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Community-Acquired Urinary Tract Infections Due to *Corynebacterium amycolatum*, Including Multidrug-Resistant and Bla Gene-Positive Strains

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ABSTRACT

Corynebacterium amycolatum has been increasingly reported as an etiologic agent of mild to severe nosocomial infections. However, further studies concerning community-acquired and nosocomial infections due to multidrug-resistant (MDR) *C. amycolatum* remain necessary, especially in developing countries, including South America. In this study, *C. amycolatum* strains were identified as etiologic agents of 38 cases of community-acquired urinary tract infections in patients, mostly female, attended at a Brazilian ambulatorial unit: 63.16% adults (18 to ≤ 59 years old), 28.95% elderly (≥60 years old); 7.89% children (<18 years old). Most of *C. amycolatum* strains (n=33, 86.84%) expressed heterogenic MDR profiles. Interestingly, seven MDR *C. amycolatum* strains presented the *bla* gene encoding class A beta-lactamase. Therefore, additional studies must be conducted to define the clonal nature and dissemination of MDR *C. amycolatum* strains in community and hospital environment units in Brazil.

INTRODUCTION

Non-diphtheria toxin (DT)-producing *Corynebacterium* spp. have been increasingly related to different types of human infections in both immunocompetent and immunocompromised individuals. Cases of severe infections and nosocomial outbreaks due to multidrug-resistant (MDR) strains have been reported in industrialized and developing countries. Clinical relevance has been also recognized, due to the increased number of immunocompromised patients and improvements in microbiological identification procedures [1, 2, 3, 4, 5, 6, 7, 8, 9].

During the last decades, *Corynebacterium amycolatum* has been isolated from nosocomial infections, mainly in immunocompromised patients, including endocarditis, bacteremia, septicemia, catheter-related infection, otitis, and mastitis [7, 10, 11, 12, 13, 14, 15]**.** However, there are only a few reports in the literature of community-acquired and nosocomial urinary tract infections (UTIs) due to *C. amycolatum*. Recently, a study from Poland showed *C. amycolatum* as the third isolated pathogen (11.8%) in nosocomial UTIs, mostly from transplanted female patients [16].

Most of the reported cases of nosocomial infections due to *Corynebacterium* spp. were related to clinical isolates expressing MDR phenotypes [7, 11, 13]. MDR *C. amycolatum* strains have been associated with nosocomial infections with high mortality and morbidity rates [11]. Geographical variations in the frequency of *C. amycolatum* infections and MDR profiles have been reported [5, 7, 12, 15, 17, 18]. *C. amycolatum* strains have demonstrated varied resistance phenotypes, presenting resistance to the following antimicrobial agent groups: beta-lactams, aminoglycosides, macrolides, quinolones, including clindamycin and rifampin [11, 18, 19]**.**

Consequently, further studies remain necessary to investigate *C. amycolatum* clinical and pathogenicity potential features. This study aimed to report clinical, epidemiological, and microbiological aspects of UTI caused by *C. amycolatum* in outpatients attended at a Brazilian ambulatorial unit.

MATERIAL AND METHODS

Study design and origin of bacterial isolates

Urine samples of 247 outpatients with clinical signs and symptoms of lower UTI, attended at a Brazilian ambulatorial unit were sent for microbiological analysis by using regular diagnostic cultures and also inoculated onto selective chocolate-tellurite agar (CTA) media at the hospital laboratory [20, 21].

Irregular Gram-positive rods (IGPRs) strains isolated from urine samples of outpatients were sent for microbiological analysis at the Experimental Microbiology Laboratory of the Nova Friburgo Institute Health (Fluminense Federal University), for one year period and used in the present study.

IGPR strains were considered potential pathogens when growth exceeded 10^4 colony-forming units per milliliter (CFU ml^{-1}) as the only isolate or > 10^5 CFU ml^{-1} as the predominant isolate [2].

Data related to outpatients (age and sex) and other Gram-positive and Gram-negative uropathogens identified during routine procedures were provided by the hospital laboratory and included in this work.

Culture conditions and phenotypic and molecular identification procedures

Urine specimens were inoculated onto selective Tellurite-chocolate-agar media-Columbia Agar Base Difco®, with 5% defibrinated sheep blood and 1% potassium tellurite (CTA) plates and sent to the Experimental Microbiology Laboratory of Nova Friburgo Institute Health for microbiological analysis. TCA plates were incubated aerobically at 37°C for 24/48 hours up to 10 days.

Bacterial colonies which were capable of reducing potassium tellurite to tellurium and, thereby producing gray-black colored colonies, were picked up and then examined by Gram staining and tested for catalase activity [21, 22].

Clinical IGPR or coccobacilli isolates were identified by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), as previously described [11, 23], using the Biotyper system's automation control and the current Bruker Biotyper 3.1 software and library.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles were determined by the disk diffusion method on a Mueller-Hinton agar (MHA) plate (Plast Labor®, Brazil) supplemented with 5% sheep blood using a bacterial inoculum in saline (0.9% NaCl) equivalent to a 0.5 McFarland standard. Results were interpreted based on criteria used for *Staphylococcus* spp. according to CLSI guidelines, except for Cefazolin, Ampicillin, Amoxicillin-clavulanic acid, Ampicillin-sulbactam and Imipenem, which were based on the criteria for *Enterobacteriaceae*, and Vancomycin, based on the criteria for *Enterococcus* spp. Intermediate results were considered resistant [1, 10, 13, 24].

Fifteen antibiotic disks (Sensifar, Cefar Diagnóstica Ltd, Brazil) were used: penicillin (10 U), ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ampicillin-sulbactam (10/10 µg), imipenem (10 µg), cefazolin (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), vancomycin (30 µg) and rifampin (30 µg). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [9, 25, 26].

Molecular detection of resistance genes

Amplification and sequencing of bla and ampC resistance genes

C. amycolatum isolates resistant to penicillin and cefazolin (n=7) were evaluated for the presence of the *bla* and *ampC* genes, encoding for a class A beta-lactamase involved in resistance to penicillins and cephalosporins, and a class C beta-lactamase, respectively, based on previously described methods for investigating other *Corynebacterium* species [27].

Briefly, bacterial isolates were cultured on tryptic soy agar overnight at 37ºC. A single colony was inoculated into 100 µL sterile water and boiled at 100°C for 10 min. Cell debris was pelleted

by centrifugation at 16,500g per 2 minutes, and the supernatant was collected and stored at -20ºC. PCR reactions were performed using the AccuPrime™ Taq DNA Polymerase System (Invitrogen, USA) for a final volume of 25 µl containing per sample: 2.5µL of AccuPrime™ 10x PCR Buffer II, 0.5µL of each primer (10µmol/µL), 0.5µL of Accuprime™ *Taq* DNA Polymerase, 19µL of DNAse free water, and 2µL of template DNA. PCR amplification was performed under the following conditions: an initial cycle of 94ºC at 3 min, and then 35 cycles of 94ºC at 1 min, 58ºC at 45 sec, and 68ºC at 45 sec, followed by a cycle of 68ºC at 5 min with primer sets *bla* e *ampC*. Primers used to amplify the above-mentioned genes are listed in **Table 1** [27]. PCR products were purified with the ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™, USA).

Purified DNA was sequenced by Sanger methodology with the primers outlined in **Table 1**, with the automatic sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems™, USA). Sequencing reactions were performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™, USA) following standard protocols. The *bla* and *ampC* genes sequences were compared to *Corynebacterium* species available in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) algorithm [28].

Accession numbers

The accession numbers of *bla* gene sequences of isolates BR-SW-04, BR-SW-14, BR-SW-23, BR-SW-25, BR-SW-27, BR-SW-28 and BR-SW-33 were deposited at GenBank under the accession ON630901, ON630902, ON630903, ON630904, ON630905, ON630906 and ON630907, respectively.

Ethical approval

This study takes part of the main project of LDCIC laboratory that includes diagnosis activities without direct human patient contact. (I) The project (CAAE 25847614.8.0000.5259) was approved by the Research Ethics Committee of Hospital Universitário Pedro Ernesto/Universidade do Estado do Rio de Janeiro. Written informed consent for participation was not required for this study following the national legislation and the institutional

requirements, and the investigated isolate was taken as a part of standard care (diagnostic purposes); (II) Moreover this collaborative study, which did not directly involve any human or animal participants, was also approved by the Research Ethics Committee of Fluminense Federal University (CAAE: 67843417.1.0000.5626), since did not contain any human or animal participants**.**

RESULTS

C. amycolatum strains recovered from infected patients

During one year of study, 63 IGPR strains were isolated from urine samples of 247 (23.9%) outpatients with clinical signs and symptoms of UTI attended at a Brazilian ambulatorial unit and submitted to laboratory diagnostic procedures.

From a total of positive bacterial cultures for IGPRs selected for further identification due to suspect UTI, analysis by MALDI-TOF identified 59(93.65%) strains as *Corynebacterium* spp., including 38 (60.31%) as *C. amycolatum* (Score $\geq$2.0).

In **Table 2** data showed *C. amycolatum* strains isolated from patients in pure culture (n=14; 36.84%), or co-isolated with other pathogens: *Escherichia coli* (n=22; 57.90%), *Klebsiella* sp. (n= 01; 2.63%) or *Enterococcus* sp. (n= 01; 2.63%). Concomitant growth of *C. amycolatum* and other IGPR species ($\geq 10^5$ CFU ml^{-1}),as *Corynebacterium* spp. (n= 04; 10.53%), *Brevibacterium* spp. (n= 02; 5.26%) or *Brevibacillus* spp. (n= 1; 2.63%), was also observed.

C. amycolatum strains were predominantly isolated from adults at working age (63.16%), followed by the elderly (28.95%) - 97.37% from females and 2.63% from males. Moreover, *C. amycolatum* strains were also isolated from children (n=3; 7.89%); in pure culture (n=1) or numerically predominant colonies in association with *Escherichia coli* (n=2).

Antimicrobial multidrug-resistance properties

Heterogeneity of antimicrobial susceptibility testing results for C. amycolatum strains obtained from outpatients with urinary tract infection

Antimicrobial susceptibility testing results of the 38*C. amycolatum* strains are presented in **Tables 2, 3** and **Table S1.** Most of them (>50%) were found resistant to penicillin, erythromycin, and trimethoprim-sulfamethoxazole. Moreover, all *C. amycolatum* strains were resistant to nitrofurantoin and expressed resistance activity to at least one of the others antibiotics tested, except vancomycin and rifampin.

Multidrug-resistance profiles

Among the 38*C. amycolatum* strains, 33 (86.84%) expressed MDR profiles. A total of 19 MDR *C. amycolatum* strains were isolated from adults, whereas 7 were obtained from elderly females and 2 from children with 3-years old and 12-years old age. MDR *C. amycolatum* strains were grown in pure culture (n=8) or in numerically predominant colonies in association with *E. coli* (n=20) and other Gram-negative and Gram-positive bacteria, such as *Enterococcus* sp. (n=1), *Brevibacterium* spp. (n=2), *Brevibacillus* sp. (n=1) and *Corynebacterium* spp. (n=5).

Heterogeneity of antimicrobial resistance profiles expressed by *C. amycolatum* strains obtained from outpatients with urinary tract infections was demonstrated in **Tables 2 and 3**.

Antimicrobial resistance to beta-lactams

Seven (18.42%) MDR *C. amycolatum* tested strains were positive for the *bla* gene encoding class A beta-lactamase involved in resistance to penicillins and cephalosporins (**Table 4**). However, all tested strains gave negative results for the *ampC* gene encoding a class C beta-lactamase.

DISCUSSION

Urinary tract infections (UTI) are one of the most common bacterial infections in both outpatient and inpatient settings and accounts for an important proportion of healthcare costs as a result of outpatient visits, diagnostic tests, and prescriptions [29, 30].

In the present study, *Corynebacterium* spp. were isolated from 23.9% of cases of UTI in outpatients attended at a Brazilian ambulatorial unit. Interestingly, most of the clinical isolates were identified as *C. amycolatum*, predominantly isolated from adults of working age, especially women, that have been reported more vulnerable to UTI, when compared to men, due to

anatomical and physiological factors [31]. Since acute uncomplicated UTIs reach approximately 10% of women at least every year, and 60% of women at least once in their lifetime, with recurrence in 5% of cases, antimicrobial therapy is frequently prescribed [32, 33].

Gram-negative bacteria found in the gut are the most common reported uropathogens, mainly *E. coli* and *Klebsiella* spp. However, Gram-positive etiologic agents of UTI in hospitalized and outpatients are also verified, such as *Enterococcus* spp.; *Streptococcus pyogenes* and *Streptococcus agalactiae*; *Staphylococcus saprophyticus*, *S. aureus*, and *S. hominis* [34, 35, 36]. Moreover, MDR bacterial genus includes a wide range of potential urinary tract pathogens. Different species expressing MDR profiles have been increasingly reported in community-acquired infections, though prevalence differs by region [6, 36, 37, 38]. Remarkably, present findings demonstrated *C. amycolatum* strains isolated from community-acquired UTI patients, including immunocompetent children, expressing an unexpectedly high number (86.84%) of MDR profiles.

During the last decades, investigations have been demonstrating an alarmingly increasing frequency of antimicrobial resistance among pathogenic *Corynebacterium* species [1, 2, 4, 7, 9, 18]. A higher number of reported cases of infections are mainly due to deeper attention given to both the pathogenic potential and taxonomy of this bacterial genus, in addition to the increased number of immuno-compromised patients [3]. However, the importance of *Corynebacterium* isolates in clinical settings is still underestimated, either because the number of organisms present is below the culture threshold of 10^3 CFU ml^{-1}, or because these bacteria grow more slowly on usual culture media and require special culture conditions, such as enriched and selective media or prolonged incubation time, or might be incorrectly classified [18, 39]. Currently, species like *C. amycolatum* have been increasingly recovered from clinical specimens, like catheter tips, urine, and pus samples, and recognized as potential pathogens, especially in debilitated patients and as hospital-acquired pathogens [7, 13, 40].

Although the acquisition of resistance is a natural process, the misuse of antimicrobials in human and veterinary practice, inadequate surveillance, and the poorly controlled regulation of antibiotics in clinical medicine and the livestock industry has exacerbated the appearance and spread of MDR microorganisms [41, 42]. Antimicrobial resistance occurs as a result of target

alteration, decreased drug accumulation, and drug modification, and it may be an innate feature of a microorganism or may result from mutation or acquisition of exogenous resistance genes [42, 43]. Hydrolysis of beta-lactam antibiotics by beta-lactamases, the most common mechanism of resistance for this class of antimicrobial agents, was also previously observed among *C. striatum* strains. Data also revealed high resistance rates to beta-lactams and a high prevalence of the *bla* and the *ampC* genes among *C. striatum* clinical isolates from a hospital unity located at Tunisia [27].

In conclusion, *C. amycolatum* strains expressing heterogenic MDR profiles, presenting *bla*gene encoding class A beta-lactamase involved in resistance to penicillins and cephalosporins, were found as the etiologic agent of community-acquired UTI in an urban area located in Brazil. Therefore, current study showed an alarming rate of *C. amycolatum* strains characterized as uropathogens with variable antimicrobial resistance patterns in South America, emphasizing the worldwide dissemination of MDR *Corynebacterium* pathogenic species. These findings also reinforce the importance of *Corynebacterium* spp. strains not be promptly discarded as contaminants even when found associated with one or more potentially pathogenic strains in a clinical sample from hospitalized and outpatients, regardless of age, gender and comorbidities. Mechanisms of multifactorial nature may directly or indirectly contribute to the virulence potential of *C. amycolatum* strains and strategies to overcome environmental and chemical challenges, including antimicrobial agents, should be further investigated. Additional studies must be conducted to define the clonal nature and dissemination of MDR *C. amycolatum* pathogenic strains in community and hospital environment units in Brazil and other countries.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: Gomes, SWG; Póvoa, HCC; Mattos-Guaraldi, AL; **Performed the experiments:** Gomes, SWG; Longo, LGA; Sant'Anna, LO; Vieira, VV; **Data analysis and draft of the manuscript were performed by** Gomes, SWG, Sant'Anna, LO, Heggendornn, LH, Schimidt, DB, Longo, LGA, Souza, C, Vieira, VV; Mattos-Guaraldi, AL; Póvoa, HCC and Santos, LS. All authors approved the final version of the manuscript for submission.

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Tables

Table No. 1: Primers used in this study for detection and sequencing of resistance genes of *Corynebacterium amycolatum* strains

Gene	Related resistance	DNA sequence (5'-3')	Annealing temp	Size (bp)	Reference
bla	beta-lactms	CAGTCTAGCCACTTCGCCAAT TGACTGCACGGATGGAGATGG	58ºC	808	Alibi et al. 2017 adapted
ampC	beta-lactms	CAATCGGATTCCTGGTCGCT TGGTTCGCGTGATGTTTTCG	58ºC	965	

Table No. 2: Epidemiological and heterogenic antimicrobial resistance profiles of 38 *Corynebacterium amycolatum* clinical strains isolated from outpatients with urinary tract infections of different age groups and sex.

Urine clinical isolates (n=38)	**Outpatients Sex/Age**	**Culture**	**Antimicrobial resistance**	
			Agents/classes	**Profiles**
BR-SW-14	F/90	*Brevibacterium* sp.*; Escherichia coli*	PEN, AMP, AMC, SAM/ CFZ/ IPM/ ERY/ CLI/ CIP/ NIT/ SXT	**MDR**
BR-SW-33	F/49	*E. coli*	PEN, AMP, AMC, SAM/ CFZ/ IPM/ ERY/ CLI/ CIP/ NIT/ SXT	**MDR**
BR-SW-23	F/53	Pure	PEN, AMP, AMC, SAM/ CFZ/ IPM/ ERY/ CIP/ NIT/ SXT	**MDR**
BR-SW-27	M/56	Pure	PEN, AMP, SAM/ CFZ/ IPM/ GEN/ ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-28	F/48	*E. coli*	PEN, AMP, AMC/ CFZ/ IPM/ ERY/ CIP/ NIT/ SXT	**MDR**
BR-SW-04	F/31	*E. coli*	PEN, AMP AMC, SAM/ CFZ/ IPM/ ERY/ NIT/ SXT	**MDR**

BR-SW-25	F/56	Pure	PEN, AMP, SAM/ CFZ/ IPM/ CIP/ NIT/ SXT	**MDR**
BR-SW-32	F/48	*Corynebacterium sp.; E coli*	PEN/ GEN/ ERY/ CLI/ TET/ CIP/ NIT/ SXT	**MDR**
BR-SW-29	F/3	*E. coli*	PEN, AMP/ ERY/ CLI/ CIP/ NIT/ SXT	**MDR**
BR-SW-01	F/12	*E. coli*	PEN, AMP/ ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-06	F/85	*Brevibacterium* sp.	PEN, AMC, SAM/ ERY/ NIT	**MDR**
BR-SW-87	F/92	*E. coli*	PEN/ ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-49	F/76	*E. coli*	TET/ ERY/ CIP/ NIT/ SXT	**MDR**
BR-SW-78	F/38	Pure	CLI/ ERY/ CIP/ NIT/ SXT	**MDR**
BR-SW-03	F/70	Pure	PEN/ ERY/ CLI/ NIT	**MDR**
BR-SW-19	F/37	*Brevibacillus* sp.	PEN/ ERY/ CLI/ NIT	**MDR**
BR-SW-10	F/27	Pure	PEN/ ERY/ NIT/ CIP	**MDR**
BR-SW-24	F/18	*E. coli*	PEN/ ERY/ NIT/ SXT	**MDR**
BR-SW-15	F/64	*E. coli*	PEN/ ERY/ CIP/ NIT	**MDR**
BR-SW-58	F/29	*E. coli*	PEN/ ERY/ NIT/ SXT	**MDR**
BR-SW-38	F/35	*Corynebacterium sp.; E coli*	ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-26	F/31	*E. coli*	ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-37	F/21	*Corynebacterium* sp.	ERY/ CLI/ NIT/ SXT	**MDR**
BR-SW-61	F/92	*E. coli*	TET/ ERY/ NIT/ SXT	**MDR**
BR-SW-51	F/42	*E. coli*	GEN/ ERY/ NIT/ SXT	**MDR**
BR-SW-17	F/33	*E. coli*	PEN/ ERY/ NIT	**MDR**
BR-SW-60	F/58	*E. coli*	PEN/ ERY/ NIT	**MDR**
BR-SW-74	F/54	Pure	PEN/ CIP/ NIT	**MDR**
BR-SW-12	F/54	*Klebsiella* sp.	PEN/ CIP/ NIT	**MDR**
BR-SW-85	F/28	*Corynebacterium* sp.*; Enterococcus sp.*	ERY/ NIT/ SXT	**MDR**
BR-SW-84	F/75	*Corynebacterium* sp.	ERY/ NIT/ SXT	**MDR**
BR-SW-16	F/30	Pure	ERY/ CLI/ NIT	**MDR**
BR-SW-21	F/75	*E. coli*	ERY/ CLI/ NIT	**MDR**
BR-SW-52	F/72	*E. coli*	CIP/ NIT/ SXT	**MDR**
BR-SW-08	F/55	Pure	NIT/ SXT	**MDS**
BR-SW-43	F/69	*Corynebacterium* sp.*; E coli*	NIT/ SXT	**MDS**
BR-SW-20	F/44	Pure	NIT	**MDS**
BR-SW-42	F/3	Pure	NIT	**MDS**

Legend: PEN penicillin, AMP ampicillin, AMC amoxicillin-clavulanate, SAM ampicillin-sulbactam, CFZ cefazolin, IMP Imipenem ERY erythromycin, CLI clindamycin, CIP ciprofloxacin, SXT trimethoprim-sulfamethoxazole, MDR Multidrug-resistant, NIT nitrofurantoin, GEN gentamicin, TET tetracycline, MDS Multidrug susceptible

Table No. 3: Antimicrobial susceptibility levels of *Corynebacterium amycolatum* strains obtained from outpatients with urinary tract infection attended at a Brazilian ambulatorial unit

Antimicrobial Agents	**Resistance n (%)**	**Susceptibility n (%)**
Ampicillin	9 (23.68)	29 (76.32)
Ampicillin-sulbactam	7 (18.42)	31 (81.58)
Amoxicillin-clavulanic acid	6 (15.79)	32 (84.21)
Cefazolin	7 (18.42)	31 (81.58)
Ciprofloxacin	14 (36.84)	24 (63.16)
Clindamycin	15 (39.47)	23 (60.53)
Erythromycin	30 (78.95)	8 (21.05)
Gentamicin	3 (7.89)	35 (92.11)
Imipenem	7 (18.42)	31 (81.58)
Nitrofurantoin	38 (100.0)	0 (0.00)
Penicillin	22 (57.89)	16 (42.11)
Rifampin	0 (0)	38 (100)
Trimethoprim-sulfamethoxazole	25 (65.79)	13 (34.21)
Tetracycline	3 (7.89)	35 (92.11)
Vancomycin	0 (0)	38 (100)

Table No. 4: Presence of genes encoding beta-lactamase involved in mechanisms of resistance to penicillins and cephalosporins among six multidrug-resistant *Corynebacterium amycolatum* strains acting as the etiologic agent of urinary tract infections in outpatients

Clinical strains	**Antimicrobial resistance**							**beta-lactamase genes***
	beta-lactams	**Agents**						
BR-SW-14	PEN/ AMP/ AMC/ SAM/ CFZ/ IPM	ERY	CLI	-	CIP	-	SXT	*bla*
BR-SW-33	PEN/ AMP/ AMC/ SAM/ CFZ/ IPM	ERY	CLI	-	CIP	NIT	SXT	*bla*
BR-SW-23	PEN/ AMP/ AMC/ SAM/ CFZ/ IPM	ERY	-	-	CIP	NIT	SXT	*bla*
BR-SW-27	PEN/ AMP/ SAM/CFZ/ IPM	ERY	CLI	GEN	-	NIT	SXT	*bla*
BR-SW-28	PEN/ AMP/ AMC/ CFZ/ IPM	ERY	-	-	CIP	NIT	SXT	*bla*
BR-SW-04	PEN/ AMP/ SAM/CFZ/ IPM	ERY	-	-	-	NIT	SXT	*bla*
BR-SW-25	PEN/ AMP/ SAM/CFZ/ IPM	-	-	-	CIP	NIT	SXT	*bla*

Legend: MDR multidrug-resistant, ERY erythromycin, CLI clindamycin, GEN gentamicin, NIT nitrofurantoin, SXT trimethoprim-sulfamethoxazole, PEN penicillin, AMP ampicillin, SAM ampicillin-sulbactam, CFZ cefazolin, IPM imipenem, CIP ciprofloxacin, AMC amoxicillin-

clavulanic acid. *All tested strains gave negative results for the *ampC* gene encoding a class C beta-lactamase.

Supplemental material

Table S1 - Antimicrobial agents zone diameter (mm) of *Corynebacterium amycolatum* clinical isolates evaluated in this work

Legend: NIT nitrofurantoin, CIP ciprofloxacin, RIF Rifampin, AMC amoxicillin-clavulanic acid, IPM imipenem, VAN Vancomycin, CFZ cefazolin, AMP ampicillin, PEN penicillin, GEN gentamicin, TET tetracycline, CLI clindamycin, ERY erythromycin, SXT trimethoprim-sulfamethoxazole, SAM ampicillin-sulbactam.

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Supplemental material

Table S1 - Antimicrobial agents zone diameter (mm) of *Corynebacterium amycolatum* clinical isolates evaluated in this work.

	ANTIMICROBIAL AGENTS ZONE DIAMETER (mm)														
C. amycolatum strains	NIT	CIP	RIF	AMC	IPM	VAN	CFZ	AMP	PEN	GEN	TET	CLI	ERY	SXT	SAM
BR-SW-14	0	0	39	14	0	27	0	0	0	33	28	0	12	12	0
BR-SW-33	0	0	34	0	0	25	0	0	0	28	28	0	16	0	0
BR-SW-23	0	0	40	17	14	27	0	0	0	30	32	23	19	0	0
BR-SW-27	0	22	40	26	0	23	0	0	0	0	21	0	18	0	0
BR-SW-28	9	0	47	16	0	26	0	0	0	33	35	22	21	0	18
BR-SW-04	10	21	36	0	18	26	0	0	0	27	31	25	20	0	0
BR-SW-25	0	20	40	32	0	26	0	0	0	34	28	28	40	0	0
BR-SW-32	0	13	32	27	36	22	30	25	23	0	16	0	15	0	24
BR-SW-29	0	0	41	26	34	26	30	0	0	16	22	0	14	0	27
BR-SW-01	0	31	31	31	35	24	21	0	0	20	20	19	19	17	25
BR-SW-06	0	40	37	0	30	26	27	24	24	28	28	25	19	16	0
BR-SW-87	0	24	46	36	50	32	40	31	27	30	40	0	19	0	38
BR-SW-49	0	15	42	42	42	26	38	32	30	32	16	22	17	0	32
BR-SW-78	10	0	46	34	40	24	36	34	31	17	36	0	21	0	34
BR-SW-03	0	40	30	23	29	24	24	24	20	25	27	20	13	22	22
BR-SW-19	0	22	40	31	40	27	30	28	26	15	24	0	0	20	28
BR-SW-10	0	20	37	27	34	24	26	23	22	28	32	23	20	16	29
BR-SW-24	0	22	40	33	38	27	32	26	28	30	36	27	22	0	30

BR-SW-15	0	19	36	34	39	28	32	29	27	17	31	26	22	0	28
BR-SW-58	0	21	48	28	26	33	33	29	19	35	42	25	11	0	26
BR-SW-38	0	40	42	31	40	27	34	28	30	31	35	0	14	0	35
BR-SW-26	0	40	44	44	52	26	40	48	40	28	31	0	11	0	40
BR-SW-37	0	41	42	36	44	28	38	34	36	30	34	20	19	0	34
BR-SW-61	0	23	45	45	46	27	40	36	32	34	16	28	21	0	41
BR-SW-51	0	24	46	43	48	28	40	38	32	13	36	22	22	0	37
BR-SW-17	0	22	30	26	42	29	28	28	26	34	32	24	20	16	26
BR-SW-60	0	27	46	36	42	25	36	26	0	23	36	31	20	22	31
BR-SW-74	14	18	34	35	46	29	34	40	28	38	40	26	25	20	28
BR-SW-12	12	16	43	35	37	29	31	28	28	26	36	29	27	17	22
BR-SW-85	0	42	50	20	50	28	42	41	36	31	46	26	22	0	50
BR-SW-84	0	24	46	42	20	32	42	40	36	34	40	36	20	0	38
BR-SW-16	0	34	34	40	41	27	39	32	32	32	36	0	0	22	34
BR-SW-21	0	28	50	20	50	26	42	42	40	29	36	20	14	26	38
BR-SW-52	0	20	44	36	42	28	33	30	30	36	38	26	25	0	32
BR-SW-08	0	40	50	36	40	28	40	30	30	40	40	30	40	0	22
BR-SW-43	0	40	48	42	56	31	48	20	42	34	40	30	40	11	20
BR-SW-20	0	50	40	50	20	24	20	50	42	32	40	24	24	24	30
BR-SW-42	9	40	46	48	60	29	47	43	36	33	38	30	40	20	20

Legend: NIT nitrofurantoin, CIP ciprofloxacin, RIF Rifampin, AMC amoxicillin-clavulanic acid, IPM imipenem, VAN Vancomycin, CFZ cefazolin, AMP ampicillin, PEN penicillin, GEN gentamicin, TET tetracycline, CLI clindamycin, ERY erythromycin, SXT trimethoprim-sulfamethoxazole, SAM ampicillin-sulbactam.

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