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Antimicrobial susceptibility of clinical isolates of *Actinomyces* and related genera reveals an unusual clindamycin resistance among *Actinomyces urogenitalis* strains

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ABSTRACT

Objectives: Patterns of antimicrobial susceptibility in *Actinomyces* and related genera are very limited in the literature. Data of predominant susceptibility profiles could contribute to the establishment of an accurate empirical treatment.
Methods: A total of 113 isolates from clinical samples were included in this study. Each isolate was identified using phenotypic methods and MALDI-TOF/MS. When discrepancies were observed, 16S rRNA gene sequencing was performed. The minimum inhibitory concentrations (MICs) of nine antimicrobial agents (penicillin, ceftriaxone, linezolid, tetracycline, clindamycin, erythromycin, ciprofloxacin, levofloxacin and vancomycin) were tested against the species *Actinotignum schaalii* ($n = 23$), *Actinomyces turicensis* ($n = 18$), *Actinomyces europaeus* ($n = 13$), *Actinomyces naeslundii*/*Actinomyces viscosus* group ($n = 12$), *Actinomyces urogenitalis* ($n = 11$), *Actinomyces radingae* ($n = 11$), *Actinomyces neuii* ($n = 9$), *Actinomyces odontolyticus* ($n = 8$), *Bifidobacterium scardovii* ($n = 3$), *Actinomyces graevenitzii* ($n = 2$), *Alloscardovia omnicolens* ($n = 2$) and *Varibaculum cambriense* ($n = 1$).
Results: All of the isolates were susceptible to penicillin, ceftriaxone, vancomycin and linezolid. Almost all of the *A. urogenitalis* isolates (8/11) were resistant to clindamycin and showed susceptibility to erythromycin, suggesting an L-phenotype, however no determinants of clindamycin resistance (*lnu* and *lsa* genes) were detected by PCR. High MIC values to quinolones were observed in 54/113 isolates (47.8%). All of the *A. urogenitalis* isolates were highly resistant to ciprofloxacin and levofloxacin.
Conclusions: These data highlight the importance of ongoing surveillance to provide relevant information for empirical management of infections caused by these organisms.

1. Introduction

The genus *Actinomyces* is a heterogeneous group of Gram-positive, catalase-negative pleomorphic rods that are part of the commensal microbiota of the oral and gastrointestinal tract. Despite their low virulence, a wide range of species is being increasingly associated with infections [1,2].

The vast majority of infections caused by *Actinomyces* are polymicrobial and are located within their natural habitat (oral cavity, pharynx, gut, genitourinary tract and skin). However, they are also involved in other systemic and monomicrobial infections, both in immunocompromised and immunocompetent patients [1–3].

Routine laboratory identification, which requires many biochemical tests to correctly identify the species or genus, together

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with the clinical diagnosis are often troublesome [3]. Undoubtedly, sequencing of the 16S rRNA gene could contribute as a major tool in the taxonomy of these bacteria [4]. *Actinomyces* spp. have been described as susceptible to a wide range of antimicrobial agents [3]. However, in view of the lack of data in this field, an antimicrobial susceptibility study with 113 strains of *Actinomyces* and other related genera from clinical isolates was performed. We consider that this characterisation is relevant since it will contribute to recognition of the predominant susceptibility profiles of *Actinomyces* spp. Moreover, it will provide data that can be used in the choice of the most accurate empirical treatment.

2. Materials and methods

2.1. Bacterial strains

A total of 113 clinical isolates from a culture collection assembled from 2010–2015 at the university teaching hospital Hospital de Clínicas 'José de San Martin' of the Universidad de Buenos Aires (Buenos Aires, Argentina) were used in this study. All of the samples were from clinically relevant samples. Bacterial strains previously preserved at −70 °C in stock medium (BD Bacto™ brain–heart infusion broth supplemented with 20% glycerol; Becton Dickinson & Co., Franklin Lakes, NJ) were subcultured twice prior to testing on 5% sheep blood agar (bioMérieux, Marcy-l'Étoile, France) and were incubated for 48–72 h in a 5% CO_2 atmosphere at 35 °C.

The strains were from urine (n = 30), soft tissue and bone infections (n = 27), genital abscess (n = 27), head and neck abscess (n = 11), abdominal abscess and peritoneal fluid (n = 9), breast abscess (n = 4), blood (n = 2), catheter (n = 1) and sputum (n = 2) (Table 1).

All isolates were identified by conventional phenotypic methods as described previously [3] and were retrospectively identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Bacterial isolates were identified by the direct colony on-plate extraction method as previously described [5–7]. The MALDI Biotyper library v.3.0 and MALDI Biotyper software v.3.1 (Bruker Daltonik GmbH, Bremen, Germany) were used. Lowered cut-off scores for identification were used (>1.5 for genus level and >1.7 for species level). A score of <1.5 was considered as resulting in no reliable identification as suggested by Barberis et al. [8]. A minimum difference of 10% between the top and next closest score was required for a different genus or species [5].

When there was no agreement between both methods, a 16S rRNA gene sequencing strategy was used. PCR reactions were performed as previously described [9]. Sequencing of the PCR products was performed on both DNA strands using an ABI Prism® 3100 Bioanalyzer (Applied Biosystems/Hitachi, Seoul, South Korea) at the Macrogen Inc. sequencing facility (Seoul, South Korea). The sequences were analysed using BLAST v.2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/). A >99.0% (16S rRNA gene) similarity cut-off was required for species identification.

2.2. Susceptibility testing

The minimum inhibitory concentrations (MICs) of nine antibiotics (penicillin, ceftriaxone, linezolid, vancomycin, tetracycline, clindamycin, erythromycin, ciprofloxacin and levofloxacin) were determined by agar dilution in Mueller–Hinton broth with 5% sheep blood (bioMérieux) in a 5% CO_2 at 35 °C for 48–72 h. MIC breakpoints for *Corynebacterium* spp. and coryneforms were applied for all of the antimicrobial agents using Clinical and Laboratory Standards Institute (CLSI) criteria [10]. Since there are no breakpoints for levofloxacin, criteria for *Staphylococcus* spp. were used according to the CLSI [11]. Plates were inoculated with a bacterial suspension in saline of turbidity equivalent to a 0.5 McFarland standard. Quality assurance was performed using *Staphylococcus aureus* ATCC 29213.

Antimicrobial susceptibility to lincomycin and tiamulin was determined by MIC agar dilution method in a subset of clindamycin-resistant/erythromycin-susceptible isolates using *Streptococcus pneumoniae* ATCC 49619 as a control strain. As there are no CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for lincomycin, the Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM) lincomycin breakpoints for Gram-positive cocci (susceptible, ≤2 mg/L; resistant, >8 mg/L) were utilised to define lincomycin susceptibility.

Furthermore, in these isolates, lincomycin (2 μg) and clindamycin (2 μg) disks were placed at the sides of an erythromycin (15 μg) disk (Oxoid Ltd., Basingstoke, UK) 20 mm apart on Mueller–Hinton agar with 5% sheep blood (bioMérieux) for detection of inducible clindamycin resistance (iMLS$_B$ phenotype) by D-test. A lincosamide resistance mechanism was detected when an isolate

Table 1
Actinomyces and related genera according to clinical samples.

Source	No. of isolates											
	A. neuii (n = 9)	*A. viscosus* (n = 12)	*A. urogenitalis* (n = 11)	*A. europaeus* (n = 13)	*A. turicensis* (n = 18)	*A. radingae* (n = 11)	*A. odontolyticus* (n = 8)	*A. graevenitzii* (n = 2)	*Actinotignum schaalii* (n = 23)	*Bifidobacterium scardovii* (n = 3)	*Varibaculum cambriense* (n = 1)	*Alloscardovia omnicolens* (n = 2)
Blood							2					
Bone/soft tissues	1	4	7	5	2	5	1		1		1	
Catheter[a]		1										
Sputum		1						1				
Breast abscess	1				3							
Head/neck abscess		2		5			3		1			
Abdominal abscess/ peritoneal fluid	1	3			2	1	2					
Genital abscess	3	1	3	3	7	5		1	4			
Urine	3		1		4				17	3		2

[a] Peritoneal catheter.

showed erythromycin susceptibility but clindamycin resistance in the D-test screening.

In addition, the modified Hodge test (MHT) phenotypic method was used to determine the presence of enzymatic inactivation by adenylation of lincosamides [12].

The presence of the *lnu* resistance gene variants *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D) and *lnu*(E) coding for the lincosamide nucleotidyltransferase enzyme was determined by PCR as described previously [13–15]. PCR to detect *lsaA*,*lsaB*, *lsaC* and *lsaE* variants was performed using the primers described previously. *lsa* genes encode ATP-binding proteins that have been classified as class 2 ABC transporters [16,17].

3. Results

From March 2010 to March 2015, 113 strains of *Actinomyces* and other related genera were isolated from clinical samples (Table 1).

Antimicrobial susceptibility test results for the 113 *Actinomyces* spp. and other related genera tested are shown in Table 2. All isolates were susceptible to β-lactams (penicillin, ceftriaxone), linezolid and vancomycin.

The percentage of strains showing intermediate susceptibility or resistance to quinolones was 47.8%. However, when the levels of resistance were analysed by species, higher levels were observed among *Actinomyces urogenitalis* strains, with all of them being resistant to quinolones. Intermediate or resistant MICs were observed in 12 of 23 *Actinotignum schaalii*, 6 of 8 *Actinomyces odontolyticus* and 10 of 18 *Actinomyces turicensis.*

Almost all of the isolates were susceptible to erythromycin and clindamycin (78.8%). No isolate resistant to erythromycin but susceptible to clindamycin exhibited the $iMLS_B$ phenotype. However, despite the fact that some isolates showed resistance to erythromycin and clindamycin (21.2%), almost all the *A. urogenitalis* showed susceptibility to erythromycin and resistance to clindamycin. The tiamulin MIC of *A. urogenitalis* with L-phenotype was 32 μg/mL, whilst the lincomycin MIC was 1–2 μg/mL. No clindamycin resistance determinants (*lnu* and *lsa*) were detected by PCR, and all of the strains gave a negative MHT result.

4. Discussion

4.1. Identification and clinical approaches

Many species of *Actinomyces* are involved in clinical infections. In many reports, a considerable number of well recognised species have been associated with infections both in immunocompetent and immunocompromised individuals. Infections caused by *Actinomyces* were considered as uncommon diseases that could affect subjects of all ages; [2,18] however, they are involved in infections such as oral, genitourinary, abdominal, pulmonary and other infections, and most of them are expected to be polymicrobial [2,18].

Identification of *Actinomyces* or other related species has been difficult to achieve using biochemical tests [3]. However, there are a number of species differences in antimicrobial susceptibility profiles suggesting that identification may have an impact on clinical outcome, therefore correct identification could be a challenge [18]. Use of MALDI-TOF/MS allows successful identification of most of the species in a rapid, less costly and easily performed procedure compared with conventional phenotypic methods [5–8]. However, sequencing of the 16S rRNA gene is an alternative method with greater precision [4]. Although this method is extremely powerful and can be relied upon for genus-level identification, some validly published species have highly similar 16S rRNA sequences. In the *Actinomyces naeslundii*/*Actinomyces viscosus* group, 16S rRNA gene sequence analysis does not allow unequivocal identification of some of the recently described members of this group [19].

4.2. Clinical antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *Actinomyces* spp. is scarcely performed in clinical microbiology laboratories, in particular because it is assumed that strains are generally susceptible to β-lactams. Although most of the strains are non-resistant to β-lactams, some may exhibit higher MICs [20].

There are currently no CLSI or EUCAST standards for testing and reporting of antibiotic susceptibility results for *Actinomyces* spp. Susceptibility testing of *Actinomyces* spp. is not an important concern so far since they are susceptible to β-lactams agents. Whenever isolates originate from serious invasive or monomicrobial infections, antimicrobial susceptibility testing should be indicated. The MIC gradient diffusion method is the most appropriate choice [21]. On the other hand, susceptibility cannot be estimated because there may be variation between species [22].

In agreement with other authors, in this study almost all of the isolates had lower MICs to β-lactams (≤1 μg/mL) [22–24], except for a single *A. schaalii* strain (ceftriaxone MIC of 2 μg/mL) and one *A. odontolyticus* isolate (penicillin MIC of 2 μg/mL and ceftriaxone MIC of 2 μg/mL). Penicillin may not be successful in mixed infections and also it would fail in cases caused by *Actinomyces* spp. since this agent does not penetrate in abscesses or lesions [23].

It is known that fluoroquinolones generally have poor activity against *Actinomyces* spp. [25]. However, differences between susceptibility patterns among species were observed in the current study. Except for all *Actinomyces radingae* ($n = 11$), *Actinomyces graevenitzii* ($n = 2$) and the single isolate of *Varibaculum cambriense*, reduced susceptibility to fluoroquinolones was observed in the different species included. All of the *A. urogenitalis* isolates were resistant to both quinolones assayed. Data regarding the antimicrobial susceptibility of *A. urogenitalis* are very scarce; even so, the susceptibility profile of three *A. urogenitalis* strains previously described also showed high MICs to fluoroquinolones [23,26,27]. For isolates of *A. odontolyticus*, six of eight isolates demonstrated decreased susceptibility to ciprofloxacin and levofloxacin.

Nevertheless, of the 18 *A. turicensis* isolates only 1 strain showed decreased susceptibility to both fluoroquinolones, with levofloxacin being more active. Smith et al. have shown reduced susceptibility to ciprofloxacin in *A. turicensis* strains, whereas *Actinomyces europaeus* strains were susceptible [22]. In the current study, many *A. europaeus* strains had MICs above the upper limit of susceptibility.

Regarding *A. schaalii*, reduced susceptibility to ciprofloxacin was observed in 14 of 23 isolates. These results are consistent with those published by Reinhard et al., since reduced susceptibility was seen with ciprofloxacin (MIC_{90}, 4 μg/mL) [28]. However, all isolates except two were susceptible to levofloxacin. In other study by Cattoir, 100% of strains were resistant to ciprofloxacin but only 10% were resistant to levofloxacin [29].

These differences in antimicrobial susceptibility indicate the importance of correct species identification [30].

Overall, an incidence of 21.2% (24/113 strains) of resistance to both macrolides and lincosamides was observed. *Actinotignum schaalii* and *A. europaeus* showed higher rates of resistance (ca. 40%).

Smith et al. showed in their study that 87 tested isolates exhibited good susceptibility to erythromycin and clindamycin with the exception of one *A. europaeus* strain (MIC_{90}, ≥256 μg/mL)

Table 2
Susceptibility of *Actinomyces* and related genera to nine antimicrobial agents.

Species	Antimicrobial agent	MIC (μg/mL)		
		Range	MIC_{50}	MIC_{90}
Actinotignum schaalii (*n* = 23)	Penicillin	<0.063–1	<0.063	0.5
	Ceftriaxone	<0.063–2	<0.063	0.5
	Erythromycin	<0.063–128	0.125	128
	Clindamycin	<0.063–>128	0.063	64
	Tetracycline	0.063–2	0.5	1
	Ciprofloxacin	0.063–8	2	8
	Levofloxacin	0.063–2	0.5	2
	Linezolid	0.125–2	0.5	1
	Vancomycin	0.063–1	0.125	0.5
Actinomyces turicensis (*n* = 18)	Penicillin	0.063–0.5	0.063	0.25
	Ceftriaxone	<0.063–0.25	<0.063	0.25
	Erythromycin	<0.063–1	0.063	0.5
	Clindamycin	<0.063–0.063	<0.063	0.063
	Tetracycline	0.063–4	0.125	1
	Ciprofloxacin	0.063–8	2	8
	Levofloxacin	0.063–2	1	1
	Linezolid	0.25–0.5	0.5	0.5
	Vancomycin	0.125–0.5	0.25	0.5
Actinomyces europaeus (*n* = 13)	Penicillin	<0.063–1	<0.063	1
	Ceftriaxone	<0.063–1	<0.063	1
	Erythromycin	<0.063–128	0.5	128
	Clindamycin	<0.063–>128	0.125	8
	Tetracycline	0.063–4	0.5	1
	Ciprofloxacin	0.063–8	0.5	4
	Levofloxacin	0.063–4	0.25	2
	Linezolid	0.125–1	0.5	1
	Vancomycin	0.063–1	0.125	0.5
Actinomyces viscosus (*n* = 12)	Penicillin	<0.063–0.5	0.125	0.25
	Ceftriaxone	<0.063–0.5	<0.063	0.125
	Erythromycin	<0.063–64	<0.063	64
	Clindamycin	<0.063–8	0.125	1
	Tetracycline	0.063–1	0.25	0.5
	Ciprofloxacin	0.063–4	0.5	2
	Levofloxacin	0.063–2	0.5	1
	Linezolid	0.125–0.5	0.5	0.5
	Vancomycin	0.125–1	0.5	1
Actinomyces urogenitalis (*n* = 11)	Penicillin	0.25–1	0.5	1
	Ceftriaxone	<0.063	<0.063	<0.063
	Erythromycin	<0.063–1	0.063	0.125
	Clindamycin	0.25–8	1	4
	Tetracycline	0.25–16	1	16
	Ciprofloxacin	2–32	4	8
	Levofloxacin	1–16	4	4
	Linezolid	0.25–2	0.5	1
	Vancomycin	0.125–1	0.5	0.5
Actinomyces radingae (*n* = 11)	Penicillin	<0.063–0.5	<0.063	0.5
	Ceftriaxone	<0.063–1	0.063	0.5
	Erythromycin	<0.063–128	0.063	128
	Clindamycin	<0.063–128	0.063	64
	Tetracycline	<0.063–0.5	0.125	0.25
	Ciprofloxacin	0.032–1	0.125	1
	Levofloxacin	0.032–1	0.125	1
	Linezolid	0.5–1	0.5	1
	Vancomycin	<0.032–0.5	0.125	0.5
Actinomyces neuii (*n* = 9)	Penicillin	<0.063–0.25		
	Ceftriaxone	<0.063–<0.063		
	Erythromycin	<0.063–32		
	Clindamycin	0.032–32		
	Tetracycline	0.032–1		
	Ciprofloxacin	0.25–2		
	Levofloxacin	0.125–1		
	Linezolid	0.25–1		
	Vancomycin	<0.063–1		
Actinomyces odontolyticus (*n* = 8)	Penicillin	<0.063–2		
	Ceftriaxone	<0.063–2		
	Erythromycin	<0.063–4		
	Clindamycin	<0.063–32		

Table 2 (*Continued*)

Species	Antimicrobial agent	MIC (μg/mL)		
		Range	MIC_{50}	MIC_{90}
	Tetracycline	0.063–1		
	Ciprofloxacin	<0.063–8		
	Levofloxacin	<0.063–4		
	Linezolid	0.5–2		
	Vancomycin	<0.125–1		
Bifidobacterium scardovii (*n* = 3)	Penicillin	0.125–0.5		
	Ceftriaxone	0.125–1		
	Erythromycin	<0.063–0.25		
	Clindamycin	<0.063–0.125		
	Tetracycline	0.125–0.5		
	Ciprofloxacin	0.25–2		
	Levofloxacin	0.125–2		
	Linezolid	0.5–2		
	Vancomycin	0.5–1		
Alloscardovia omnicolens (*n* = 2)	Penicillin	<0.063–0.25		
	Ceftriaxone	0.25–0.5		
	Erythromycin	<0.063–<0.063		
	Clindamycin	<0.063–<0.063		
	Tetracycline	1–2		
	Ciprofloxacin	0.125–8		
	Levofloxacin	0.125–4		
	Linezolid	0.5		
	Vancomycin	0.125–1		
Actinomyces graevenitzii (*n* = 2)	Penicillin	0.063		
	Ceftriaxone	0.063		
	Erythromycin	0.063–8		
	Clindamycin	0.063–2		
	Tetracycline	0.063–4		
	Ciprofloxacin	0.063–0.5		
	Levofloxacin	0.063–1		
	Linezolid	0.5		
	Vancomycin	0.125		
Varibaculum cambriense (*n* = 1)	Penicillin	<0.063		
	Ceftriaxone	0.25		
	Erythromycin	>128		
	Clindamycin	>128		
	Tetracycline	0.5		
	Ciprofloxacin	0.5		
	Levofloxacin	0.5		
	Linezolid	0.5		
	Vancomycin	0.25		

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MICs that inhibit 50% and 90% of the isolates, respectively.

[22]. Steininger and Willinger reported resistance rates similar for all *Actinomyces* spp. (*n* = 367) of which 66% were susceptible, except for higher resistance rates in *A. europaeus* as in the current study. However, erythromycin was not included in their study [31].

In contrast, in another study it was reported that in 34 studied strains, erythromycin was the most active antimicrobial agent and clindamycin also showed very good activity in vitro [22].

Among *A. schaalii* isolates that showed reduced susceptibility to clindamycin and erythromycin, other authors have also reported high MICs to clindamycin (MIC ≥ 128 μg/mL) [24,32]. Hays et al. revealed an $iMLS_B$ resistance phenotype due to the *erm*(X) gene in these isolates [32].

The importance of being aware of macrolide and lincosamide resistance in these organisms is assumed as it is increasingly reported as skin and soft-tissue infections and other invasive infections [29,33].

Even more surprisingly, almost all of the *A. urogenitalis* strains (8/11) showed higher MICs to clindamycin but no resistance to erythromycin. Here we reported eight strains of *A. urogenitalis* that were resistant to clindamycin. This mechanism of resistance is usually due to enzymatic inactivation of lincosamides (L-phenotype) mediated by acquisition of *lnu* genes encoding nucleotidyltransferases [12,13].

More recently, another phenotype conferring cross-resistance to lincosamides, streptogramin A and pleuromutilins (LSAP phenotype) has been described in group B streptococci [17]. The *lsa*(A) gene has also been described for intrinsic resistance to lincosamides and streptogramin A in *Enterococcus faecalis*, but none of these genes has been described in *Actinomyces* spp. or other related genera [34].

We attempted to characterise the isolates with high MIC levels to clindamycin and lincomycin but susceptible to erythromycin; however, none of the *lnu* or *lsa* variants were detected. Further research is needed to fully determine whether this mechanism of resistance could be related to mutations in 23S rDNA or in ribosomal proteins.

In conclusion, *Actinomyces* strains can be present in many infections that may require long treatment. The use of rapid methods such as MALDI-TOF/MS for bacterial identification could improve the recognition of this species even in polymicrobial infections. It is important to perform susceptibility testing even though there are no guidelines for these organisms. Although they

are susceptible to many antibiotics including β-lactams, many other antimicrobial agents such as quinolones or lincosamides could be used empirically, leading to a failure treatment.

These data highlight the importance of ongoing surveillance to provide clinically relevant information to clinicians to adjust the empirical management of infections caused by these organisms.

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Competing interests

None declared.

Ethical approval

Not required.

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