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Antimicrobial susceptibility and molecular mechanisms of acquired resistance in *Actinotignum* (*Actinobaculum*) *schaalii* isolated in patients with hidradenitis suppurativa

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Introduction

- ✓ Hidradenitis suppurativa (HS) or Verneuil's disease is a skin disease characterized by recurrent nodules or abscesses and chronic suppurating lesions.
- ✓ Actinomycetes are found in 24% of abscesses or nodules and in 87% of chronic suppurating lesions, including *Actinotignum schaalii* (formerly *Actinobaculum schaalii*).
- ✓ *A. schaalii* is an emerging pathogen, who has been responsible for numerous urinary tract infections, mainly in elderly (usually >60 years) and patients with underlying urological conditions.
- ✓ The combination clindamycin-rifampicin (CLI-RIF) is empirically recommended as first-line in HS patients; but there is a potential risk of emerging resistance to these two drugs.

Objectives

❖ **The aim of this study was to assess the in vitro antimicrobial susceptibility of *A. schaalii isolates and to* to dissect the genetic basis of acquired resistance to macrolide-lincosamide-streptogramin (MLS) and RIF resistance.**

Methods

Bacterial strains and antimicrobial susceptibility

❖ A collection of 22 *A. schaalii* clinical isolates were isolated from skin lesions from 14 HS patients (Dermatology department, Necker Hospital, Paris). Identification was obtained by using MALDI-TOF mass spectrometry technology (Microflex; Bruker Daltonics, Bremen , Germany).

❖ MICs of 19 antibiotics were determined using the agar dilution method on Mueller-Hinton agar plates supplemented with 5% lysed horse blood and 20 mg/L β-NAD: amoxicillin, piperacillin, ceftriaxone, imipenem, chloramphenicol, erythromycin, clindamycin, quinupristin/dalfopristin (Q/D), linezolid, tetracycline, tigecycline, ciprofloxacin, levofloxacin, moxifloxacin, vancomycin, teicoplanin, gentamicin, cotrimoxazole and rifampicin). Tested concentrations ranged from 0.002 to 256 mg/L.

Molecular techniques

❖ Screening for MLS resistance genes was performed by PCR: *erm*(A) including *erm*(TR), *erm*(B), *erm*(C), *erm*(F), *erm*(G) and *erm*(X).

❖ The rifampicin-resistance determining region (RRDR) of the *rpoB* gene coding for the β-subunit of the RNA polymrase was amplified and sequenced using specific primers (389-bp product):

-rpoB-AS-F (5'-GTGAGCTCATCCAGAACCAG-3')
-rpoB-AS-R (5'-AGATCAATTCCTTCGGCTTC-3')

Results

① Antimicrobial susceptibility

Table 1. In vitro activity of 19 antimicrobial agents against 22 clinical isolates of *A. schaalii*.

Antibiotics	MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}
Amoxicillin	0.06-0.12	0.12	0.12
Pipéracillin	0.03-0.12	0.06	0.06
Ceftriaxone	0.008-0.03	0.016	0.016
Imipenem	0.002-0.03	0.03	0.03
Chloramphénicol	0.5-4	1	1
Erythromycin	0.008->256	>256	>256
Clindamycin	0.002->256	>256	>256
Q/D	0.006-0,5	0.25	0.5
Linezolid	0.25-1	1	1
Tetracycline	0.25-0.5	0.5	0.5
Tigecycline	0.016-0.12	0.12	0.12
Ciprofloxacin	2	2	2
Levofloxacin	0.5-2	1	1
Moxifloxacin	0.5-1	0.5	1
Vancomycin	0.12-0.5	0.25	0.25
Teicoplanin	0.03-0.25	0.25	0.25
Gentamicin	0.25-1	1	1
Cotrimoxazole	4	4	4
Rifampicin	<0.002-128	<0.002	128

- ✓ All 22 isolates exhibited low MICs for amoxicillin, piperacillin, ceftriaxone, imipenem, vancomycin, teicoplanin, quinupristin-dalfopristin, tetracycline and tigecycline.
- ✓ MICs of ciprofloxacin, levofloxacin, moxifloxacin, linezolid, gentamicin and cotrimoxazole were slightly higher.
- ✓ 16 isolates (80%) were highly resistant to both erythromycin (MICs >256 mg/L) and CLI (MICs from 32 to >256 mg/L), including three isolates (15%) also resistant to RIF (MICs at 128 mg/L).

② Molecular analysis of resistance

Figure 1. Amino acid sequence comparison of RpoB RRDRs (AA 502-551 in *E. coli* numbering) of *A. schaalii* isolates.

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                          509          529  531
                           ↓            ↓    ↓
A.schaalii-16     IKEFFGTFQLSQFMDQNNPLAGLTHKRHLSALGPGGLSRDRAGMEVRDVHP
A.scaalii-4       IKEFFGTFQLSQFMDQNNPLAGLTHKRHLSALGPGGLSRDRAGMEVRDVHP
A.schaalii-wt     IKEFFGTSQLSQFMDQNNPLAGLTHKRRLSALGPGGLSRDRAGMEVRDVHP
A.schaalii-20     IKEFFGTSQLSQFMDQNNPLAGLTHKRRLLALGPGGLSRDRAGMEVRDVHP
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- ✓ All MLS-resistant isolates only harboured the *erm*(X) resistance gene.
- ✓ All RIF-resistant isolates possessed mutations in the RRDR of *rpoB* (*Escherichia coli* numbering): a double mutation (Ser509Phe + Arg529His) for two isolates and a unique mutation (Ser531Leu) for the last isolate.

Conclusions

- ❑ **This study shows a high prevalence (80%) of MLS resistance among *A. schaalii* recovered from HS patients for which CLI is commonly used.**
- ❑ **As previously described, it is due to Erm(X), suggesting that *A. schaalii* could be an important reservoir for this resistance determinant.**
- ❑ **Also, this is the first report of RIF resistance in this species with characterization of the corresponding molecular mechanism.**
- ❑ **Taken together, this confirms the risk of emerging resistance to both CLI and RIF in HS patients, which may be due, at least partially, to the antagonistic effect between these two molecules.**