

The background of the slide is a microscopic image showing numerous small, purple-stained cocci (bacteria) arranged in various patterns, including chains and clusters. The background has a light blue/teal tint.

**Gram-positive anaerobic cocci:
clinical relevance, changed
taxonomy, identification and
antibiotic resistance**

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Introduction

Gram-positive anaerobic cocci

Peptostreptococcus sp. (formerly)

New species and taxonomy

Identification

Antibiotic resistance

Virulence *F. magna*

GPAC in general

Clinical relevance: \pm 30% of all anaerobes recovered from human clinical specimens

Most GPAC:

- mixed infections
- susceptible for antibiotics usually used to treat anaerobic infections

Metronidazole-resistance:

- strict anaerobic cocci are generally sensitive
- micro-aerophilic cocci are generally resistant

Introduction

Gram-positive anaerobic cocci (GPAC):

Peptococcus

P.niger

Seldomly isolated from clinical material

Ruminococcus

Faecal microbiota

Seldomly isolated from clinical material

Insufficient culture methods?

Coprococcus

Isolated from faecal samples

Peptostreptococcus

The genus *Peptostreptococcus* contained 13 species.

Clinically most relevant (literature):

P. anaerobius

P. asaccharolyticus

P. magnus

P. micros

New species, changed taxonomy (in chronological order)

In 1997 3 new species were added to the genus
Peptostreptococcus

P. harej

P. ivorii

P. octavius

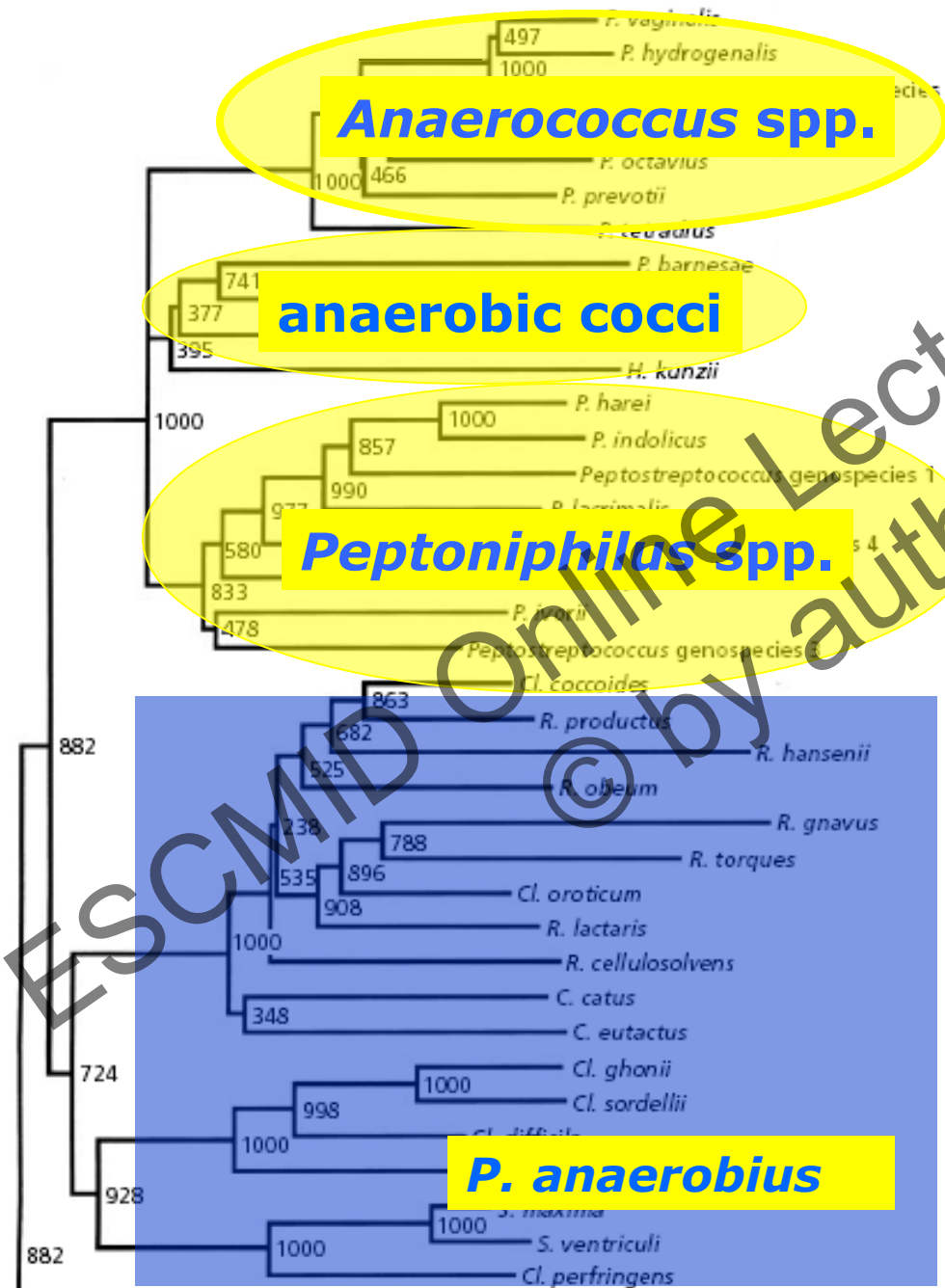
Clinical relevance unknown

Description of Three New Species of the Genus *Peptostreptococcus* from Human Clinical Specimens: *P. harej* sp. nov., *P. ivorii* sp. nov., and *P. octavius* sp. nov., D. A. Murdoch et al., Int. J. Syst. Bact. 1997

New taxonomy

Reclassification of *Peptostreptococcus magnus* (Prevot 1933) Holdeman and Moore 1972 as *Finegoldia magna* comb. nov. and *Peptostreptococcus micros* (Prevot 1933) Smith 1957 as *Micromonas micros* comb. nov. Murdoch et al., *Anaerobe*, 5, 1999

Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus* Ezaki et al., *Int J syst Evol Microbiol*, 51, 2001



Anaerococcus spp.

anaerobic cocci

Peptoniphilus spp.

P. anaerobius

Anaerobic cocci:

Fingoldia magna

Parvimonas micra

Gallicola barnesea

Helcococcus kunzii

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A rollercoaster of new species (1)

Species name	year	origin
<i>Peptostreptococcus stomatis</i>	2006	Human oral cavity
<i>Peptoniphilus allenii</i>	2006	Human clinical specimen
<i>Anaerococcus murdochii</i>	2007	Human clinical specimen
<i>Peptoniphilus gorbachii</i>	2007	Human clinical specimen
<i>Peptoniphilus olsenii</i>	2007	Human clinical specimen
<i>Murdochiella asaccharolytica</i>	2010	Human clinical specimen
<i>Peptostreptococcus russellii</i>	2011	Swine manure
<i>Peptoniphilus methioninivorax</i>	2011	Retail ground beef
<i>Peptoniphilus koenoeniae</i>	2011	Human clinical specimen
<i>Peptoniphilus duerdenii</i>	2011	Human clinical specimen

A rollercoaster of new species (2)

Species name	year	origin
<i>Peptostreptococcus canis</i>	2012	Oral dog
<i>Anaerococcus obesiensis</i>	2012	Human faeces
<i>Anaerococcus senegalensis</i>	2012	Human faeces
<i>Peptoniphilus coxii</i>	2012	Human clinical specimen
<i>Peptoniphilus tyrelliae</i> (senegalensis)	2012	Human clinical specimen
<i>Peptoniphilus grossensis</i>	2012	Human faeces
<i>Peptoniphilus timonensis</i>	2012	Human faeces
<i>Anaerococcus pacaensis</i>	2013	Human clinical specimen
<i>Peptoniphilus obesi</i>	2013	Human faeces

A rollercoaster of new species (3)

Species name	year	origin
<i>Peptoniphilus rhinitidis</i>	2014	Human clinical specimen
<i>Peptoniphilus mikwangii</i>	2014	Human clinical specimen
<i>Anaerococcus degenerii</i>	2015	Human clinical specimen
<i>Anaerococcus nagyae</i>	2016	Human clinical specimen
<i>Anaerococcus urinomassiliensis</i>	2016	Human clinical specimen
<i>Anaerococcus rubiinfantis</i>	2016	Human faeces
<i>Peptoniphilus catoniae</i>	2016	Human faeces

Peptostreptococcus

4 species

Anaerococcus

14 species

Peptoniphilus

19 species

Phenotypic identification

Enzymatic tests derived from the API 32A (BioMerieux) → Table Wadsworth manual

Reliable identification: - *F. magna*
- *P. micra*

P. harei – *P. asaccharolyticus* → similar biochemical features

The addition of new species made available schemes for the identification of GPAC less useful



Identification of GPAC by MALDI-TOF MS

Bruker system

- 54% of GPAC encountered in human clinical specimens is identified by MALDI-TOF MS (estimated)
- On target extraction with 70% formic acid is recommended
- *A. vaginalis* is misidentified as *A. hydrogenalis*
- *P. harei* is correctly identified
- New species are not represented in de MALDI-TOF MS database

The optimization and validation of the Biotyper MALDI-TOF MS database for the identification of gram positive anaerobic cocci

A.C.M Veloo¹, E.D. de Vries¹, H. Jean-Pierre^{3,4}, U.S. Justesen⁵, T. Morris⁶, E. Urban⁷, I. Wybo⁸, A.J. van Winkelhoff^{1,2}, on behalf of the ENRIA workgroup

Clin Microbiol Infect 2016; doi: 10.1016/j.cmi.2016.06.016

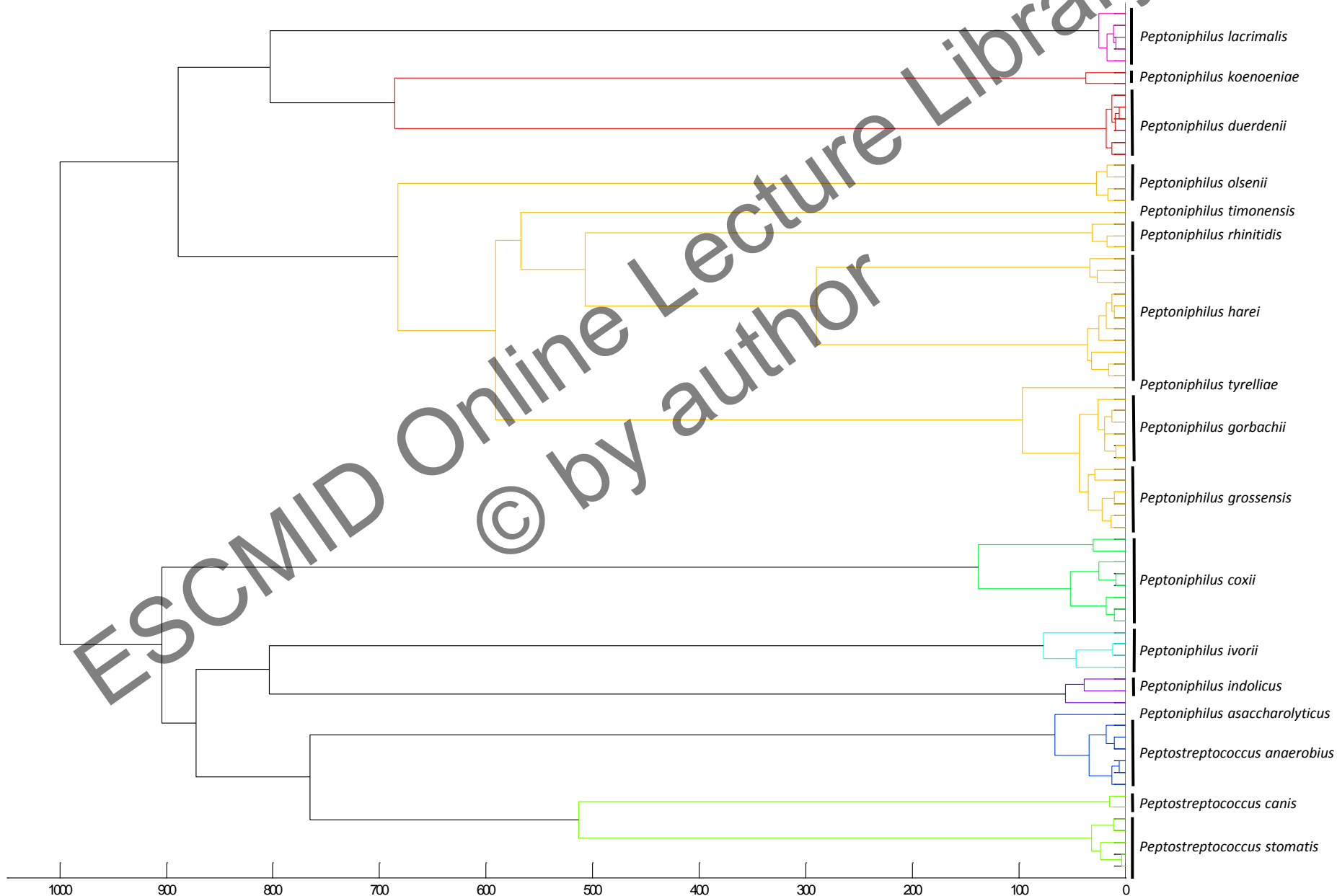
Added to database:

Main Spectral Profiles of 108 clinical GPAC isolates, representing 17 different species

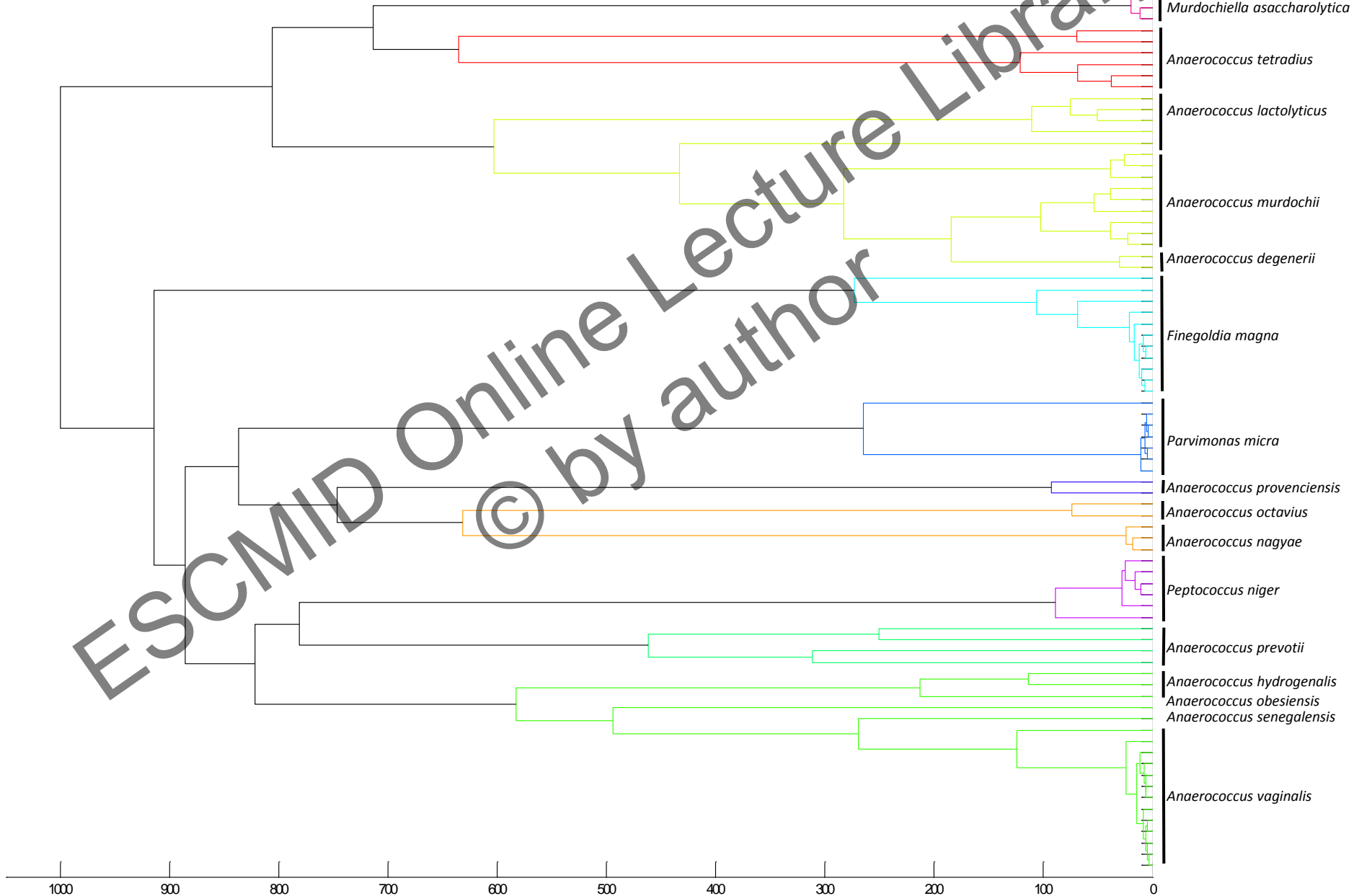
Validation database:

140 clinical GPAC isolates

Dendrogram of MSPs created of strains belonging to the genera *Peptoniphilus* and *Peptostreptococcus*



Dendrogram of MPSs created of strains belonging to the genera *Anaerococcus*, *Murdochiella*, *Peptococcus*, *Finegoldia* and *Parvimonas*

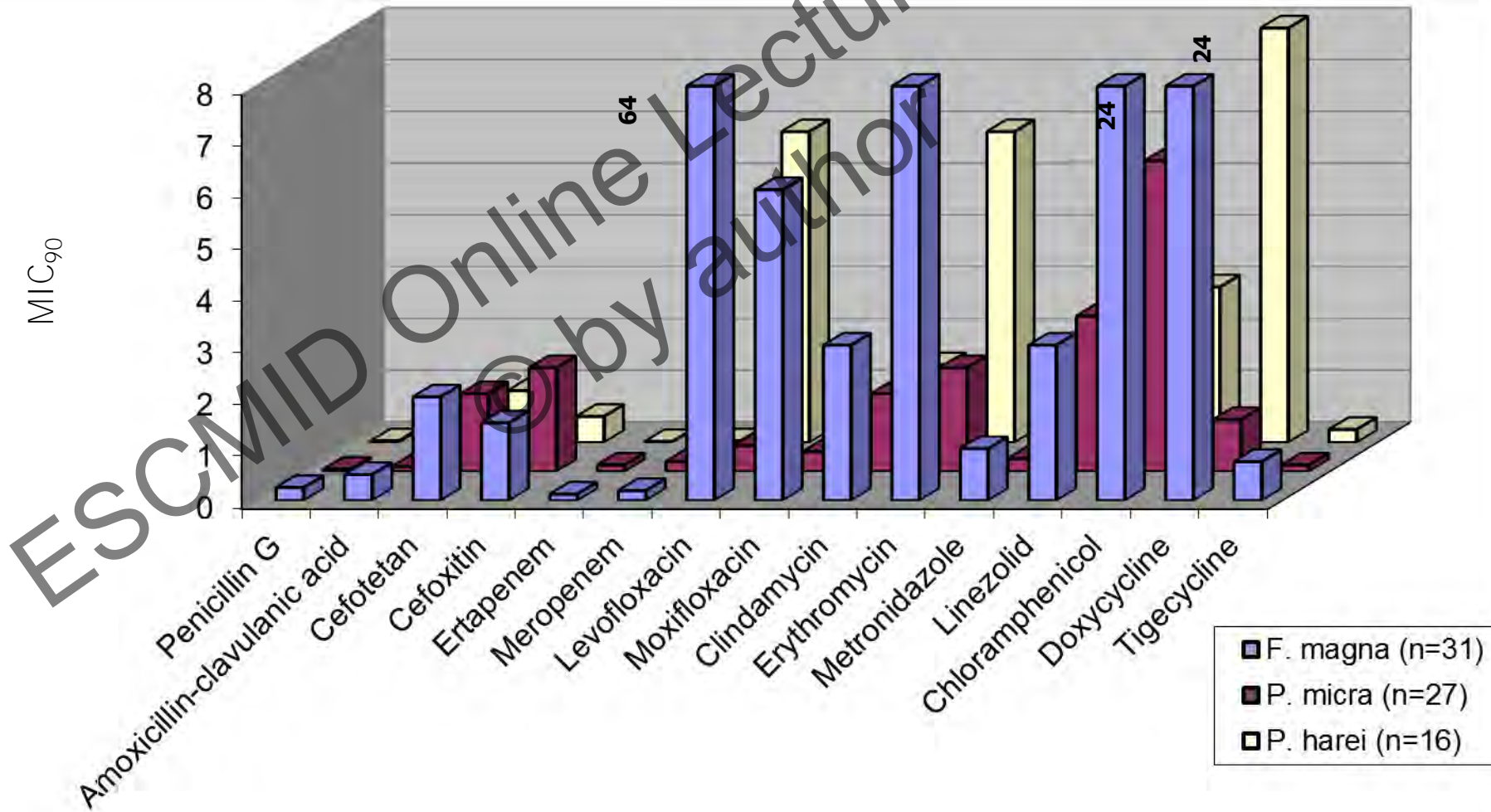


Results validation

species ID	Bruker database + ENRIA GPAC database						no. of strains of which the log score increased
	Bruker database			Bruker database + ENRIA GPAC database			
	score ≥ 2	score $>1.7 - <2$	score ≤ 1.7	score ≥ 2	score $>1.7 - <2$	score ≤ 1.7	
<i>P. harei</i> (n=28)	19	8	1	28	0	0	26
<i>F. magna</i> (n=25)	16	8	1	16	8	1	0
<i>P. micra</i> (n=27)	25	2	0	25	2	0	0
<i>A. vaginalis</i> (n=8)	0	2	6	8	0	0	8
<i>A. murdochii</i> (n=5)	4	1	0	5	0	0	1
<i>A. hydrogenalis</i> (n=2)	0	0	2	0	2 ^e	0	2
<i>A. obesiensis</i> (n=2)	0	0	2	0	0	2	0
<i>P. anaerobius</i> (n=8)	7	1	0	8	0	0	8
<i>P. stomatis</i> (n=2)	0	0	2	1	1	0	2
<i>M. asaccharolytica</i> (n=3)	0	0	3	3	0	0	3
<i>P. coxii</i> (n=6)	0	0	6	5	1	0	6
<i>P. lacrimalis</i> (n=3)	0	0	3	3	0	0	3
<i>P. grossensis</i> (n=2)	0	1	1	1	1	0	2
<i>P. lactolyticus</i> (n=2)	1	0	1	2	0	0	1
<i>P. gorbachii</i> (n=2)	1	0	1	2	0	0	2
<i>P. duerdenii</i> (n=2)	0	0	2	2	0	0	2
different GPAC species (n=4)	0	0	4	4	0	0	4
GPAC (9)	2	0	7	2	1	6	2
Total (n)	75	23	42	115	16	9	72
% of all strains	53.6%	16.4%	30.0%	82.1%	11.4%	6.4%	51.4%

Resistance GPAC in the Netherlands 2002-2004

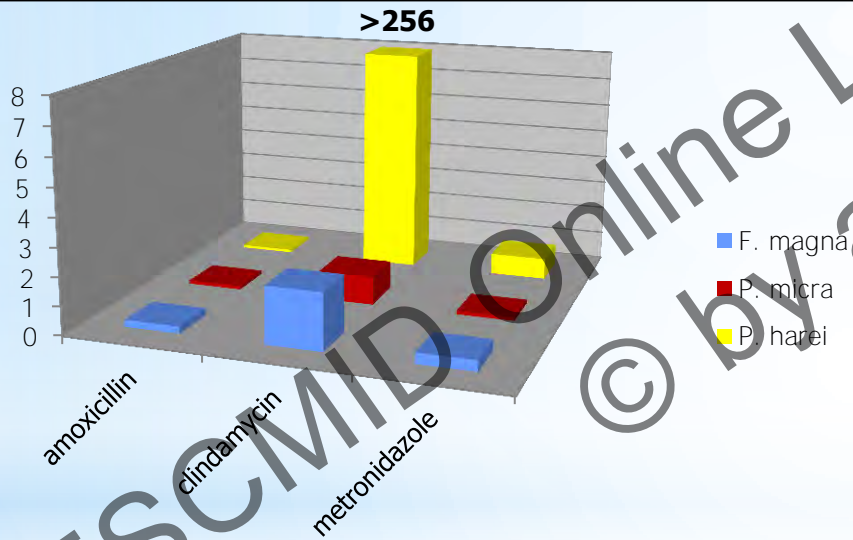
Antimicrobial susceptibility of clinically relevant gram-positive anaerobic cocci, collected in a 3-year period in The Netherlands
Veloo et al., Antimicrob Agent Chemother, 2011;55:1199-1203



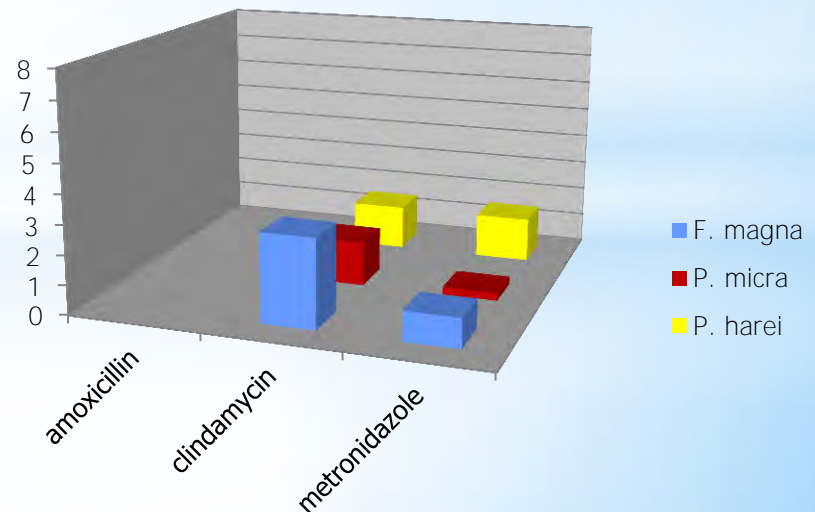
Resistance GPAC in The Netherlands 2011-2013

Antibiotic susceptibility profiles of anaerobic bacteria in the Netherlands
Veloo ACM, van Winkelhoff AJ
Anaerobe 2015; 31:19-24

2011-2013 MIC₉₀



2002-2004 MIC₉₀



**Evaluation of antibiotic susceptibility of gram-positive anaerobic cocci isolated from cancer patients of the N. N. Blokhin Russian cancer research center
Shilnikova et al. J Pathogens 2015; article ID 648134**

81 GPAC strains collected from 2004-2014

Variable resistance of the different species for:

- penicillin G
- amoxicillin/clavulanic acid
- metronidazole
- ciprofloxacin
- levofloxacin

Two metronidazole resistant *P. micra* strains

One multi-drug resistant (penG, cipro, levo, amoxi/clav, metro) *F. magna* strain

Antibiotic susceptibilities of gram-positive anaerobic cocci: results of a sentinel study in England and Wales

Brazier JS et al. *J Antimicrob Chemother* 2003; 52:224-228

113 GPAC strains collected in 2002

Antibiotic	% resistance
PenicillinG	7.1
Tetracycline	41.6
Erythromycin	27.4
Cefoxitin	0
Clindamycin	7.1
Chloramphenicol	0
Imipenem	0
Amoxicillin/clavulanic acid	3.5
Piperacillin/tazobactam	0
Metronidazole	0

F. magna

Infections in which anaerobic bacteria play a role:

30 % GPAC

→ 30 % *F. magna*

Can be isolated in pure culture. Only *Bacteroides fragilis* is more often encountered in pure culture.

Part of the commensal microbiota:

- vagina
- faeces
- skin

Not present in the oral microbiota

Pathogenesis *F. magna*

Virulence factor

Function

Collagenase

breakdown collagen

PAB

binding to human serum albumin

Protein L

Immunoglobulin (Ig)-binding protein

release of "de novo-synthesized mediators"

SufA

degradation of fibrinogen

degradation of antibacterial peptides

release of FAF from bacterial cell wall

Pathogenesis *F. magna*

Virulence factor

Function

FAF

mediation of bacterial aggregation
binding with BM40, present on the skin
blocking of an antibacterial peptide
inactivation of MIG/CXCL9 (=antibacterial peptide)

Virulence and “site of infection”

Correlation between collagenase production and site of infection

Krepel et al. (1991, 1992):

	<i>F. magna</i> (n)
- 222 intra-abdominal samples	11
- 58 non-puerperal breast abscesses	21
- 56 diabetic foot infections	18

Strains isolated from abdominal infections had the lowest enzymatic activity

F. magna is the most common anaerobe isolated from non-puerperal breast abscesses

Virulence and “site of infection”

Correlation between protein L producing *F. magna* strains and bacterial vaginosis

Kastern et al. Infect immun 58: 1217, 1990

Total: 30 *F. magna* strains

- 19 from healthy women
- 4 from healthy men
- 7 from women with bacterial vaginosis

↳ 4 strains express protein L

Virulence and “site of infection”

de Château et al. J Biol chem 271: 26609, 1996

Expression of protein L and PAB in 48 *F. magna* strains

- 30 strains from suppurative infections

1 strain protein L, 16 strains PAB

- 8 strains isolated from bacterial vaginosis

5 strains protein L, no strains PAB

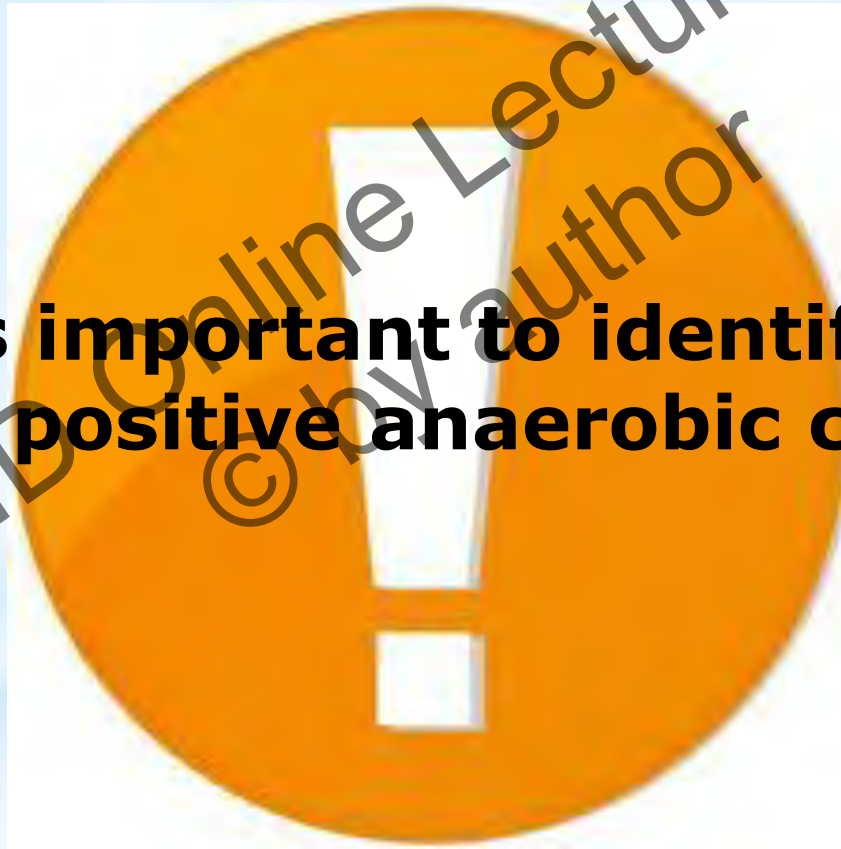
- 10 commensal strains

No expression of protein L or PAB

Protein L and PAB were not expressed at the same time.

Message

It is important to identify gram-positive anaerobic cocci



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