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In vitro susceptibility of *Alloscardovia omnicolens* to 18 antimicrobial agents and molecular analysis of fluoroquinolone resistance

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Introduction

- ✓ *Alloscardovia omnicolens* is a catalase-negative, facultatively anaerobic Gram-positive rod that belongs to the *Bifidobacteriaceae* family
- ✓ Because of its recent description (2007) and the unreliability of conventional methods for its identification, data on *A. omnicolens* are very limited
- ✓ Particularly, no data on its in vitro antimicrobial susceptibility are available.

Objectives

❖ **The aim of the study was to assess the in vitro susceptibility of *A. omnicolens* to 18 antimicrobial agents as well as to dissect the genetic basis of acquired fluoroquinolone (FQ) resistance**

Material and methods

Bacterial strains and antimicrobial susceptibility

A total of 36 clinical isolates of *A. omnicolens* as well as the type strain CCUG 31649^T were studied. Bacterial identification was carried out using the MALDI-TOF technology (Microflex, Bruker Daltonics) and 16S rRNA sequencing.

MICs of 18 antibiotics were determined using the E-test method on Mueller-Hinton agar plate supplemented with lysed horse blood (5%) and β-NAD (20 mg/L), except for fosfomycin (agar dilution method). Interpretation of results was made according to 2014 EUCAST breakpoints.

Table 1. Primers used in the study

Primer	Nucleotide sequence (5'-3')[b]	Purpose
gyrA1-F	TGCTTAGGCGCTGACCATTA	Detection of mutation in QRDRs
gyrA1-R	CTGTTGAGCAATGGGGAGAT	
gyrA2-F	CCGTTTTCTCCTTCATCGAG	
gyrA2-R	ATACATGGCACGCAAACG	
parC1-F	TGAGTTTAGCTTAACAAAAGCACTG	
parC1-R	GATGCTGTGCCAAAGGTTGT	
parC2-F	CTGCAGCTGAGTGTGCTTGT	
parC2-R	CCTGACAGATTGTATTAAAGGGTAGA	

Whole genome sequencing

Complete genome of *A. omnicolens* CCUG 31649^T was obtained by high-throughput sequencing (Illumina MiSeq, ProfilXpert, Lyon). Data computing was performed using the CLC Workbench software (Qiagen, USA) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

PCR assays

For PCR assays, bacterial genomic DNA was extracted using the easyMAG® NucliSens extractor (bioMérieux) according to manufacturer's instructions. Mutations in quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes were screened using specific primers (Table 1). Sequencing was performed with the Sanger sequencing method in both directions using the same sets of primers (GATC Biotech, Konstanz, Germany).

Results

① Antimicrobial susceptibility

Out of the 37 strains, **all were susceptible to β-lactams** (i.e. penicillins, cephalosporins and carbapenems), **glycopeptides** (i.e. vancomycin and teicoplanin), **linezolid** and **cotrimozaxole**.

Noteworthy, **14.5%** and **81.8% of strains were resistant to nitrofurantoin** and **fosfomycin**, respectively, two antimicrobials usually recommended in empirical treatment of lower UTIs.

Surprisingly, **daptomycin** was only **active** against **37.8%** of tested isolates.

All isolates appeared intrinsically **resistant** to **gentamicin** and **metronidazole**.

Interestingly, **one strain was highly resistant to FQs** (MICs of ciprofloxacin and levofloxacin ≥32 mg/L).

Finally, **one strain exhibited high-level resistance to erythromycin and clindamycin** (MICs ≥256 mg/L).

Table 2. Antimicrobial susceptibility of the 36 *A. omnicolens* clinical isolates

Antimicrobials	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	Susceptibility breakpoint (mg/L)	% susceptible
Penicillin G	0.047	0.094	≤0.016-0.19	≤0.25	100
Amoxicillin	0.19	0.25	0.032-0.75	≤2	100
Cefotaxime	0.19	0.38	0.047-0.5	≤1	100
Imipenem	0.047	0.094	0.008-0.125	≤2	100
Gentamicin	32	≥256	4-≥256	≤2	0
Amikacin	≥256	≥256	8-≥256	≤8	2.9
Ciprofloxacin	0.75	1.5	0.094-≥32	≤0.5	48.6
Levofloxacin	0.5	1.5	0.19-≥32	≤1	83.8
Erythromycin	≤0.016	≤0.016	≤0.016-≥256	≤1	96.7
Clindamycin	≤0.016	0.023	≤0.016-≥256	≤4	96.7
Vancomycin	0.38	0.5	0.125-0.75	≤2	100
Teicoplanin	0.064	0.094	0.016-0.125	≤2	100
Daptomycin	2	12	≤0.016-16	≤1	37.8
Linezolid	0.25	0.75	0.094-1	≤4	100
Cotrimoxazole	0.023	0.047	≤0.002-0.094	≤2	100
Nitrofurantoin	0.5	128	0.064-≥512	≤64	86.5
Fosfomycin	128	512	4-2,048	≤32	18.2
Metronidazole	≥32	≥32	≥32	≤4	0

① Molecular analysis of FQ resistance

All isolates that were susceptible or low-level resistant to FQs showed identical GyrA and ParC amino acid QRDR sequences. In contrast, the unique isolate exhibiting high-level FQ resistance (i.e. ***A. omnicolens* 12342**) possessed a **unique mutation in ParC (Ser80Phe)** (*Escherichia coli* numbering) whereas no mutation was present in GyrA.

Figure 1. Amino acid sequence comparison of GyrA and ParC QRDRs (AA 67-105 and 64-102, respectively, in *E. coli* numbering) of *A. omnicolens* and other related species.

GyrA

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                                        83  87
M. criceti                  ARVVGEVMGKLHPHGDSAIYEAMVRLAQPFALRLPLVDG
B. longum                   SRVVGDVMGKYHPHGDSAIYDTLVRMAQSWSMRNMLVDG
G. vaginalis                SRVVGDVMGKYHPHGDSAIYDTLVRMAQSWSMRYMLVDG
L. delbrueckii              ARIVGDVMGKYHPHGDSSIYLAMAHMAQDFAYRYMLVDG
A. omnicolens DSM21503      ARVVGEVMGKLHPHGDSAIYEAMVRLAQPFAMRLPLVDG
A. omnicolens 12342         ARVVGEVMGKLHPHGDSAIYEAMVRLAQPFAMRLPLVDG
E. coli                     ARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDG
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ParC

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                                        80  84
M. criceti                  SRVVGDVMGKYHPHGDSAIYDTLVRMAQSWSMRYMLVDG
B. longum                   ARVVGEVMGKLHPHGDSAIYEAMVRLAQPFAMRLPLVDG
G. vaginalis                ARVVGEVMGKLHPHGDSAIYEAMVRLAQPFSLRLPLVDG
L. delbrueckii              AKAVGNIMGNYHPHGDSSIYGALVFLSQDWKMREPLIEM
A. omnicolens DSM21503      SRVVGDVMGKYHPHGDSAIYDTLVRMAQSWSMRYLLVDG
A. omnicolens 12342         SRVVGDVMGKYHPHGDFAIYDTLVRMAQSWSMRYLLVDG
E. coli                     ARTVGDVLGKYHPHGDSACYEAMVLMAQPFSYRYPLVDG
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Conclusions

Since *A. omnicolens* is likely an emerging uropathogen, it is important to note that this species is poorly susceptible to fosfomycin or nitrofurantoin, antibiotics both commonly used for the treatment of UTIs as well as there is a risk of acquired high-level FQ resistance. β-lactams, glycopeptides and cotrimozaxole should be preferred therapeutic options.