

Multidrug-resistant strains of non-diphtheritic corynebacterias nosocomial emerging, isolated from hospitalized patients undergoing endotracheal intubation procedures with ability to biofilm production on surfaces polyvinyl chloride and silicone

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Abstract

Non-diphtheritic corynebacteria are aerobic and anaerobic, nonacid fast, pleomorphic, nonbranching, gram-positive rods that do not form spores. Hence when isolated from clinical specimens they are often neglected as skin contaminants. But recent reports of increased rate of isolation evidenced their potential as emerging nosocomial pathogens among immunocompromized patients, inpatients on medical device, patients receiving broad spectrum antimicrobial therapy and after invasive procedures. Some of the species like *C. jeikeium*, *C. striatum* and *C. urealyticum* can cause infections among immune-competent persons and are true pathogens. Multidrug-resistant (MDR) *Corynebacterium striatum* has been cited with increased frequency as an emergence pathogen of invasive infections including nosocomial outbreak. In this study, cases of catheter-related and endotracheal intubation infections following a nosocomial outbreak predominantly caused by MDR *C. striatum* isolates was verified in a hospital of Rio de Janeiro. Different clinical isolated *C. striatum* from blood, catheter segments and tracheal secretion were identified by Maldi-tof methods. All isolates were submitted to antimicrobial susceptibility testing. The ability to biofilm formation on the surface of PVC and silicone catheter was demonstrated by previously described tests and scanning electron microscopy. All samples from the related sites showed a multidrug resistance profile. Scanning electron microscopy illustrated the ability of MDR blood, Catheter and endotracheal intubation isolate partaker of the epidemic clone (PFGE profile I) to produce mature biofilm on the surface of polyurethane and silicone catheter. Different strains of *C. striatum* strains remain in the nosocomial environment,

continue to persist and establish the etiologic agents of invasive infections as emerged. However, samples isolated from blood, catheter and endotracheal intubation appear to be predominant among patients with invasive and secondary infections. The high level of multidrug resistance associated with biofilm formation capacity observed in MDR *C. striatum* is a case of concern.

Keywords

Antimicrobial multiresistance; Biofilm; Catheter-related; Blood and endotracheal tubes infection; *C. striatum*; Nosocomial outbreak.

Introduction

Health-care Associated Infections (HAIs) occur in every health care institution, in every country, both developed and developing, and affect 1.4 million patients per year worldwide [1]. HAIs result in higher rates of morbidity and mortality, prolonged hospital stays, long-term disability, increased resistance of microorganisms to antimicrobials, higher costs for patients, families and health care systems, and preventable deaths [1].

Non-diphtheritic corynebacteria are aerobic and anaerobic, nonacid fast, pleomorphic, nonbranching, gram-positive rods that do not form spores. Hence when isolated from clinical specimens they are often neglected as skin contaminants. But recent reports of increased rate of isolation evidenced their potential as emerging nosocomial pathogens among immunocompromised patients, inpatients on medical device, patients receiving broad spectrum antimicrobial therapy and after invasive procedures [2,3]. Some of the species like *Corynebacterium jeikeium*, *Corynebacterium striatum* and *Corynebacterium macginleyi*, can cause infections among immune-competent persons and are true pathogens [4-8]. *Corynebacterium striatum* has been increasingly associated with severe infections in both immunocompetent and immunocompromised hosts [9]. However, *C. striatum* isolates have been increasingly included among the emergent etiologic agents of bacteria with different infections for world including nosocomial outbreaks [3].

Prolonged duration of hospitalization, advanced stage of chronic obstructive pulmonary disease, recent administration of antibiotics and exposure to an invasive diagnostic procedure have been highlighted as commonly found risk factors for acquiring MDR *C. striatum* infections [10,11]. Empirical antibiotic therapy may select MDR Gram-positive skin flora that may become the etiologic agent of nosocomial invasive diseases [2]. The emergence of MDR *C. striatum* and its involvement in nosocomial infections require appropriate interpretive criteria to the selection of the adequate antibiotic therapy [10].

Most reports of nosocomial infections and outbreaks caused by *C. striatum* mainly encompassed the respiratory tract [12,3]. On the other hand, few studies have investigated invasive infections by *C. striatum* [9,13,14]. In a Brazilian tertiary care hospital located at Rio de Janeiro metropolitan area, a nosocomial outbreak caused by MDR *C. striatum* mostly isolated from tracheal aspirates samples was initially verified in 2009 [12]. Subsequently, cases of bloodstream, catheter endotracheal tubes-related infections and other invasive infectious caused by *C. striatum* isolates were noticed in the same hospital [15]. In the present

study, we aimed to investigate the relationship of antimicrobial susceptibility profiles and ability of biofilm formation in materials associated with medical device such as polyvinyl chloride and silicone, related to invasive *C. striatum* infections.

Methods

Epidemiological and microbiological features of four partially studied [12] *C. striatum* BR-RJ/2009 strains representative of Two different PFGE types (2023/I, 2369/II) used in this investigation are displayed in Table 1.

***C. striatum* molecular identification by MALDI-TOF proteomic and 16S rRNA and *rpoB* gene amplification comparison**

MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-Of-Flight, Bruker Daltonics™). Each bacterial colony were tested in duplicate onto a 98-target plate to verify reproducibility and achieve proper identification. Identification criteria recommended by equipment manufacturer were as follows: score ≥ 2.000 , species-level; 1.700 - 1.999, genus level; ≤ 1.700 , no identification and 16S rRNA and *rpoB* gene amplification and sequencing assays for one strain [16].

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles were determined by the disk diffusion and by minimum inhibitory concentration (MIC) using E-test strips methods in cation-adjusted Mueller-Hinton agar supplemented with 5% sheep blood using inoculum equivalent to a 0.5 McFarland standard. Seven antibiotic disks (Oxoid, Hampshire, United Kingdom) were used: clindamycin (2 μg), moxifloxacin (5 μg), gentamicin (10 μg), rifampicin (5 μg) and vancomycin (5 μg), according Brazilian Committee on Antimicrobial Susceptibility Testing – BrCAST [17]. Another three antimicrobials imipenem (10 μg), erythromycin (15 μg) and chloramphenicol (30 μg) were interpreted in accordance to criteria defined by BrCAST for *Staphylococcus* spp. These antibiotics are not considered in Clinical and Laboratory Standards Institute (CLSI) and BrCAST/EUCAST guidelines for *Corynebacterium* spp. Linezolid 30 μg was interpreted in accordance to criteria defined by CLSI [18] for *Staphylococcus* spp. The BrCAST document recommends the use of linezolid 10 μg for antimicrobial susceptibility testing, but this is not found commercially available in Brazil.

Semi-quantitative analyses of biofilm formation on polyurethane and silicone catheter surfaces

Sterile 4 cm segments of Silicone catheters were immersed in TSB containing 10^6 CFUml⁻¹ of *C. striatum* and incubated at 37°C for 48 h then Maki's semi-quantitative roll plate technique were performed. Basically, after washing (three times) with phosphate buffered saline (PBS) 0.1 M pH 7.2, contaminated abiotic substrates were rolled up on Columbia agar plates supplemented with 5% sheep blood (Oxoid, Germany) for 48 h at 37°C were analyzed presence of bacterial carpet [9,13-15].

Quantitative tests of biofilm formation on substrates polyvinyl chloride and Silicone abiotic surfaces

Quantitative analysis of viable sessile cells of representative isolates of PFGE profile I (2023) and II (2369) was evaluated by quantitative tests based on previously described methods [9,13-15] and using the following abiotic substrates: Hydrophilic surfaces of fragment of 0,5 cm polyvinyl chloride and Silicone catheters (Intracath; Deseret Pharmaceutical Co., Sandy, Utah). Each experiment was carried out in triplicate and repeated three times. Results of the viable cell counts of experiments performed with the 2023 and 2038 strains were compared using one-way analysis of variance (ANOVA) and Tukey's multiple-comparison post-test. The values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software, San Diego, CA).

Morphological aspects of biofilm formation polyurethane and silicone catheters

The biofilm production (48 h incubation) on the surface of *in vitro* prepared on fragments of polyvinyl chloride and silicone catheters by *C. striatum* isolates from, 2023 (PFGE profile I) and 2369 (PFGE profile II), were demonstrated by *Scanning Electron Microscopy (SEM)*. The substrates were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and dehydrated in a graded series of ethanol. Subsequently, catheter segments were subjected to critical point drying with carbon dioxide, covered with a 10 nm layer of gold palladium and examined with a JEOL JSM 5310 scanning electron microscope. Sterile unused polyurethane catheters were also processed by SEM directly upon removal from commercial packaging. Specimen preparation and staining protocol for SEM were performed as described by Souza and co-workers [9,13-15]. Catheter segments infected *in vitro* with *C. striatum* 1987 (PFGE profile I), 1961 (PFGE profile III) and 1954 (PFGE profile IV) isolates were used as positive controls [12,13].

Results

C. striatum MALDI-TOF identification

All *C. striatum* strains were isolated and confirmed by different molecular identification methods.

Antimicrobial profiles

Antimicrobial susceptibility profiles *C. striatum* isolates are displayed in Table 1. All *C. striatum* isolates, regardless of their PFGE profiles and hospital settings, showed non-susceptibility to at least one agent in three or more antimicrobial categories and were consequently identified as MDR pathogens. *C. striatum* isolates related to tracheal secretion and catheter-related infections, representative of different PFGE profiles, showed susceptibility only to tetracycline, vancomycin, linezolid and daptomycin.

Semi-quantitative analyses biofilm produces *in vitro* model of catheter infection

Colonization by *C. striatum* 2023/PFGE profile I and 2369/PFGE profile II isolates was observed on fragments of silicone catheters. Results of the semi-quantitative roll plate method (>15 CFU) showed extensively adherent viable sessile forms. Both *C. striatum* strains were also able to multiply on catheter

surfaces, as illustrated in Figure 1a,1b.

Biofilm formation and survival on different abiotic surfaces

Results of quantitative analyses of viable sessile cells of *C. striatum* isolates 2023 and 2369 (PFGE profiles I and II, MDR isolates, respectively) are shown in Figure 1. Viable sessile bacterial cells were detected 48 h post-infection on surfaces of all types of abiotic substrates tested, but at different levels.

Initially, we observed that all samples analyzed were capable of producing PVC biofilm, at varying intensities, represented by the averages of viable bacterial counts (CFU/ml) extracted by abrasion of the biofilm formed after 48 hours of interaction.

The *C. striatum* 2369 MDR strain, isolated from tracheal secretion, showed the highest viable counts ($1.333 \times 10^9 + 2.055 \times 10^8$ CFU/ml) differing significantly, presenting viable counts about three times higher than the *C. striatum* 2023 isolated of blood strain ($P = 0.0002$) Figure 1.

Morphological aspects of biofilm formation on polyvinyl chloride hydrophobic and silicone catheters evaluated by SEM

Micrographs illustrating biofilm formation on the surface abiotic surfaces hydrophilic (silicone catheters) and polyvinyl chloride hydrophobic surface catheters by *C. striatum* 2023/PFGE profile I (Figure 2b-2d) and 2038/PFGE profile II (Figure 2e, 2f) isolates demonstrated by SEM are displayed in Figure 2b-2f.; Figure 2c showed microcolony formation (a hallmark of biofilm formation) by auto aggregative *C. striatum* on polyurethane surface. SEM assays also evidenced the presence of *hollow voids*, and extracellular matrix indicative of mature biofilm formation on surfaces of polyurethane (Figure 3d, 3e) and silicone (Figure 3f) catheters.

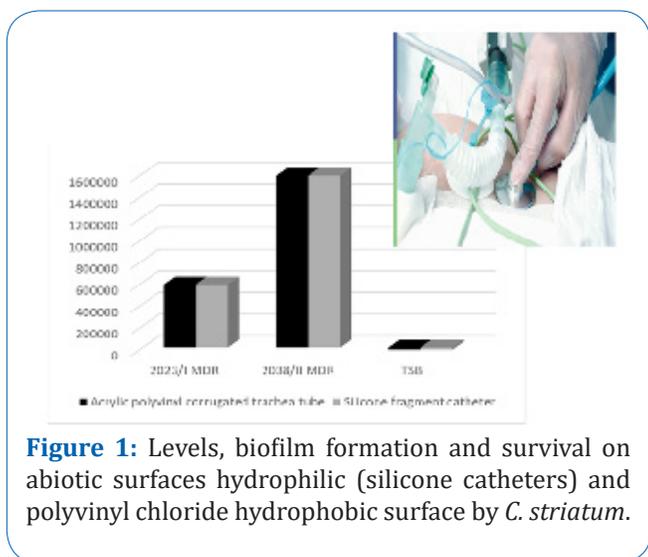


Figure 1: Levels, biofilm formation and survival on abiotic surfaces hydrophilic (silicone catheters) and polyvinyl chloride hydrophobic surface by *C. striatum*.

Table 1: Epidemiological and clinical-microbiological features of *Corynebacterium striatum* strains isolated from infected patients attended at the university hospital - HUPE/UERJ, Rio de Janeiro, Brazil

Antimicrobial resistance Profiles	Date of isolated	Strains/clone	Clinical Samples Hospital	
			Sites	Wards
MDR	2010	2023/I	Blood	General ICU and ICU II
MDR	2010	2369/II	Tracheal aspirate	General ICU

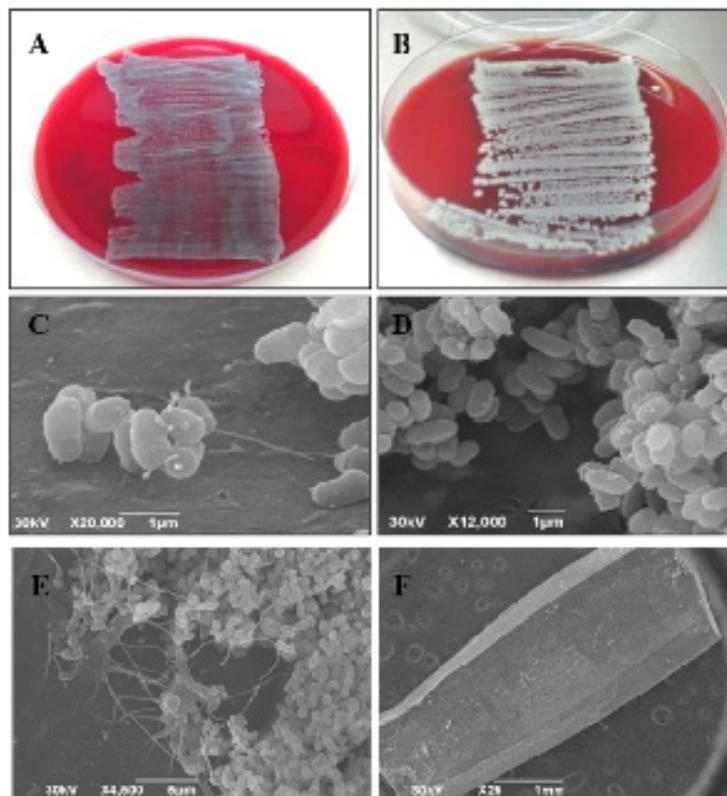


Figure 2: Biofilm formation on the surface of a silicone catheter and circular fragments of polyvinyl chloride by *Corynebacterium striatum* observed by the (A, B) semiquantitative culture technique (rolling method) recommended by Maki and (C-F) microphotographs of Scanning Electron Microscopy (ME V). Quantitative differences in the culture of viable sessile bacteria on the abiotic silicone surface subsequent to the rolling of the catheter tip segment on sheep blood agar plate were observed between (A) the multidrug-resistant sample (MDR) 2023BR-RJ/PFGE- type I (isolated from blood) characterized as strongly (+++) biofilm producer and (B) sample MDR 2038BR-RJ/PFGE-type II (isolated from catheter) characterized as moderately (++) biofilm producing. (C-F): Microphotographs illustrating steps of biofilm formation on the surface of a silicone catheter observed by SEM using the MDR 2023BR-RJ/PFGE-type I sample: (C), initial adhesion to the surface and formation of microcolonies; (D), union of several microcolonies to each other, (E), Mature biofilm surrounded by extracellular matrix and exchange channels between bacteria and the environment (hollow voids) (F), thick mature biofilm accumulated in the catheter lumen.

Discussion

Hospital-acquired infections are responsible for significantly higher mortality rates, length of stay and hospital costs, being an increasing cause for concern in healthcare worldwide. During the Covid 19 Pandemic, all this problem was aggravated, it was possible to verify the fragility of the world health system, both in terms of the scarcity of alternatives to control infections by opportunistic microorganisms as well as highlighting the deficiencies in logistics and management. aimed at coping with serious systemic events [11,19] *C. striatum* has increasingly been certified as a potentially pathogenic microorganism during the last few decades. During various period, very studies related to *C. striatum* human infections and nosocomial outbreaks were reported including catheter e and endotracheal tubes infections associated [3]. Nevertheless, routine procedures for laboratory identification of *Corynebacterium* spp. remain uncommonly undertaken in many countries or IGPR clinical isolates are frequently considered as contaminants and/or underestimated by health professionals [9,13-15].

The use of indwelling medical or transient catheters (e.g., catheters, respiratory aids devices) in current therapeutic practice is associated with most hospital-acquired systemic and deep tissue infections. New knowledge in the pathogenesis of medical device infections may lead to advances in the prevention and management of these infections [9,13-15]. In the Brazilian hospital investigated in this study, the vast majority of *C. striatum* were isolated from medical device procedures [12].

Wong and co-workers (2010) also demonstrated that *C. striatum* may also be responsible for serious hospital-acquired respiratory infection (empyema, pneumonia or acute bronchitis) and community-acquired pneumonia (CAP). Only 50% of patients who received antibiotics active against the bacterium survived the infection. Most of their patients received ventilatory support using non-invasive ventilators and/or invasive mechanical ventilators. This study was emphasized that *C. striatum* should not be simply discarded as a contaminant, especially if it is isolated as pure growth in chronic debilitated patients with multiple invasive medical devices [20].

In Brazil there were isolation of *C. striatum* from representative different epidemiological and clinical characteristics of hospitalized patients with signs and symptoms of infection [12,14,15]. In recent study, we documented four PFGE profiles during a nosocomial outbreak caused by *C. striatum* in Rio de Janeiro, Brazil. PFGE profiles I and II related MDR clones were predominantly, mostly isolated from tracheal aspirates samples of patients observed endotracheal intubation procedures. In that opportunity, only two isolates of PFGE I and II profiles were isolated from blood samples [12,16]. Due to the subsequent increased number of cases of systemic catheter-related and other medical device infections caused by *C. striatum* isolates in HUPE, current investigation revealed that PFGE I and II clones showed the ability to adhere and form biofilms on common materials in the composition of devices found in hospital environments such as PVC and silicone, establishing a strong relationship between this characteristic and its persistence in different health care environments [12].

The method of susceptibility by disk-diffusion is widely used by microbiology laboratories in Brazil and in other countries [9,15]. However, antimicrobial susceptibility testing remains rarely performed on *Corynebacterium* spp. by the laboratory community [15]. Moreover, CLSI does not provide breakpoints for disk-diffusion guidelines providing only provides breakpoints for corynebacterial susceptibility testing only for some antibiotic. Thus, staphylococcal breakpoints many researchers are used. In some studies, therapy with an association of at least two of the following antimicrobial agents has been reported: vancomycin, rifampin, linezolid and daptomycin. Presently, *C. striatum* isolates expressed five different MDR profiles [21,22].

It has been established that the majority of human infections in a nosocomial environment associated with biofilm was acquired during associated medical invasive procedures. Biofilms increase the cost of medical assistance and extend hospitalization [13]. Initiatives aimed at controlling and eliminating bacterial biofilms are important for the reduction of HAIs, reliable techniques for identifying, measuring and quantifying biofilms in addition to antimicrobial-drug resistance are necessary [15]. Recently, biofilm production on abiotic surfaces was verified for MDR and MDS *C. striatum* isolates PFGE profiles I to IV

isolated during the Brazilian nosocomial outbreak. *C. striatum* isolates were able to adhere to hydrophilic and hydrophobic abiotic surfaces [13].

In Japanese studies, a predominant PFGE profile was found among various MDR *C. striatum* isolates from different cultures from hospitalized patients. Nine *C. striatum* isolates were capable to produce biofilm only 72 h post-infection. In the present study, biofilm formation and survival on five abiotic surfaces were demonstrated 48 h post-infection of bacterial cells representative of MDR *C. striatum* PFGE profiles I and II isolated from patients with bloodstream infections, but at different levels: polyvinyl chloride hydrophobic surface and hydrophilic silicone catheters. Similar to *C. striatum* PFGE profile I isolated from patients undergoing endotracheal intubation procedures, PFGE profile I isolated from bloodstream, catheter-related and endotracheal tubes infections also showed a higher ability to adhere to and to survive on abiotic surfaces of medical devices including those used in invasive procedures. MDR *C. striatum* viable cells were able to multiply and to produce mature biofilms on both types of catheter surfaces [23].

Conclusions

The ability of several *C. striatum* clones to biofilm produces on different types of abiotic surfaces, especially those that are the main materials that make up various medical devices, in addition to resisting different antimicrobial agents' classes, may contribute to the pathogenicity favoring bacterial potential invasive and establishment of invasive infections, in addition to remaining in hospital environment and systemic. The virulent capacity of *C. striatum* should not be underestimated, particularly among high-risk patients. Therefore, antimicrobial susceptibility testing should be performed on clinically significant *C. striatum* isolates. Medical and surveillance programs should include control strategies in order to decrease potential risk factors of nosocomial infections and outbreaks due to *C. striatum* and consequently decrease in Health Care-Related Infections.

Declarations

Ethical approval and consent to participate: The consent to participate was not required because all the investigated isolates were taken as a part of standard care (diagnostic purposes). The samples were not collected for research purposes.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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