



# Anaerobic bacteria: clinical significance and current knowledge on antimicrobial resistance

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# Anaerobic bacteria

The questions ...

**”are anaerobic bacteria clinical significant?” and**

**”are there antimicrobial resistance?”**

**... are important to discuss before we begin to discuss antimicrobial susceptibility testing – next presentations ...**

# Anaerobic?

- Definition?
- Metronidazole susceptible?
- Cannot tolerate oxygen?
- Growth on certain media?

*“The metronidazole resistant gang”:*

- *Actinomyces* spp.
- *Lactobacillus* spp.
- *Propionibacterium/Cutibacterium* spp.
  
- *Clostridium tertium*?
- *Helicobacter pylori*?
- *Streptococcus anginosus* group?

# Anaerobic bacteria – significance?

- The majority of our normal microbiota in the oral cavity, gut and (for women) internal genital organs
- As many bacterial cells as human cells in and on a human, but X 300 as many genes!!!
- The significance of the human microbiota (primarily anaerobic bacteria) is currently a subject of intense investigation: obesity, diabetes, autism, depression but also some nice aspects...

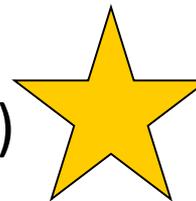
# Anaerobic bacteria – clinical significance?

## The classic anaerobic diseases:

- Lemierre's syndrome
- Gas gangrene
- Tetanus
- Fournier's gangrene
- Botulism

And not to forget (the anaerobic superstar):

- *C. difficile* (antibiotic associated diarrhea)



# Anaerobic bacteria – clinical significance?

**Most anaerobic infections are endogenous (caused by the patients own bacteria) but can also be caused by environmental bacteria after trauma (surgical or accident)**

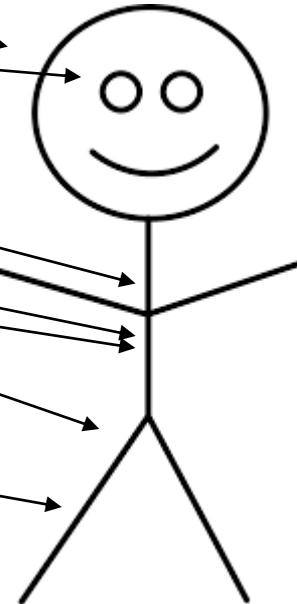
The bacteria escapes from their natural habitat – directly or via the blood stream

- Bacteraemia (*Bacteroides* spp. alone is on the TOP-10 as a cause of bacteraemia) – usually a sign of something VERY SERIOUS!
- From superficial skin infections to gangrene
- Abscesses and empyemas (often in a mix with other facultative bacteria, as e.g. *Streptococcus* spp.)

# Anaerobic bacteria – clinical significance?

Severe infections, which very often involves anaerobic bacteria

- Central nervous system
- Endophthalmitis
- Severe lung infections
- Liver abscesses
- Abdominal abscesses
- Soft tissue infections, especially gangrene
- Bone and joint infections



# Anaerobic bacteria – clinical significance?

**Because of much better diagnostics, clinical significance has increased**

- Anaerobic Gram-negative rods from blood cultures
- *Bacteroides pyogenes*?? – this must be a gut bacterium

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2011, p. 3092–3093  
0095-1137/11/\$12.00 doi:10.1128/JCM.00250-11  
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## Bacteremia with *Bacteroides pyogenes* after a Cat Bite<sup>▽</sup>

Ida Ringsborg Madsen\* and Ulrik Stenz Justesen

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Received 4 February 2011/Returned for modification 9 March 2011/Accepted 19 May 2011

**Animal bite wounds are often infected with bacteria from the animal's oral flora. We report what we believe to be the first case of bacteremia with *Bacteroides pyogenes* resulting from an infected cat bite.**

# Anaerobic bacteria – clinical significance?

- Anaerobic Gram-positive rods from blood cultures
- *Solobacterium moorei*?? – doesn't matter or what?

JOURNAL OF CLINICAL MICROBIOLOGY, July 2011, p. 2766–2768  
0095-1137/11/\$12.00 doi:10.1128/JCM.02525-10  
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## *Solobacterium moorei* Bacteremia: Identification, Antimicrobial Susceptibility, and Clinical Characteristics<sup>▽</sup>

Rune Micha Pedersen,\* Hanne Marie Holt, and Ulrik Stenz Justesen

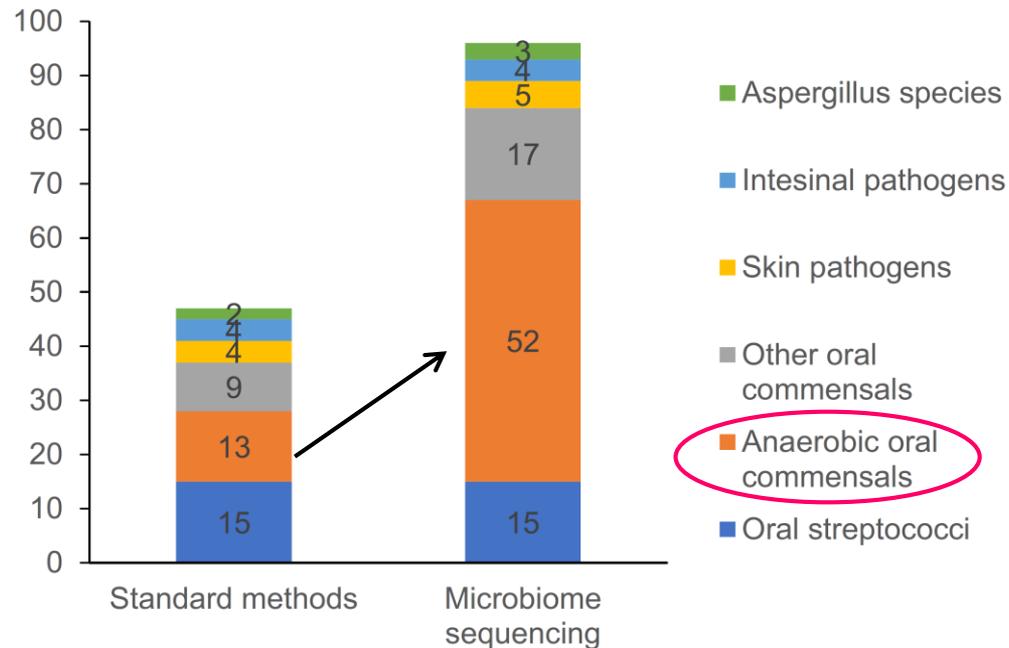
*Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark*

Received 13 December 2010/Returned for modification 9 February 2011/Accepted 13 April 2011

We present five cases of *Solobacterium moorei* bacteremia. The isolates were identified with 16S rRNA gene sequencing and were susceptible to common antibiotics used for anaerobic infections. Bacteremia with *S. moorei* seems to be associated with debilitating conditions, but the prognosis of the infection appears to be good.

# Anaerobic bacteria – clinical significance?

## Anaerobic bacteria and brain abscesses (always count on anaerobic bacteria):



36 primary brain abscesses, conventional culture, including 16S/18S sequencing versus microbiome sequencing

**Figure 3.** Significant pathogens identified by standard methods (culture and/or Sanger sequencing) (n = 47) compared to microbiome sequencing (n = 96) divided into the following groups: oral streptococci, anaerobic oral commensals, other oral commensals, skin pathogens, intestinal pathogens, and *Aspergillus* species.

# Anaerobic bacteria – clinical significance?

## Bacteraemia with anaerobic bacteria and colorectal cancer

Table 1. Percentage of cases followed by a colorectal cancer diagnosis after bacteremia

Bacteria	Within 1 Year (%)
<i>B. fragilis</i>	1.9
<i>B. ovatus</i>	6.7
<i>C. perfringens</i>	1.8
<i>C. septicum</i>	20.8
<i>Escherichia coli</i>	0.6
<i>Staphylococcus aureus</i>	0.2
Negative blood cultures	0.4

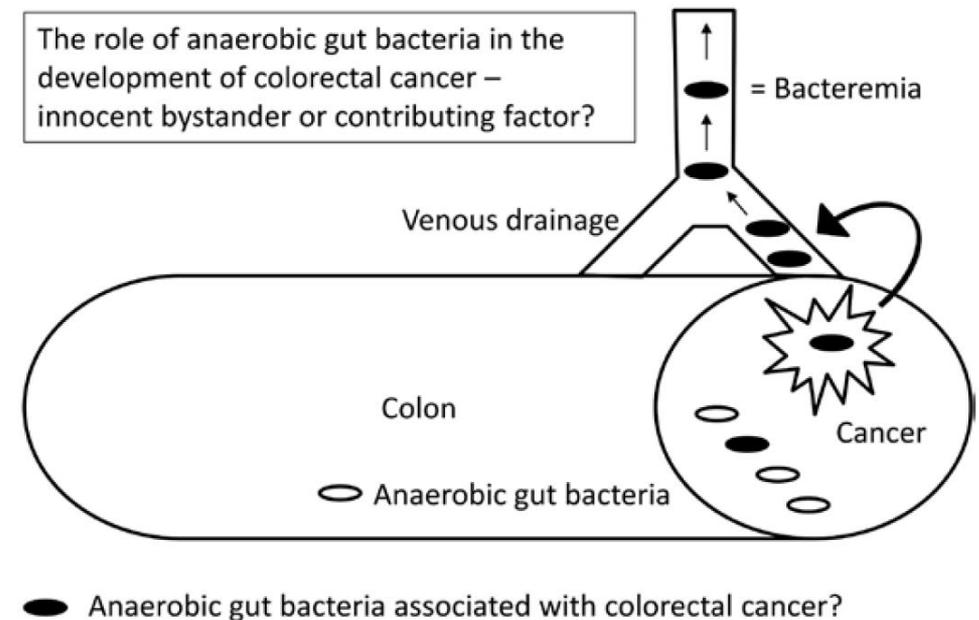
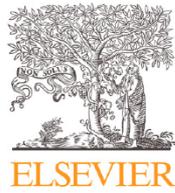


Figure 1. Anaerobic gut bacteria and their possible role in colorectal cancer.

# Anaerobic bacteria – clinical significance?



Contents lists available at [ScienceDirect](#)

Journal of Infection

journal homepage: [www.elsevier.com/locate/jinf](http://www.elsevier.com/locate/jinf)



Development and evaluation of a nanopore 16S rRNA gene sequencing service for same day targeted treatment of bacterial respiratory infection in the intensive care unit



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From ECCMID 2022

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Worked well to identify a possible bacterial cause

... but also an abundance of reads from anaerobic bacteria

# Anaerobic bacteria – antimicrobial resistance?

Anaerobic bacteria are clinically significant and should be considered when choosing empirical, prophylactic and targeted antibiotic therapy

But what about antimicrobial resistance?

Disclaimer: Data are flawed by different AST methods, breakpoints, identification methods and changes in taxonomy!!!

# Anaerobic bacteria – antimicrobial resistance

**Bacteroides spp. (n=202 faecal isolates) – European multicentre study – agar dilution**

Antimicrobial agent	% resistant	Resistance mechanism/gene
Ampicillin,	96.6	cepA/cfxA – cfiA: beta-lactamases
Amoxicillin-clavulanic acid	4.5	
Cefoxitin,	14.9	
Imipenem	2.0	cfiA: beta-lactamase
Clindamycin	47.3	ermF: modified target
Moxifloxacin	11.4	gyrA(?): modified target
Metronidazole	0	nim A-? enzyme modification of drug
Tetracycline	66.2	tetQ: modified target
Tigecycline	1.5	
Chloramphenicol	0	

IMPORTANT:  
EUCAST clinical  
disk diffusion  
breakpoints are  
calibrated to detect  
these resistance  
mechanisms

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Sóki et al. Anaerobe. 2020.

# Anaerobic bacteria – antimicrobial resistance

## Prevotella spp. (n=508 clinical isolates) – primarily European multicentre study – Etest on BBA

**Table 2**

MIC range, MIC<sub>50</sub>, MIC<sub>90</sub> and percentage of susceptible and non-susceptible *Prevotella* isolates tested.

Antimicrobial agents	Breakpoints <sup>a</sup> (mg/L)			MIC (mg/L)			Percentage (%)		
	S	I	R	Range	50%	90%	Susceptible	Non-susceptible <sup>e</sup>	Resistant
Ampicillin	≤0,5		>2	<0.016->256	1	>256	48.8	51.2	42.1
Ampicillin/sulbactam	≤4		>8	<0.016–8	0.125	2	99.6	0.4 <sup>f</sup>	0
Piperacillin/tazobactam	≤8		>16	<0.016–4	<0.016	0.064	100	0	0
Cefoxitin	≤16	32	≥64	<0.016–32	0.5	2	99.6	0.4 <sup>f</sup>	0
Imipenem	≤2		>8	<0.002–1	0.064	0.125	100	0	0
Meropenem	≤2		>8	<0.002–0.5	0.016	0.064	100	0	0
Clindamycin	≤4		>4	<0.016->256	0.016	>256	66.3	33.7	33.7
Erythromycin <sup>d</sup>	NA		NA	<0.016->256	2	>256	–	–	–
Tetracycline <sup>b</sup>	≤4	8	≥16	<0.016->256	1	32	61.6	36.8	17.7
Tigecycline <sup>c</sup>	≤4	8	≥16	<0.016–4	0.064	0.32	100	0	0
Moxifloxacin <sup>b</sup>	≤2	4	≥8	<0.002->32	0.032	8	81.7	18.3	9.4
Metronidazole	≤4		>4	<0.016–4	0.064	0.5	100	0	0

<sup>a</sup> MIC breakpoints recommended by the EUCAST.

<sup>b</sup> By the CLSI, and.

<sup>c</sup> By FDA.

<sup>d</sup> NA indicates that clinical breakpoints are unavailable in both EUCAST and CLSI guidelines.

<sup>e</sup> Intermediate and resistant categories together.

<sup>f</sup> Only intermediate resistant strains were found.

cfxA - beta-lactamase  
ermF - modified target  
tetQ - modified target  
gyrA(?) - modified target

Toprak et al. Anaerobe. 2018.

# Anaerobic bacteria – antimicrobial resistance

## *Clostridium perfringens*:

Penicillin-R not rare, PBP changes (affects pip-tazo as well) (OUH: 3%)

Clindamycin-R, frequent (OUH: 25%)

## *Clostridium innocuum* (and *Clostridium ramosum*)

Very often vancomycin-R (enzymatic change of binding site, like VRE)

Phenotypically mistaken for *C. difficile*

## *Clostridium ramosum*

Metronidazole-R -> not nim (OUH 5%)

## *Clostridium clostridioforme* group

Penicillin-R (beta-lactamase production) (>45% OUH)

# Anaerobic bacteria – antimicrobial resistance

***Clostridium difficile* metronidazole resistance (0.2%)**

Resistance: MIC > ECOFF

Freeman, J. et al. The ClosER study: results from a three-year pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes, 2011-2014  
*Clin. Microbiol. Infect. Dis.* **24**, 724–731 (2018).

**Plasmid pCD-METRO -> mechanism unknown?**

**Haemin dependant resistance in certain ribotypes???!?**

OUH: vancomycin-"R" or metronidazole-"R" = 0% ( $\approx$  3000 isolates)

# Anaerobic bacteria – antimicrobial resistance

## Gram-positive anaerobic cocci – GPAC

*Peptoniphilus* spp., *Peptostreptococcus anaerobius*, *Finegoldia magna*, *Parvimonas micra* (*Micromonas micros*)

Change of names makes it difficult!!!

*Pepto(Streptococcus) intermedius*  
metronidazole resistance!?

Penicillin-R: 0-7% depending on species

Clindamycin-R: 5-50% depending on species



GPACs: Inducible clindamycin resistance can be detected by the D-test (rare)

# Anaerobic bacteria – antimicrobial resistance

## Special cases:

### **Fusobacterium necrophorum:**

Penicillin-R? Rare. One from Tromsø – Arnfinn please help!

Clindamycin-R: Reported 15% (OUH 4%)

Metronidazole?

### **Cutibacterium/Propionibacterium:**

Penicillin rare (OUH 0%)

Clindamycin a few % (OUH 1%)

# Summary

Anaerobic bacteria are clinically significant and should be considered when choosing empirical, prophylactic and targeted antibiotic therapy

We need to know whether anaerobic bacteria are susceptible to our antibiotic therapy

Usually susceptible to metronidazole

**ESCMID Study Group on Anaerobic Infections (ESGAI) study on:**

*“European antimicrobial resistance surveillance of Bacteroides fragilis from blood cultures”*

- Methods
- EUCASTs disk diffusion method
- Explanatory methods document
- Method development

### Method document in quality control

Method documents in quality control are available [here](#).

### Explanatory methods documents

Visa 50 rader

Kod	Titel
Anaerobes	Anaerobic bacteria and resistance
Enterococcus spp.	Detection of glycopeptide-resistance in enterococcal isolates
Staphylococcus spp.	Detection of methicillin resistance in staphylococci
Streptococcus pneumoniae	Detection of reduced penicillin susceptibility in Streptococcus pneumoniae
CPE detection	Enterobacterales: Detection of carbapenemases
ESBL/AmpC detection	Enterobacterales: Detection of classical ESBL (ESBLA) and plasmid-mediated AmpC (ESBLM-C)
Haemophilus	Haemophilus influenzae and beta-lactam resistance
Enterococci and viridans streptococci	High level aminoglycoside resistance in enterococci and viridans group streptococci
Staphylococci and streptococci	Inducible clindamycin-resistance in staphylococci and streptococci
Pseudomonas	Pseudomonas aeruginosa og karbapenemaser (ESBLCARBA)
CPE screening	Screening for carbapenemase-producing Enterobacteriaceae (CPE)
ESBL screening	Screening for ESBL and pAmpC producing Enterobacterales (EPE) carriage
MRSA	Screening for Methicillin resistant Staphylococcus aureus (MRSA)
VRE	Screening for Vancomycin-resistant Enterococci (VRE) carriage

Visar 1 till 14 av totalt 14 rader



### Nyheter Metoder

- [New recommendations for quality control of gradient tests](#)  
Erika Matuschek | 2021-05-11
- [New agents for broth microdilution at reference laboratory in Karlskrona and Växjö](#)  
Erika Matuschek | 2021-01-27
- [EUCAST evaluation of Mueller-Hinton agar published](#)  
Erika Matuschek | 2020-09-21
- [EUCAST panel of phenotypically defined strains](#)  
Erika Matuschek | 2020-08-13
- [New extended BMD panel for staphylococci and enterococci at EDL](#)  
Erika Matuschek | 2019-03-26

**Will be updated shortly!**

# Questions?