-resistant bacteria, which can be transferred to humans and cause clinical diseases with limited treatment options (47).

## Biofilm formation

A wide variety of foodborne pathogens are capable of attaching to and colonizing surfaces and forming biofilms (34). Bacterial biofilms in commercial establishments can be sources of pathogens, which can contribute to food contamination (50). In the present study, all strains of *S. aureus* and *Salmonella* and 95.6% of *E. coli* strains were biofilm producers (Fig. 6). Of these, 42.9% (6 of 14) and 57.2% (8 of 14) of *S. aureus* isolates from goat meat samples acquired from street fairs and commercial establishments, respectively, were strong biofilm producers. This information is important because the food processing environment provides ideal conditions for the development of biofilms, which compromise food safety and put consumer health at risk (3) because equipment and contact surfaces can provide a persistent source of contamination (34).

According to Zhao et al. (54), biofilm formation is mainly influenced by surface properties such as roughness and hydrophobic interactions. In general, biofilms can develop on any type of surface (38); however, the use of wooden boards for food handling purposes is not recommended (13). Sekoai et al. (39) stated that numerous pathogenic

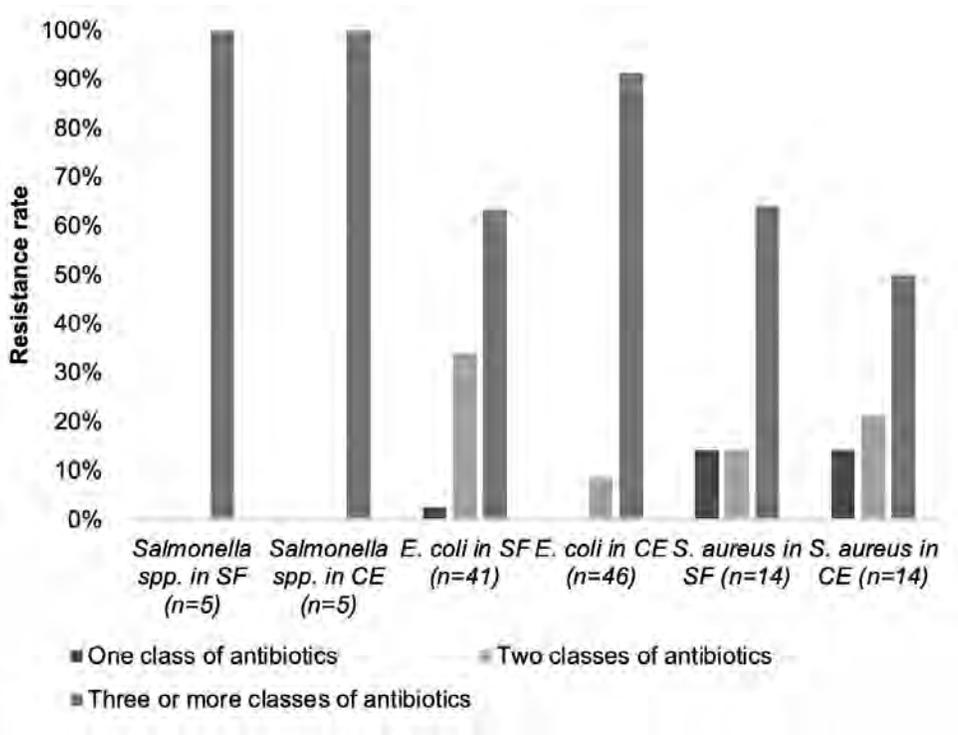


Figure 5. Resistance to drugs tested in isolates of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* obtained from meat sold in street fairs (SF) and commercial establishments (CE) in Petrolina, Pernambuco, Brazil.

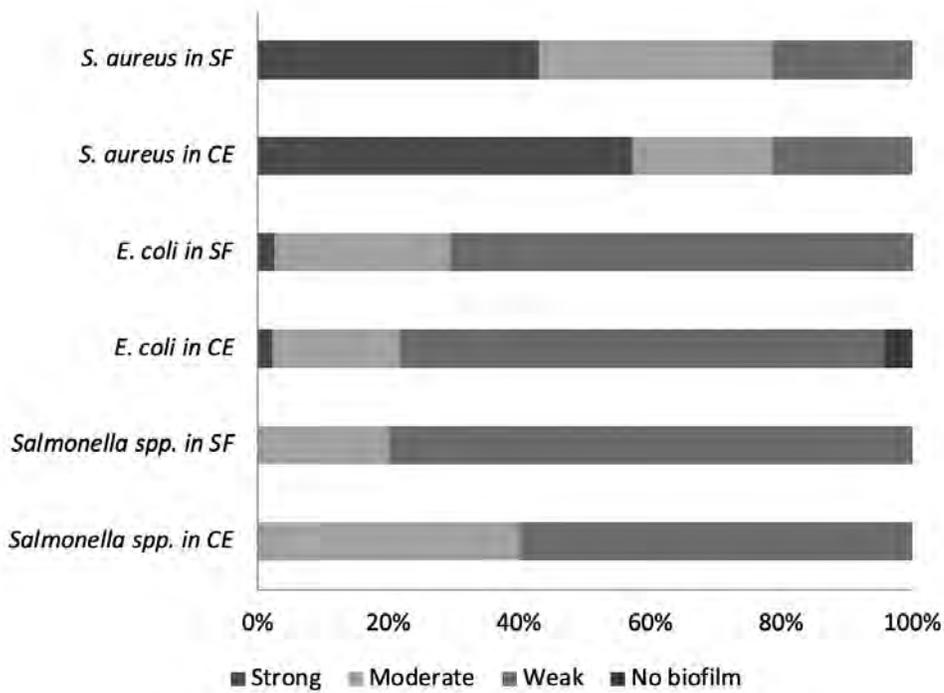


Figure 6. Biofilm production by isolates of *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* obtained from goat meat sold in street fairs (SF) and commercial establishments (CE) in Petrolina, Pernambuco, Brazil.

species have been isolated from wooden boards used in the processing of raw meat in Hong Kong markets probably due to the porosity and hydrophilicity of these surfaces and the availability of nutrients, which provide favorable conditions for the growth of biofilm-forming bacterial communities.

Although stainless steel surfaces are considered safer than wooden surfaces, Souza et al. (42) found that many bacterial strains can adhere to and form biofilms on stainless steel surfaces under different growing conditions. Kusumaningrum et al. (26) found that *Salmonella* Enteritidis, *S. aureus*, and *Campylobacter jejuni* remained viable on dry stainless steel surfaces for hours (*C. jejuni*) or days (*Salmonella* Enteritidis and *S. aureus*) after initial contamination and thus could be easily transferred from these surfaces to food. Although biofilms can form on both wooden and stainless steel surfaces, the latter is more recommended because it is easier to clean and sanitize.

Our results revealed that goat meat marketed in Petrolina, Pernambuco, Brazil, regardless of whether it is sold in street fairs or in commercial establishments, is heavily contaminated with bacterial pathogens of extreme public health importance. The antibiotic resistance profiles revealed a high prevalence of resistance to multiple drugs in the isolates from meat sold both in street fairs and in commercial establishments. The ability of almost all isolates to form biofilms indicates the importance of proper hygiene for food surfaces to prevent formation of biofilms, which can adversely affect the hygienic quality of food and the health of consumers.

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