

Cellular Interaction Potential of Clinical *Corynebacterium Striatum* Isolates With Human Pneumocytes

Arize Duarte Vieira

Universidade do Estado do Rio de Janeiro

Guilherme Goulart Cabral de Oliveira

Universidade do Estado do Rio de Janeiro

St phanie Rocha Vieira Elexias

Universidade do Estado do Rio de Janeiro

C ntia Silva dos Santos

Universidade do Estado do Rio de Janeiro

Fellipe de Oliveira Cabral

Fund     Oswaldo Cruz

Cynara Luziet Lages do Nascimento

Universidade do Estado do Rio de Janeiro

Cassius de Souza

Instituto Federal Fluminense (IFF) - Campus Maric 

Ana Luiza de Mattos Guaraldi

aguaraldi@gmail.com

Universidade do Estado do Rio de Janeiro

Research Article

Keywords: *Corynebacterium striatum*, Infection, Biofilm, Healthcare-associated infections (HAIs), Pneumocytes, Pulmonary alveolus

Posted Date: August 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10624614/v1>

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Additional Declarations: No competing interests reported.

Abstract

Healthcare-Associated Infections (HAIs) represent a major epidemiological concern in both public health and hospital care settings. Currently, several studies have associated different *Corynebacterium* species with these infections, particularly among patients admitted to intensive care units (ICUs) and those using invasive medical devices, with *Corynebacterium striatum* being the species most frequently reported. This study aimed to elucidate the interaction of *C. striatum* strains isolated from severe invasive infections in patients hospitalized at a tertiary care hospital in the city of Rio de Janeiro, Brazil. Bacterial isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Cell interaction profiles were evaluated using bacterial adherence, invasion, intracellular internalization, and viable cell quantification assays. The results demonstrated that all isolates exhibited distinct patterns of cellular adherence. Furthermore, all tested isolates were able to survive in the presence of A549 human pneumocytes and persist within the intracellular environment for up to 24 hours, although with varying intensities. Among them, *C. striatum* strains 1961BR-RJ and 2351BR-RJ displayed the most prolonged interaction profile. Overall, the findings of this study expand the current knowledge regarding the pathogenicity of *C. striatum* by demonstrating that these clinical isolates are capable of adhering to and surviving within human pneumocytes.

INTRODUCTION

The genus *Corynebacterium* is described as a commensal microorganism of the human skin and mucosal surfaces, with *Corynebacterium diphtheriae* being the species of greatest medical relevance due to its ability to produce diphtheria toxin and cause diphtheria (DONOVAN; BRAMPKAMP, 2014; ZHOU *et al.*, 2019). However, recent studies have highlighted the pathogenic potential and clinical relevance of other *Corynebacterium* species, particularly in healthcare-associated infections affecting immunocompromised patients and individuals undergoing prolonged hospitalization (SILVA *et al.*, 2021).

Among the species most frequently associated with hospital-acquired infections, *C. striatum* has been extensively recognized because of its multidrug-resistant phenotype and its ability to form biofilms on a wide range of surfaces. These characteristics promote bacterial persistence in the hospital environment, hinder microbial eradication, and negatively affect both patient treatment and clinical outcomes (MAGIORAKOS, 2012; YANG *et al.*, 2018; SILVA, 2021). In recent years, reports of severe invasive infections and hospital outbreaks caused by *C. striatum* strains have increased in several countries worldwide (OTSUKA *et al.*, 2006; RENOM *et al.*, 2007; ADDERSON *et al.*, 2008; CAMPANILE *et al.*, 2009; MARTINS *et al.*, 2009; WANG *et al.*, 2019).

Nosocomial pneumonia develops within the hospital setting and is considered the second most common healthcare-associated infection in the United States (TORRES *et al.*, 2021). Cases of pneumonia caused by *C. striatum* have already been described in the literature, revealing infections that are often difficult to diagnose and treat. One of the major determinants of disease severity is the

microorganism's ability to adhere to host cells and maintain intracellular viability, thereby evading host immune defenses (SOUZA *et al.*, 2022).

Previous laboratory studies have demonstrated that *Corynebacterium* species are capable of adhering to, invading, and persisting within human cells. The interaction between bacterial isolates and host cell surfaces may therefore be directly associated with the persistence of infection and its degree of pathogenicity (HIRATA *et al.*, 2002; PEIXOTO *et al.*, 2014). Nevertheless, despite the growing clinical and epidemiological importance of *C. striatum*, only a limited number of studies have investigated the interaction of this species with human respiratory cells. Therefore, the present study aimed to elucidate the interaction between *C. striatum* strains isolated from severe invasive infections and human pneumocytes.

MATERIALS AND METHODS

Origin and Identification of Bacterial Isolates

In the present study, four *Corynebacterium striatum* isolates recovered from different clinical specimens were obtained from patients admitted to Hospital Universitário Pedro Ernesto, Rio de Janeiro, Brazil. The bacterial isolates had been preserved at -70°C in Skim Milk medium (Difco) in the Culture Collection of the Laboratory of Diphtheria and Corynebacteria of Clinical Importance (LDCIC), Department of Microbiology and Immunology, Faculty of Medical Sciences, State University of Rio de Janeiro (FCM/UERJ). Before the experiments, the isolates were reactivated, and their identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Human Pneumocytes

The A549 human pneumocyte cell line was cryopreserved at -80°C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% cryoprotectant (dimethyl sulfoxide [DMSO]; Sigma-Aldrich, St. Louis, MO, USA), 50 $\mu\text{g/mL}$ gentamicin (Thermo Fisher Scientific, USA), 100 U/mL penicillin (Sigma-Aldrich, São Paulo, Brazil), and 5% fetal bovine serum (FBS; Gibco, Brazil), previously heat-inactivated for 30 minutes at 60°C . For cell recovery, frozen aliquots were centrifuged (Eppendorf, Germany) at 12,000 rpm for 2 minutes to remove the cryoprotectant. The resulting cell pellet was resuspended in DMEM supplemented with FBS, L-glutamine, and antibiotics (10% FBS, 500 mg/mL L-glutamine, penicillin, and gentamicin; Sigma-Aldrich, USA), hereafter referred to as **complete DMEM**. Cells were subsequently incubated at $35 \pm 2^{\circ}\text{C}$ under a humidified atmosphere containing 5% CO_2 in 25- cm^2 (Exim Lab, Brazil) or 75- cm^2 (Equipar, Brazil) polystyrene culture flasks, according to experimental requirements (WU *et al.*, 2017; HIRATA *et al.*, 2004).

Adherence Assays by Microscopic Analysis

Bacterial adherence assays were performed according to the methodology and classification previously described by Hirata *et al.* (2004), replacing the HEp-2 epithelial cell line with the type II human pneumocyte cell line (A549).

Briefly, bacterial isolates were initially cultured on tryptic soy agar (TSA) plates for 48 h at $35 \pm 2^\circ\text{C}$. Colonies were then inoculated into 3 mL of tryptic soy broth (TSB) and incubated for 16–18 h at $35 \pm 2^\circ\text{C}$ under constant agitation (120 rpm). Bacterial suspensions were centrifuged at 4,000 rpm for 7 minutes and washed three times with sterile phosphate-buffered saline (PBS) to remove residual debris. Based on previous standardization procedures, the bacterial concentration of each isolate was adjusted to a multiplicity of infection (MOI) of 100 (approximately 100 CFU per eukaryotic cell) in DMEM without fetal bovine serum or antimicrobial agents. One milliliter of each bacterial suspension was added to A549 cells previously cultured for 48 h in 24-well tissue culture plates containing sterile glass coverslips (VidroBras; 13 mm diameter and 0.13–0.16 mm thickness) at a density of 1×10^5 cells per well. Following 3 h and 6 h of bacterium–cell interaction, the wells were washed three times with PBS and fixed with 80% methanol (Isofar, Duque de Caxias, Rio de Janeiro, Brazil) diluted in distilled water (v/v). Coverslips were stained using the Panoptic Fast Staining Kit (Prolab, Brazil) according to the following protocol: Solution 1 for 15 s, Solution 2 for 15 s, and Solution 3 for 10 s. Subsequently, coverslips were rinsed with distilled water and mounted onto glass microscope slides using Entellan mounting medium (Merck, Darmstadt, Germany). Adherence patterns were evaluated by light microscopy (MicroNal, São Paulo, Brazil; model CBA-213) and classified according to the predominant pattern observed among the three analyzed coverslips. Images were captured using a Nikon Eclipse 80i microscope equipped with a digital camera (Nikon, Japan).

The following *Corynebacterium diphtheriae* strains were used as controls for each adherence pattern: TR241 (localized adherence), CDC-E8392 (diffuse adherence), and HC01 (aggregative adherence). Adherence profiles were classified by direct microscopic observation as follows: aggregative adherence (AA), characterized by bacterial clusters resembling stacked aggregates; diffuse adherence (DA), characterized by bacterial dispersion over the entire cell surface; and localized adherence (LA), characterized by the formation of small, compact bacterial microcolonies at discrete sites on the eukaryotic cell surface (SOUZA *et al.*, 2012).

Bacterial Adherence and Internalization Assays/Viable Cell Counts

To evaluate bacterial adherence, intracellular localization, and persistence, the methodology described by Hirata *et al.* (2002) was employed with minor modifications.

A 96-well tissue culture plate containing the A549 cell line was prepared using a cell suspension at a concentration of 2.5×10^5 cells/mL, with 200 μL added to each well. The plate was incubated for 48 h at 37°C in a humidified atmosphere containing 5% CO_2 and 95% relative humidity. For the infection assays, bacterial suspensions were added to the cell monolayers at a multiplicity of infection (MOI) of approximately 10, prepared in serum-free DMEM. Bacteria and host cells were allowed to interact for **3, 6,**

12, and 24 h at 37°C under an atmosphere of approximately 5% CO₂ and 95% relative humidity. To determine the number of cell-associated bacteria (adherent plus intracellular bacteria), culture supernatants were completely removed, and the wells were washed three times with phosphate-buffered saline (PBS) to eliminate planktonic bacteria. Subsequently, the cell monolayers were lysed with 100 µL of 0.1% Triton X-100 (USB Corporation, Cleveland, OH, USA) prepared in PBS (v/v). To evaluate the presence of intracellular bacteria, 200 µL of vancomycin (150 µg/mL) was added to cell monolayers that had previously been washed three times with PBS. Vancomycin was used because it is unable to penetrate eukaryotic cells and therefore selectively eliminates extracellular bacteria while preserving intracellular microorganisms. Following 60 min of vancomycin treatment, the wells were washed again with PBS, and the monolayers were lysed with 0.1% Triton X-100. Cell lysates were serially diluted in PBS, and 10-µL aliquots were spread onto TSA plates, which were incubated for 48 h at 37°C. After colony-forming unit (CFU) enumeration, the obtained values were multiplied by the respective dilution factor and expressed as the number of bacterial cells associated with the monolayers (HIRATA *et al.*, 2002).

For the intracellular persistence assay, vancomycin treatment was maintained for 24 h following the interaction period that yielded the highest number of intracellular bacteria. Cell monolayers were then washed six times with sterile PBS and lysed with 0.1% Triton X-100. The resulting lysates were processed for bacterial plating as previously described. Each experiment was performed in triplicate and independently repeated three times on different days.

RESULTS

Identification of the Isolates

All bacterial isolates used in this study were confirmed as *Corynebacterium striatum* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), yielding reliable identification scores (≥ 2.000). The characteristics of the bacterial isolates are summarized in **Table 1**.

Tabel 1. Origin of <i>Corynebacterium striatum</i> isolates recovered from hospitalized patients in a tertiary-care hospital located in the metropolitan region of Rio de Janeiro, Brazil, used in the present study.			
Sample	Species	Hospital/Year of Isolation	Isolation Site
1961BR/RJ	<i>Corynebacterium striatum</i>	HUPE/2009	Urine
1987BR/RJ	<i>Corynebacterium striatum</i>	HUPE/2009	Bronchoalveolar lavage
2369BR/RJ	<i>Corynebacterium striatum</i>	HUPE/2009	Tracheal aspirate
2351BR/RJ	<i>Corynebacterium striatum</i>	HUPE/2012	Tracheal secretion
Footnote: HUPE: Pedro Ernesto University Hospital; UCI: Intensive Care Unit.			

Adherence Assays by Microscopic Analysis

Microscopic evaluation of the adherence profile demonstrated an aggregative adherence (AA) pattern for isolates 1987BR/RJ, 2369BR/RJ, and 2351BR/RJ, whereas isolate 1961BR/RJ exhibited a localized adherence (LA) pattern (Fig. 1).

Legend: Representative optical microscopy images obtained at **400× magnification**. Scale bar = 35 μm .

Bacterial Adherence and Internalization Assays/Viable Cell Counts

Initially, analysis of the absolute values obtained from the bacterial interaction assays demonstrated that, under the experimental conditions employed, all *C. striatum* isolates were able to survive in the presence of A549 human pneumocytes, adhere to the cell surface, survive following interaction, and persist within the intracellular environment for up to 24 h, although with different levels of intensity. Furthermore, *C. striatum* isolates 1961BR-RJ and 2351BR-RJ, recovered from urinary (urine) and respiratory (tracheal secretion) infections, respectively, were capable of persisting and multiplying within the intracellular compartment for up to 24 h following 6 h of bacterium–cell interaction (**Graphics 1–4**).

Isolate *C. striatum* 2351BR-RJ exhibited significantly higher extracellular survival than the remaining isolates, followed by isolates 1987BR-RJ, 2369BR-RJ, and 1961BR-RJ ($P < 0.001$). Both isolates 2351BR-RJ and 1987BR-RJ displayed similar growth kinetics, with progressive increases in extracellular bacterial concentrations throughout the experimental period ($P < 0.01$), indicating that these isolates remained viable and were able to proliferate in the presence of A549 pneumocytes for up to 24 h. In contrast, isolate 2369BR-RJ exhibited a slight increase in bacterial numbers after 6 h of incubation ($P < 0.01$), followed by a modest reduction up to 12 h ($P < 0.05$), with bacterial counts remaining stable until 24 h ($P = 0.06$). Interestingly, isolate 1961BR-RJ displayed a distinct growth profile compared with the other isolates. Bacterial counts increased during the first 3 h of interaction ($P < 0.05$), decreased by 6 h ($P < 0.01$), increased again at 12 h ($P < 0.05$), and then declined markedly after 24 h of interaction ($P < 0.0001$). Nevertheless, the mean number of viable bacteria recovered from the culture supernatant after 24 h was 0.49×10^4 CFU, which remained significantly different from zero ($P < 0.05$) (**Graphic 1**).

Graphic 1. Extracellular growth of *C. striatum* isolates represented as the mean $\pm$ standard deviation of colony-forming units (CFU) recovered at each time point.

Legend: (A) *C. striatum* 1961BR-RJ; (B) *C. striatum* 1987BR-RJ; (C) *C. striatum* 2351BR-RJ; (D) *C. striatum* 2369BR-RJ. **SUP** = supernatant. Bars and dots represent bacterial counts expressed as CFU/mL at each analyzed time point (hours). Statistical analysis was performed using two-way ANOVA with multiple comparisons.

Cell-Associated Bacteria

Regarding the number of cell-associated bacteria (adherent plus intracellular bacteria), *C. striatum* isolate 2351BR-RJ exhibited the highest bacterial counts, followed by isolates 2369BR-RJ, 1987BR-RJ, and 1961BR-RJ ($P < 0.001$). Time-course analysis revealed distinct interaction profiles among the isolates.

Isolate 1961BR-RJ showed higher numbers of cell-associated bacteria at 3 h and 12 h (interaction peaks), followed by significant reductions at 6 h and 24 h (Fig. 2 and Table 6). This profile was consistent with that observed for extracellular bacterial survival; however, no direct correlation between these parameters could be established.

Isolate 1987BR-RJ exhibited a gradual increase in the number of cell-associated bacteria up to 12 h ($P < 0.0001$), followed by a slight decrease at 24 h ($P = 0.002$), indicating that the maximum interaction occurred after 12 h under the experimental conditions.

Similarly, isolate 2351BR-RJ demonstrated an increase in the number of cell-associated bacteria up to 6 h ($P < 0.0001$), after which bacterial counts remained stable until 24 h ($P = 0.781$ and $P = 0.834$).

Finally, isolate 2369BR-RJ exhibited progressive bacterial growth between 3 h and 6 h ($P < 0.0001$), remained stable from 6 h to 12 h ($P = 0.096$), and increased again by 24 h ($P < 0.0001$) (**Graphic 2**).

Graphic 2. Cell-associated *C. striatum* recovered from A549 pneumocytes, expressed as the mean $\pm$ standard deviation of colony-forming units (CFU) recovered at each time point.

Legend: (A) *C. striatum* 1961BR-RJ; (B) *C. striatum* 1987BR-RJ; (C) *C. striatum* 2351BR-RJ; (D) *C. striatum* 2369BR-RJ. **Cell-associated bacteria (BAC ASS).** Bars and dots represent bacterial counts expressed as CFU/mL at each analyzed time point (hours). Statistical analysis was performed using two-way ANOVA with multiple comparisons.

Intracellular Bacteria

As expected, isolate 2351BR-RJ exhibited the highest number of intracellular bacteria, followed by isolates 2369BR-RJ, 1987BR-RJ, and 1961BR-RJ ($P < 0.0001$). Nevertheless, no direct correlation between the numbers of cell-associated and intracellular bacteria could be established under the conditions evaluated.

Isolate 1961BR-RJ demonstrated a progressive increase in intracellular bacteria up to 12 h, representing the peak of interaction, followed by a significant reduction between 12 h and 24 h ($P < 0.05$).

Isolate 1987BR-RJ displayed a gradual increase in intracellular bacterial counts throughout the experiment, reaching the highest values after 24 h of interaction ($P < 0.0001$).

Isolate 2351BR-RJ showed increasing intracellular bacterial counts up to 6 h ($P < 0.0001$), followed by a slight decrease at 12 h, and a subsequent increase by 24 h ($P < 0.001$).

Likewise, isolate 2369BR-RJ exhibited increased intracellular bacterial counts between 3 h and 6 h ($P < 0.0001$), a slight decrease between 6 h and 12 h ($P < 0.001$), and a marked increase by 24 h ($P < 0.0001$) (**Graphic 3**).

Graphic 3. Intracellular *C. striatum* recovered from A549 pneumocytes, expressed as the mean $\pm$ standard deviation of colony-forming units (CFU) recovered at each time point.

Legend: (A) *C. striatum* 1961BR-RJ; (B) *C. striatum* 1987BR-RJ; (C) *C. striatum* 2351BR-RJ; (D) *C. striatum* 2369BR-RJ. Intracellular bacteria (BAC INT). Bars and dots represent bacterial counts expressed as CFU/mL at each analyzed time point (hours). Statistical analysis was performed using two-way ANOVA with multiple comparisons.

Intracellular Persistence

To determine whether *C. striatum* isolates were able to persist within A549 pneumocytes for prolonged periods, intracellular persistence assays were performed using all isolates after 6 h and 12 h of bacterium–cell interaction. The results demonstrated that all isolates were able to persist within pneumocytes for at least 24 h, although with different levels of persistence.

Isolate 2351BR-RJ exhibited the highest numbers of persistent intracellular bacteria, followed by isolates 1987BR-RJ, 2369BR-RJ, and 1961BR-RJ ($P < 0.0001$). Interactions involving isolates 1961BR-RJ, 1987BR-RJ, and 2369BR-RJ displayed a similar profile, characterized by a significant reduction in the number of persistent intracellular bacteria after 12 h of interaction ($P < 0.001$). In contrast, isolate 2351BR-RJ exhibited a significant increase in intracellular persistence between 6 h and 12 h ($P < 0.0001$).

After 12 h of interaction, A549 monolayers infected with isolates 2351BR-RJ and 1987BR-RJ showed visible cellular damage under light microscopy, including partial detachment of the cell monolayer. This phenomenon may have reduced the recovery of persistent bacteria because damaged cells carrying intracellular bacteria were detached and lost during the washing procedures (**Graphic 4**).

Graphic 4. Intracellular persistence of *C. striatum* in A549 pneumocytes following 24 h of vancomycin treatment, expressed as the number of colony-forming units (CFU) recovered at each interaction time point.

Legend: (A) *C. striatum* 1961BR-RJ; (B) *C. striatum* 1987BR-RJ; (C) *C. striatum* 2351BR-RJ; (D) *C. striatum* 2369BR-RJ. Persistent intracellular bacteria (BAC PERS). Bars and dots represent bacterial counts expressed as CFU/mL at each analyzed time point (hours). Statistical analysis was performed using two-way ANOVA with multiple comparisons.

DISCUSSION

The incidence of lower respiratory tract infections caused by *Corynebacterium striatum* has increased in recent years, particularly following the COVID-19 pandemic (OROSZ *et al.*, 2022; LI *et al.*, 2025). This

species has gained increasing clinical relevance as an etiological agent of severe secondary infections in immunocompromised patients and has been recognized as one of the major pathogens associated with aspiration pneumonia, together with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans* (DIBARDINO; WUNDERINK, 2015; NEILL; DEAN, 2019; XU *et al.*, 2024; LI *et al.*, 2025).

Recent studies have demonstrated that *C. striatum* is an emerging nosocomial pathogen capable of causing hospital outbreaks, highlighting its remarkable ability to produce biofilms, particularly on medical devices (SOUZA *et al.*, 2022). Biofilm formation may promote the emergence of multidrug-resistant (MDR) strains by limiting the activity of antimicrobial agents commonly used to treat bacterial infections. Consequently, this characteristic facilitates the establishment and dissemination of healthcare-associated infections, especially among patients requiring invasive medical devices (CAMPANILE *et al.*, 2009; WONG *et al.*, 2010; RAMOS *et al.*, 2019; SOUZA *et al.*, 2020, 2022).

Although *C. striatum* has been recognized as a highly pathogenic species, it has historically been neglected, and relatively few studies have investigated its virulence factors, pathogenic potential, and mechanisms of host cell damage in human tissues and organs (OROSZ *et al.*, 2022). Du *et al.* (2025) demonstrated that all isolates evaluated in their study were capable of intracellular invasion and induced different degrees of apoptosis and necrosis in infected cells.

In the present study, *C. striatum* isolates 2351BR-RJ, 2369BR-RJ, and 1961BR-RJ exhibited an aggregative adherence (AA) pattern, whereas isolate 1987BR-RJ displayed a localized adherence (LA) pattern on A549 pneumocytes. To the best of our knowledge, no previous studies have investigated the molecular composition of the bacterial factors responsible for the adherence patterns displayed by *C. striatum*, preventing direct comparison with the present findings.

The aggregative adherence pattern has been associated with an increased ability of bacterial strains to migrate into the intracellular compartment of host cells, a mechanism closely related to immune evasion and the development of invasive infections, including endocarditis (HIRATA *et al.*, 2004; HUITEMA *et al.*, 2021; WORLEY, 2023). In the present study, isolates exhibiting an aggregative adherence pattern demonstrated different levels of cellular invasion throughout the 24-hour observation period. In contrast, the isolate displaying a localized adherence pattern showed a progressive increase in intracellular invasion over time, reaching its highest level after 24 hours. These findings suggest that the bacterial adherence pattern may influence the efficiency of host-cell invasion.

Du *et al.* (2025) further demonstrated that strongly invasive isolates induced significantly higher rates of host cell death than moderately invasive isolates, suggesting an association between invasive potential and bacterial virulence. Consequently, considerable effort has been devoted to elucidating the pathogenic mechanisms of *C. striatum*. In addition, genomic analyses of *C. striatum* isolates have identified several genes involved in adhesion, biofilm formation, and intracellular survival, indicating that multiple virulence factors may act synergistically during bacterium–host interactions (JESUS *et al.*, 2023).

Although biofilm formation is widely recognized as an important virulence determinant, no direct relationship has yet been established between the intensity of biofilm production and the ability of *C. striatum* to induce cellular damage in pneumocytes (DU *et al.*, 2025). This gap in knowledge is particularly relevant in respiratory infections, where biofilm formation may promote prolonged bacterial adhesion to the pulmonary epithelium; however, this relationship remains poorly understood. Nevertheless, all isolates evaluated in the present study have previously been shown to produce biofilms on different substrates, thereby favoring the dissemination of this pathogen within the hospital environment (SOUZA *et al.*, 2020, 2022). When these findings are considered together with the observed adherence, invasion, and intracellular persistence profiles, they suggest that biofilm formation may be mechanistically associated with these virulence-related characteristics.

Some bacterial species are capable of replicating within the cytoplasm of host cells while simultaneously evading host immune responses (RESKO *et al.*, 2024). In the present study, *C. striatum* isolate 2351BR-RJ demonstrated the ability to replicate within A549 pneumocytes under the experimental conditions employed (Fig. 4). The remaining isolates persisted within the intracellular compartment without evidence of intracellular proliferation. In general, bacteria internalized by host cells are directed to lysosomes for degradation. However, several intracellular pathogens evade this process by disrupting lysosomal trafficking, remodeling specialized vacuoles, escaping from the phagolysosome into the cytosol, or surviving under acidic conditions (RESKO *et al.*, 2024). Considering that *C. striatum* was able to survive and, in one isolate, proliferate within pneumocytes, it is plausible that this species possesses mechanisms enabling survival within early endosomes, avoidance or neutralization of lysosomal degradation, and persistence within the cytosol, thereby temporarily escaping host immune recognition.

One possible explanation for this phenomenon may be the presence of mycolic acids in the mycomembrane of the *Corynebacterium* cell wall. Mycolic acids are α -alkyl, β -hydroxylated fatty acids composed of a short α -alkyl side chain and a meromycolate chain containing approximately 12–18 carbon atoms in *Corynebacterium* species. These molecules are covalently linked to the arabinogalactan–peptidoglycan complex of the bacterial cell wall and, at the opposite end, to specific sugars that form glycolipids located in the outer layer of the mycomembrane. One of the most relevant glycolipids is trehalose dimycolate (TDM), which has been implicated in host–pathogen interactions involving *Mycobacterium tuberculosis* and *Rhodococcus equi* and is therefore considered an important determinant of bacterial pathogenicity (OTT *et al.*, 2017; BURKOVSKI, 2018).

Therefore, the present study contributes to the understanding of *C. striatum* pathogenicity by demonstrating, through qualitative and quantitative analyses, its ability to adhere to, invade, and persist within human pulmonary pneumocytes. These virulence-associated traits appear to play an important role in intracellular survival and may contribute to the occurrence and persistence of nosocomial outbreaks.

CONCLUSION

All *Corynebacterium striatum* isolates evaluated in the present study were able to interact with the A549 human pneumocyte cell line. Isolates 1961BR-RJ, 2351BR-RJ, and 2369BR-RJ exhibited an aggregative adherence (AA) pattern, whereas isolated 1987BR-RJ displayed a localized adherence (LA) pattern. All isolates were capable of surviving within the intracellular compartment of pulmonary cells, suggesting that intracellular persistence may represent one of the mechanisms employed by this microorganism to evade the host immune response.

Given the increasing number of reports describing *C. striatum* in nosocomial outbreaks and healthcare-associated infections (HAIs), together with its ability to form biofilms and exhibit multidrug resistance, the additional capacity to invade, persist within, and, in some cases, proliferate inside pneumocytes further reinforces its clinical significance. In this context, a comprehensive understanding of the pathogenic mechanisms of *C. striatum* may provide valuable insights for the development of more effective strategies aimed at the prevention, surveillance, and control of infections caused by this emerging pathogen.

Declarations

Conflict of Interest: *The authors declare no conflict of interest*

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Arize Duarte, Cíntia Silva dos Santos, Fellipe de Oliveira Cabral. The first draft of the manuscript was written by Arize Duarte and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brasil) – Finance code 001 ; the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) - Processo nº E-26/200.398/2023 and E-26/210.049/2023, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), nº 311233/2022-8.

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Table 6

Table 6 is not available with this version

Graphic 1 To 4

Graphic 1 To 4 are available in the Supplementary Files section.

Figures

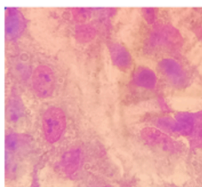
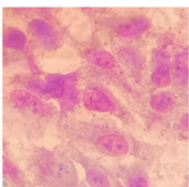
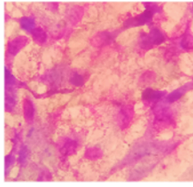
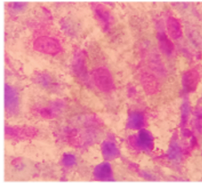
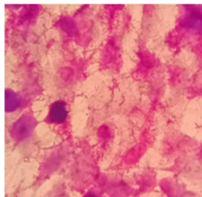
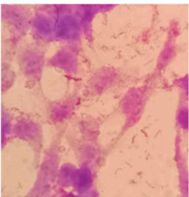
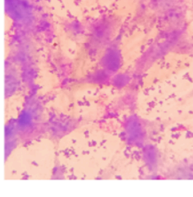
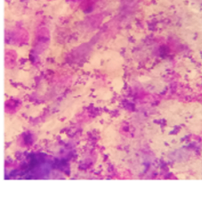
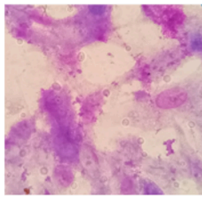
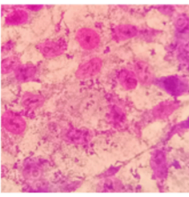
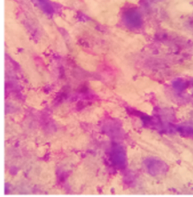
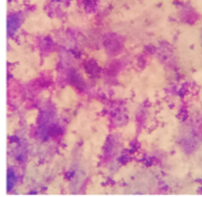
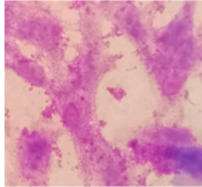
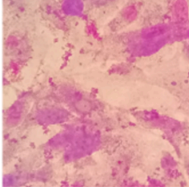
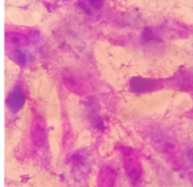
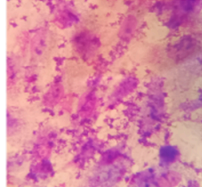
Sample (adhesion pattern)	Hours of interaction		Sample (adhesion pattern)	Hours of interaction	
	3h	6h		3h	6h
Negative control			<i>C. striatum</i> 1961 BR-RJ (Aggregative AA)		
<i>C. diphtheriae</i> CDC-E8392 (Diffuse control)			<i>C. striatum</i> 1987 BR-RJ (Localized AL)		
<i>C. diphtheriae</i> TR241 (Localized control)			<i>C. striatum</i> 2351 BR-RJ (Aggregative AA)		
<i>C. diphtheriae</i> HC01 (Aggregative control)			<i>C. striatum</i> 2369 BR-RJ (Aggregative AA)		

Figure 1

Adherence patterns of *Corynebacterium* spp. to A549 pneumocytes and the negative control

Representative optical microscopy images obtained at **400× magnification**. Scale bar = 35 μ m.

Supplementary Files

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