

## Research Note

# Antibiotic Resistance and Phylogenetic Characterization of *Acinetobacter baumannii* Strains Isolated from Commercial Raw Meat in Switzerland

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## ABSTRACT

The spread of antibiotic-resistant bacteria through food has become a major public health concern because some important human pathogens may be transferred via the food chain. *Acinetobacter baumannii* is one of the most life-threatening gram-negative pathogens; multidrug-resistant (MDR) clones of *A. baumannii* are spreading worldwide, causing outbreaks in hospitals. However, the role of raw meat as a reservoir of *A. baumannii* remains unexplored. In this study, we describe for the first time the antibiotic susceptibility and fingerprint (repetitive extragenic palindromic PCR [rep-PCR] profile and sequence types [STs]) of *A. baumannii* strains found in raw meat retailed in Switzerland. Our results indicate that *A. baumannii* was present in 62 (25.0%) of 248 (CI 95%: 19.7 to 30.9%) meat samples analyzed between November 2012 and May 2013, with those derived from poultry being the most contaminated (48.0% [CI 95%: 37.8 to 58.3%]). Thirty-nine strains were further tested for antibiotic susceptibility and clonality. Strains were frequently not susceptible (intermediate and/or resistant) to third- and fourth-generation cephalosporins for human use (i.e., ceftriaxone [65%], cefotaxime [32%], ceftazidime [5%], and cefepime [2.5%]). Resistance to piperacillin-tazobactam, ciprofloxacin, colistin, and tetracycline was sporadically observed (2.5, 2.5, 5, and 5%, respectively), whereas resistance to carbapenems was not found. The strains were genetically very diverse from each other and belonged to 29 different STs, forming 12 singletons and 6 clonal complexes (CCs), of which 3 were new (CC277, CC360, and CC347). Rep-PCR analysis further distinguished some strains of the same ST. Moreover, some *A. baumannii* strains from meat belonged to the clonal complexes CC32 and CC79, similar to the MDR isolates responsible for human infections. In conclusion, our findings suggest that raw meat represents a reservoir of MDR *A. baumannii* and may serve as a vector for the spread of these pathogens into both community and hospital settings.

Food may represent a source of antibiotic-resistant bacteria, and the spread of such food contaminants is becoming a major public health concern (27, 28, 30). For instance, *Enterobacteriaceae* resistant to clinically important antibiotics (e.g., third-generation cephalosporins) are frequently found in retail poultry meat (14, 22, 24). These multidrug-resistant (MDR) organisms can be clonally related to isolates that cause diseases in the community or in hospitalized patients. Thus, it is speculated that the food chain might have an important role in the dissemination of difficult-to-treat clinically relevant pathogens (29).

*Acinetobacter baumannii* is responsible for hospital-acquired infections with a high attributable morbidity and mortality (23). In addition to broad intrinsic resistance, *A. baumannii* is capable of acquiring further mechanisms, making some *A. baumannii* strains virtually resistant to all major classes of antibiotics (11, 25, 31). MDR *A. baumannii*

isolates are commonly reported in the hospital environment (7, 32), and they often exhibit clonal relatedness (9, 35).

The reservoirs of *A. baumannii* are very diverse and include humans, animals, and the environment (1, 26). Due to the ability of this organism to spread and to adapt to the nosocomial environment, it is important to identify and monitor the possible sources and routes of transmission to clinics. Within this context, we note that *A. baumannii* has been isolated from food products, such as milk (18), meat, fish, and raw vegetables (2, 15, 19). Among these, vegetables have been suspected to introduce *A. baumannii* into the clinical environment (2). However, none of these investigations elucidated the link with the clinical context, and the *A. baumannii* strains were only tested for antibiotic resistance and not for their fingerprint (2, 15, 18, 19). Overall, data about the clonality of *A. baumannii* from food are lacking, which precludes any speculation about the eventual exchange of *A. baumannii* clones between food and clinical settings.

In this study, the presence of *A. baumannii* in meat was investigated during a campaign that examined raw meat retailed in Switzerland for the presence of third-generation

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TABLE 1. MICs of 21 antimicrobials for 39 of 62 *Acinetobacter baumannii* strains isolated from raw meat in Switzerland<sup>a</sup>

Antibiotic(s) <sup>b</sup>	Breakpoints (µg/ml) and interpretation criteria <sup>c</sup>		No. of strains for the corresponding MIC (µg/ml):													MIC <sub>50</sub> s and MIC <sub>90</sub> s and interpretation in percent				
	S	R	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC <sub>50</sub>	MIC <sub>90</sub>	S %	I %	R %
Piperacillin/Taz	≤16/4	≥128/4						22		9	6	1		1		2	16	95	2.5	2.5
Ceftriaxone	≤8	≥64								14	20	5				16	32	35	65	
Cefpodoxime	NA	NA							4	20	11	3		1		8	16			
Cefotaxime	≤8	≥64								5	22	11	1			8	16	68	32	
Cefotaxime/Cla	NA	NA	1				1	2	10	15	9	1				8	16			
Ceftazidime	≤8	≥32						4	19	14	1	1				4	8	95	2.5	2.5
Ceftazidime/Cla	NA	NA	2				1	1	10	10	12	3				4	8			
Cefepime	≤8	≥32				11			17	9	1	1				2	4	97.5	2.5	
Imipenem	≤4	≥16				39										0.25	0.25	100		
Meropenem	≤4	≥16				39										0.5	0.5	100		
Ciprofloxacin	≤1	≥4	2			8	19	7	1	1				1		0.5	0.5	95	2.5	2.5
Nalidixic acid	NA	NA							29	7	1			2		2	8			
Colistin	≤2	≥4					37			2						2	2	95		5
Gentamicin	≤4	≥16							39							0.5	1	100		
Kanamycin	NA	NA							38	1						4	4			
Streptomycin	NA	NA								1	4	14	8	10	1		16	64		
Chloramphenicol	NA	NA								1	1	2	8	27		128	128			
Florfenicol	NA	NA								1	1	2	15	20		128	128			
Tetracycline	≤4	≥16			4				21	10	2			2		2	4	90	5	5
Trimethoprim	NA	NA			1				1	2	11	16	5	3		16	32			
Sulfamethoxazole	NA	NA								29	7	1		2		8	16			

<sup>a</sup> The number of isolates that exhibited the corresponding MICs are reported. The concentration ranges tested for each antibiotic are those contained within the rectangles. The values above this range indicate MICs higher than the highest concentration in the range. The values below the lowest concentration tested indicate MICs smaller than or equal to the lowest concentration in the range. S, susceptible; R, resistant; I, intermediate; NA, not available.

<sup>b</sup> Taz, tazobactam (concentration, 4 µg/ml); Cla, clavulanic acid (4 µg/ml).

<sup>c</sup> Susceptibility was interpreted according to the Clinical Laboratory Standards Institute criteria (5).

cephalosporin-resistant *Escherichia coli* (29, 33). To our knowledge, we describe here for the first time the antibiotic susceptibility and fingerprint patterns of *A. baumannii* strains isolated from poultry, pork, veal, and beef.

**MATERIALS AND METHODS**

**Sampling, isolation, and cultivation of strains.** Meat (*n* = 248; i.e., veal, *n* = 50; beef, *n* = 50; pork, *n* = 50; chicken, *n* = 94; and turkey, *n* = 4) was purchased in Bern, Switzerland, at different intervals from 12 retail stores belonging to three convenience store groups (I, II, and III) between November 2012 and May 2013. All the chicken, beef, veal, and pork samples originated from Swiss meat production and were processed in seven geographically distant Swiss meat packing plants (MPPs), including two that processed chicken (MPPA and MPPB), four that processed beef (MPPC, MPPD, MPPE, and MPPF), three that processed pork (MPPC, MPPE, and MPPF), and three that processed veal (MPPC, MPPE, and MPPG). Turkey meat came from foreign production and was processed in one MPP located in Germany (MPPI) and in one (MPPK) located within the European Union (country not specified). The package stickers provided information about MPP and country of origin of the meat, but not about the farms.

Approximately 25 g of meat was homogenized in 25 ml of Luria-Bertani broth in a stomacher (Stomacher 400 Circulator, Seward, Norfolk, UK) for 1 min and was incubated overnight at 37°C with agitation. From this overnight culture, 10 µl was streaked onto selective Chrom ID ESBL agar (bioMérieux, Marcy-l'Étoile, France), which does not suppress growth of *A. baumannii* (20), and was incubated at 37°C. All white colonies (presumptive *A. baumannii*) were transferred onto tryptone soy agar plates containing 5% sheep blood (BD, Franklin Lakes, NJ) and were incubated overnight at 37°C. The colonies were identified using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (Microflex LT, Bruker Daltonics, Bremen, Germany) (16).

**Antimicrobial susceptibility tests.** The MICs of 21 antibiotics were determined for 39 isolates using ESBF1 and EUMVS2 microdilution plates (Trek Diagnostic Systems, East Grinstead, UK) following the Clinical and Laboratory Standards Institute guidelines and interpretation criteria (5, 6).

**Analysis of genetic diversity.** Relatedness between the *A. baumannii* strains was determined by multilocus sequence typing (MLST) (www.pasteur.fr/mslt) (10) and repetitive extragenic palindromic PCR (rep-PCR) (13). The rep-PCR band patterns

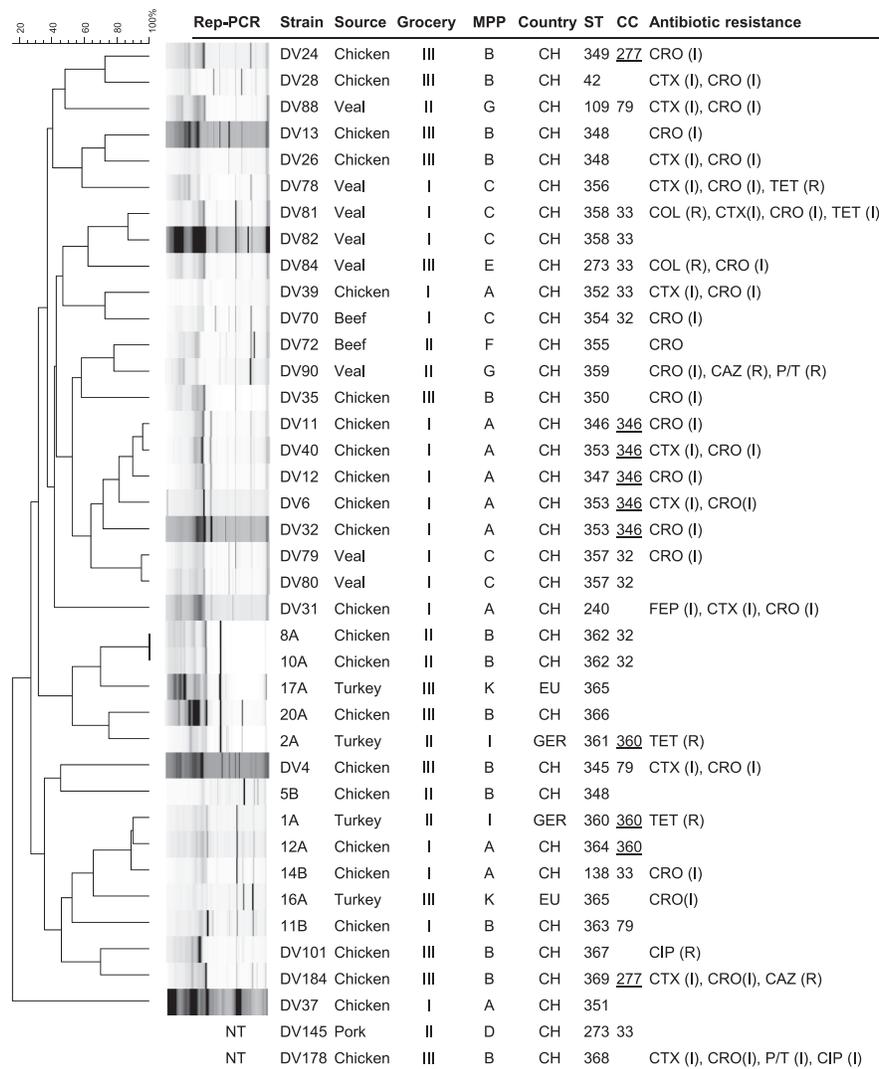


FIGURE 1. Relatedness of the *A. baumannii* strains isolated from retail raw meat in Switzerland, as established by repetitive extragenic palindromic PCR (rep-PCR). The tree was generated by unweighted pair group method with arithmetic mean using Bionumerics 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) with the following comparison settings: Dice, optimization 1.0%, position tolerance 1.0%. A similarity  $\geq 95\%$  was interpreted as clonality. Novel clonal complexes defined in this study are underlined. MPP, meat packing plant; Country, country of origin of the meat and MPP; CH, Switzerland; GER, Germany; EU, European Union (country not specified); ST, sequence type; CC, clonal complex; I, intermediate; R, resistant; CRO, ceftriaxone; CTX, cefotaxime; TET, tetracycline; COL, colistin; CAZ, ceftazidime; P/T, piperacillin-tazobactam; FEP, cefepime; CIP, ciprofloxacin; NT, not typeable by rep-PCR.

were interpreted according to the Dice coefficient of similarity and unweight pair group method with arithmetic mean, using Bionumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) (13). Isolates showing  $\geq 95\%$  genetic identity were defined as clonally related (9, 13). Cluster analysis of the obtained sequence types (STs) was performed with eBURSTv3 software (www.eburst.mlst.net/v3) (17), using the database of the Institute Pasteur MLST (www.pasteur.fr/mlst).

**Statistical analysis.** Prevalence was calculated using R version 3.0.1 (The R foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

## RESULTS

*A. baumannii* was found in 62 (25.0%) of 248 raw meat samples (CI 95%: 19.7 to 30.9%). Poultry meat was the most frequently contaminated, with 47 (48.0%) of the 98 analyzed samples being positive (CI 95%: 37.8 to 58.3%)

(43 [45.7%] of 94 chicken samples [CI 95%: 35.4 to 56.3%] and 4 of 4 turkey meat samples). Next most frequently contaminated were veal, with 9 (18%) of 50 samples positive (CI 95%: 8.6 to 31.4%); beef, with 3 (6%) of 50 positive (CI 95%: 1.3 to 16.5%); and pork, with 3 (6%) of 50 positive (CI 95%: 1.3 to 16.5%). Thirty-nine isolates (24 from chicken, 8 from veal, 4 from turkey, 2 from beef, and 1 from pork) were selected to perform phenotypic tests and fingerprint analyses.

Among these 39 isolates, resistance to piperacillin-tazobactam, ceftazidime, and ciprofloxacin was sporadic (2.5%), as it was for colistin and tetracycline (5%) (Table 1). Resistance to colistin and tetracycline was found in the isolates from veal, with one of the isolates exhibiting resistance to both (Fig. 1). Resistance to tetracycline was observed in two isolates from turkey meat, whereas resistance to ciprofloxacin and ceftazidime

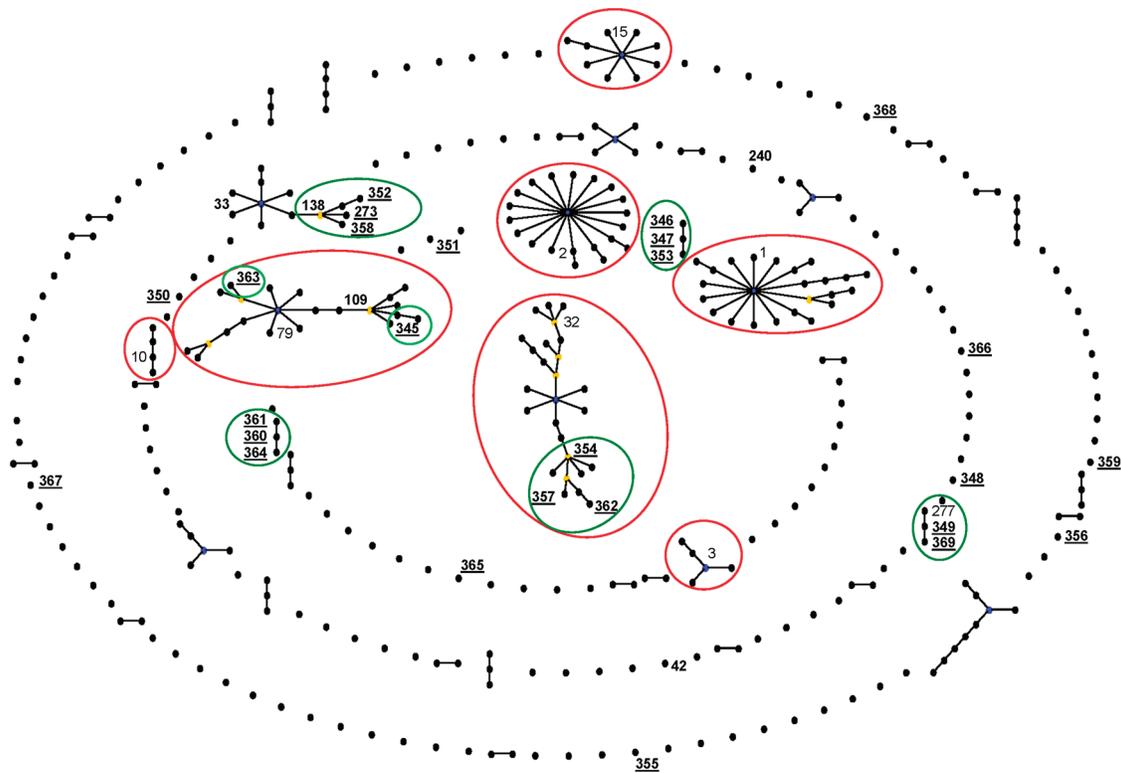


FIGURE 2. *eBURST* analysis of *A. baumannii* strains including those isolated from retailed raw meat in Switzerland and those available from the database of the Institute Pasteur MLST ([www.pasteur.fr/mlst](http://www.pasteur.fr/mlst); accessed 5 November 2013). Sequence types (STs) corresponding to the founder of a group are represented in blue and the founders of subgroups in yellow. The STs characterized in this study are reported in bold and underlined. Green circles highlight the STs found in this study and clustering together. Groups constituted by STs associated with multidrug-resistant strains are highlighted in red.

was observed only in the isolates from chicken meat. A large number of strains (25 and 12 strains, respectively) were not susceptible, with intermediate MICs to the cephalosporins ceftriaxone (MIC<sub>90</sub>, 32 µg/ml) and cefotaxime (MIC<sub>90</sub>, 16 µg/ml), and one strain was not susceptible to cefepime. We did not observe resistance to carbapenems and gentamicin.

Based on the rep-PCR analysis (Fig. 1), most of the *A. baumannii* strains were sporadic, and only three clusters of clonally related isolates were found. Two strains could not be typed by rep-PCR. The MLST analysis revealed a high diversity among the 39 strains, identifying 29 different STs, of which 24 were novel. Strains belonging to the same STs were further distinguished by different rep-PCR profiles, except for two pairs of strains (i.e., DV79 and DV80, both of ST357; 8A and 10A, both of ST362) that displayed two clonal rep-PCR profiles each (Fig. 1). These two pairs of strains were each isolated from the same lot of chicken and veal meat purchased on the same days and at the same stores, but from different packages. Overall, ST348 and ST353 were the most frequent clones (Fig. 1) and were only detected in chicken meat.

Among the 29 identified STs, 12 (i.e., 42, 240, 348, 350, 351, 355, 356, 359, 365, 366, 367, and 368) were singletons, and 17 were grouped into five clonal complexes (CCs) (Fig. 2). ST349 was a single-locus variant of the previously deposited ST277 and ST369, thus constituting a

novel CC: CC277. Similarly, ST360, ST361, and ST364 and ST346, ST347, and ST353 formed two novel clonal complexes, here named CC360 and CC346, respectively (Figs. 1 and 2). The remaining STs belonged to previously delineated CCs. In particular, ST109, which has previously been reported to be associated with clinical isolates, as well as ST345 and ST363, belonged to CC79. ST354, ST357, and ST362 belonged to CC32, which is constituted of STs reported from the human setting. Finally, ST138, ST273, ST352, and ST358 clustered together into CC33, also made up of STs linked to clinics (Fig. 2).

## DISCUSSION

In the present study, almost one quarter of the 248 Swiss meat samples examined were found to be contaminated with *A. baumannii*. In particular, poultry meat was the most frequently contaminated meat, representing the main food safety concern, especially when considering other recent reports regarding the high prevalence of cephalosporin-resistant *Enterobacteriaceae* in poultry (12, 22, 24, 28).

In contrast to *Enterobacteriaceae*, the *A. baumannii* isolates found in meat were generally susceptible to clinically relevant antibiotics. However, a few isolates displayed resistance to colistin, ciprofloxacin, tetracycline, ceftazidime, and piperacillin-tazobactam. This latter is still among the drugs of choice for the treatment of *A. baumannii* infections in humans in Switzerland. The introduction into

the clinics of resistant isolates via the food chain would jeopardize therapeutic options. Additionally, these sporadic resistance phenotypes may confer a selective advantage in the presence of antibiotics. For instance, isolates displaying resistance to colistin, tetracycline, and/or piperacillin-tazobactam were all from veal, and these classes of drugs are used to treat infections in calves (3).

For the first time, we determined the clonality of *A. baumannii* detected in meat by MLST. Part of the detected STs constituted three novel clonal complexes (i.e., CC277, CC360, and CC346), which were all poultry isolates, suggesting a possible new reservoir as well as the clonal diffusion of *A. baumannii* in the food chain setting. Rep-PCR typing allowed the further discrimination of isolates belonging to the same ST and identification of small clusters constituted by strains that were isolated from different meat samples of the same lot and MPP, suggesting a common source of contamination. The point at which (i.e., breeding, slaughter, or meat processing and packing) the spread of these clonally related strains occurred remains to be determined. For this aim, rep-PCR may represent a useful, simple tool to identify sources of contamination and track clones.

Another group of STs clustered into CC33, for which the founder ST33 was originally isolated from a human clinical setting (i.e., an infected wound) and did not present an MDR phenotype (10). One strain belonged to ST42, a ST that has also been linked to an isolate from a bloodstream infection of a horse in The Netherlands; this isolate was susceptible to antibiotics (10).

Interestingly, three STs (i.e., ST354, ST357, and ST362) were found to cluster into CC32. A recent report by Da Silva et al. (8) has noted that members of this CC have epidemic potential. Indeed, clones belonging to CC32 have been spreading by outbreaks, and some of these clones are resistant to carbapenems. In our study, we found that the *A. baumannii* isolates of ST357 and ST362 were single-locus variants of ST331 and ST336, clustering into CC32. These two latter STs have been reported from hospital settings in China and in different cities but have not been associated with particular MDR patterns (34). The global spread of clones of CC32 in different geographical regions and in different settings emphasizes the dissemination potential of these clones, and their presence in the food chain may also contribute to the spread of this clonal lineage. Considering the susceptibility of the strains associated with these STs, the success of this clone may not be related to antibiotic resistance (1, 26).

The remaining STs (i.e., ST109, ST345, and ST363) clustered into CC79, which mainly includes STs of MDR *A. baumannii* isolates that are spreading in Europe and Brazil (35). Of note, isolates of ST109 have been associated with the spread of OXA-23-like carbapenemase enzymes (21). This finding is of major importance, unveiling for the first time that an ST associated with nosocomial infections and resistance to clinically important antibiotics is also present in food. This suggests that susceptible strains may be able to adapt to the hospital environment and easily acquire antibiotic resistance traits. Clones belonging to CC1, CC2,

and CC3, which also include MDR isolates, have been detected worldwide (35). Our study highlights that other CCs may have the potential for global spread.

In conclusion, this study demonstrated that retail meat constitutes a reservoir for *A. baumannii*, including strains resistant to piperacillin-tazobactam, ceftazidime, ciprofloxacin, colistin, and/or tetracycline. The presence of *A. baumannii* in meat may represent an additional concern for public health because meat may serve as a vehicle for *A. baumannii*, which could spread to the community and clinics. In this latter setting, adaptation and the development of antibiotic resistance in new clones may represent an additional concern, as occurred with extended-spectrum  $\beta$ -lactamase- and carbapenemase-producing *Enterobacteriaceae* (4, 28).

A systematic clonal typing of *A. baumannii* strains resident in nonclinical settings may undoubtedly contribute to a better understanding of the population evolution of this important pathogen and may predict new possible scenarios for the clinical setting. Nevertheless, stricter hygiene and hazard analysis and critical control point principles should be implemented along the entire meat production chain up to the retail setting to identify sources of contamination and prevent potentially pathogenic bacteria from reaching consumers.

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