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Objectives: Facultative-anaerobic, non-motile Gram-positive rod, *Alloscardovia omnicolens* is now considered as an emerging uropathogen. Since it is still poorly studied, the aim of the work was to assess its *in vitro* susceptibility to 18 antimicrobial agents as well as to dissect the genetic basis of acquired FQ resistance.

Methods: A total of 36 clinical isolates of *A. omnicolens* collected from urine specimens as well as the type strain CCUG 31649^T were included. Identification was carried out by MALDI-TOF mass spectrometry and 16S rRNA sequencing. MICs of 18 antibiotics were determined using E-test strips 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 EUCAST clinical breakpoints. The quinolone-resistance-determining regions (QRDRs) of *gyrA* and *parC* genes were also identified and sequence analysis was performed for all strains.

Results: All 37 isolates were susceptible to penicillin G, amoxicillin, cefotaxime, imipenem, vancomycin, teicoplanin, linezolid and cotrimoxazole. One strain was highly resistant to erythromycin and clindamycin (MICs ≥ 256 mg/L). Whereas 86.5% of isolates were categorized as susceptible to nitrofurantoin, only 18.2% were susceptible to fosfomycin. Surprisingly, daptomycin was only active against 37.8% of tested isolates. All isolates appeared intrinsically resistant to aminoglycosides and metronidazole. While FQs seemed to have a moderate activity against most of the strains, one strain was highly resistant exhibiting MICs of ciprofloxacin and levofloxacin ≥ 32 mg/L. 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 possessed a unique mutation in ParC, Ser80Phe (*Escherichia coli* numbering), whereas no mutation was present in GyrA.

Conclusions: Since *A. omnicolens* is mainly an uropathogen, it is important to note that fosfomycin is not active against most of isolates and there is a risk of acquired high-level FQ resistance, and then β -lactams, cotrimoxazole or glycopeptides should be preferred. Finally, reduced susceptibility to daptomycin must be noted.

Antimicrobials	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	MIC range (mg/L)	Susceptibility breakpoint (mg/L)	Percentage 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