One Health 17 (2023) 100591

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

# Clonal dissemination of highly virulent *Serratia marcescens* strains producing KPC-2 in food-producing animals

Tiago Barcelos Valiatti [a,*], Francisco Ozório Bessa-Neto [a,b], Fernanda Fernandes Santos [a], Ramon Giovanni Brandão Silva [a,b], Ruanita Veiga [a], Dandara Cassu-Corsi [a], Tuane Carolina Ferreira Moura [c], Amalia Raiana Fonseca Lobato [c], Antonio Carlos Campos Pignatari [a,d], Cintya Oliveira Souza [c], Danielle Murici Brasiliense [c], Rodrigo Cayô [a,b], Ana Cristina Gales [a,d,*], GUARANI Network

[a] *Universidade Federal de São Paulo (UNIFESP), Laboratório Alerta, Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina (EPM), São Paulo, SP, Brazil*
[b] *Universidade Federal de São Paulo (UNIFESP), Laboratório de Imunologia e Bacteriologia (LIB), Setor de Biologia Molecular, Microbiologia e Imunologia, Departamento de Ciências Biológicas (DCB), Instituto de Ciências Ambientais, Químicas e Farmacêuticas (ICAQF), Diadema, SP, Brazil*
[c] *Seção de Bacteriologia e Micologia, Instituto Evandro Chagas (IEC), Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde (SCTIE), Ministério da Saúde, Ananindeua, PA, Brazil*
[d] *Universidade Federal de São Paulo (UNIFESP), Laboratório Especial de Microbiologia Clínica (LEMC), Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina (EPM), São Paulo, SP, Brazil*

ARTICLE INFO

*Keywords:*
Antimicrobial resistance
Genomic surveillance
One health
Food-production-animal
Amazon region

ABSTRACT

*Serratia marcescens* is a Gram-negative bacterium presenting intrinsic resistance to polymyxins that has emerged as an important human pathogen. Although previous studies reported the occurrence of multidrug-resistance (MDR) *S. marcescens* isolates in the nosocomial settings, herein, we described isolates of this extensively drug-resistant (XDR) species recovered from stool samples of food-producing animals in the Brazilian Amazon region. Three carbapenem-resistant *S. marcescens* strains were recovered from stool samples of poultry and cattle. Genetic similarity analysis showed that these strains belonged to the same clone. Whole-genome sequencing of a representative strain (SMA412) revealed a resistome composed of genes encoding resistance to β-lactams [$bla_{KPC\text{-}2}$, $bla_{SRT\text{-}2}$], aminoglycosides [*aac(6′)-Ib3*, *aac(6′)-Ic*, *aph(3′)*-VIa], quinolones [*aac(6′)-Ib-cr*], sulfonamides [*sul2*], and tetracyclines [*tet(41)*]. In addition, the analysis of the virulome demonstrated the presence of important genes involved in the pathogenicity of this species (*lipBCD*, *pigP*, *flhC*, *flhD*, *phlA*, *shlA*, and *shlB*). Our data demonstrate that food-animal production can act as reservoirs for MDR and virulent strains of *S. marcescens*.

## 1. Introduction

*Serratia* spp. is a genus of Gram-negative bacteria (GNB) belonging to the order *Enterobacteriales*. Most of the 30 species classified under this genus have been described as environmental species, however, *Serratia marcescens* has been considered a human pathogen causing serious nosocomial infections [1], including sepsis and urinary tract infections [1–3]. In addition, this pathogen has been associated with a large number of hospital outbreaks. [3,4]. It seems that the success of *S. marcescens* as a nosocomial pathogen is related, in part, to its impressive adaptation features that favor its maintenance and dissemination in the hospital environment [5,6].

The European Center for Disease Prevention and Control (ECDC) reported *Serratia* spp. was the sixth most frequent bacterial pathogen causing nosocomial pneumonia, and the ninth pathogen isolated from the bloodstream and urinary tract infections, respectively [7]. In addition, according to the latest bulletin of the National Health Surveillance Agency (ANVISA), *Serratia* spp. ranked as the tenth most frequent pathogen (2.85%) causing catheter-associated bloodstream infections (CLABSI) among Brazilian adult intensive care units (ICU) in 2021, among which, 44.8% were resistant to carbapenems [8]. Such data is worrisome since carbapenems are considered the most effective

* Corresponding authors at: Laboratório ALERTA, Universidade Federal de São Paulo - UNIFESP, Rua Pedro de Toledo, 781, 6th floor, Vila Clementino, 04039-032 São Paulo, SP, Brazil.
*E-mail addresses:* tiago.valiatti@unifesp.br (T.B. Valiatti), ana.gales@unifesp.br (A.C. Gales).

https://doi.org/10.1016/j.onehlt.2023.100591
Received 22 December 2022; Received in revised form 17 June 2023; Accepted 20 June 2023
Available online 21 June 2023
2352-7714/© 2023 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

antimicrobials against 3rd and 4th generation cephalosporin-resistant GNB [9]. However, the global spread of carbapenem-resistant GNB pathogens has become one of the greatest public health concerns [10], and this resistance phenotype occurs mainly due to the production of carbapenemases, which are the most potent β-lactamases [11]. In addition, *S. marcescens* is its intrinsic resistance to several antimicrobials, including polymyxins. This fact is very worrisome because drastically reduces the therapeutic options available for the treatment of carbapenem-resistant *S. marcescens* infections. Currently, in Brazil, the resistance to carbapenems among *S. marcescens* clinical isolates has been mainly associated with the production of KPC-2 and, to a lesser extent, NDM-1 [3,12–14]. Interestingly, basically, all the published data regarding the resistance mechanisms found in *S. marcescens* strains are related to isolates recovered from nosocomial settings, highlighting a gap concerning the occurrence of this MDR pathogen in other ecological niches. Herein, we describe for the first time, the occurrence of *S. marcescens* strains carrying $bla_{KPC-2}$ and $bla_{SRT-2}$ in farm animals located in the Brazilian Amazon region.

## 2. Material and methods

### 2.1. Bacterial isolates

As part of a Brazilian surveillance study performed by the GUARANI network aiming to detect antimicrobial resistance mechanisms at the human-animal interface [15], three carbapenem-resistant *S. marcescens* strains (SMA371, SMA412, SMA433) were selected for this study. These isolates were recovered from stool samples of a bovine and a poultry from property 1, and of a poultry from property 2, both located in the municipality of Castanhal, Pará state, in the Brazilian Amazon region.

### 2.2. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of ampicillin/sulbactam, aztreonam, ceftriaxone, ceftazidime, cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, tigecycline, and minocycline (Sigma-Aldrich, St. Louis, USA) were determined by agar dilution method according to the European Antimicrobial Susceptibility Testing Committee (EUCAST) recommendations (www.eucast.org/). The susceptibility profile to ceftazidime/avibactam and sulfamethoxazole/trimethoprim were tested by disk diffusion, while the MICs for fosfomycin and moxifloxacin were determined by *E*-test® gradient strips. The results were interpreted following the Brazilian Committee on Antimicrobial Susceptibility (BrCAST/EUCAST) (http://brcast.org.br/), which is affiliated with the EUCAST. The *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control for the antimicrobial susceptibility tests.

### 2.3. Screening for genes encoding for carbapenemases and Extended-Spectrum β-Lactamases (ESβL)

The samples were screened by PCR for the presence of genes encoding for cephalosporinases of classes A and C ($bla_{TEM}$-like, $bla_{SHV}$-like, $bla_{CTX-M}$-like, $bla_{GES}$-like, $bla_{SRT}$-like) and for carbapenemases ($bla_{KPC}$-like, $bla_{NDM}$-like, $bla_{IMP}$-like, $bla_{VIM}$-like, $bla_{SPM}$-like, $bla_{GIM}$-like, $bla_{SIM}$-like, $bla_{OXA-48}$-like).

### 2.4. Bacterial typing

To determine the genetic similarity of *S. marcescens* strains, pulsed-field gel electrophoresis (PFGE) was performed using the restriction enzyme *Spe*I (New England Biolabs, Ipswich, UK). The electrophoresis was carried out using a CHEF-DR® II system (Bio-Rad Laboratories, USA). The PFGE band profiles were analyzed using the BioNumerics® version 5.0 software package (Applied Maths, Kortrijk, BE).

### 2.5. Galleria mellonella as a in vivo model for evaluation of S. marcescens virulence

The pathogenic potential of *S. marcescens* strains was evaluated using the in vivo infection model of *G. mellonella*, as previously described [16]. Briefly, larvae weighing between 0.25 and 0.35 g were infected with $10^5$ CFU of each *S. marcescens* strain, and the mortality rate was assessed for 96 h using three groups of *G. mellonella* containing five larvae each per strain. The *E. coli* MNEC RS218 strain associated with meningitis/sepsis was used as a positive control, and larvae inoculated with 0.85% saline solution were used to verify that *G. mellonella* would not be killed by physical trauma. Given the lack of a known highly virulent *S. marcescens* strain to be used in the virulence test, we included for comparison a *S. marcencens* strain (SMA133) recovered from bloodstream infection and carrying the virulence-encoding genes *lipB*, *pigP*, *flhD*, *phlA*, and *shlA*.

### 2.6. Whole genome sequencing

Total bacterial DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. The DNA libraries were prepared using the Nextera® XT kit (Illumina® Inc., San Diego, USA) and sequenced at MicrobesNG of the University of Birmingham (UK) on the Illumina® HiSeq™ 2500 System 2 × 250 bp paired-end mode platform. The data obtained from the readings were assembled using the SPAdes software version 3.9.1 [17] and the annotation was performed using Prokka version 1.12 [18]. The genome assembly metric was calculated using QUAST (http://quast.sourceforge.net/). All software was used with the default settings. The resistome of the isolates was determined by ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/) and plasmid replicons by PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/), both belonging to the Center for Genomic Epidemiology (CGE) platform (http://www.genomicepidemiology.org/). Virulence determinants were analyzed by the Pathosystems Resource Integration Center (PATRIC) platform using the PATRIC_VF database (https://www.patricbrc.org/). Additionally, the Basic Local Alignment Search Tool (BLAST) was used for the search of *lipBCD* (Extracellular secretion of lipase), *pigP* (positive regulator of prodigiosin and serratamolide production), *flhC* and *flhD* (flagellar production regulators), *phlA* (phospholipase A with hemolytic activity), *shlA* (pore-forming toxin with hemolytic activity), and *shlB* (activation and secretion of ShlA) genes, given their importance for *S. marcescens* virulence. The search for phages in the SMA412 genome was carried out using the PHASTER web server (https://phaster.ca/). Phylogenetic analysis was performed using the phylogenetic tree tool of PATRIC. For the construction of the phylogenetic tree, 48 genomes of *S. marcescens* were selected through the Similar Genome Finder service of PATRIC considering the following parameters: *P*-value threshold of 0.001 and distance of 0.01. The phylogenetic tree was built employing the Codon Tree method with 1,000 single-copy genes using the RAxML program, on the PATRIC platform. The final phylogenetic tree was generated with iTOL v.5.5. (https://itol.embl.de).

## 3. Results

High resistance rates for the 19 antimicrobials tested were observed for all three *S. marcescens* isolates. The minimal inhibitory concentrations (MICs) for the antimicrobials tested were as following as: ceftazidime (MICs, 32–64 μg/mL), ceftriaxone (MICs, 256 - > 256 μg/mL), cefepime (MICs, 256 - >256 μg/mL), ertapenem (MICs, 256 - >256 μg/mL), imipenem (MICs, 128–256 μg/mL), meropenem (MICs, 256 μg/mL), gentamycin (MICs, 64–128 μg/mL), amikacin (MICs, 64–128 μg/mL), tobramycin (MIC s,32–64 μg/mL), ciprofloxacin (MICs, >64 μg/mL), levofloxacin (MICs, 32–64 μg/mL), moxifloxacin (MICs, 2–4 μg/mL), minocycline (MICs,64–128 μg/mL), and fosfomycin (MICs, 128 μg/mL). These isolates were resistant to sulfamethoxazole/trimethoprim

(9–11 mm) but susceptible to ceftazidime/avibactam (19–21 mm; CAZ-AVI). In fact, CAZ-AVI was the only antimicrobial showing activity against the tested *S. marcescens* isolates, which were classified as XDR. The *Spe*I-PFGE analysis demonstrated that the three *S. marcescens* strains belonged to the same clonal group and based on this fact, a single isolate, SMA412, was selected for whole genome sequencing. Intriguingly, the properties from which the strains were isolated were geographically distant from one another and bore no interrelation, thereby bolstering the hypothesis that this specific clone is widely dispersed within the region under study.

The total size of the SMA412 genome was 5,484,808 bp distributed in 109 contigs with a G + C content of 59.12% and the largest contig being 780,505 bp. The $N_{50}$ and $N_{75}$ values were 392,502 bp and 196,864 bp, while the values for $L_{50}$ and $L_{75}$ were 5 and 10 contigs, respectively. In addition, a total of 82 tRNA genes, 14 rRNA genes, and 5459 coding sequences (CDS) were obtained. The search for antimicrobial resistance genes (ARGs) in the SMA411 genome revealed the presence of determinants that are responsible for conferring resistance to β-lactams [$bla_{KPC-2}$, $bla_{SRT-2}$], aminoglycosides [*aac(6′)-Ib3*, *aac(6′)-Ic*, *aph(3′)*-VIa], quinolones [*aac(6′)-Ib-cr*], sulfonamides [*sul2*], and tetracyclines [*tet(41)*]. In addition, three incompatibility groups (IncC, IncP6, and IncQ1) were detected. Interestingly, the $bla_{KPC-2}$ was located in the same contig in which the IncP6 replicon was found and inserted in a Tn*3*-like transposon composed by the genetic arrangement Δ*tnpA*-IS*Apu1*-*orf*-IS*Apu2*-Tn*3*-Δ*tnpA* + Tn*3*-*tnpR* + IS*Kpn27* + Δ$bla_{TEM-1}$ + $bla_{KPC-2}$ + IS*Kpn6*. The results revealed that this contig had 100% identity and 99% coverage with larger plasmids carried by *Enterobacter hormaechei* (accession number CP047966.1), *Klebsiella pneumoniae* (accession number MH909348.1), and *Aeromonas hydrophila* (accession number GI. CP028566.1) from China, *Aeromonas veronii* from Japan (accession number AP022283.1), *Citrobacter freundii* from Spain (accession number LT992437.1), and *Escherichia coli* from Vietnam (accession number GI. CP018968.1). The search for phage sequences in the SMA412 genome identified the presence of five phage regions, with sizes ranging from 12.3 Kb to 35.8 Kb (Table 1).

Phylogenetic analysis grouped the strain SMA412 in a cluster with other *S. marcescens* clinical strains recovered in a Brazilian tertiary hospital located in the city of São Paulo (Fig. 1). In general, all *S. marcescens* strains showed a similar β-lactam resistome background, with a predominance of $bla_{KPC-2}$, $bla_{OXA-1}$, and $bla_{SRT-2}$ genes (Fig. 1).

In addition, the key virulence genes *lipBCD*, *pigP*, *flhC*, *flhD*, *phlA*, *shlA*, and *shlB* were verified in the genome of SMA412, indicating a highly virulent genotype. We observed 100% mortality of the *G. mellonella* larvae after 12 h of *S. marcescens* infection. In contrast, the control strains, SMA133 and *E. coli* RS218 killed all *G. mellonella* larvae within 24 h of infection (Fig. 2).

## 4. Discussion

Given the increasing reports of ARGs in different environments, antimicrobial resistance surveillance studies focused on the human-animal-environment interface (One Health) have been encouraged, since the epidemiology of antimicrobial resistance is complex with the

**Table 1**
Phages found in the SMA412 genome.

| Region | Size (Kb) | Status | Most Common Phage/Accession Number | GC (%) |
|---|---|---|---|---|
| 1 | 35.8 | Intact | PHAGE_Klebsi_3LV2017_NC_047817 (28) | 56.74 |
| 2 | 34.9 | incomplete | PHAGE_Pseudo_B3_NC_006548(17) | 56.41 |
| 3 | 43.7 | Questionable | PHAGE_Burkho_BcepMu_NC_005882 (31) | 56.18 |
| 4 | 16.7 | Intact | PHAGE_Klebsi_phiKO2_NC_005857 (7) | 55.32 |
| 5 | 12.3 | Questionable | PHAGE_Salmon_SJ46_NC_031129(3) | 53.89 |

interconnection of all ecological niches [19,20]. Currently, although many studies monitor antimicrobial resistance in different environments, they generally are carried out targeting “indicators” species such as *E. coli* and *K. pneumoniae* [21–24]. However, less frequent Gram-negative species like *S. marcescens* may also colonize animals and play a role in the maintenance and spread of clinically significant ARGs. To the best of our knowledge, this is the first report of KPC-2-producing *S. marcescens* strains isolated from farm animals. Interestingly, the *S. marcescens* isolates were recovered from different food-producing animals and properties, suggesting possible clonal dissemination in such a geographic region.

In Brazil, previous reports investigating the occurrence of carbapenem-resistant *S. marcescens* isolates in intensive care units have already been conducted in distinct Brazilian geographic regions [25 – 30], and to a large extent, the main carbapenem resistance mechanism was the production of KPC-2. In contrast, a few studies have demonstrated the production of unusual carbapenemases by *S. marcescens* isolates in Brazil, such as SME-4 [31], GES-16 [28], and GES-5 [32].

In the present study, the $bla_{KPC-2}$ was associated with an IncP-6 plasmid, which is naturally found in *P. aeruginosa*. However, the presence of IncP-6 plasmid carrying $bla_{KPC-2}$ in different clinical and environmental GNB species has been increasingly observed, demonstrating the versatility of these plasmids [33]. Furthermore, the genetic context of $bla_{KPC-2}$ in *S. marcescens* strains is different from what is commonly described in the Brazilian territory, which is predominantly associated with the Tn*4401* and its variants.

The phylogenetic analysis revealed that the strain SMA412 showed a genomic kinship and resistome to β-lactams very similar to *S. marcescens* strains recovered from a tertiary hospital in the city of São Paulo (Southeast region), which is 2800 km from Castanhal city (North region), and where the *S. marcescens* strains were isolated, suggesting the wide spread of this clonal lineage throughout the Brazilian territory. In addition, we also observed the occurrence of a virulence arsenal that confers to bacterial cells the ability to colonize/infected and invade the host's immune system [34]. In some bacterial species, these mechanisms are widely known [35]; however, there are few studies dealing with their pathogenic mechanisms in *Serratia* spp. The study conducted by Kurz et al. [36] demonstrated that genes involved in lipopolysaccharide (LPS) biosynthesis, iron absorption, and hemolysin production are directly involved in the virulence of *S. marcescens*. Furthermore, this species also produces a diversity of enzymes (chitinase, lipase, chloroperoxidase, among others) that act as virulence factors [1,34,37]. Many of these enzymes are secreted into the extracellular medium by Type I Secretion System (SSTI), also called Lip, which is encoded by the operon *lipBCD* [38–40], which interestingly was present in the SMA412 genome. Additionally, the presence of the genes *pigP*, *flhC*, *flhD*, *phlA*, *shlA*, and *shlB* were also found in the SMA412 genome, suggesting a high pathogenic profile, since these genes are associated with important virulence mechanisms in *S. marcescens* clinical isolates. PigP works by regulating the production of prodigiosin that directly impacts swarming and hemolysis via serratamolide production [38,41], while FlhDC has been associated with flagellar biogenesis, biofilm production, and expression of virulence factors during swarming [42–45]. On the other hand, Hemolysin ShlA is responsible for the formation of pores in cells, contributing to cell invasion. In large quantities, ShlA can promote vacuolization of the cytoplasm leading to cell lysis [46,47]. PhlA has also been linked to hemolytic and cytotoxic activities [48], mainly due to the production of lysophospholipids that damage the cell membrane, leading to hemolysis and cell death [34]. We emphasize that our findings corroborate the results of Ferreira et al. [6], since 98.2% of *S. marcescens* clinical isolates carried the virulence-encoding genes mentioned above.

In view of the virulence encoding genes carried by our *S. marcescens* isolates, we decided to use the *G. mellonella* larvae infection model to understand the virulence of such isolates. All larvae infected by *S. marcescens* isolates died within 12 h of infection. Previous studies have

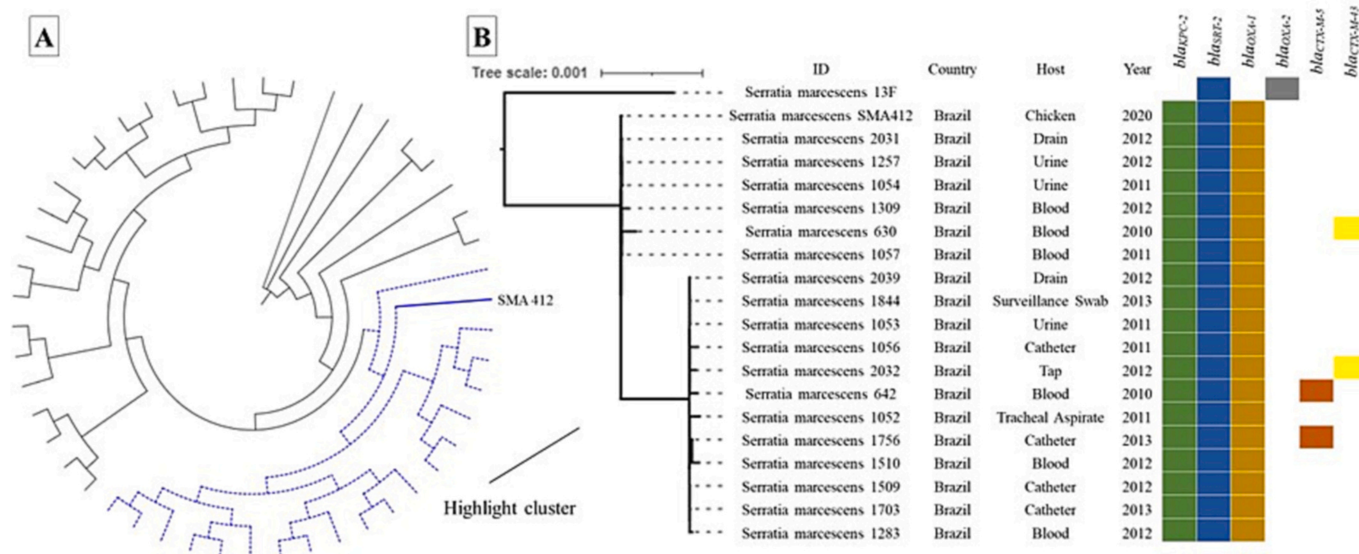


**Fig. 1.** Phylogenetic analysis between SMA412 strain and 48 public *S. marcescens* genomes using the codon tree method for 1000 unique protein copies in the maximum likelihood-based matrix (RAxML). The SMA412 strain was organized into the cluster highlighted in blue (A). The highlighted cluster includes a spatial representation of the tree, containing country, source, and year of isolation, as well as β-lactamase-encoding genes(B). The phylogenetic tree was generated with iTOL v.5.5 (https://itol.embl.de). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

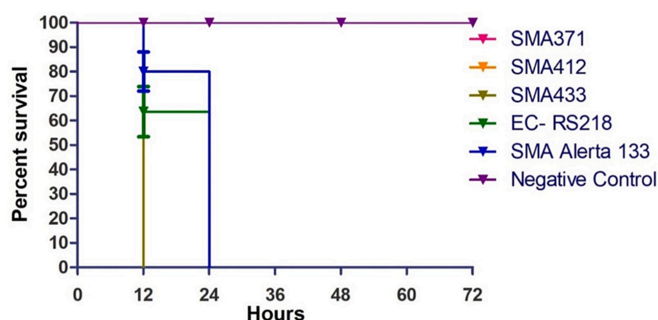


**Fig. 2.** Kaplan-Meier survival curves of *G. mellonella* infected with $10^5$ CFU/larvae of KPC-2-producing *S. marcescens* strains (SMA371, SMA412, and SMA433) comparing with virulent control strains (SMA133 and EC-RS218). Solution of 0.85% NaCl was used as a negative control.

also used this model to determine the pathogenicity of *S. marcescens*, but none of them observed 100% mortality. Gruber et al. [49] analyzed an NDM-1-producing *S. marcescens* strain and found 62% of mortality within 24 h of infection with an inoculum of $10^5$ CFU, while González et al. [50] reported mortality rates varying from 20% to 60% after 48 h of infection.

## 5. Conclusions

Our findings suggest that farm animals in the Brazilian Amazon region are functioning as reservoirs for virulent *S. marcescens* strains carrying a diversity of ARGs of clinical importance. Additionally, our phylogenetic analysis has indicated that this particular KPC-2-producing *S. marcescens* clone is closely related with other clinical isolates circulating at Brazilian nosocomial settings. Therefore, the data presented herein underscore the necessity to broaden the spectrum of GNB species considered in antimicrobial resistance surveillance studies within the One Health context performed in Brazil.

## Guarani network

Regional University of Blumenau (FURB), Blumenau - SC: Alessandro Conrado de Oliveira Silveira and Eleine Kuroki Anzai. Universidade Federal da Grande Dourados (UFGD), Laboratório de Pesquisa em Ciências da Saúde, Dourados - MS: Gleyce Hellen de Almeida Souza, Márcia Soares Mattos Vaz and Simone Simionatto. Postgraduate Program in Medical Microbiology, Group of Applied Medical Microbiology, Federal University of Ceará (UFC), Fortaleza - CE: Débora de Souza Collares Maia Castelo-Branco and Glaucia Morgana de Melo Guedes. Laboratory of Molecular Biology of Microorganisms, University São Francisco (USF), Bragança Paulista - SP: Lúcio Fábio Caldas Ferraz and Walter Aparecido Pimentel Monteiro. Universidade Federal de São Paulo (UNIFESP), Laboratório Especial de Microbiologia Clínica (LEMC), Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina (EPM), São Paulo - SP, Brazil: Carlos Roberto Veiga Kiffer. Bioinformatics Laboratory, National Laboratory of Scientific Computing (LNCC), Rio de Janeiro, Rio de Janeiro, Brazil: Fabíola Marques de Carvalho, Leandro Nascimento Lemos, Ana Tereza Ribeiro de Vasconcelos.

## Authors' contributions

ACG, RC, ACCP, TBV: Conceptualization. TBV, FFS, RGB, FOB-N, RV, DDC, COS, DMB, ARFL, TCFM: Formal analysis. TBV and FFS: Writing-original draft. TBV, FFS, RC: Writing - review & editing. ACG, ACCP, DMB, COS and RC: Supervision and final revision of the manuscript. All authors accepted the final form of the manuscript

## Ethical approval

Ethics approval for this study was obtained from the Research Ethics Committee (CEP) and the Committee on Ethics in the Use of Animals (CEUA) of the Universidade Federal de São Paulo (UNIFESP) (process numbers 3.116.383 and 2,607,170,119, respectively). This project was also registered by the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (process number AA1668A).

## Funding

This study was supported by the National Council for Science and

Technological Development (CNPq) and the Bill & Melinda Gates Foundation (process numbers 402659/2018-0, 443805/2018-0, and OPP1193112). Under the grant conditions of the Bill & Melinda Gates Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission. We are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants to TBV, RGBS, FFS (PNPD) and to the CNPq for providing grants to FOB-N, RFB-N., RV, and ACG (Process number: 312066/2019-8).

## Accession number

The whole-genome sequence was deposited in GenBank database under the accession number JAOWIM000000000 BioProject PRJNA888994.

## Declaration of Competing Interest

A.C.G. has recently received research funding and/or consultation fees from bioMérieux, Eurofarma, MSD, Pfizer, Roche, Sandoz, and United Medical. Other authors have nothing to declare. This study was not financially supported by any Diagnostic/Pharmaceutical company.

## Data availability

Genomic sequences are deposited at the NCBI

## References

[1] A. Iguchi, Y. Nagaya, E. Pradel, T. Ooka, Y. Ogura, K. Katsura, K. Kurokawa, K. Oshima, M. Hattori, J. Parkhill, M. Sebaihia, S.J. Coulthurst, N. Gotoh, N. R. Thompson, J.J. Ewbank, T. Hayashi, Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen, Genome Biol. Evol. 6 (8) (2014) 2096–2110, https://doi.org/10.1093/gbe/evu160.

[2] F. Grimont, P.A.D. Grimont, The genus *Serratia*, in: M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer, E. Stackebrandt (Eds.), The Prokaryotes, Springer, New York, 2006, pp. 219–244.

[3] D.M. Ghaith, M.M. Zafer, D.K. Ismail, M.H. Al-Agamy, M.F.F. Bohol, A. Al-Qahtani, M.N. Al-Ahdal, S.M. Elnagdy, I.Y. Mostafa, First reported nosocomial outbreak of *Serratia marcescens* harboring $bla_{IMP-4}$ and $bla_{VIM-2}$ in a neonatal intensive care unit in Cairo, Egypt, Infect. Drug Resist. 11 (2018) 2211–2217, https://doi.org/10.2147/IDR.S174869.

[4] M.L. Cristina, M. Sartini, A.M. Spagnolo, Serratia marcescens infections in neonatal intensive care units (NICUs), Int. J. Environ. Res. Public Health 16 (4) (2019) 610, https://doi.org/10.3390/ijerph16040610.

[5] P. Gastmeier, *Serratia marcescens*: an outbreak experience, Front. Microbiol. 5 (2014) 81, https://doi.org/10.3389/fmicb.2014.00081.

[6] R.L. Ferreira, G.S. Rezende, M. Damas, M. Oliveira-Silva, A. Pitondo-Silva, M. Brito, E. Leonardecz, F.R. Góes, E.B. Campanini, I. Malavazi, A.F. Cunha, M.C. S. Pranchevicius, Characterization of KPC-producing *Serratia marcescens* in an intensive care unit of a Brazilian tertiary hospital, Front. Microbiol. 11 (2020) 956, https://doi.org/10.3389/fmicb.2020.00956.

[7] European Centre for Disease Prevention and Control, Healthcare-Associated Infections Acquired in Intensive Care Units, Annual Epidemiological Report for 2017.https://www.ecdc.europa.eu/sites/default/files/documents/AER_for_2017-HAI.pdf, 2019 (Accessed 26 November 2022).

[8] The Brazilian Health Regulatory Agency - ANVISA, Boletim Segurança do Paciente e Qualidade em Serviços de Saúde n° 25: Avaliação dos Indicadores Nacionais de Infecções Relacionadas à Assistência à Saúde (IRAS) e Resistência Microbiana (RM), Ano 2021. Brasília, 2022, 2021. Available: https://app.powerbi.com/view?r=eyJrIjoiZDIwZjYyMzUtMmYxZS00MTRjLTk0NWMtZWE2ZDUzOGRjOTVjIiwidCI6ImI2N2FmMjNmLWMzZjMtNGQzNS04MGM3LWI3MDg1ZjVlZGQ4MSJ9. Accessed 30 Aug 2021.

[9] M. Bassetti, G. Poulakou, E. Ruppe, E. Bouza, S.J. Van Hal, A. Brink, Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach, Intensive Care Med. 43 (2017) 1464–1475, https://doi.org/10.1007/s00134-017-4878-x.

[10] P. Kannian, P. Mahanathi, V. Ashwini, M. Vaishnavi, C. Priya, Carbapenem-resistant gram negative bacilli are predominantly multidrug or Pan-drug resistant, Microb. Drug Resist. 27 (2021) 1057–1062, https://doi.org/10.1089/mdr.2020.0294.

[11] M.I. El-Gamal, I. Brahim, N. Hisham, R. Aladdin, H. Mohammed, A. Bahaaeldin, Recent updates of carbapenem antibiotics, Eur. J. Med. Chem. 131 (2017) 185–195, https://doi.org/10.1016/j.ejmech.2017.03.022.

[12] K.E. Silva, R. Cayô, C.G. Carvalhaes, F.P.C. Sacchi, F. Rodrigues-Costa, A.C.R. da Silva, J. Croda, A.C. Gales, S. Simionatto, Coproduction of KPC-2 and IMP-10 in carbapenem-resistant *Serratia marcescens* isolates from an outbreak in a Brazilian teaching hospital, J. Clin. Microbiol. 53 (7) (2015) 2324–2328, https://doi.org/10.1128/JCM.00727-15.

[13] M. Biagi, A. Shajee, A. Vialichka, M. Jurkovic, X. Tan, E. Wenzler, Activity of imipenem-relebactam and meropenem-vaborbactam against carbapenem-resistant, SME-producing *Serratia marcescens*, Antimicrob. Agents Chemother. 64 (4) (2020), https://doi.org/10.1128/AAC.02255-19 e02255–19.

[14] Á. Tóth, A. Makai, L. Jánvári, I. Damjanova, M. Gajdács, E. Urbán, Characterization of a rare $bla_{VIM-4}$ metallo-β-lactamase-producing *Serratia marcescens* clinical isolate in Hungary, Heliyon. 6 (6) (2020), e04231, https://doi.org/10.1016/j.heliyon.2020.e04231.

[15] F.M. Carvalho, T.B. Valiatti, F.F. Santos, A.C.O. Silveira, A.P.C. Guimarães, A. L. Gerber, et al., Exploring the bacteriome and resistome of humans and food-producing animals in Brazil, Microbiol Spectr. 10 (2022), https://doi.org/10.1128/spectrum.00565-22.

[16] A.C.M. Santos, R.M. Silva, T.B. Valiatti, F.F. Santos, J.F. Santos-Neto, R. Cayô, et al., Virulence potential of a multidrug-resistant *Escherichia coli* strain belonging to the emerging clonal group ST101-B1 isolated from bloodstream infection, Microorganisms 30 (2020), https://doi.org/10.3390/microorganisms8060827.

[17] A. Bankevich, S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V. M. Lesin, S.I. Nikolenko, S. Phan, A.D. Prjibelski, A.V. Pyshkin, A.V. Sirotkin, N. Vyahhi, G. Tesler, M.A. Alekseyev, P.A. Pevzner, SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (5) (2012) 455–477, https://doi.org/10.1089/cmb.2012.0021.

[18] T. Seemann, Prokka: rapid prokaryotic genome annotation, Bioinformatics. 30 (14) (2014) 2068–2069, https://doi.org/10.1093/bioinformatics/btu153.

[19] T.R. Walsh, A one-health approach to antimicrobial resistance, Nat. Microbiol. 3 (2018) 854–855, https://doi.org/10.1038/s41564-018-0208-5.

[20] S.A. McEwen, P.J. Collignon, Antimicrobial resistance: a one health perspective, Microbiol. Spectr. 6 (2) (2018), https://doi.org/10.1128/microbiolspec.ARBA-0009-2017.

[21] L. Salinas, F. Loayza, P. Cárdenas, C. Saraiva, T.J. Johnson, H. Amato, J.P. Graham, G. Trueba, Environmental spread of extended Spectrum Beta-lactamase (ESBL) producing *Escherichia coli* and ESBL genes among children and domestic animals in Ecuador, Environ. Health Perspect. 129 (2) (2021) 27007, https://doi.org/10.1289/EHP7729.

[22] J. Li, Z. Bi, S. Ma, B. Chen, C. Cai, J. He, S. Schwarz, C. Sun, Y. Zhou, J. Yin, A. Hulth, Y. Wang, Z. Shen, S. Wang, C. Wu, L.E. Nilsson, T.R. Walsh, S. Börjesson, J. Shen, Q. Sun, Y. Wang, Inter-host transmission of carbapenemase-producing *Escherichia coli* among humans and backyard animals, Environ. Health Perspect. 127 (10) (2019), 107009, https://doi.org/10.1289/EHP5251.

[23] T. Leangapichart, K. Lunha, J. Jiwakanon, S. Angkititrakul, J.D. Järhult, U. Magnusson, M. Sunde, Characterization of *Klebsiella pneumoniae* complex isolates from pigs and humans in farms in Thailand: population genomic structure, antibiotic resistance and virulence genes, J. Antimicrob. Chemother. 76 (8) (2021) 2012–2016, https://doi.org/10.1093/jac/dkab118.

[24] D.B. Nobrega, A.P. Calarga, L.C. Nascimento, C.G.C. Vasconcelos, E.M. de Lima, H. Langoni, M. Brocchi, Molecular characterization of antimicrobial resistance in *Klebsiella pneumoniae* isolated from Brazilian dairy herds, J. Dairy Sci. 104 (6) (2021) 7210–7224, https://doi.org/10.3168/jds.2020-19569.

[25] A.C.C. Guimarães, A.C.S. Almeida, A.G. Nicoletti, M.A. Vilela, A.C. Gales, M.M. C. Morais, Clonal spread of carbapenem-resistant *Serratia marcescens* isolates sharing an IncK plasmid containing $bla_{KPC-2}$, Int. J. Antimicrob. Agents 42 (4) (2013) 369–370, https://doi.org/10.1016/j.ijantimicag.2013.05.017.

[26] E. Margate, V. Magalhães, L.C. Fehlberg, A.C. Gales, A.C. Lopes, KPC-producing *Serratia marcescens* in a home-care patient from Recife, Brazil, Rev. Inst. Med. Trop. Sao Paulo 57 (4) (2015) 359–360, https://doi.org/10.1590/S0036-46652015000400016.

[27] A.P. Streling, P.P. Barbosa, M.F. Marcondes, A.G. Nicoletti, R.C. Picão, E.C. Pinto, E.A. Marques, V. Oliveira, A.C. Gales, Genetic and biochemical characterization of GES-16, a new GES-type β-lactamase with carbapenemase activity in *Serratia marcescens*, Diagn. Microbiol. Infect. Dis. 92 (2) (2018) 147–151, https://doi.org/10.1016/j.diagmicrobio.2018.05.003.

[28] V.B. Ribeiro, L.N. Andrade, A.R. Linhares, J. Barin, A.L.D.C. Darini, A.P. Zavascki, A.L. Barth, Molecular characterization of *Klebsiella pneumoniae* carbapenemase-producing isolates in southern Brazil, J. Med. Microbiol. 62 (11) (2013) 1721–1727, https://doi.org/10.1099/jmm.0.062141-0.

[29] F.F. Tuon, K. Cordova, T.M. Dario, L.S. Nunes, A.L. Barth, A.F. Martins, *Klebsiella pneumoniae* carbapenemase-producing *Serratia marcescens* outbreak in a university hospital, Am. J. Infect. Control 45 (6) (2017) 700–702, https://doi.org/10.1016/j.ajic.2017.03.002.

[30] R. Cayô, R.C. Leme, A.P. Streling, A.P. Matos, C.S. Nodari, J.R. Chaves, J.L. F. Brandão, M.F. Almeida, V. Carrareto, M.A.C. Pereira, J.P.A. Almeida, D. C. Ferreira, A.C. Gales, *Serratia marcescens* harboring SME-4 in Brazil: a silent threat, Diagn. Microbiol. Infect. Dis. 87 (4) (2017) 357–358, https://doi.org/10.1016/j.diagmicrobio.2017.01.008.

[31] C.S. Nodari, M. Siebert, U.D.S. Matte, A.L. Barth, Draft genome sequence of a GES-5-producing *Serratia marcescens* isolated in southern Brazil, Braz. J. Microbiol. 48 (2) (2017) 191–192, https://doi.org/10.1016/j.bjm.2016.08.002.

[32] B. Ghiglione, M.S. Haim, P. Penzotti, F. Brunetti, G.D.A. González, J. Di Conza, R. F. Espinoza, L. Nuñez, M.T.P. Razzolini, B. Fuga, F. Esposito, M.V. Horden, N. Lincopan, G. Gutkind, P. Power, M. Dropa, Characterization of emerging pathogens carrying $bla_{KPC-2}$ gene in IncP-6 plasmids isolated from urban sewage in Argentina, Front. Cell. Infect. Microbiol. 11 (2021), 722536, https://doi.org/10.3389/fcimb.2021.722536.

[33] C. Aggarwal, S. Paul, V. Tripathi, B. Paul, M.A. Khan, Characterization of putative virulence factors of *Serratia marcescens* strain SEN for pathogenesis in *Spodoptera*

*litura*, J. Invertebr. Pathol. 143 (2017) 115–123, https://doi.org/10.1016/j.jip.2016.12.004.

[34] L. Diacovich, J.P. Gorvel, Bacterial manipulation of innate immunity to promote infection, Nat. Rev. Microbiol. 8 (2) (2010) 117–128, https://doi.org/10.1038/nrmicro2295.

[35] Â. Kurz, S. Chauvet, C. Le, M. Aurouze, I. Vallet, G.P.F. Michael, M. Uh, J. Celli, A. Filloux, S.D. Bentzmann, I. Steinmetz, J.A. Hoffmann, B.B. Finlay, J.P. Gorvel, D. Ferrandon, J.J. Ewbank, Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by in vivo screening, EMBO J. 22 (2003) 1451–1460, https://doi.org/10.1093/emboj/cdg159.

[36] A. Hejazi, F.R. Falkiner, Serratia marcescens, J. Med. Microbiol. 46 (11) (1997) 903–912, https://doi.org/10.1099/00222615-46-11-903.

[37] R.M.Q. Shanks, N.A. Stella, K.M. Hunt, K.M. Brothers, L. Zhang, P.H. Thibodeau, Identification of *SlpB*, a cytotoxic protease from *Serratia marcescens*, Infect. Immun. 83 (2015) 2907–2916, https://doi.org/10.1128/IAI.03096-14.

[38] H. Akatsuka, E. Kawai, K. Omori, T. Shibatani, The three genes *lipB, lipC*, and *lipD* involved in the extracellular secretion of the *Serratia marcescens* lipase which lacks an N-terminal signal peptide, J. Bacteriol. 177 (22) (1995) 6381–6389, https://doi.org/10.1128/jb.177.22.6381-6389.1995.

[39] K. Omori, A. Idei, H. Akatsuka, *Serratia* ATP-binding cassette protein exporter, lip, recognizes a protein region upstream of the C terminus for specific secretion, J. Biol. Chem. 276 (2001) 27111–27119, https://doi.org/10.1074/jbc.M101410200.

[40] E.D.P.C. Fineran, H. Slater, L. Everson, K. Hughes, G.P. Salmond, Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia:* integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production, Mol. Microbiol. 56 (6) (2005) 1495–1517, https://doi.org/10.1111/j.1365-2958.2005.04660.x.

[41] M. Givskov, L. Eberl, G. Christiansen, M.J. Benedik, S. Molin, Induction of phospholipase- and flagellar synthesis in *Serratia liquefaciens* is controlled by expression of the flagellar master operon flhD, Mol. Microbiol. 15 (3) (1995) 445–454, https://doi.org/10.1111/j.1365-2958.1995.tb02258.x.

[42] G.M. Fraser, C. Hughes, Swarming motility, Curr. Opin. Microbiol. 2 (6) (1999) 630–635, https://doi.org/10.1016/s1369-5274(99)00033-8.

[43] G.S. Chilcott, K.T. Hughes, Coupling of flagellar gene expression to flagellar assembly in *salmonella enterica* serovar typhimurium and *Escherichia coli*, Microbiol. Mol. Biol. Rev. 64 (4) (2000) 694–708, https://doi.org/10.1128/mmbr.64.4.694-708.2000.

[44] C.S. Lin, J.T. Horng, C.H. Yang, Y.H. Tsai, L.H. Su, C.F. Wei, C.C. Chen, S.C. Hsieh, C.C. Lu, H.C. Lai, RssAB-FlhDC-ShlBA as a major pathogenesis pathway in *Serratia* marcescens, Infect. Immun. 78 (11) (2010) 4870–4881, https://doi.org/10.1128/IAI.00661-10.

[45] E. Schiebel, H. Schwarz, V. Braun, Subcellular location and unique secretion of the hemolysin of *Serratia marcescens*, J. Biol. Chem. 264 (27) (1989) 16311–16320.

[46] L.M. Petersen, L.S. Tisa, Friend or foe? A review of the mechanisms that drive *Serratia* towards diverse lifestyles, Can. J. Microbiol. 59 (2013) 627–640, https://doi.org/10.1139/cjm-2013-0343.

[47] K. Shimuta, M. Ohnishi, S. Iyoda, N. Gotoh, N. Koizumi, H. Watanabe, The hemolytic and cytolytic activities of Serratia marcescensphospholipase A (PhlA) depend on lysophospholipid production by PhlA, BMC Microbiol. 9 (2009) 261, https://doi.org/10.1186/1471-2180-9-261.

[48] T.M. Gruber, S. Göttig, L. Mark, S. Christ, V.A. Kempf, T.A. Wichelhaus, A. Hamprecht, Pathogenicity of pan-drug-resistant *Serratia marcescens* harbouring $bla_{\text{NDM-1}}$, J. Antimicrob. Chemother. 70 (4) (2015) 1026–1030, https://doi.org/10.1093/jac/dku482.

[49] G.M. González, A. Andrade, H. Villanueva-Lozano, C.L. Campos-Cortés, M. A. Becerril-García, A.M. Montoya, A.S. González, A. Bonifaz, R.F. Cendejas, L.E. L. Jácome, R.J.T. Rangel, Comparative analysis of virulence profiles of *Serratia marcescens* isolated from diverse clinical origins in Mexican patients, Surg. Infect. 21 (7) (2020) 608–612, https://doi.org/10.1089/sur.2020.029.