

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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Contents

Introduction

Gram-positive anaerobic cocci

Peptostreptococcus sp. (formerly)

New species and taxonomy

Identification

Antibiotic resistance

Virulence *F. magna*



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

Introduction

Atopobium

Faecal microbiota and clinical material

Sarcina

Soil, abdominal samples, faecal samples from vegetarians and patients with gastro-intestinal abnormalities

Unique cell morphology:

Groups of 8 cells which might produce spores





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



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. harei

P. ivorii

P. octavius

Clinical relevance unknown

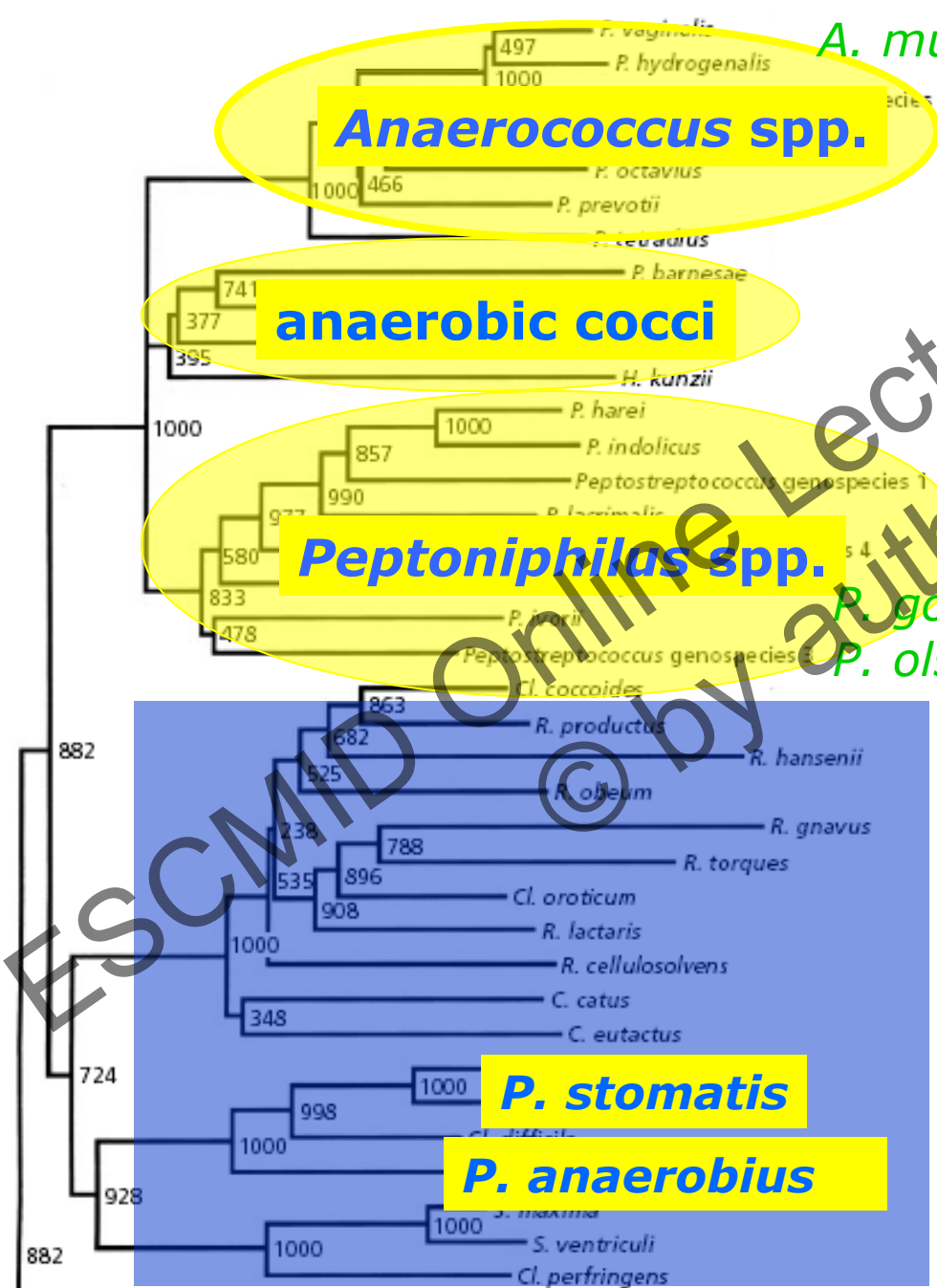
Description of Three New Species of the Genus *Peptostreptococcus* from Human Clinical Specimens: *P. harei* sp. nov., *P. ivorii* sp. nov., and *P. octavius* sp. nov., D. A. Murdoch et al., Int. J. Syst. Bact. 1997



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



A. murdochii

Anaerococcus spp.

anaerobic cocci

Peptoniphilus spp.

P. gorbachii

P. olsenii

P. stomatis

P. anaerobius

Anaerobic cocci:

Fingoldia magna

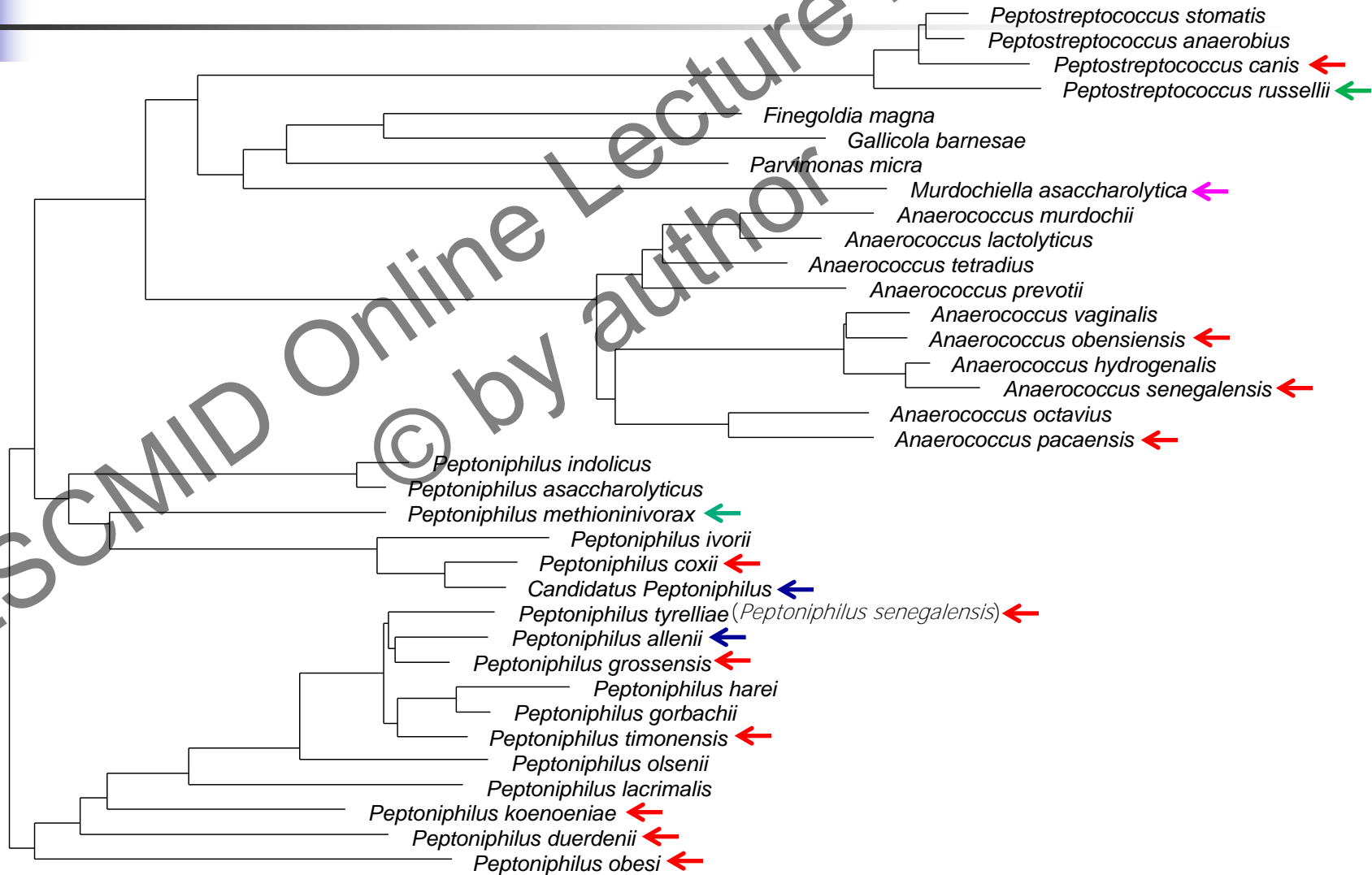
Parvimonas micra

Gallicola barnesea

Helcococcus kunzii

Peptostreptococcus stomatis sp. nov., isolated from the human oral cavity. J. Downes et al. Int. J. Syst. Evol. Microbiol. 2006

New genus and 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 GPAC using 16S rRNA sequencing

presented in NCBI

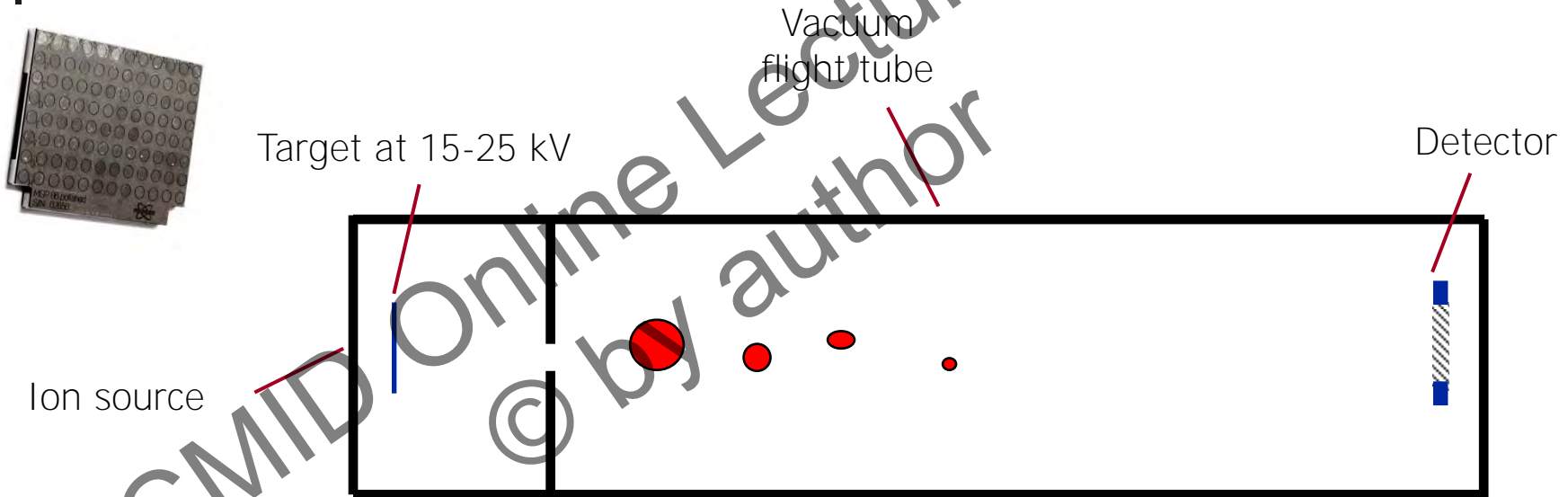
Species	Nucleotide collection	16S rRNA sequences Bacteria and Archaea	remark
<i>A. murdochii</i>	only strain nr	no	
<i>A. tetradius</i>	species name	yes	
<i>A. lactolyticus</i>	species name	yes	
<i>A. octavius</i>	species name	yes	
<i>A. prevotii</i>	species name	yes	
<i>A. vaginalis</i>	species name	yes	
<i>A. hydrogenalis</i>	species name	no	sequence similarity with <i>A. senegalensis</i> is >99%
<i>A. pacaensis</i>	only strain nr	no	link with accession number yields species name
<i>A. senegalensis</i>	only strain nr	no	sequence similarity with <i>A. hydrogenalis</i> is >99% link with accession number yields species name
<i>A. obesiensis</i>	species name	no	
<i>P. coxii</i>	species name	no	
<i>P. tyrelliae</i>	species name	no	sequence similarity with <i>P. senegalensis</i> is >99%
<i>P. harei</i>	species name	yes	
<i>P. ivorii</i>	species name	yes	also in NCBI under the species name <i>P. ivoricus</i>
<i>P. olsenii</i>	only strain nr	no	type strain only mentioned with WAL number in NCBI clinical isolate with species name present in NCBI
<i>P. gorbachii</i>	only strain nr	no	type strain only mentioned with WAL number in NCBI clinical isolate with species name present in NCBI
<i>P. duerdenii</i>	species name	no	
<i>P. koenoeniae</i>	species name	yes	
<i>P. timonensis</i>	species name	no	

Identification GPAC using 16S rRNA sequencing

presented in NCBI

Species	Nucleotide collection	16S rRNA sequences Bacteria and Archaea	remark
<i>P. grossensis</i>	species name	no	
<i>P. lacrimalis</i>	species name	yes	
<i>P. asaccharolyticus</i>	species name	yes
<i>P. indolicus</i>	species name	yes	
Candidatus <i>P. massiliensis</i>	species name	no	this species is not officially described
Candidatus <i>P. allenii</i>	only strain nr	no	this species is not officially described
<i>P. rhinitidis</i>			no sequence of 16S rRNA gene available
<i>P. senegalensis</i>	only strain nr	no	sequence similarity with <i>P. tyrelliae</i> is >99% link with accession number yields species name
<i>P. obesi</i>	species name	no	
<i>P. micra</i>	species name	yes	
<i>F. magna</i>	species name	yes	
<i>P. anaerobius</i>	species name	yes	
<i>P. stomatis</i>	species name	yes	
<i>M. asaccharolytica</i>	species name	no	

Matrix Assisted Laser Desorption/Ionization time-of-flight Mass Spectrometry (MALDI-TOF MS)



- **“Time of flight” of individual proteins is converted into mass information.**
- **Spectrum is produced**
- **Database is built**

Identification of GPAC by MALDI-TOF MS

Bruker system

- 75% 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

Identification of Gram-positive anaerobic cocci by MALDI-TOF mass spectrometry

Veloo et al. Syst Appl Microbiol 2011; 34:58-62

Shimatzu/BioMerieux MS system

Construction database:

sequenced clinical isolates (n=77) and reference strains (n=12)

reference strain *P. harei* is different compared to the clinical isolates *P. harei*

Validation of constructed database:

107 genotypically identified clinical isolates of GPAC

Validation database

Strain (n)	MALDI-TOF ID (n)	
	correct ID	no ID
<i>F. magna</i> (32)	29	3
<i>A. vaginalis</i> (3)	2	1
<i>P. ivorii</i> (3)	1	2
<i>P. octavius</i> (1)		1
<i>Bacterium</i> N14-24 (1)		1
GPAC (3)		3

1 strain sequence similarity < 98 %
 sequence similarity < 98 %
 Not present in database

All other GPAC were correctly identified:

P. micra (29)

P. harei (15)

A. murdochii (6)

P. anaerobius (4)

P. gorbachii (3)

A. parvulum (3)

P. niger (1)

A. lactolyticus (1)

A. tetradius (1)

P. lacrimalis (1)



Correctly identified: 96 strains

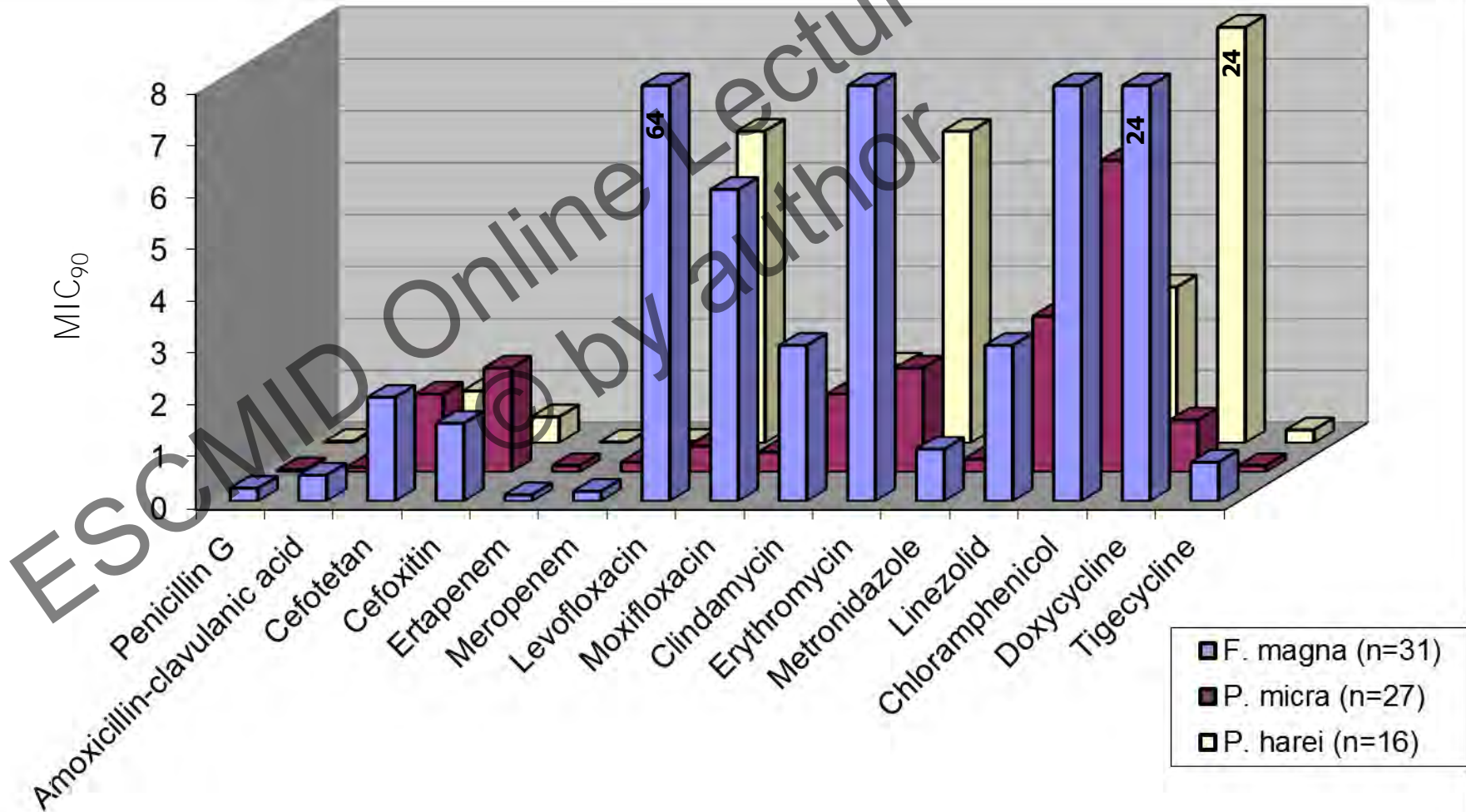
No identification: 11 strains

- 2 strains, no reference spectra present in database
- 6 strains, <98 % sequence similarity with closest relative
- 3 strains, unknown

90 % correctly identified

Resistance GPAC 2002-2004

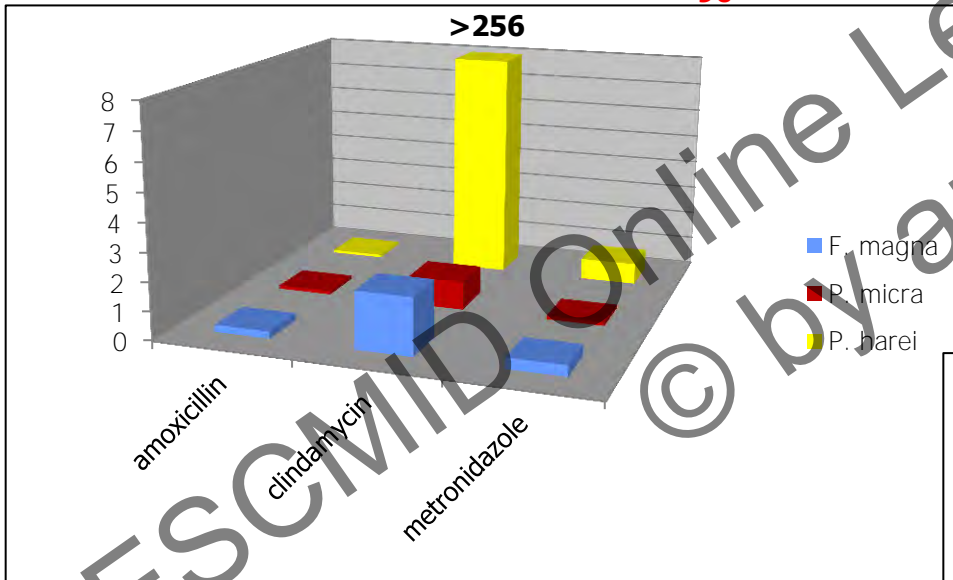
Veloo et al., Antimicrob Agent Chemother, 2011;55:1199-1203



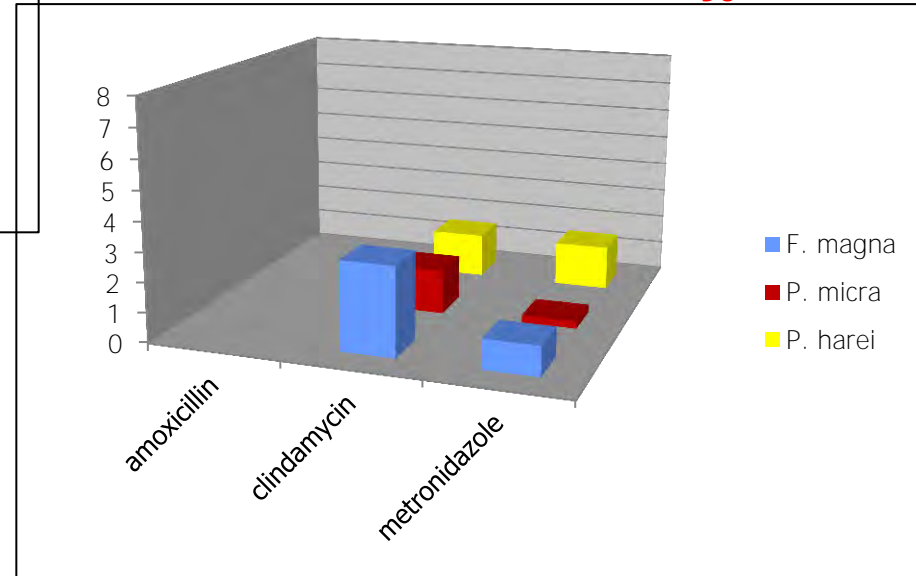
Resistance GPAC 2011-2013

Veloo et al., Anaerobe, 2014 in press

2011-2013 MIC₉₀



2002-2004 MIC₉₀





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