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## Introduction

- The genus *Helcococcus* : catalase-negative, facultatively-anaerobic Gram-positive cocci
- 5 species described to date: *H. kunzii* (1993), *H. ovis* (1999), *H. sueciensis* and *H. pyogenica* (2004), and *H. seattlensis* (2013)
- H. kunzii* : rarely described, mainly isolated from chronic wounds of lower limbs, particularly in diabetic patients

- Objectives of the study : - Determine clinical significance,
- Compare methods of identification,
- Evaluate antimicrobial susceptibility of *H. kunzii* clinical isolates

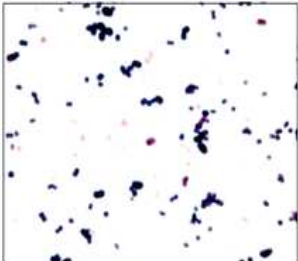


Fig. 1. Gram of *H. kunzii* (x1000)



Fig. 2. Colonies of *H. kunzii* (blood agar plate / 48h with 5% CO<sub>2</sub>)

## Methods

- From 2008 to 2013, 46 clinical isolates of *H. kunzii*, recovered from 42 patients, in 4 French hospitals (Cahors, Toulouse, Caen and Alençon) and 1 institution in Switzerland
- Clinical data were obtained for each patient : age, gender, predisposing conditions, site of isolation, clinical presentation
- Identification was carried out by ID-GP card of Vitek2 (bioMérieux) and by MALDI-TOF mass spectrometry with the Microflex LT instrument (Bruker Daltonics)
- Identifications were confirmed by sequencing of the *sodA* and *rrs* (16S rRNA) genes
- MIC values of 19 antibiotics were determined for 40 isolates by the microdilution method in Mueller-Hinton broth with lysed horse blood (5 %) and β-NAD (20 mg/L)
- Screening for the following resistance genes was performed by PCR : *erm(A)*, *erm(TR)*, *erm(B)*, *erm(C)*, *mef(A)*, *tet(L)*, *tet(M)* and *tet(O)*

## Results

### Identification

Phenotypic		Genotypic
ID-GP card of Vitek 2	Microflex LT	<i>sodA</i> / <i>rrs</i> genes
<b>Correct identification to the species level with the 3 methods</b>		
93-99% of probability	Score : 2.01 à 2.49 Including 90% : > 2.21	98-100 % nucleotide sequence identity with the type strain

### References

- Collins MD, Facklam RR, Rodrigues UM, Ruoff KL. Phylogenetic analysis of some Aerococcus-like organisms from clinical sources: description of *Helcococcus kunzii* gen. nov., sp. nov. Int J Syst Bacteriol. 1993 Jul;43(3)
- Leclercq R. Mechanisms of resistance to macrolides and lincosamides : nature of the resistance elements and their clinical implications. Clin Infect Dis Off Publ Infect Dis Soc Am. 2002 Feb 15;34(4)
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## Clinical data

Tab. 1 : Clinical data for a sample of 14 patients (28 patients of the study not presented here)

Age (yr) / Sex	Underlying comorbidity(ies)	Site of isolation	Clinical presentation	Concomitant organism(s)
57/M	Diabetes	Foot	Infected diabetic foot ulcer complicated by osteoarthritis	<i>S. aureus</i> , <i>P. aeruginosa</i>
84/M	PAOD, coronary artery disease, stroke, hypertension, overweight, alcoholism	Foot	Arterial ulcer complicated by osteitis	<i>S. aureus</i> , <i>Corynebacterium</i> sp.
91/M	PAOD, coronary artery disease, hypertension	Toe	Critical ischemia with purulent gangrene extending to the forefoot	<i>E. coli</i> , <i>Acinetobacter radioresistens</i> , Anaerobic flora
52/M	Diabetes, overweight	Buttock abscess	Large abscess of the buttock with cellulitis	Anaerobic flora
74/M	Diabetes, PAOD, hypertension	Foot (liquid drainage)	Infected diabetic foot ulcer complicated by osteoarthritis	<i>Enterococcus</i> sp., CNS
37/M	Paraplegia	Sacral pressure ulcer	Infected pressure ulcer	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>Actinobacillus</i> sp., CNS
57/M	Diabetes, paraplegia, alcoholism	Sacral pressure ulcer	Severe sepsis caused by infected sacral pressure ulcer	<i>S. aureus</i> , <i>E. coli</i> , <i>Corynebacterium</i> sp., Blood cultures : <i>E. coli</i> (identical profil to ulcer)
69/M	Diabetes, Charcot foot, PAOD, coronary artery disease, venous insufficiency, hypertension, overweight	Foot (isolated in 3 samples at intervals of several months)	Infected diabetic foot ulcer complicated by osteitis	<i>S. agalactiae</i> , <i>C. koseri</i> , <i>Corynebacterium</i> sp.
84/M	PAOD, coronary artery disease, congestive heart failure	Blood culture	Septic shock caused by infected foot ulcers	<i>S. aureus</i> , <i>Proteus</i> sp., <i>Providencia</i> sp., <i>F. nucleatum</i>
49/M	Diabetes, PAOD	Toes (deep pus)	Purulent gangrene 3rd, 4th and 5th toes	<i>S. agalactiae</i>
82/M	Diabetes, hypertension	Foot (bone biopsy)	Osteitis after amputation of the 5th metatarsal	None
47/F	Radiotherapy in iliac region for a desmoid tumor -> radiodermatitis	Hip (deep pus)	Osteo-arthritis of the hip	<i>S. aureus</i> , <i>S. dysgalactiae</i>
63/M	Diabetes, hypertension	Foot (tissue biopsy)	Osteitis	<i>Enterococcus</i> sp.
63/M	Diabetes, venous insufficiency	Toe (tissues biopsies x3 : <i>H. k.</i> : 3/3+)	Septic bone necrosis of 5th toe	<i>S. aureus</i>

PAOD : peripheral arterial occlusive disease ; CNS : coagulase-negative staphylococcus

Fig. 3 : Sites of isolation for the 46 isolates

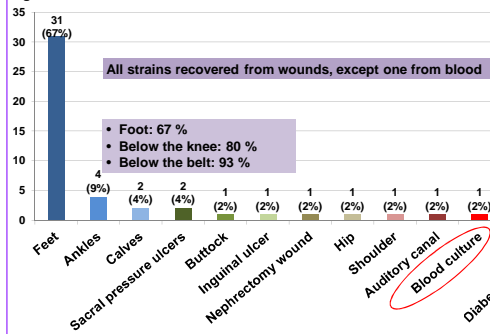


Fig. 4 : Origin of the wound

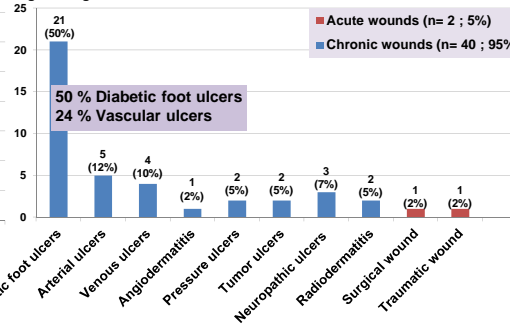
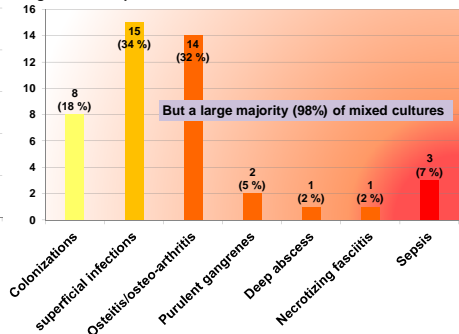


Fig. 5 : Clinical presentation



## Antimicrobial susceptibility

Tab. 2 : MIC distributions of 40 isolates

Antibiotics	MIC <sub>50</sub>	MIC <sub>90</sub>	Ranges	% susceptible isolates *
Penicillin G	0.03	0.06	0.01-0.12	100
Amoxicillin	0.06	0.12	0.01-0.25	100
Cefotaxim	0.25	0.5	0.01-1	97.5
Imipenem	0.01	0.03	0.01-0.03	100
Ciprofloxacin	2	4	1-32	17
Levofloxacin	1	2	0.25-32	88
Erythromycin	1	>256	0.25->256	14.5
Clindamycin	0.25	>256	0.03->256	75.5
Quinolipristin-Dalopristin	0.25	1	0.12-2	97.5
Gentamicin	2	4	0.5-64	10
Vancomycin	0.5	0.5	0.25-1	100
Teicoplanin	0.12	0.25	0.03-0.25	100
Tetracycline	0.12	0.25	0.05-16	95
Tigecycline	<0.01	<0.01	<0.01	100
Daptomycin	0.5	0.5	0.25-1	100
Linezolid	2	2	1-2	100
Rifampicin	0.03	0.12	<0.01-2	85.5
Cotrimoxazole	0.5	1	0.12-8	90
Colistin	>256	>256	>256	0

\* Accordant to breakpoints of EUCAST for streptococcus

- > Macrolides-Lincosamides-Streptogramins (MLS): Acquired high-level of resistance
  - MIC erythromycin and MIC clindamycin ≥256 mg/L : 10 strains (25 %) → due to the presence of the *ermTR* gene [subclass of *erm(A)*]
  - MIC erythromycin ≥ 256 mg/L and clindamycin = 0.25 mg/L (with antagonism) : 1 strain → negative for the screened genes

- > Tetracyclines: 2 strains (5 %) resistant to tetracycline → due to the *tet(M)* gene

- > Fluoroquinolones:
  - Low-level resistance to ciprofloxacin and levofloxacin
  - Acquired high-level resistance: 2 strains (5 %)

### Antibiotic treatment:

- Most antibiotics used to treat usual pathogens isolated in chronic wounds, also appear to be effective against *H. kunzii*
- If clindamycin is used, susceptibility testing is required, due to the incidence of high-level resistance, especially mediated through the acquisition of *erm(TR)*
- Avoid the use of fluoroquinolones

## Conclusion

- > To date, this is the largest case series of infections caused by *H. kunzii*, an emerging pathogen usually recovered from polymicrobial samples
- > *H. kunzii* colonizes chronic wound, especially those of lower limbs in patients suffering from trophic disorders; from this initial skin entry, it can be involved in deep and/or systemic infections