Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

# Evaluation of the antimicrobial activity of *Eucalyptus radiata* essential oil against *Escherichia coli* strains isolated from meat products

## Avaliação da atividade antimicrobiana do óleo essencial de *Eucalipto radiata* contra cepas de *Escherichia coli* isoladas de produtos cárneos

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**Abstract**

The present study sought to evaluate the antimicrobial and anti-adherent potential of *Eucalyptus radiata* essential oil against food-borne strains of *Escherichia coli*. The study was performed using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). In addition, the disk diffusion technique was used to evaluate the association of *Eucalyptus radiata* essential oil with synthetic antimicrobials. The Minimum Inhibitory Adherence Concentration (MIC) was also performed. The results revealed that E. radiata showed antimicrobial activity against the *E. coli* strains tested, with MIC values ranging from 500 µg/mL to 1000 µg/mL and MBC values ranging from 500 µg/mL to 1,024 µg/mL. As for the associations, it was observed that *E. radiata* oil exhibited a synergistic effect for some antibiotics, especially Ceftriaxone, with greater interference from the essential oil. Furthermore, it was effective in inhibiting the adherence of bacterial strains of *E. coli*, showing a more significant antibiofilm effect than the antibacterial agent 0.12% chlorhexidine digluconate. In summary, the essential oil of *E. radiata* showed antimicrobial potential against strains of *E. coli* of food origin, and can therefore, through in-depth studies, be used alone or in association with synthetic antimicrobials to combat infections caused by this pathogen.

**Keywords:** microbiology, phytotherapy, unique health.

**Resumo**

O presente estudo buscou avaliar o potencial antimicrobiano e antiaderente do óleo essencial de *Eucalipto radiata* frente às cepas de veiculação alimentar de *Escherichia coli*. O estudo foi realizado usando a Concentração Inibitória Mínima (CIM) e Concentração Bactericida Mínima (CBM). Além disso, a técnica de difusão em disco foi usada para avaliar a associação do óleo essencial de *Eucalipto radiata* com antimicrobianos sintéticos. A Concentração Inibitória Mínima de Aderência (CIMA) também foi realizada. Os resultados revelaram que *E. radiata* apresentou atividade antimicrobiana contra as cepas de *E. coli* testadas, com valores de CIM variando entre 500 µg/mL a 1000 µg/mL e valores de CBM variando entre 500 µg/mL a 1,024 µg/mL. Quanto às associações, observou-se que o óleo de *E. radiata* exibiu efeito sinérgico para alguns antibióticos, sobretudo a Ceftriaxona, apresentando maior interferência do óleo essencial. Além disso, foi eficaz na inibição a aderência das cepas bacterianas de *E. coli*, mostrando um efeito antibiofilme mais significativo que o agente antibacteriano digluconato de clorexidina a 0.12%. Em resumo, o óleo essencial de *E. radiata* apresentou potencial antimicrobiano contra cepas de *E. coli* de origem alimentar, podendo assim, mediante estudos aprofundados, ser utilizado isoladamente ou em associação com antimicrobianos sintéticos para combater infecções causadas por esse patógeno.

**Palavras-chave:** microbiologia, fitoterapia, saúde única.

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Received: December 12, 2023 – Accepted: February 14, 2024

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## 1. Introduction

Meat products are widely consumed by the public and have seen a global increase in recent years, as they represent an important component of the human diet (Ursachi et al., 2020). In addition, their practicality in preparation and low cost make products such as meat sausages convenient and increasingly present in the diet of the Brazilian population (Trentini and Macedo, 2019; IBGE, 2020).

In this context, the issue of food safety is one of the aspects that deserve attention, since microorganisms such as foodborne pathogens affect food safety because food is subject to contamination during its production stages, triggering the development of probable foodborne illnesses (Flores and Melo, 2015; Abebe et al., 2020). These diseases include various types of foodborne illnesses and have a high incidence, most of which are caused by pathogenic microorganisms that cause major public health problems (Ferrari and Fonseca, 2019; Melo et al., 2018).

Among the most prominent agents causing foodborne illness are bacteria. In recent years in Brazil, the bacteria Salmonella spp., *Escherichia coli* and *Staphylococcus aureus* have been among the main agents involved in outbreaks of food-borne diseases (Brasil, 2020). Cavalcanti (2021), analyzing the microbiological quality of meat products (sausage and mortadella) sold in supermarkets and mini-markets located in the cities of Patos, Sousa, Princesa Isabel and Itaporanga, Paraíba, Brazil, found that *E. coli* bacteria were detected in 6 (10%) samples out of a total of 60 samples.

Seen as one of the most frequent bacterial agents in food-borne illnesses (Macedo et al., 2018), the *Escherichia coli* species is well known and studied due to its pathogenic potential, being the most common among the species. It is a Gram-negative bacillary bacterium belonging to the Enterobacteriaceae family, it is a facultative anaerobic pathogen, therefore it does not require oxygen for its growth, however it shows greater growth in aerobic conditions, being found in the intestinal microbiota of humans and warm-blooded animals (Paredes-Espinoza, 2022; Chile, 2017).

*Escherichia coli* has important resistance mechanisms and infections caused by these bacteria are more difficult to treat than those caused by non-resistant bacteria (WHO, 2015). In this circumstance, the emergence of microorganism resistance to antimicrobials is mainly due to the indiscriminate use of this medication, making it increasingly difficult to treat bacterial diseases, being a Single Health Problem and becoming one of the greatest threats to global health today (Costa and Silva Junior, 2017).

Studies show that antimicrobials can be replaced by other natural components, such as essential oils (Gebreyes et al., 2017). These compounds derived from medicinal plants are volatile, highly concentrated liquids with a characteristic and persistent odor, secondary metabolites extracted from various parts of plants and which have great antimicrobial and antioxidant potential. They have emerged as a viable and promising alternative, since they are able to act as a natural antimicrobial agent in food products and, above all, be a substitute for synthetic products that can cause severe adverse effects (Manion and Widder, 2017; Tongnuanchan and Benjakul, 2014; Ribeiro-Santos et al., 2017; Aquino et al., 2010).

Species of the Eucalyptus genus, a plant belonging to the Myrtaceae family, are examples of natural products with great potential for pharmaceutical use. Among other activities, essential oils such as *Eucalyptus radiata* have been reported to have nematicidal, acaricidal, insecticidal, antimicrobial (Chagas et al., 2002; Gilles et al., 2010; Barbosa et al., 2016) and hypoglycemic (Capetti et al., 2020). As a result, the use of essential oils in the pharmaceutical industry continues to be widely investigated and discussed, especially their properties as anti-inflammatory, antimicrobial, antiviral, anticancer and improvement of cell permeability (Aziz et al., 2018).

Therefore, given the existence of antimicrobial-resistant bacteria such as *Escherichia coli* and the frequent food-borne illnesses, it is necessary to look for new therapeutic alternatives to meet the demands of food safety and control the spread of resistant bacterial strains. Based on this, this study evaluated the antimicrobial potential of *Eucalyptus radiata* essential oil against *E. coli* strains isolated from meat products.

## 2. Materials and Methods

### *2.1. Study location*

The laboratory tests were carried out at the Microbiology Laboratory of the Central Laboratories of the Biological Sciences Academic Unit (UACB) of the Federal University of Campina Grande (UFCG)/Patos - PB.

### *2.2. Obtaining the essential oil*

The essential oil of *Eucalyptus radiata* (*Eucalipto radiata*) was purchased from Terra Flor (Ponta Grossa - Paraná). To carry out pharmacological tests, the compound was solubilized in the presence of the dispersants Tween 80 and dimethylsulfoxide (DMSO), and diluted in distilled water (Allegrini et al., 1973).

### *2.3. Bacterial strains*

A standard strain of *Escherichia coli* (CCCD-E003) and clinical strains (Ec 41, Ec 42, Ec 44, Ec 45) isolated from meat products of animal origin from the Microbiology laboratory of the Central Laboratory of the UACB - UFCG were used.

These strains were kept on Muller-Hinton Agar (MHA) at 4 °C. The inocula were obtained from overnight cultures on MHA at 35 ± 2 °C and diluted in 0.9% sterile saline solution to obtain a final concentration of approximately 1.5 x 108 colony-forming units per mL (CFU/mL), adjusted for turbidity by comparing with a suspension of barium sulphate and sulphuric acid on the McFarland 0.5 scale (Bona et al., 2014).

### *2.4. Antimicrobials*

Ampicillin (10 µg/mL), gentamicin (10 µg/mL), ceftriaxone (30 µg/mL) and ciprofloxacin (5 µg/mL) were used according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2018).

*2.5. Culture media*

The culture media used in the tests were Mueller Hinton broth and solid Mueller Hinton Agar. The culture media was purchased from Difco® and prepared according to the manufacturer's instructions.

*2.6. Determination of the Minimum Inhibitory Concentration (MIC)*

The MIC was determined using microdilution technique in a 96-well plate with a U-shaped bottom. Initially, in each well, 100 µL of Mueller Hinton broth, doubly concentrated, and 100 µL of the studied product (*Eucalyptus radiata*) were added to the plate performing a serial dilution (ratio of two), obtaining the concentrations of 1000, 500, 250, 125, 62.5, and 31.2 µg/mL. Determination of the MIC was conducted using 10 µL of the microorganism suspension in each well, being approximately 1.5 x $10^8$ CFU/mL. In the penultimate well, the sterile control was performed containing 100 µL of Muller Hinton broth alone, and in the final well, the growth control was performed, containing only 10 µL of the microorganism suspension in 100 µL of broth. The assay was performed in duplicate. The plates were incubated at 35 ± 2 °C for 24 hours, and after this appropriate bacterial incubation time, the first reading of the results was performed. 20 µL of sodium resazurin solution (SIGMA) was then added, being previously solubilized in sterilized distilled water, at a concentration of 0.01% (w/v). Resazurin is well recognized as a colorimetric oxide-reduction indicator for bacteria. Afterwards, a new incubation at 35 ± 2 °C was performed. The reading was done visually for the absence or presence of microorganism growth through formation of a cluster of cells (button), as well as observing changes in solution color, from blue to pink to indicate growth. The MIC was determined as the lowest concentration of the compound inhibiting visible growth of the microorganism, as verified through the change of the solution color, from blue to pink, which indicates microorganism growth (Palomino et al., 2002; Ostrosky et al., 2008; CLSI, 2012; Bona et al., 2014).

*2.7. Determination of the Minimum Bactericidal Concentration (MBC)*

To perform the MBC, inoculations (10 µL) of dilutions from the MIC were performed in Mueller Hinton broth medium (100 µL/cavity) in a sterile microdilution plate, and subsequently, incubation was performed at 35 ± 2 °C for 24 hours. After incubation, 20 µL of resarzurin was added, and a new incubation was performed at 35 ± 2 °C to confirm the concentration capable of total growth inhibition of the bacterial species, which would be verified by no indicator dye color change (Ncube et al., 2008; Guerra et al., 2012).

*2.8. Study of the association of Eucalyptus radiata oil with synthetic antimicrobials*

For the study of the product's association with antimicrobials, the disk diffusion technique was used on solid media using filter paper disks (Bauer et al., 1966; Oliveira et al., 2006). Using a sterile swab, a volume of approximately 1mL of each bacterial suspension was seeded onto the solid surface of the Muller Hinton agar (MHA) contained in flat sterile plates. Subsequently, the paper discs (impregnated with the antimicrobials) were applied onto MHA with the bacterial suspension. Soon afterwards, a 20µL aliquot (MIC) of the tested compound was transferred to the discs containing the antimicrobials. A negative control containing only the bacterial suspension with antimicrobials discs was also performed. The plates were incubated at 35 ± 2 °C for 24-48h, followed by reading. The effect was considered synergistic if the microbial growth inhibition halo formed by the association (oil + antimicrobial) presented a diameter ≥ than 2mm, when compared to the inhibition halo formed by the action of the antimicrobial alone. When the formation of the inhibition halo resulting from the combined action (oil + antimicrobial) was smaller in diameter than that developed by the isolated action of the antimicrobial, it was considered an antagonistic effect. The effect was considered indifferent when the halo of inhibition resulting from the combined application (oil + antimicrobial) presented the same diameter as that resulting from the isolated application of the antimicrobial (Cleeland and Squires, 1991; Oliveira et al., 2006). All tests were performed in duplicate.

*2.9. Determination of the Minimum Inhibitory Concentration for Adherence (MICA)*

The Minimum Adherence Inhibitory Concentration (MIC) of the compound was determined in the presence of 5% sucrose, according to Albuquerque et al. (2010) with modifications, using concentrations corresponding to the compound up to a dilution of 1:128. After bacterial growth, the bacterial strain was cultured at 37 °C in Mueller Hinton broth, then 0.9 mL of the subculture was poured into test tubes and 0.1 mL of the solution corresponding to the dilutions of the compound was added. Incubation was carried out at 37 °C for 24 hours with the tubes tilted at 30º. The reading was taken by visually observing the bacteria adhering to the walls of the tube after shaking it. The test was carried out in duplicate. The same procedure was carried out for the positive control, 0.12% chlorhexidine digluconate (Riohex Gard, Rioquímica, São José do Rio Preto, São Paulo). CIMA was considered to be the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

*2.10. Statistical analysis*

The study used descriptive statistical analysis, using the Microsoft® Word program version 2312 Build 16.0.17126.20132.

## 3. Results

*3.1. Minimum Inhibitory Concentration (MIC)*

The results of the minimum inhibitory concentration for the essential oil of *Eucalyptus radiata* against strains of

*Escherichia coli* are shown in Table 1. Activity was measured in terms of the presence of microorganism growth with a MIC 50 of 1000 µg/mL.

### 3.2. Minimum Bactericidal Concentration (MBC)

The results of the Minimum Bactericidal Concentration of *E. radiata* essential oil against *E. coli* are shown in Table 2. When analyzing the results, it can be seen that for most strains the MBC was greater than 1000µg/mL.

### 3.3. Association of E. radiata oil with synthetic antimicrobials

The inhibition halos (mm) resulting from the association of *E. radiata* essential oil with synthetic antimicrobials for *E. coli* strains are shown in Table 3. Analyzing the results, it

**Table 1.** Minimum Inhibitory Concentration (MIC) in µg/mL of *Eucalyptus radiata* against *E. coli* strains isolated from meat sausages.

| | ***Eucalyptus radiata*** | | | | |
|---|---|---|---|---|---|
| | **CCCD- E003** | **Ec 41** | **Ec 42** | **Ec 44** | **Ec 45** |
| 1000 µg/mL | + | + | + | + | - |
| 500 µg/mL | + | - | - | - | - |
| 250 µg/mL | - | - | - | - | - |
| 125 µg/mL | - | - | - | - | - |
| 62.5 µg/mL | - | - | - | - | - |
| 31.25 µg/mL | - | - | - | - | - |

**Legend:** (+) Inhibited bacterial growth (-) Did not inhibit bacterial growth.

**Table 2.** Minimum Bactericidal Concentration (MBC) in µg/mL of *Eucalyptus radiata* against *E. coli* strains isolated from meat sausages.

| | ***Eucalyptus radiata*** | | | |
|---|---|---|---|---|
| | **CCCD-E003** | **Ec 41** | **Ec 42** | **Ec 44** |
| 1000 µg/mL | + | - | - | - |
| 500 µg/mL | + | - | - | - |
| 250 µg/mL | - | - | - | - |
| 125 µg/mL | - | - | - | - |
| 62.5 µg/mL | - | - | - | - |
| 31.25 µg/mL | - | - | - | - |

**Legend:** (+) Inhibited bacterial growth (-) Did not inhibit bacterial growth.

**Table 3.** Interference of *Eucalyptus radiata* essential oil in association with synthetic antimicrobials for *E. coli* strains.

| **Antibiotics** | | **Microorganisms** | | | | |
|---|---|---|---|---|---|---|
| | | ***E. coli*** | ***E. coli*** | ***E. coli*** | ***E. coli*** | ***E. coli*** |
| | | **Ec 41** | **Ec 42** | **Ec 44** | **Ec 45** | **CCCD-E003** |
| Ampicilin | AIH | 18 mm | 20 mm | 32 mm | 30 mm | 18 mm |
| | AIH w/ ER | 22 mm ↑ | 14 mm ↓ | 30 mm ↓ | 30 mm * | 18 mm * |
| Gentamicin | AIH | 24 mm | 26 mm | 18 mm | 18 mm | 16 mm |
| | AIH w/ ER | 28 mm ↑ | 24 mm ↓ | 18 mm * | 18 mm * | 20 mm ↑ |
| Ciprofloxacin | AIH | 40 mm | 38 mm | 26 mm | 24 mm | 38 mm |
| | AIH w/ ER | 40 mm * | 36 mm ↓ | 26 mm * | 28 mm * | 40 mm ↑ |
| Ceftriaxone | AIH | 34 mm | 34 mm | 24 mm | 24 mm | 38 mm |
| | AIH w/ ER | 36 mm ↑ | 34 mm * | 24 mm * | 26 mm ↑ | 40 mm ↑ |

**Legend:** AIH: antimicrobial inhibition halo; ER: *Eucalyptus radiata*; Synergistic effect (↑); antagonistic effect (↓); indifferent effect (*).

**Table 4.** Minimum Inhibitory Concentration for Adherence (MICA) in µg/mL of *Eucalyptus radiata* essential oil and 0.12% chlorhexidine digluconate against the *Escherichia coli* strain (Ec 44).

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Eucalyptus radiata*** | | | | | | | | |
| µg/mL | **1:1** | **1:2** | **1:4** | **1:8** | **1:16** | **1:32** | **1:64** | **1:128** |
| | - | - | - | + | + | + | + | + |
| **0.12% Chlorhexidine digluconate** | | | | | | | | |
| µg/mL | **1:1** | **1:2** | **1:4** | **1:8** | **1:16** | **1:32** | **1:64** | **1:128** |
| | + | + | + | + | + | + | + | + |

**Legend:** (+) adhesion to the pipe wall (-) no adhesion to the pipe wall.

can be seen that *E. radiata* oil showed synergism in most strains when combined with ceftriaxone.

*3.4. Minimum Inhibitory Concentration for Adherence (MICA)*

Table 4 shows the results of the Minimum Adhesion Inhibitory Concentration (MIC) of the essential oil of *E. radiata*, as well as a comparison with the positive control (chlorhexidine 0.12%) against the *Escherichia coli* strain (Ec 44). checking the results, it can be seen that the essential oil of *E. radiata* was able to inhibit biofilm adhesion at a ratio of 1:4, thus showing antibiofilm effects against the strain studied.

## 4. Discussion

Given the increase in bacterial resistance to current antimicrobials, the need for new agents with antibacterial activity is explicit. In this sense, natural products turn out to be a viable therapeutic alternative, since they are well accepted and more accessible (Khadake et al., 2021; Bezerra et al., 2017). Certain characteristics make natural products, especially essential oils, a promising alternative for the treatment of bacterial infections, since some plants contain safe and effective compounds with antibacterial action (Nakagawa et al., 2020). In this way, essential oils become an interesting object of study, due to the existence of different mechanisms of action against bacteria (Cutrim et al., 2019).

According to the results of the Minimum Inhibitory Concentration (MIC), it was observed that the essential oil of *E. radiata* showed a MIC with values ranging from 500 µg/mL to 1000 µg/mL for most strains against the growth of most of the diferente strains of *Escherichia coli*. For antimicrobial activity to be considered strong, it must have a MIC of up to 500 µg/mL, MICs of 600 to 1500 µg/mL are considered moderate, and weak activity presents MICs above 1500 µg/mL according to Sartoratto et al. (2023). Thus, the standard strain (CCCD-E003) showed strong antimicrobial activity and the other strains showed moderate antimicrobial activity.

The oil showed Minimum Bactericidal Concentration (MBC) values ranging from 500 µg/mL to 1000 µg/mL, demonstrating bacteriostatic activity for four of the five strains tested. It was found that for one of the strains tested, the CBM value was equal to the MIC value, which is an indicator of the bactericidal activity of this essential oil. According to Hafidh et al. (2011), a compound is considered bactericidal or bacteriostatic and, for this, the ratio of CBM and CIM is analyzed. When this ratio is between 1:1 and 2:1, the compound is considered bactericidal and, to be considered bacteriostatic, the ratio must be greater than 2:1.

Thus, for the standard strain (CCCD-E003) it was possible to observe that the essential oil of *E. radiata* had a strong, bactericidal effect and for the clinical strains it had a moderate, bacteriostatic effect.

The results presented of moderate and strong inhibitory activity and bactericidal and bacteriostatic effect of *E. radiata* essential oil against *E. coli* strains may be associated with the significant concentration of the compound 1,8-cineole (eucalyptol), known to have antimicrobial properties (Paranagrama, 1991; Bizzo et al., 2009). Furthermore, authors suggest that the abundance of this compound and the presence of monoterpene alcohols, such as α-terpineol, may be related to the antibacterial activity against these bacteria (Ameur et al., 2021).

Another terpene compound that can also be found in the *E. radiata* species is limonene. Gupta et al. (2021) confirm that the unique nature of this compound makes it a promising antibacterial agent against multidrug-resistant bacterial pathogens such as *E. coli*. Therefore, this bioactivity of *E. radiata* may be due to these terpenes and their cooperative action.

In this situation, a study carried out by Luís et al. (2016) in order to analyze the antimicrobial effect of the essential oils of *E. globulus* and *E. radiata*, revealed that the essential oil of *E. radiata* showed significant antibacterial activity against different strains of Gram-negative bacteria, including the bacterium *Escherichia coli* of human origin, since it obtained lower MIC values than those obtained by *E. globulus*. In addition, research reveals that the essential oil of *E. radiata* has shown remarkable antimicrobial activity against a broad spectrum of Gram-positive, Gram-negative and yeast pathogens (Mahumane et al., 2016).

The results of the interference of the essential oil of *E. radiata* on the antibacterial action of synthetic antibiotics (Table 3) takes into account the comparison of the diameters of the halos of inhibition of bacterial growth in the tests with the antibiotics alone and in association with the essential oil. In some interactions, it is possible to observed that the essential oil interfered with the antibacterial power of the antibiotics (Oliveira et al., 2006). In the study in question, a synergistic effect was observed for some of the antibiotics, especially ceftrioaxone, which showed greater interference from the essential oil, exhibiting synergism for most of the bacterial strains under study. Their confirmed synergistic activity suggests that using a combination of these synthetic antibiotics with the compound under study could be a viable technique for reducing antibiotic consumption, which would overcome antibiotic resistance or delay its onset (Mayyas et al., 2021).

The minimum adherence inhibitory concentrations (MICs) of *E. radiata* are shown in Table 4. Looking at the results, the essential oil of *E. radiata* was effective in inhibiting the adherence of bacterial strains of *E. coli* in the presence of sucrose, showing an eminent effect when compared to the antibacterial agent, since there was no inhibition in the adhesion effect against *E. coli* by 0.12% chlorhexidine digluconate.

Previous research has suggested that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria (Snoussi et al., 2018; Bordoni et al., 2019). However, in the present research, the product being tested in this study was effective in inhibiting the adherence of *Escherichia coli.* This corroborates the findings of Ramalho et al. (2020), who observed that the essential oil of *Eucalyptus globulus* had an anti-adherent activity of 1:64, showing a positive result at a lower concentration of the oil compared to the result obtained by chlorhexidine digluconate 0.12%, which showed inhibition at a higher concentration of 1:8, confirming that this plant species is also effective in inhibiting biofilm formation against clinical strains of *E. coli* of human origin.

## 5. Conclusion

It can be concluded that the essential oil of *Eucalyptus radiata* is effective in inhibiting the growth of the Gram-negative bacterium *Escherichia coli* isolated from food. *E. radiata* oil also potentiated the antibacterial activity of synthetic antimicrobials against *E. coli* strains, showing synergism against some antibiotics, especially ceftriaxone. In addition, it was effective in inhibiting the adherence of bacterial strains of *E. coli*, showing a more significant effect than 0.12% chlorhexidine digluconate, an antibacterial agent. In summary, *E. radiata* oil showed antibacterial potential against food-borne *E. coli* strains and, following further studies, can be used alone or in combination with synthetic antimicrobials to combat infections caused by this pathogen.

## Acknowledgements

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for its financial support, as well as the Federal University of Campina Grande (UFCG).

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