Research Article

Trends in the prevalence and antibiotic susceptibility of anaerobic Gram-negative bacteria causing clinical infections in Estonia

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Abstract

Objective: The aim of our study was to describe the species distribution and changes in the resistance profile of anaerobic bacteria isolated at Tartu University Hospital.

Methods: The data for 2010, 2016 and 2020 were analysed retrospectively. The strains were identified by Vitek2 (2010) and MALDI-TOF MS (2016, 2020); and the MIC values for the antibiotics were determined using gradient tests. Resistance was interpreted using EUCAST breakpoints.

Results: The average number of anaerobic cultures received during the 10 years increased from 1551 to 5983; (386%).. The most common pathogens were Gram negative anaerobic rod-shaped bacteria (*Bacteroides fragilis, Bacteroides* spp., *Fusobacterium* spp. and *Prevotella* spp.). The percentage of susceptible strains in the three years studied was stable for the majority of drug-bug combinations tested, except beta-lactam antibiotic sensitivity (*Bacteroides* spp. sensitivity to imipenem and ampicillin-sulbactam, *Fusobacterium* spp. and *Prevotella* spp. to penicillin).

There were significant differences in the MIC values ($p < 0.05$) when comparing 2020 with 2016. The MIC was higher among *Bacterioides* spp. ampicillin-sulbactam, *Bacteriodes fragilis* for imipenem and *Prevotella* for imipenem, ampicillin-sulbactam and clindamycin in 2020 than in 2016. The same was observed in 2020 *vs* 2010 among *Prevotella* spp. MIC for metronidazole, penicillin and cefoxitin. In contrast, the MIC values were surprisingly lower in 2020 than in 2010 for other Gram-negative rods.

Conclusions: There was no shift in the spectrum of microbial groups as causative agents of clinical infections during the 2010-2020 period. However, due to the improvement of identification methods, the number of identified species increased. The resistance pattern of anaerobes was stable, but the changes in MIC values may indicate a further steady increase in

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resistance. The surveillance of antibiotic resistance of anaerobes is important to predict the efficiency of empirical treatment.

Keywords: *Gram-negative anaerobes, taxonomy, antimicrobial susceptibility*

Introduction

Anaerobic bacteria belong to the normal human microbiota, colonising the mucosa and, to a lesser extent, the skin.[1,2] The entry of endogenous anaerobic bacteria from the mucous membranes into sterile areas of the body can lead to opportunistic infections.[3,4] Anaerobic infections can be severe and even life threatening, with an increasing incidence of complicated underlying diseases.[5] Studies show that the lack of appropriate therapy has a negative effect on treatment outcomes.[6]

The diagnosis of anaerobic infections is time-consuming, requires special growth conditions and is therefore more expensive than culturing aerobic bacteria, which may cause laboratories to make cost-based choices.[7] Empirical treatment of anaerobic infections is therefore often initiated without the routine determination of sensitivity to extended-spectrum antibiotics.[8] Empirical treatment is based most often on published data, but the spectrum of agents, the antibiotics used, and the susceptibility of bacteria to them may vary from region to region.[9–12] Empiric treatment is further complicated by changes in anaerobic resistance over time and taxonomic rearrangements, where, for example, the former taxonomic family is subdivided into separate taxonomic units.[2,11]

Monitoring drug resistance in clinically important anaerobic bacteria is not a part of routine monitoring practice; only a few relevant studies are available. Anaerobic microbes have been studied in Estonia since the 1990s, and published data are available from 2003.[13] Anaerobic culturing technique and determination of antibiotic susceptibility have not significantly changed during this period. However, the identification of isolates has changed since the introduction of Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI – TOF) in the Laboratory of Microbiology of the Tartu University Hospital in 2014 and taxonomic evaluations based on the SNOMED (SNOMED Clinical Terms) database. The susceptibility assessment criteria changed in 2010 when the Laboratory of Microbiology switched from the American Clinical and Laboratory Standards Institute's (CLSI) criteria to that of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

The aim of this study was to describe the species distribution and antibiotic susceptibility of Gram-negative anaerobic bacteria, based on routine analyses performed in the Laboratory of Microbiology at Tartu University Hospital during the last ten years.

Methods

We retrospectively analysed the species composition and antibiotic susceptibility of anaerobic bacteria isolated in the Laboratory of Microbiology of Tartu University Hospital, Estonia, in 2010, 2016 and 2020. The patient samples studied were blood (2010: n = 602; 2016: n = 836; 2020: n = 1376) and wound exudate, tissue and body fluid/punctate (2010: n = 945; 2016: n = 3422; 2020: n = 4607).

Anaerobic culture techniques and determination of antimicrobial resistance were based on standard laboratory procedures.[6,12,14] The automatic microbial identification systems used for

strain identification were Vitek2 (bioMérieux, Marcy I'Etoile, France) in 2010 and MALDI-TOF mass spectrometry (Bruker Daltonics, Germany) in 2016 and 2020.

Antibiotic susceptibility was determined using gradient tests (Liofilchem s.r.l., Roseto degli Abruzzi, Teramo, Italy) on Wilkins–Chalgren Agar (Oxoid Limited, Basingstoke, United Kingdom) with 5% sheep blood in an anaerobic atmosphere. The minimum inhibitory concentration (MIC) was determined for ampicillin-sulbactam, clindamycin, metronidazole, and penicillin in 2010. Starting from 2016, imipenem and cefoxitin were added. The MIC was evaluated according to EUCAST criteria. The criteria for assessing anaerobes were unchanged during the study period.

Data collection was conducted using , the electronic laboratory programme of Tartu University Hospital OLAP (Online Inquiry System)., The PAST 4.03 programme was used for statistical analysis. The MIC values of the antibiotics were compared using the Mann–Whitney U test, and P values < 0.05 were considered statistically significant.

Results

The spectrum of anaerobic bacteria

Over ten years, the number of anaerobic cultures analysed in our laboratory increased from 1551 to 5983, i.e. an additional 4432 cultures (386%) per 10 years. The percentage of positive cultures did not differ significantly, being 12.5%, 9.1% and 10.4% in 2010, 2016 and 2020 respectively. We detected 139 different Gram-negative anaerobe isolates in 2010, 203 in 2016, and 490 in 2020 , as presented in Table 1A, B, and C.

Three genera of Gram-negative anaerobes dominated: *Bacteroides, Fusobacterium* and *Prevotella*. Over the 10 year study period, the proportion of *Bacteroides* spp. was 28.0%, 58.7% and 58.3% respectively, *Prevotella* spp. was 54.5%, 31.5% and 29.2%, respectively, and that of *Fusobacterium* spp. was 16.1%, 7.7% and 9.7%, respectively. The most populous species in the MALDI-TOF era were *Bacteroides fragilis, Bacteroides ovatus, Bacteroides thetaiotaomicron, Bacteroides vulgatus, Fusobacterium nucleatum, Prevotella bivia, Prevotella buccae* and *Prevotella melaninogenica*.

Susceptibility of anaerobic bacteria

The percentages of susceptible strains in 2010, 2016 and in 2020, the MIC ranges and MIC50/MIC90 are presented in Table 2.

Table 1A. Distribution and frequency of gram-negative anaerobic microbes (*Bacteroides* spp.)

Species	2010			2016			2020		
		G- (n=143)	G-/G+ (n=194)		G- (n=286)	G-/G+ (n=361)		G- (n=472)	G-/G+ (n=585)
	No	%	%	No	%	%	No	%	%
Bacteroides fragilis	4	2.8	2.1	67	23.4	18.6	113	23.9	19.3
Bacteroides sp.	8	5.6	4.1	3	1.0	1.0	0	0	0
Bacteroides caccae	0	0	0	2	0.7	0.6	1	0.2	0.2
Bacteroides ovatus	3	2.1	1.5	27	9.4	7.5	58	12.3	9.9
Bacteroides pyogenes	0	0	0	5	1.7	1.4	7	1.5	1.2
Bacteroides stercoris	23	16.1	11.9	0	0	0	1	0.2	0.2
Bacteroides thetaiotaomicron	2	1.4	1.0	33	11.5	9.1	52	11.0	8.9
Bacteroides urealyticus	0	0	0	1	0.3	0.3	1	0.2	0.2
Bacteroides uniformis	0	0	0	7	2.4	1.9	10	2.1	1.7
Bacteroides vulgatus	0	0	0	17	5.9	7	24	5.1	4.1
Odoribacter splanchnicus (*Bacteroides splanchnicus*)	0	0	0	0	0	0	1	0.2	0.2
Parabacteroides distasonis (*Bacteroides distasonis*)	0	0	0	2	0.7	0.6	7	1.5	1.2
Parabacteroides goldsteinii	0	0	0	4	1.4	1.1	0	0	0
***Bacteroides* spp.**	**40**	**28.0**	**20.6**	**168**	**58.7**	**46.5**	**275**	**58.3**	**47.0**

G – Gram-negative anaerobes G -/+ Gram negative and Gram-positive anaerobes

Table 1B. Distribution and frequency of gram-negative anaerobic microbes (*Bilophila* spp. , *Dialister* spp., *Fusobacterium* spp., *Porphyromonas* spp.)

Species	2010			2016			2020		
		G- (n=143)	G-/G+ (n=194)		G- (n=286)	G-/G+ (n=361)		G- (n=472)	G-/G+ (n=585)
	No	%	%	No	%	%	No	%	%
Bilophila sp.	0	0	0	1	0.3	0.3	0	0	0
***Bilophila* spp.**	**0**	**0**	**0**	**1**	**0.3**	**0.3**	**0**	**0**	**0**
Dialister micraerophilus	0	0	0	0	0	0	1	0.2	0.2
Dialister pneumosintes	0	0	0	0	0	0	1	0.2	0.2
***Dialister* spp.**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0.4**	**0.3**
Fusobacterium sp.	0	0	0	3	1.0	0.8	0	0	0
Fusobacterium canifelinum	0	0	0	1	0.3	0.3	3	0.6	0.5
Fusobacterium gonidiaformans	0	0	0	1	0.3	0.3	5	1.1	0.9
Fusobacterium mortiferum	3	2.1	1.5	0	0	0	0	0	0
Fusobacterium naviforme	0	0	0	0	0	0	2	0.4	0.3
Fusobacterium necrophorum	3	2.1	1.5	4	1.4	1.1	6	1.3	1.0
Fusobacterium nucleatum	17	11.9	8.8	9	3.1	2.5	28	5.9	4.8
Fusobacterium periodonticum	0	0	0	3	1.0	0.8	2	0.4	0.3
Fusobacterium varium	0	0	0	1	0.3	0.3	0	0	0
***Fusobacterium* spp.**	**23**	**16.1**	**11.9**	**22**	**7.7**	**6.1**	**46**	**9.7**	**7.9**
Porphyromonas somerae	0	0	0	5	1.7	1.4	1	0.2	0.2
***Porphyromonas* spp.**	**0**	**0**	**0**	**5**	**1.7**	**1.4**	**1**	**0.2**	**0.2**

G - Gram negative anaerobes G -/+ Gram negative and Gram positive anaerobes

Table 1C. Distribution and frequency of gram-negative anaerobic microbes (*Prevotella* spp., *Veillonella* spp.)

Species	2010			2016			2020		
		G- (n=143)	G-/G+ (n=194)		G- (n=286)	G-/G+ (n=361)		G- (n=472)	G-/G+ (n=585)
	No	%	%	No	%	%	No	%	%
Prevotella sp.	4	2.8	2.1	1	0.3	0.3	1	0.2	0.2
Prevotella amnii	0	0	0	1	0.3	0.3	1	0.2	0.2
Prevotella baroniae	0	0	0	0	0	0	2	0.4	0.3
Prevotella bergensis	0	0	0	2	0.7	0.5	3	0.6	0.5
Prevotella bivia	29	20.3	14.9	12	4.2	3.3	24	5.1	4.1
Prevotella buccae	2	1.4	1.0	19	6.6	5.3	25	5.3	4.3
Prevotella buccalis	0	0	0	1	0.3	0.3	0	0	0
Prevotella dentalis	0	0	0	0	0	0	1	0.2	0.2
Prevotella denticola	0	0	0	8	2.8	2.2	14	3.0	2.4
Prevotella disiens	14	9.8	7.2	7	2.4	1.9	15	3.2	2.6
Prevotella heparinolytica	0	0	0	1	0.3	0.3	0	0	0
Prevotella intermedia	5	3.5	2.6	5	1.7	1.4	10	2.1	1.7
Prevotella loescheii	0	0	0	0	0	0	1	0.2	0.2
Prevotella melaninogenica	9	6.3	4.6	11	3.8	3.0	26	5.5	4.4
Prevotella nanceiensis	0	0	0	1	0.3	0.3	0	0	0
Prevotella nigrescens	0	0	0	12	4.2	3.3	6	1.3	1.0
Prevotella oralis	13	9.1	6.7	0	0	0	1	0.2	0.2
Prevotella oris	2	1.4	1.0	4	1.4	1.1	5	1.1	0.9
Prevotella pallens	0	0	0	1	0.3	0.3	0	0	0
Prevotella salivae	0	0	0	3	1.0	0.8	1	0.2	0.2
Prevotella veroralis	0	0	0	1	0.3	0.3	2	0.4	0.3
***Prevotella* spp.**	**78**	**54.5**	**40.2**	**90**	**31.5**	**24.9**	**138**	**29.2**	**23.6**
Veillonella sp.	2	1.4	1.0	0	0	0	0	0	0
Veillonella atypica	0	0	0	1	0.3	0.3	3	0.6	0.5
Veillonella dispar	0	0	0	2	0.7	0.7	1	0.2	0.2
Veillonella parvula	0	0	0	2	0.7	0.7	6	1.3	1.0
***Veillonella* spp.**	**2**	**1.4**	**1.0**	**5**	**1.7**	**1.7**	**10**	**2.1**	**1.7**

G - Gram negative anaerobes G -/+ Gram negative and Gram positive anaerobes

The percentages of susceptible strains in the three years studied were not similar.

As shown in Table 2, the percentages of susceptible strains are not similar when comparing drug-microbe combinations. *Bacteroides* spp. were more resistant to ampicillin-sulbactam and imipenem in 2010. Penicillin sensitivity also differed, whereas *Fusobacterium* spp. strains became more sensitive and *Prevotella* spp. strains more resistant.

The differences could be discerned when comparing the MIC values. The MIC values for metronidazole, penicillin and cefoxitin in 2010 and 2020 were statistically similar for *Bacteroides fragilis* and *Bacteroides* non fragilis but not for *Prevotella* spp. and *Fusobacterium* spp. As *Porphyromonas* spp. was not found in 2010, these bacteria were excluded from the comparison. The MIC values of ampicillin-sulbactam for *Bacteroides fragilis*, *Bacteroides* non fragilis and *Prevotella* spp. did not differ, nor did clindamycin for *Bacteroides*-non fragilis or imipenem and clindamycin for *Prevotella* spp. and metronidazole for *Fusobacterium* spp. The differences in MIC values are presented in Table 3.

Table 2. Antibiotic susceptibility of anaerobes in 2010, 2016 and 2020

Genus	Antimicrobial agent	2010 n = 143			2016 n = 286			2020 n = 472		
		% S	MIC ranges	MIC 50/90 mg/L	% S	MIC ranges	MIC 50/90 mg/L	% S	MIC ranges	MIC 50/90 mg/L
Bacteroides fragilis	Ampicillin + sulbactam	75	0.5 - 256	1.5/256	97	0.023 - 256	0.25/1.5	89	0.047 - 256	0.75/6
	Imipenem	50	0.125 - 32	1/32	97	0.002 - 32	0.94/0.38	92	0.008 - 32	0.125/1
	Clindamycin	75	0.5 - 256	1/256	82	0.016 - 256	0.25/16	86	0.016 -256	0.19/256
	Metronidazole	100	0.5 - 4	1.5/4	96	0.032 - 256	0.75/2	97	0.032 -256	0.5/2
	Penicillin	0	32	32/32	2	0.006 - 32	32/32	0	3 - 32	32/32
	Cefoxitin	NR	8 - 24	8/24	NR	0.032 -256	8/32	NR	0.032 - 256	32/32
Bacteroides non *fragilis*	Ampicillin + sulbactam	85	0.016 - 256	1/8	89	0.016 - 256	0.38/8	79	0.016 - 256	0.125/256
	Imipenem	68	0.016 - 32	0.75/32	95	0.002 - 32	0.19/1	95	0.008 - 32	0.19/1.5
	Clindamycin	80	0.023 - 256	0.75/256	71	0.016 - 256	1/256	77	0.016 - 256	0.5/256
	Metronidazole	98	0.023/256	0.5/2	95	0.023 - 256	0.75/3	96	0.016 - 256	0.75/2
	Penicillin	10	0.006 - 32	32/32	5	0.002 - 32	32/32	3	0.004 - 32	32/32
	Cefoxitin	NR	0.016 - 96	12/32	NR	0.032 - 256	8/64	NR	0.032 - 256	16/256
Fusobacterium spp.	Ampicillin + sulbactam	96	0.016 - 256	0.19/4	96	0.016 - 256	0.032/0.25	96	0.016 - 256	0.032/0.38
	Imipenem	96	0.002 - 32	0.094/0.75	96	0.004 - 32	0.032/0.094	94	0.004 - 32	0.032/0.38
	Clindamycin	96	0.016 - 12	0.094/1	100	0.016 - 2	0.094/0.25	98	0.016 - 256	0.047/0.38
	Metronidazole	91	0.016 - 256	0.125/0.75	100	0.016 -1.5	0.099/0.38	100	0.016 - 1	0.094/0.38
	Penicillin	48	0.002 - 32	0.5/32	85	0.006 - 32	0.016/32	87	0.003 - 32	0.016/2
	Cefoxitin	NR	0.016 - 6	0.75/3	NR	0.023 - 256	0.125/1	NR	0.016 - 256	0.125/1.5
Prevotella spp.	Ampicillin + sulbactam	97	0.016 - 6	0.125/1	100	0.016 - 3	0.023/0.094	97	0.016 - 64	0.064/1
	Imipenem	100	0.002 -1.5	0.047/0.5	100	0.002 - 0.38	0.032/0.064	97	0.002 - 8	0.047/0.19
	Clindamycin	95	0.016 - 256	0.064/1	84	0.016 - 256	0.023/256	78	0.016 - 256	0.047/256
	Metronidazole	100	0.016 - 2	0.125/1	100	0.016 - 4	0.25/1.5	99	0.016 - 256	0.5/1.5
	Penicillin	74	0.002 - 32	0.25/32	39	0.002 - 32	2/32	42	0.003 - 32	4/32
	Cefoxitin	NR	0.064 - 64	0.25/3	NR	0.016 - 6	0.5/2	NR	0.016 - 256	0.75/4
Porphyromonas spp.	Ampicillin + sulbactam	Microbes were not isolated			100	0.016 - 0.125	n=5*	100	0.023	n=1*
	Imipenem	Microbes were not isolated			100	0.003 - 0.064	n=5*	100	0.003	n=1*
	Clindamycin	Microbes were not isolated			80	0.023 - 256	n=5*	100	0.023	n=1*
	Metronidazole	Microbes were not isolated			100	0.016 - 0.5	n=5*	100	0.023	n=1*
	Penicillin	Microbes were not isolated			20	0.016 - 32	n=5*	100	0.012	n=1*
	Cefoxitin	Microbes were not isolated			NR	0.016 - 0.5	n=5*	NR	0.023	n=1*

S - susceptible strains; *small number; NR – No rating

Table 3. Differences in MICsof selected antibiotics for anaerobes in 2010, 2016 and 2020

Genus	Antimicrobial agent	Median (MIC values)			P value	
		2010	2010	2020	2010 vs 2020	2016 vs 2020
Bacteroides fragilis	Ampicillin + sulbactam	2.75	0.25	0.75	> 0.05	**0.0001**
	Imipenem	2	0.09	0.125	**0.035592**	**0.0044**
	Clindamycin	1.25	0.25	0.19	**0.046785**	> 0.05
	Metronidazole	1.75	0.75	0.5	> 0.05	> 0,05
	Penicillin	32	32	32	> 0.05	> 0,05
	Cefoxitin	small number	8	12	> 0.05	> 0,05
Bacteroides non *fragilis*	Ampicillin + sulbactam	1	0.5	1	> 0.05	**0.001**
	Imipenem	0.75	0.25	0.25	**0.0073156**	> 0.05
	Clindamycin	0.625	2	1	> 0.05	> 0.05
	Metronidazole	0.5	0.75	0.75	> 0.05	> 0.05
	Penicillin	32	32	32	> 0.05	> 0.05
	Cefoxitin	12	24	16	> 0.05	> 0.05
Fusobacterium spp.	Ampicillin + sulbactam	0.22	0.032	0.032	**0.00095708**	> 0.05
	Imipenem	0.094	0.032	0.032	**0.016367**	> 0.05
	Clindamycin	0.094	0.094	0.047	**0.027855**	> 0.05
	Metronidazole	0.125	0.1095	0.094	> 0.05	> 0.05
	Penicillin	0.5	0.016	0.016	**0.000082107**	> 0.05
	Cefoxitin	0.75	0.1575	0.125	**0.013325**	> 0.05
Prevotella spp.	Ampicillin + sulbactam	0.125	0.023	0.064	> 0.05	**0.0001**
	Imipenem	0.047	0.032	0.047	> 0.05	**0.0072**
	Clindamycin	0.064	0.0275	0.047	> 0.05	**0.0039**
	Metronidazole	0.125	0.25	0.5	**0.0000011996**	> 0.05
	Penicillin	0.25	2	4	**0.02736**	> 0.05
	Cefoxitin	0.25	0.5	0.75	**0.044681**	> 0.05

A statistically significant difference was observed in the case of *Prevotella* spp. for metronidazole (p = 0.0000012), penicillin (p = 0.02736) and cefoxitin (p = 0.044681), where the MIC values were higher in 2020 than in 2010.

The MIC values of *Fusobacterium* spp. were surprisingly lower in 2020 than in 2010 (ampicillin-sulbactam p = 0.00095708, imipenem p = 0.016367, penicillin p = 0.000082107, cefoxitin p = 0.013325 and clindamycin p = 0.027855). A statistically significant difference was noted for imipenem (*Bacteroides fragilis* p = 0.03559, *Bacteroides* non fragilis p = 0.0073156), and clindamycin (*Bacteroides fragilis* p = 0.046785), where MIC values were lower in 2020.

The MIC values for metronidazole, penicillin and cefoxitin in 2016 and 2020 were statistically not significant for *Bacteroides fragilis*, *Bacteroides* non fragilis, *Prevotella* spp. and *Fusobacterium* spp. ($p > 0.5$). As *Porphyromonas* spp. were found in low numbers, these bacteria were excluded from the comparison.

The clindamycin MICs in the case of *Bacteroides fragilis*, *Bacteroides* non fragilis and *Fusobacterium* spp. did not differ, nor did *Bacteroides* non fragilis and *Fusobacterium* spp. imipenem values.

A statistically significant difference in MIC values was observed for ampicillin-sulbactam (*Bacteroides fragilis*, *Bacteroides* non fragilis, *Prevotella* spp, imipenem (*Bacteroides fragilis* and *Prevotella* spp.) and clindamycin (*Prevotella* spp.) that were higher in 2020 than in 2016.

Discussion

This study revealed that the taxonomical structure of anaerobic bacteria found from clinical materials at the group level did not change significantly. Although the pattern of antibiotic susceptibility of anaerobes on the susceptible/resistant scale has been stable during the last 10 years, some changes in MIC values can be noted in the observed years, which may indicate a possible increase in resistance in the future.

Tartu University Hospital is a regional and teaching hospital in the south-eastern part of Estonia with 962 beds and 38774 patients treated in 2020.

During the ten-year study period, the average number of anaerobic cultures analysed in the said laboratory increased from 1551 to 5983, i.e. an increase of 386%. , Additionally, the proportion of blood cultures received also doubled. These changes can be associated with an increase in the overall workload during the said years as many smaller hospitals joined Tartu University Hospital but they could also be attributed to an increase in awareness of anaerobic infections among doctors.

Due to the working group of microbiologists of the Estonian Laboratory Medicine Association, the situation in Estonia has contributed towards improving the quality of antimicrobial sensitivity testing. This includes an increase in the list of antibiotics incorporated for testing and switching to EUCAST for interpretation criteria.

In 2010, when the Vitek2 system was used, the *Fusobacterium nucleatum* and *Prevotella bivia* species prevailed. After the introduction of MALDI-TOF, the possibilities for species differentiation improved. The same applies to *Fusobacterium* spp. whereas the distribution of *Prevotella* spp. did not increase significantly .

Although the species of bacteria determined by different methods are not unequivocally comparable, it is worth noting the following tendency: while *Fusobacterium* spp. and *Prevotella* spp dominated in 2010, new genera and species (especially *Bacteroides* spp.) were added in 2016 and 2020. It is unlikely that the spectrum of anaerobic pathogens changed in just 10 years, but the differences in diagnostic systems did change. This result calls into question the diagnostic capabilities of the laboratories. While sequencing would be the most accurate solution, it would also be too time-consuming for a conventional laboratory. Therefore, MALDI-TOF could be considered a standard method for use in clinical laboratories.

Next, the antibacterial susceptibility of Gram-negative anaerobes were compared over time. The methods of antibiotic susceptibility determination did not change during the study period. During the observation period, the clinical susceptibility (susceptible/resistant) of anaerobes remained almost unchanged, similar to the previous study of 2003.[13] However, changes in MIC values were observed over time for some antibiotics and microbial groups although the sensitivity testing methods were unchanged. However, the given MIC values all fell within the susceptible range. Although there was a change in microbial identification during this period (Vitek2 vs. MALDI-TOF), this should not have significantly affected the susceptibility results .

From 2010 to 2021, the EUCAST rules did not give established evaluation criteria and quality control requirements, referring only to the need to follow the manufacturer's recommendations. Nevertheless, fulfilling the manufacturer's instructions should have ensured obtaining the correct results. The reason for decreasing MIC values during the ten-year period may be associated with possible changes in antibiotic treatment policies.

EUCAST are currently developing a method for the disk diffusion tests of anaerobes,[15,16] which would be cheaper and better standardised with a precise methodology and quality control.

In general, anaerobes were comparatively sensitive to the tested antibiotics, but resistant strains were also present in each group. Unlike in 2020, there were no ampicillin-sulbactam or imipenem-resistant *Prevotella* spp. and clindamycin-resistant *Fusobacterium* spp. in 2016. Clindamycin resistance was high among anaerobes in the *Bacteroides* non fragilis group, which may be an important reason for the possible failure of empirical treatment. Such a trend was not observed in Estonia as previously reported in 2003 by this group.[13]

There is sufficient data in the literature on the increase in resistance of anaerobic bacteria[17, 18], which is indirectly confirmed by the current results. The link between the use of antibiotics and the development of resistance is difficult to prove due to the short period of time and relatively limited data. Ampicillin-sulbactam is used in the treatment of both aerobic and anaerobic infections according to the Estonian Guidelines for Antibiotic Therapy, and some increase in beta-lactam use has also been observed.[19] Lass et al. found the same when studying Estonian data.[20] However, the changes in the MIC values of ampicillin-sulbactam were not yet initially realised as resistance to this antibiotic.

Conclusions.

Awareness of the importance of anaerobic infections and the practice of ordering anaerobic cultures by physicians have improved over the years.

The taxonomical structure of anaerobic bacteria at the group level has not changed significantly, although the number of identified species has increased due to improvement of bacterial identification by introduction of the MALDI–TOF methodology. Although the pattern of antibiotic susceptibility of anaerobes on the susceptible/resistant scale has been stable during the last 10 years, some changes in MIC values are noted in the observed years, which may indicate a possible increase in resistance in the future. In view of the above, it would make sense to monitor changes in the susceptibility of anaerobic bacteria that cause clinical infections at longer intervals.

Declarations

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