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Phenotypic and genotypic characterization of *Serratia marcescens* from a Neonatal Unit in Belém, Pará State, Brazil

Caracterização fenotípica e genotípica de Serratia marcescens provenientes de Unidade Neonatal de Referência em Belém, Pará, Brasil

La caracterización fenotípica y genotípica de *Serratia marcescens* proveniente de la Unidad de Neonatología de Referencia de Belém (Pará, Brasil)

Raimundo Gladson Corrêa Carvalho
Curso de Pós-Graduação em Análises Clínicas, Centro Universitário do Pará, Belém, Pará, Brasil
Laboratório de Patologia Clínica e Laboratorial Dr. Paulo Azevedo, Belém, Pará, Brasil

Irna Carla do Rosário Souza Carneiro
Departamento de Medicina, Universidade Federal do Pará, Belém, Pará, Brasil

Marcelo Sena Pinheiro
Curso de Pós-Graduação em Análises Clínicas, Centro Universitário do Pará, Belém, Pará, Brasil

Surama da Costa Pinheiro
Laboratório de Patologia Clínica e Laboratorial Dr. Paulo Azevedo, Belém, Pará, Brasil

Paulo Sérgio Roffe Azevedo
Laboratório de Patologia Clínica e Laboratorial Dr. Paulo Azevedo, Belém, Pará, Brasil

Schirley Dias dos Santos
Curso de Pós-Graduação em Análises Clínicas, Centro Universitário do Pará, Belém, Pará, Brasil
Seção de Bacteriologia e Micologia, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Ana Roberta Fusco da Costa
Seção de Bacteriologia e Micologia, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Francisco Lúzio de Paula Ramos
Seção de Bacteriologia e Micologia, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

Karla Valéria Batista Lima
Seção de Bacteriologia e Micologia, Instituto Evandro Chagas/SVS/MS, Ananindeua, Pará, Brasil

ABSTRACT

Serratia marcescens has been reported as an important agent of health care-related infections and has been highlighted for presenting a high level of intrinsic resistance to antimicrobials used in neonatology, besides persisting in hospital environments for long periods. In this work, *S. marcescens* was recovered from colonies in the gastrointestinal tract or late sepsis in newborn infants hospitalized in a Neonatal Unit in Belém. The identification of *S. marcescens* and the sensitivity test was carried out using a Vitek (BioMérieux) automated system; susceptibility to ertapenem was assessed using e-test strips (Oxoid). Genotyping was executed by ERIC-PCR using the primers ERIC1 (5'-TGAATCCCCAGGAGCTTACAT-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'). Twenty-two strains of *S. marcescens* were recovered: 15 from hemocultures and seven from surveillance (rectal swab culture). All presented resistance to ampicillin, ampicillin-sulbactam, gentamicin and cephalothin. There were no indications of resistance to ciprofloxacin, imipenem, meropenem or ertapenem. The susceptibility profiles varied for other antibiotics. Eleven amplification patterns by ERIC-PCR were obtained, and two were shared by 14 isolates. It was possible to observe a characteristic polymorphic pattern in the strains from gastrointestinal colonization, except for two cases, which presented genotypic patterns related to cases of sepsis. The data obtained in this work confirm the high level of resistance of *S. marcescens* against antimicrobials; however, all isolates displayed sensitivity to ciprofloxacin and carbapenemics. Antibiogram and ERIC-PCR typing suggest a dispersion of clones associated with colonization or sepsis among the wards of the Neonatal Unit in the surveyed hospital.

Keywords: *Serratia marcescens*; Bacterial Typing Techniques; Polymerase Chain Reaction; Bacterial Drug Resistance.

Correspondência / Correspondence / Correspondencia:
Karla Valéria Batista Lima
Instituto Evandro Chagas, Seção de Bacteriologia e Micologia
Rodovida BR316, km 7, s/n°, Levilândia
CEP: 67030-000 Ananindeua-Pará-Brasil
E-mail: karlalima@iec.pa.gov.br
Translated by / Traduzido por / Traducido por:
American Journal Experts

INTRODUCTION

Serratia marcescens, which belongs to the Enterobacteriaceae family, has been reported to be an important agent of health care-related infections (HCRI), specifically standing out due to its dissemination potential and its high level of intrinsic resistance to the drugs used in neonatology, and to antiseptic agents. This pathogen persists for long periods in the hospital environment because it colonizes the skin and the gastrointestinal tract of adults and newborns[11,2,6]. Therefore, after reporting infections, it is necessary to determine the origin of the pathogen and maintain surveillance, in order to effectively control and/or eradicate the cases.

Many methods have been used for typing epidemic strains of *S. marcescens*. They involve both phenotypic and genotypic characterizations, and are based on the assumption that related organisms have unique characteristics that distinguish them from non-related organisms. The phenotypic characteristics (e.g., biochemistry, antimicrobial resistance, serotyping, and phage typing) may not be discriminatory, so the use of molecular methods is always necessary for clonal confirmation. The techniques that use pulsed field gel electrophoresis (PFGE) and ribotyping have good discriminatory ability but have the disadvantage of being time-consuming, labor- intensive, and require expensive equipment and reagents [9].

Molecular techniques based on the amplification of nucleic acids, such as *Enterobacterial Repetitive Intergenic Consensus* (ERIC), have been used due to their ease of use, reproducibility, and their agreement with the results obtained by ribotyping[5,2,12].

In this study, strains of *S. marcescens*, isolated during colonization or late sepsis from the Neonatal Unit (NU) of Belém, were evaluated in regards to their antimicrobial resistance profile and genetic characterization.

MATERIALS AND METHODS

DESCRIPTION OF THE INSTITUTION AND SAMPLES

The study was conducted at a NU with a capacity of 103 beds, located at a high complexity hospital in the city of Belém, Pará. The unit consists of an internal nursery (for infants who were born at the hospital), a neonatal intensive care unit (NICU), and an external nursery (EN). The internal nursery is divided in five separate wards: special care (SC), intermediate care (IC), semi-intensive (SI), and transition room (TR). Most newborns referred to the unit are premature with very low weight.

During the study, 675 blood cultures and 75 surveillance cultures were isolated to evaluate the colonization of the intestinal tract by *S. marcescens* strains.

COLLECTION AND SELECTION OF SAMPLES

Blood cultures were performed on 3 mL of venous blood from each newborn using the automated BACTEC 9120 (Becton Dickinson) system. Surveillance of the colonizing agent within the newborns was performed by cultivating rectal swabs, which were obtained starting on the seventh day of neonate hospitalization and repeated weekly until hospital discharge. The positive primary cultures were subcultured in blood agar (Difco) and MacConckey agar (Difco), and incubated in a bacteriological incubator (Fanen) at 37° C for 24 h. The resulting colonies of fermenting (oxidase negative) Gram-negative bacilli were suspended in 0.45% saline solution, standardized in a colorimeter (BioMerieux) to a concentration equivalent to a 0.5 tube of the Mac-Farland scale (1.5 10^6 UFC/ mL) and incubated in GN cards for identification by the automated VITEK (BioMérieux) system. The species *S. marcescens* was selected for this study.

The *S. marcescens* strain ATCC 8100 and samples not related to the outbreak were used as controls during the genotyping.

SENSITIVITY TEST

The susceptibility profile was evaluated by the automated VITEC (BioMérieux) system, following the manufacturer's recommendations. The susceptibility of *S. marcescens* to ampicillin (AM), ampicillin-sulbactam (SAM), amikacin (AN), aztreonam (ATM), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), cephalothin (KF), ciprofloxacin (CIP), gentamicin (GN), piperacillin-tazobactam (PTZ), sulfa-trimethoprim (STX), imipenem (IPM), and meropenem (MEM) was evaluated. The sensitivity to ertapenem was evaluated with the aid of a disk containing 10 μg of the drug (Oxoide). Inhibition zone readings were performed using a millimeter ruler.

GENOTYPING OF STRAINS BY ERIC-PCR

The bacterial DNA was extracted using the boiling and freezing method (15 min each) and subjected to amplification by polymerase chain reaction (PCR), using the ERIC1 (5'-TGAATCCCCAGGAGCTTACAT-3') and ERIC2 (5'- AAGTAAGTGACTGGGGTGAGCG-3') primers, according to the protocol originally described by Liu et al[5]. Restriction fragment length polymorphism analysis was conducted after electrophoresis on a 2% agarose gel, followed by visualization on a UV transilluminator.

The development of this study was approved by the Research Ethics Committee of the Instituto Evandro Chagas, protocol CEP/ IEC n° 003/07, on July 14, 2007.

RESULTS

We obtained 22 cultures of *S. marcescens*, which were recovered from episodes of late neonatal sepsis and colonization. Fifteen of the samples were from neonatal blood cultures, while 7 came from surveillance cultures. All samples showed resistance to AM, SAM, GN, and KF. There was no resistance to CIP, IPM, ertapenem, or MEM. The susceptibility profiles for the other antibiotics tested were variable (Table 1).

We obtained 11 amplification patterns by ERIC-PCR. Eight showed patterns of five or more amplifications between 100 and 3,000 bp (Figure 1), while four samples showed patterns with less than five amplifications (patterns 17, 19, 21). Two polymorphic patterns were shared by 14 isolates (Table 1, Figure 1).

ID	Specimen	Isolation Date	Ward	Antibiogram*	ERIC-PCR
1	blood	March 7	SC14	AM/SAM/AN/KF/GN/PTZ/STX	I
2	blood	April 20	SC15	AM/SAM/AN/KF/GN/PTZ/STX	I
3	blood	March 21	IC12	AM/SAM/AN/KF/GN/PTZ/STX	I
4	blood	April 11	NICU20	AM/SAM/AN/ATM/CTX/KF/GN/PTZ	I
5	blood	April 17	SC 14	AM/SAM/AN/ATM/FOX/KF/GN/PTZ/STX	I
6	intestinal	April 26	SC 08	AM/SAM/AN/KF/GN	I
7	blood	April 11	NICU 13	AM/SAM/AN/KF/GN	I
8	blood	March 23	NICU 20	AM/SAM/AN/KF/GN/STX	I
9	blood	April 18	SC 06	AM/SAM/AN/ATM/KF/GN/PTZ/STX	I
10	intestinal	April 26	IC 07	AM/SAM/ATM/FEP/CTX/KF/GN/PTZ	II
11	intestinal	April 26	IC 02	AM/SAM/ATM/FEP/CTX/KF/GN/PTZ	II
12	intestinal	April 26	IC 17	AM/SAM/ATM/FEP/CTX/KF/GN/PTZ	II
13	blood	March 29	NICU 04	AM/SAM/ATM/FEP/CTX/KF/GN/PTZ	II
14	intestinal	April 7	NICU 01	AM/SAM/FEP/CTX/CAZ/KF/GN/PTZ	II
15	blood	March 20	NICU10	AM/SAM/AN/KF/GN/PTZ/STX	III
16	blood	March 24	NICU16	AM/SAM/ATM/FEP/CTX/KF/PTZ	IV
17	blood	March 20	IC 23	AM/SAM/AN/CTX/KF/GN/PTZ/STX	V
18	blood	March 16	NICU 18	AM/SAM/AN/KF/GN/PTZ	VI
19	intestinal	March 11	NICU 3	AM/SAM/AN/KF/GN/STX	VII
20	blood	March 20	NICU 5	AM/SAM/FEP/FOX/KF/GN	VIII
21	blood	April 19	SC 14	AM/SAM/AN/KF/GN/STX	IX
22	intestinal	April 12	NICU 9	AM/SAM/AN/KF/GN/PTZ/STX	X

* Antibiogram expressed as a resistance pattern based on tests with ampicillin (AM), ampicillin-sulbactam (SAM), amikacin (AN), aztreonam (ATM), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), cephalothin (KF), gentamicin (GN), piperacillin-tazobactam (PTZ), and sulfa-trimethoprim (STX);

NICU = Intensive Care Unit; SC = special care; IC = Intermediate care.

Table 1 – General, phenotypic, and genotypic characteristics of *S. marcescens* isolates from blood or intestinal colonization

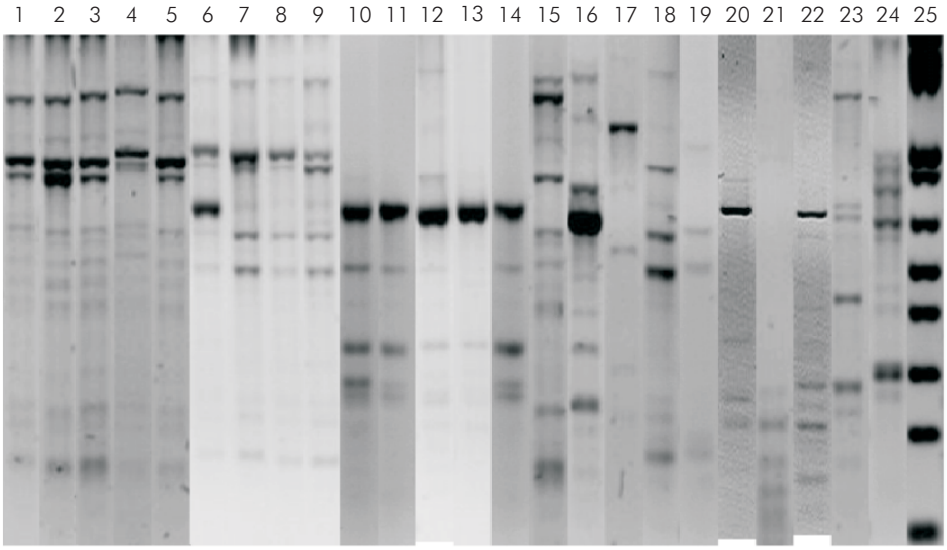

Figure 1 – The polymorphic pattern of PCR products obtained with ERIC 1 and 2 *primers* from the DNA of *Serratia marcescens*, analyzed by electrophoresis on a 2% agarose gel. Pattern 1 to 22: polymorphisms observed in the isolates studied; Pattern 23: strain unrelated to the outbreak; Pattern 24: ATCC 8100 strain of *Serratia marcescens*; Pattern 25: molecular marker 1 Kb plus (Invitrogen)

In group I, eight strains were from the blood cultures of newborns hospitalized in different wards of the NU, while the *S. marcescens* strain designated by the number 6 was recovered from intestinal colonization. In group II, strains 10, 11, 12, and 14 came from rectal swabs (surveillance), while strain 13 was from blood cultures.

DISCUSSION

Serratia marcescens may be associated with sporadic or epidemic infections. It has a high level of resistance to the antibiotics recommended for neonatal therapy[11,6] and the antiseptic agents used, which guarantees the survival of this pathogen for long periods in the hospital environment. An important characteristic of this agent is its ability to produce β-lactamase, which gives it resistance to extended-spectrum β-lactam antibiotics and complicates the therapy.

The empirical treatment of HCRI in a NU depends on the time of onset of the infection (early: those seen in the first 48 h of life of the newborn; or late: clinical and laboratory evidence of infection after 48 h of life of the newborn), the previous performance of invasive procedures, the knowledge of local microbes, and the bacterial resistance profile in each hospital[1]. The protocols may be defined as forms of structured support of technical management, which include definitions of therapeutic goals, temporal sequence of care, and diagnostic and therapeutic strategies.

In this study, all samples of *S. marcescens* showed resistance to ampicillin and to the associated ampicillin/sulbactam mix. The index of resistance to the aminoglycosides gentamicin and amikacin was also high (Table 1). When evaluating 90 samples of isolated *S. marcescens* in 1994 at the Hospital of the School of Medicine of Ribeirão Preto, Martinez et al[7] observed 99% resistance to ampicillin, 41% resistance to gentamicin, and 21% resistance to amikacin.

In this study, more than 70% (16) of the *S. marcescens* strains evaluated showed resistance to the β-lactam piperacillin associated with the β-lactamase-inhibitor tazobactam. The combination piperacillin-tazobactam has been a therapeutic alternative for the HCRI caused by Gram-negative rods and β-lactamase-producing anaerobic bacteria present in NICUs, and more recently in NICUs when these microorganisms are isolated during late infections. At the NU evaluated, the combination piperacillin-tazobactam will not be considered for treatment of infections caused by *S. marcescens*.

The groupings observed after analysis of the polymorphisms obtained by ERIC-PCR for strains obtained from patients hospitalized in different wards configured the spread between rooms of the SC, IC, and NICU. In general, we observed a characteristic polymorphic pattern for the strains originating from gastrointestinal colonization. However, in two cases, strains originating from colonization were also related to cases of sepsis (Table 1). We did not evaluate risk factors associated with septicemia.

Considering that there was no evolution of the infection in newborns with strains 10, 11, 12, and 14, a combination of risk factors is necessary for the development of sepsis, since 80% of them did not present clinical symptoms of septicemia.

Genotyping based on the amplification of repeated elements, similar to ERIC-PCR, has been successfully used for the characterization of various organisms, such as *Mycobacterium tuberculosis*[10], *Campylobacters*[8], and *Vibrio cholerae*[3].

In this study, we evaluated 22 samples of *S. marcescens*. Seven samples were recovered from the sputum of patients on mechanical ventilators during a pneumonia outbreak, while fifteen were not related to the outbreak[5]. The data from the ERIC-PCR typing showed two groupings of strains related to the outbreak and distinct profiles among the unrelated strains. These data were confirmed by ribotyping and the profiles of antibiotic resistance.

In this study, differences were observed between the data from ERIC-PCR typing and the pattern of antimicrobial resistance. There was agreement between the typing methods for strains 1, 2 and 3 (Group I); 6 and 7 (Group I); and 10, 11, 12, and 13 (Group II). In other words, 64.29% of the evaluated samples were in agreement. Several factors may justify such a result. First, the susceptibility pattern was not determined following the methods of disk-diffusion, which is standardized and currently recommended by the *Clinical and Laboratory Standards Institute* (CLSI)[4]. This technique is important because it is the most fully described and standards have been developed for interpretation of patterns supported by laboratory and clinical data, whose difficulties and improvements are constantly being discussed. Secondly, the profile of bacterial susceptibility may change in a shorter period of time than changes in the patterns of ERIC-PCR. The polymorphic patterns presented in this study retained excellent reproducibility, indicating good discrimination between related and unrelated strains in the outbreak.

In all groupings generated by ERIC-PCR, the sensitivity profiles allowed for subdivision of strains with greater variation of susceptibility to the antibiotics aztreonam, cefotaxime, and cefoxitin. However, this finding is restricted to strains with a high resistance rate.

The *S. marcescens* strains 8, 19, and 21 showed the same pattern of resistance but differed in the polymorphisms obtained by ERIC-PCR. The low resolution phenotype may originate from the low level of resistance found for these samples, where 100% of the strains showed resistance to the antimicrobials AM, SAM, NA, KF, and GN. Strains 8, 19, and 21 also showed resistance to STX, and was observed in 50% of the evaluated samples. In other words, typing by resistance profile characterization may indicate transmission; however, other methods must be evaluated for confirmation.

We observed the need to increase the number of samples to better evaluate the typing by antibiogram analysis and ERIC-PCR, in addition to developing secondary genotyping of the samples by PFGE, which is currently considered the "golden standard".

In the case of the combination of strains originating from surveillance, *S. marcescens* acts only as an indicator of transmission. Like *S. marcescens*, it is possible for other pathogens to be transmitted horizontally and concurrently, which may have been overlooked since it did not represent the focus of the research.

The data obtained in this study confirm the high levels of resistance of *S. marcescens* strains; however, all evaluated strains showed sensitivity to carbapenems and ciprofloxacin. Typing by ERIC-PCR allowed for grouping of strains, which suggested clones associated with colonization or sepsis spread between the NU wards of the hospital studied.

Caracterização fenotípica e genotípica de *Serratia marcescens* provenientes de Unidade Neonatal de Referência em Belém, Pará, Brasil

RESUMO

A *Serratia marcescens* tem sido relatada como importante agente de infecções relacionadas à saúde, destacando-se por apresentar elevado nível de resistência intrínseca aos antimicrobianos usados em neonatologia, além de persistir por longos períodos no ambiente hospitalar. Neste trabalho foram avaliadas, por métodos fenotípicos e moleculares, *S. marcescens* recuperadas a partir de colonização do trato gastrointestinal ou sepse tardia em neonatos internados em Unidade Neonatal em Belém. A identificação das *S. marcescens* e o teste de sensibilidade foram realizados por meio de sistema automatizado Vitek (BioMérieux); a suscetibilidade ao ertapenem foi avaliada com auxílio de disco contendo 10 μg da droga (Oxoide). A genotipagem foi feita por ERIC-PCR usando os *primers* ERIC1 (5'-TGAATCCCCAGGAGCTTACAT-3') e ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'). Foram obtidas 22 cepas de *S. marcescens*, sendo 15 recuperadas de hemoculturas, e sete de vigilância (*swab* retal); todas apresentaram resistência a: ampicilina, ampicilina-sulbactam, gentamicina e cefalotina. Não foi observada resistência a: ciprofloxacina, imipenem, meropenem e ertapenem. Quanto aos demais antibióticos avaliados, o perfil de suscetibilidade foi variável. Foram obtidos 11 padrões de amplificação por ERIC-PCR, dois foram compartilhados por 14 isolados. Foi possível observar um padrão polimórfico característico para as cepas provenientes de colonização gastrointestinal, exceto em dois casos, que apresentaram padrões genotípicos relacionadas a casos de sepse. Os dados obtidos neste trabalho confirmam o elevado índice de resistência da *S. marcescens* aos antimicrobianos; no entanto, todos os isolados apresentaram sensibilidade à ciprofloxacina e aos carbapenêmicos. A tipagem por meio de antibiograma e ERIC-PCR sugere dispersão de clones associados à colonização ou sepse entre alas na Unidade Neonatal do hospital estudado.

Palavras-chave: *Serratia marcescens*; Técnica de Tipagem Bacteriana; Reação em Cadeia da Polimerase; Resistência Microbiana a Medicamentos.

La caracterización fenotípica y genotípica de *Serratia marcescens* proveniente de la Unidad de Neonatología de Referencia de Belém (Pará, Brasil)

RESUMEN

La *Serratia marcescens* ha sido considerada como un agente importante de las infecciones relacionadas con la salud (IRAS, por sus siglas en portugués), y se ha destacado su alto nivel de resistencia intrínseca a los antimicrobianos utilizados en neonatología, además de persistir durante largos períodos de tiempo en el ambiente hospitalario. En este trabajo fueron evaluados a través de métodos fenotípicos y moleculares *S. marcescens* recuperados a partir de la colonización del tracto gastrointestinal o sepsis de aparición tardía en recién nacidos hospitalizados en la Unidad de Neonatología (UN) de Belém. La identificación de *S. marcescens* y las pruebas de sensibilidad se realizaron mediante el sistema automatizado Vitek (Biomerieux); la susceptibilidad al ertapenem se evaluó mediante la prueba de epsilometría (Oxoid). El genotipado se hizo mediante ERIC-PCR, utilizando partidores ERIC1 (5'-TGAATCCCCAGGAGCTTACAT-3') y ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'). Se obtuvieron 22 cepas de *S. marcescens*, 15 recuperadas de hemocultivos y siete de seguimiento (hisopado rectal); todas presentan resistencia a la ampicilina, ampicilina-sulbactam, gentamicina y cefazolina. No se presentó resistencia a la ciprofloxacina, imipenem, meropenem y ertapenem. En cuanto a los demás antibióticos evaluados, el perfil de susceptibilidad fue variable. Se obtubieron 11 patrones de amplificación por ERIC-PCR, dos de ellos compartidos por 14 aislamientos. Fue posible observar un patrón característico de las cepas polimórficas de la colonización gastrointestinal, excepto en dos casos que presentaron patrones de genotipos relacionados con los casos de sepsis. Los datos de este estudio confirman el alto nivel de resistencia de *S. marcescens* a los antibióticos, aunque todas las cepas fueron sensibles a ciprofloxacina y carbapenémicos. El tipaje a través de antibiograma y ERIC-PCR sugieren la dispersión de clones asociados con la colonización o la sepsis entre las salas de la Unidad de Neonatología del hospital estudiado.

Palabras clave: *Serratia marcescens*; Técnica de Tipificación Bacteriana; Reacción en Cadena de la Polimerasa; Farmacorresistencia Bacteriana.

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