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Short communication

A reddish problem: Antibiotic-resistant *Serratia marcescens* in dairy food commercialized in Rio de Janeiro

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Abstract

Until a few decades ago, *Serratia marcescens* was considered a harmless microorganism. Although it is known that this bacteria has clinical importance and can cause infections and diarrhea in neonates and immunodeficient patients, its presence in food is often neglected, when compared to classic Gram-negative pathogens. In this study, we analyzed different samples of the most consumed dairy products in Rio de Janeiro, Brazil, and fifty eight isolates belonging to Enterobacteriaceae family were identified. Among them, twenty isolates presented a characteristic reddish color and its identification as *S. marcescens* was confirmed by an automated microbial identification system (VITEK 2). All isolates expressed resistance to at least one of the antibiotic tested and 11(55%) of them were multidrug resistant (MDR). Using a chromogenic media for culture-based screening, 4 (20%) isolates expressed the phenotype for reduced susceptibility to carbapenems and 10 (50%) for the production of extended spectrum β-lactamase. Biofilm formation was also investigated by Congo red agar method and seventeen isolates (85%) were shown to be producers. Since *S. marcescens* can also be transmitted by food ingestion, and as dairy products are commonly consumed, this works reports a possible risk of transmission of MDR isolates to consumers, specially, to those immunocompromised.

Introduction

The presence of *S. marcescens* and its red pigment prodigiosin in food has been cause for amazement and celebration when they were probably associated with a miracle in the thirteenth century (Bennett and Bentley, 2000).

However, nowadays, the presence of *S. marcescens* in a food should be considered a cause for concern because this bacterium, which has been once commonly regarded as harmless to the gastrointestinal tract, has been identified in recent studies as a potential pathogen, capable of causing effects of cytotoxicity, inflammation, and invasion, similar to those produced by classic enteric pathogens (Ochieng *et al.*, 2014).

S. marcescens is associated as a causative agent of infection outbreaks in neonatal ICUs and diarrhea in HIV-positive patients (Ivády *et al.*, 2014; Ochieng *et al.*, 2014) and these infections can be acquired through contaminated hospital equipment, contact of medical staff, or by the ingestion of contaminated food. Some studies have also reported the transmission of *S. marcescens* through foods like breast milk (Del Valle and Salinas, 2014) and parenteral nutrition (Gupta *et al.*, 2014).

We have recently found that *Serratia* spp. may be commonly present in commercialized dairy foods, together with other classical pathogens such as *Escherichia coli, Acinetobacter* sp., and *Salmonella enterica*, which were always the targets of the studies. In this work, our objective was to isolate *S. marcescens* found in dairy food purchased in Rio de Janeiro, and investigate the resistance to antibiotics and qualitative expression of biofilm formation.

Materials and Methods

Sample collection

Samples of ten often consumed convenience dairy products in the city of Rio de Janeiro (high moisture cheeses, pasteurized milk and fermented milk drink) were obtained from different commercial establishments, such as supermarkets, bakeries and dairies. Each sample was conditioned in coolers containing reusable ice packs, in the original packaging of each product and delivered to analysis at most within two hours after purchase. Samples

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were diluted into sterile peptone water 0.1% (wt/vol) (Himedia, India). After homogenization, serial dilutions were prepared in test tubes containing the same diluent. Aliquots were inoculated on plates containing selective media EMB agar (eosin methylene blue, Himedia, India) and MacConkey agar (Merck, Germany). The plates were incubated at 37°C for 18-24h. About 50% of the number of colonies obtained per plate were selected and individually transferred to plates containing Casoy agar (Himedia, India).

Bacterium identification and storage

Isolates presenting pinkish or reddish color on Casoy agar plates were selected and submitted to Gram stain and oxidase test. Gram and oxidase-negative isolates were identified by the automated identification system VITEK 2 Compact model (BioMerieux, France). For maintenance of the isolates, bacteria were spread on Casoy agar plates and incubated at 37°C for 18-24h. The growth of each isolate was harvested and transferred to cryotubes containing 1.5 ml of Casoy broth (wt/vol) (Merck, Germany) followed the addition of 40% glycerol (vol/vol) (Merck, Germany) mixed by inversion and stored at -20°C until use.

Antibiotic resistance profile

The determination of antimicrobial resistance profile of the isolates was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2015) for bacteria of the Enterobacteriaceae family. Seventeen antibiotics (Laborclin, Paraná), belonging to 11 classes, were used: amikacin (30 µg), amoxicillin-clavulanic acid (20/10 µg), ampicillin (10 µg), aztreonam (30 µg), cephalothin (30 µg); ceftazidime (30µg) cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), norfloxacin (10 µg), tetracycline (30 µg), tobramycin (10 µg) and trimethoprim (5 µg). *Escherichia coli* ATCC 29522 was used as control.

Analysis of reduced susceptibility to carbapenems and ESBL phenotypes

To evaluate the expression of *Klebsiella pneumoniae* carbapenemase (KPC) and production of extended spectrum β-lactamase (ESBL) phenotypes, chromogenic culture media Chromagar KPC™ and Chromagar ESBL™ were employed. For each isolate, a colony obtained after isolation in Casoy agar (Himedia, India) was streaked in each of chromogenic media. The plates were incubated at 37°C for 18-24h.

Qualitative biofilm production

S. marcescens isolates studied in this work were screened for a qualitative production of biofilm, using a simple and rapid method based on growth on Congo red agar plates as described by Freeman and coworkers (1989). *Salmonella enterica* ATCC14028 was used as positive control.

Results and Discussion

From a total of 58 isolates, twenty (35%) presented a pinkish to reddish color after incubation, suggestive of *Serratia marcescens*. These colorful isolates were transferred to new plates of Casoy agar and incubated at room temperature, protected from the light for 18h and then, an intense red colour was observed in all isolates. The 20 isolates were confirmed as *S. marcescens* by VITEK system.

All isolates expressed resistance to at least one antibiotic of the 17 tested (Table 1). The most frequently observed resistance was to cephalothin. Resistance to this drug was observed in 100% of the isolates. The second most commonly observed resistance was against ampicillin, which 85% of isolates were resistant to this agent. For amoxicillin, 45% of isolates were resistant. Resistance to aztreonam, cefotaxime, chloramphenicol, imipenem, meropenem, tetracycline and trimethoprim was not detected (Table 1).

Eleven (55%) *S. marcescens* isolates expressed multidrug resistance to the antibiotic tested, since they were resistant to one or more antibiotics of at least three different classes (Magiorakos *et al*., 2012). The isolated E8, for example, was resistant to six antibiotics divided into 5 different classes (Table 1).

To evaluate the expression of the phenotypes KPC and ESBL, chromogenic culture media Chromagar KPC™ and Chromagar ESBL™ are considered reliable and fast for confirming the expression of the resistance phenotypes, and facilitates the identification and screening of resistant strains (Hornsey *et al*., 2013).

Six isolates were KPC positive, even showing sensitivity to imipenem and carbapenem by antibiogram, and 18 isolates were ESBL positive. Although the detection of KPC and /or ESBL phenotypes has been evidenced in tests with the chromogenic media, only isolate E8 showed resistance to the 3rd generation cephalosporins (ceftazidime), directly relating this phenotype with the antibiotic tested. This suggests that the remaining isolates may be resistant to other antibiotic(s) from these classes, though not tested in this study.

According to Tekiner and Özpinar (2016), studies

Table 1. Characteristics of *Serratia marcescens* isolates studied in this work.

Source	Isolates	Resistance profile	KPC	ESBL	Biofilm production
Pasteurized milk	E4*	CEP/AMC/AMP	-	+	+
	E5*	CEP/AMC/AMP	-	-	+
	E6*	CEP/AMC/AMP/STR	+	+	+
	E7*	CEP/AMC/AMP/STR	-	+	+
	E8*	CEP/CAZ/AMC/AMP/AMI/TOB	+	+	+
	E9*	CEP/AMC/AMP/GEN	+	+	+
	E10	CEP/AMP	-	+	+
	E12	CEP/AMP	-	-	+
	E13	CEP/AMP	-	+	+
	E14*	CEP/AMP/TOB	-	+	+
	E15	CEP/AMP	-	+	+
	E16	CEP/AMP	-	+	+
	E17*	CEP/AMC/AMP/STR	-	+	+
	E19	CEP/AMP	+	+	+
	E20*	CEP/CIP/AMC/AMP	+	+	+
	L6*	CEP/AMC/AMP	+	+	+
Minas Frescal Cheese	Q1E2*	CEP/AMC/AMP	-	+	+
	R7	CEP	-	+	-
	R9	CEP/STR	-	+	-
	R19	CEP	-	+	-

Legend: AMP, ampicillin; AMC, amoxicillin-clavulanate; AMI, Amikacin; CAZ, ceftazidime; CEP, cephalothin; CIP, ciprofloxacin; GEN, Gentamicin; STR, streptomycin; TOB, tobramycin; ESBL, Extended-spectrum β-lactamase production; KPC, *Klebsiella pneumoniae* carbapenemase; *, MDR isolates; +, positive result; -, negative result.

involving MDR bacteria in foods are still very scarce and need to be further investigated. In dairy food, specifically, these MDR bacteria can originate from contaminated milk (bovine mastitis cases, for example), pasteurization problems or even from a post-pasteurization contamination.

Antibiotics are often used excessively and indiscriminately to treat bacterial infections, and prevent infections (Fleming *et al*., 2010; Murphy *et al*., 2016) and this routine may be causing great impact on the food industry (Rolain, 2013). The use of antibiotics for growth promotion in animals is still very debatable. Some lines of thought argue that the consumption of products derived from these animals do not pose risks to human health, but others question the possibility of strains with resistance genes are transmitted by food. These line also emphasize the need for more studies about the consequences of the abusive use of antibiotics in veterinary consumption to better assess their potential effects on consumers health (Van Boeckel *et al*., 2015).

The biofilm formation can contribute to this contamination by providing protection against sanitization procedures. It has been demonstrated that even in the presence of chemical compounds and high temperatures, bacteria within biofilms may persist for longer time in the dairy environment (Cherif-Antar *et al*., 2016).

Seventeen isolates (85%) produced biofilm, appearing as black colonies, while non-producing isolates have become depigmented or red. Coincidently, all the MDR isolates were able to produce biofilm by growth on the Congo red agar plates (Table 1). This method is based on increasing the production of exopolysaccharides and although there are other methods for biofilm detection, such as the tube grip and microtiter plate, both involving staining with crystal violet, several studies reported that Congo red is a rapid, less expensive, more sensitive and reproducible (Hedayati *et al*., 2014; Moraes *et al*., 2015).

Conclusion

It should be noted that there is an increasing number of immunocompromised consumers, as in cases of immune deficiency (HIV, cancer, tuberculosis, etc.) and immunosuppression, for example, transplant recipients undergoing chemotherapy and patients with autoimmune diseases (Uyttendaele *et al.*, 2016). These conditions further predispose affected individuals to infectious diseases. Thus, the contact of these vulnerable consumer groups with multidrug-resistant Enterobacteriaceae, such as those found in this study, is a worrying fact and should be studied more.

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References

Bennett, J. W. and Bentley R. 2000: Seeing red: The story of prodigiosin. Advances in Applied Microbiology 47: 1-32.

Cherif-Antar, A., Moussa–Boudjemâa, B., Didouh, N., Medjahdi, K., Mayo, B. and Flórez, A. B. 2016. Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant. Dairy Science and Technology 96: 27-38.

CLSI. 2015. Performance standards for antimicrobial susceptibility testing: Twenty-fifth informational supplement. Document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

Del Valle, C. A. and Salinas, E. T. 2014. Pink Breast Milk: Serratia marcescens colonization. American Journal of Perinatology Reports 4(2): e101–e104.

Fleming, L. R., Bolzan, D. N. and Nascimento, J. S. 2010. Antimicrobial substances produced by coliform strains active against foodborne pathogens. Foodborne Pathogens and Disease 7(3): 243-247.

Freeman, D. J., Falkiner, F. R. and Keane, C. T. 1989. New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology 42: 872-874.

Gupta, N., Hocevar, S. N., Moulton-Meissner, H. A., Stevens, K. M., McIntyre, M. G., Jensen, B., Kuhar, D. T., Noble-Wang, J. A., Schnatz, R. G., Becker, S. C., Kastango, E. S., Shehab, N. and Kallen A. J. 2014. Outbreak of *Serratia marcescens* bloodstream infections in patients receiving parenteral nutrition prepared by a compounding pharmacy. Clinical Infectious Diseases 59(1): 1-8.

Hedayati, S., Eftekhar, F. and Hosseini, S. M. 2014. Biofilm formation by bacteria isolated from intravenous catheters. Journal of Medical Bacteriology 3(4): 26-31.

Hornsey, M., Phee, L., Woodford, N., Turton, J., Meunier, D., Thomas, C. and Wareham, D. W. 2013. Evaluation of three selective chromogenic media, CHROMagar ESBL, CHROMagar CTX-M and CHROMagar KPC, for the detection of *Klebsiella pneumoniae* producing OXA-48 carbapenemase. Journal of Clinical Pathology 66(4): 348-350.

Ivády, B., Szabó, D., Damjanova, I., Pataki, M., Szabó, M. and Kenesei, E. 2014. Recurrent outbreaks of *Serratia marcescens* among neonates and infants at a pediatric department: an outbreak analysis. Infection 42(5): 891-898.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E, Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. and Monnet, D. L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18(3): 268-281.

Moraes, M. S., Araújo, B. C., Costa, L. E. O. and Nascimento, J. S. 2015. Shigella in baby bottles of a Brazilian newborn nursery. Journal of Infection in Developing Countries 9(6): 679-681.

Murphy, C. P., Fajt, V. R., Scott, H. M., Foster, M. J., Wickwire, P. and Mcewen, S. A. 2016. Scoping review to identify potential non-antimicrobial interventions to mitigate antimicrobial resistance in commensal enteric bacteria in North American cattle production systems. Epidemiology and Infection 144(1): 1-18.

Ochieng, J. B., Boisen, N., Lindsay, B., Santiago, A., Ouma, C., Ombok, M., Fields, B., Stine, O. C. and Nataro, J. P. 2014. *Serratia marcescens* is injurious to intestinal epithelial cells. Gut Microbes 5(6): 729-736.

Rolain, J. M. 2013. Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Frontiers in Microbiology 4(173): 1-10.

Tekiner, I. H. and Özpinar, H. 2016. Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. Brazilian Journal of Microbiology 47(2): 444-451.

Uyttendaele, M., Franz, E. and Schlüter, O. 2016. Food Safety, a global challenge. International Journal of Environmental Research and Public Health 13(1): 67.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A. and Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences 112(18): 5649-5654.