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Diagnostic and epidemiological landscape of anaerobic bacteria in Europe, 2020–2023 (ANAEuROBE)

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ABSTRACT

Introduction: Despite being implicated in a wide spectrum of community- and healthcare-acquired infections, anaerobes have not yet been incorporated into systematic surveillance programs in Europe.
Methods: We conducted a multicentre retrospective observational study analysing all anaerobic strains isolated from blood cultures in 44 European Hospital Centres over a 4-y period (2020–2023). Diagnostic approach, epidemiology, and antimicrobial susceptibility according to EUCAST v. 15.0 were investigated.
Results: Our study included 14,527 anaerobes, most of which were Gram-positive (45%) or Gram-negative (40%) bacilli. MALDI-TOF coupled to mass spectrometry was the most widely used tool for species identification (98%). Antimicrobial susceptibility testing was performed in the vast majority of centres, using mostly gradient diffusion strip (77%) and disk diffusion (45%) methods according to EUCAST guidelines. The most prevalent species were *Cutibacterium acnes* (18.7%), *Bacteroides fragilis* (16.3%), *Clostridium perfringens* (5.3%), *Bacteroides thetaiotaomicron* (4.2%), *Fusobacterium nucleatum* (3.5%), and *Parvimonas micra* (3.4%). *C. acnes* showed high resistance to benzylpenicillin (18%), clindamycin (39%), and imipenem (19% and 13% by MIC methods and disk diffusion, respectively). *B. fragilis* showed high resistance to amoxicillin/clavulanate (24%), piperacillin/tazobactam (22% and 14% by MIC methods and disk diffusion, respectively), clindamycin (22% by both MIC methods and disk diffusion), meropenem (13%), and metronidazole (10%, only by disk diffusion). A similar resistance pattern was observed in *B. thetaiotaomicron, Bacteroides ovatus*, and *Parabacteroides distasonis. C. perfringens* showed high resistance to clindamycin (69% and 45% by MIC methods and disk diffusion, respectively), while benzylpenicillin and metronidazole maintained over 90% activity. *F. nucleatum* showed high resistance to benzylpenicillin (11%), while *Fusobacterium necrophorum* showed alarming rates of resistance to clindamycin (12%), meropenem (16%) and metronidazole (11%).
Conclusions: This study presented an up-to-date analysis of the diagnostics and epidemiology of anaerobic bacteria in Europe, providing insights for future comparative analyses and the development of antimicrobial diagnostic and management strategies, as well as the optimization of current antibiotic treatments.

1. Introduction

Anaerobic bacteria are implicated in a wide spectrum of community- and healthcare-acquired infections causing significant morbidity and mortality [1,2]. Among anaerobic species, antimicrobial resistance is variable according to region [3–5] and may increase the risk of worse clinical outcomes [6–8]. The burden of antimicrobial resistance in anaerobes is considered the result of several factors such as species, ribotype, country, hospital centre, antibiotic consumption, and sample type [9]. Over the last two decades, rates of resistance to β-lactam/β-lactamase inhibitor combinations, clindamycin and carbapenems in *Bacteroides*/*Parabacteroides* species, β-lactams in *Prevotella* species, clindamycin in anaerobic cocci, and metronidazole in *Clostridioides difficile, Bacteroides/Parabacteroides* species, and *Clostridium perfringens* have been on the rise [9–11]. An international cluster of multidrug-resistant *Bacteroides fragilis* was also identified from clinical specimens of several countries [12]. However, anaerobes are underrepresented in the literature and notably absent from current surveillance programs in Europe. Many clinical laboratories are not equipped to perform proper culture for anaerobic species, underestimating their aetiological relevance, hampering appropriate targeted therapy, and increasing broad-spectrum antibiotics consumption [13]. In addition, the methods used for identifying anaerobes and performing antimicrobial susceptibility tests may vary enormously between laboratories and countries [14,15], even moving away from official guidelines and adapting to the local contexts [16]. To date, knowledge of the epidemiology and antimicrobial resistance burden of anaerobic species is fragmented, based on some local [17–28], species-specific [4], or pre-COVID-19 pandemic multicentre studies [3,5]. Furthermore, EUCAST has recently implemented new breakpoints for frequently isolated anaerobes [29,30] supplemented by the guidance on 'When there are no breakpoints in breakpoint tables?' [31] for all the other species. The Committee of the Antibiogram of the French Society of Microbiology introduced an area of technical uncertainty to meet FDA criteria for disk diffusion [32,33] maintaining a certain degree of autonomy from EUCAST guidelines. How European laboratories have adapted to these novelties is still a field of study and there are no published data on the topic. Recognizing the challenges in diagnosing and treating anaerobic bacteria infections, our study sought to provide novel insights to address the issue of antimicrobial resistance in Europe. We investigated the diagnostic approach, epidemiology, and antimicrobial susceptibility of anaerobic species isolated from blood cultures during the last 4 y in a large cohort of European hospitals.

2. Methods

2.1. Study design

We conducted a multicentre retrospective observational study including all consecutive anaerobic species isolated from blood cultures from 44 European hospital centres (located in 22 countries; 60,000 overall hospital beds, Fig. 1) collected from 1 January 2020 to 31 December 2023. Duplicate isolates obtained within a 20-d interval from the same patient were considered part of a single positive blood culture event and thus excluded from the analysis.

2.2. Survey on the diagnostic approach to anaerobic species from blood cultures

We conducted a survey to assess microbiological diagnostic practices for anaerobic bacteria in the European centres participating in the study. The study coordinating centre designed a questionnaire, which was distributed to all laboratories involved. This questionnaire comprised 40 questions and covered various aspects, including the type of centre (e.g. hospital type, number of hospital beds), laboratory activities (such as the number of anaerobic bacteria isolates tested and the methods used for identification and antimicrobial susceptibility testing), and microbiologists' attitudes on anaerobic bacteria diagnostics.

Fig. 1. ANAEuROBE collaborative centres (top) and contribution in terms of number of isolates (bottom). AU, Austria; BE, Belgium; BU, Bulgaria; CRO, Croatia; CZ, Czech Republic; DK, Denmark; FR, France; GE, Germany; GR, Greece; HU, Hungary; ICU, intensive care unit; IR, Ireland; IT, Italy; NED, The Netherlands; NO, Norway; PL, Poland; PT, Portugal; RO, Romania; SK, Slovak Republic; SLO, Slovenia; SP, Spain; SW, Sweden; SWI, Switzerland.

2.3. Anaerobic species identification and susceptibility testing

For each anaerobic species positive blood culture episode, the following data were recorded: bacterial species identification, year of detection, clinical setting in which the pathogen was isolated (emergency, medical, surgical, ICU, or paediatrics), and method of antimicrobial susceptibility testing. We recorded the results of susceptibility testing along with the species identification method and the clinical breakpoints used by each institution during the study period. Antimicrobial susceptibility testing results (MICs or inhibition zone diameters) were interpreted in accordance with the guidelines provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version 15.0 [34] or EUCAST guidance on 'When there are no breakpoints in breakpoint tables?' [31]), the Clinical & Laboratory Standards Institute (CLSI M100 ED35:2025 – Performance Standards for Antimicrobial Susceptibility Testing, 35th Edition) or Committee of the Antibiogram of the French Society of Microbiology (CA-SFM 2024 V.1.0 June) [33].

2.4. Statistics

We presented descriptive data using absolute counts (n) and relative percentages (%) for categorical variables. Summary statistics for MIC values included MIC_{50} and MIC_{90}. Summary statistics for inhibition zone diameter values included median and interquartile range. Data analysis was performed using Microsoft Excel (Office 365), SPSS v. 25.0 (IBM Corp., Armonk, NY, USA), and Python 3.10.

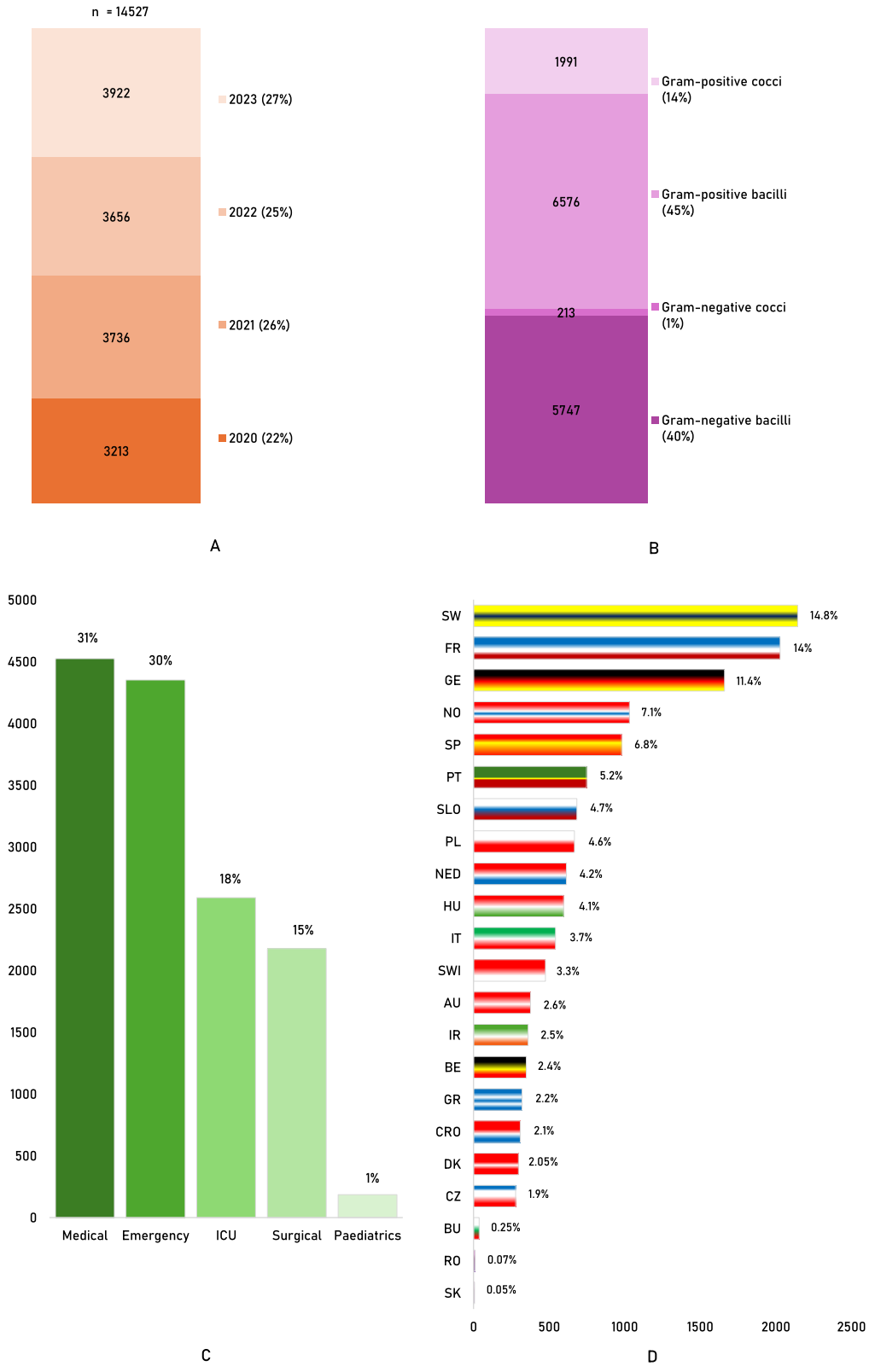
3. Results

3.1. Diagnostic approach to anaerobic species around Europe

Most microbiologists interviewed believed that the burden of infections caused by anaerobic bacteria is underestimated in the literature (93%) and identified diagnostic limitations (73%) and low clinical interest (34%) as the main reasons for this underrepresentation (Fig. S1). Likewise, they were predominantly aware (61%) that they routinely underdiagnosed anaerobes from blood cultures mainly for technical reasons (41%). However, they considered the microbiological diagnostics of anaerobic bloodstream infections a relevant chapter of their work (75%), feeling an active part of the care process both when communicating the identification of species (100%) and the antimicrobial susceptibility profile (89%). Most of them were convinced that the workflow for detecting anaerobic bacteria from blood culture in their laboratory could be improved (73%), possibly by improving the quality of diagnostics tools (72%) and the number and/or training of the staff (28%). Most laboratories did not have an area dedicated exclusively to anaerobic diagnostics (75%), did not routinely process subculture in anaerobic chambers (59%), and did not routinely use anaerobic blood culture bottles for paediatric patients (53%). Laboratories usually took <10 min (36%), 10–20 min (50%), and >20 min (14%) for the processing of blood cultures on the open bench, routinely using anaerobic environment control indicators (89%). The most used anaerobic incubation systems were gas-generating envelopes (59%) followed by anaerobic workstations (23%). The most used solid media for anaerobes isolation were Schaedler (52%) and Columbia Blood Agar (34%). Anaerobic species identification was predominantly conducted using MALDI-TOF coupled to mass spectrometry (98%; Vitek MS, bioMérieux, Marcy l'Étoile, France; Bruker Biotyper, Bruker Daltonics, Bremen, Germany, Table S1). 16S rRNA gene sequencing was used in five centres (11%) as identification method in case of unreliable result by routine methods. In addition, in parallel with conventional microbiological workflow, 39% of the laboratories had a molecular test for rapid diagnostics directly from positive blood cultures. The molecular systems implemented were BIOFIRE Blood Culture Identification 2 Panel (bioMérieux, France) and HYBCELL (Cube Dx, Austria). Among the microorganisms identified by these instruments, the anaerobic species were *B. fragilis* detected by both and *Fusobacterium necrophorum, Fusobacterium nucleatum*, and *Finegoldia magna* identified only by HYBCELL. Laboratories obtained susceptibility testing results through various methods, including gradient diffusion strip method (77%; ETEST, bioMérieux, Marcy l'Étoile, France; MIC Test Strip, Liofilchem, Roseto degli Abbruzzi, Italia), disk diffusion (45%; Thermo Scientific Oxoid Antimicrobial Susceptibility disks, Thermo Fisher Scientific, Waltham, MA, USA), and broth microdilution commercial systems (9%; MICRONAUT-S Anaerobes MIC, Merlin Diagnostika, Germany; Sensititre ARIS HiQ, Thermo Fisher Scientific, Waltham, MA, USA; ATB ANA, bioMérieux, Marcy l'Étoile, France), following the recommendations provided by the respective manufacturers. None of the labs used the reference agar dilution method. Fastidious Anaerobic Agar (34%) and Brucella Blood Agar (34%) were the most commonly used solid media for antimicrobial susceptibility testing, while the EUCAST guidelines were the most followed (84%). Fifty per cent of centres read the results of susceptibility tests within 16–20 h for frequently isolated anaerobes and up to 48 h for the others, while some read them within 48–72 h (16%), 16–20 h for frequently isolated anaerobes and up to 24 h for the others (14%), and whenever they were readable even if before or after the time stipulated in the guidelines (11%). When susceptibility testing results were not readable at the set time, laboratories predominantly reported the readable ones and waited for the others to become available (59%), while some reported the readable ones and did not report the others (23%) or repeated the test (11%). Antimicrobial susceptibility testing was carried out in all cases (61%), depending on each case (30%), or in frequent species only (2%). When faced with *Clostridia* identified from positive blood culture, laboratories predominantly carried out and reported both species identification and antimicrobial susceptibility testing results (>73%). In contrast, when faced with *Cutibacterium* species, their attitude predominantly depended on the ratio of positive blood culture bottles/total blood culture bottles and the patient's clinical condition (66%), being the combination of these two factors that defined the concept of anaerobic contaminant according to most centres (71%). Most centres did not check the intrinsic resistance of the anaerobic species detected (54%). Similarly, they did not routinely screen Gram-positive and Gram-negative anaerobic bacteria other than *Bacteroides*/*Parabacteroides* species for β-lactamase activity (93%), nor for inducible clindamycin resistance *Bacteroides*/*Parabacteroides* species (91%). They did not routinely test carbapenem-resistant *B. fragilis* isolates for carbapenemase production (77%) and when they did, they predominantly used a MALDI-TOF mass spectrometry-based subtyping approach (40%).

3.2. Epidemiology of anaerobic species isolated from positive blood cultures

In this study, 14,527 anaerobic isolates met the inclusion criteria (Fig. 2A). According to Gram staining, Gram-positive bacilli, Gram-negative bacilli, Gram-positive cocci, and Gram-negative cocci were 45%, 40%, 14%, and 1%, respectively (Fig. 2B). Anaerobic species were predominantly identified in patients admitted to medical wards (31%), emergency (30%), ICU (18%), surgical wards (15%), and paediatrics departments (1%) (Fig. 2C). More than half of these isolates were identified in centres from five countries such as Sweden (14.8%), France (14%), Germany (11.4%), Norway (7.1%), and Spain (6.8%) (Fig. 2D). More than 300 species were identified, 128 of them with at least 10 isolates. The most prevalent species



Abbreviations: ICU: Intensive Care Unit; SW: Sweden; FR: France; GE: Germany; NO: Norway; SP: Spain; PT: Portugal; SLO: Slovenia; PL: Poland; NED: The Netherlands; HU: Hungary; IT: Italy; SWI: Switzerland; AU: Austria; IR: Ireland; BE: Belgium; GR: Greece; CRO: Croatia; DK: Denmark; CZ: Czech Republic; BU: Bulgaria; RO: Romania; SK: Slovak Republic.

Fig. 2. Distribution of anaerobes species according to year (A), Gram stain (B), ward (C), and country (D) of detection.

were *Cutibacterium acnes* (18.7%), *B. fragilis* (16.3%), *C. perfringens* (5.3%), *Bacteroides thetaiotaomicron* (4.2%), *F. nucleatum* (3.5%), and *Parvimonas micra* (3.4%) (Fig. 3). The *B. fragilis* group accounted for 25.9%. The isolates' distribution in the different wards showed that the 10 most frequent anaerobes accounted for between 54% (Emergency) and 68% (ICUs and paediatrics departments) of the total isolates (Fig. S2). *Actinomyces naeslundii, Actinomyces oris, Schaalia odontolytica*, and *F. necrophorum* were more frequent in paediatrics departments, while *Staphylococcus saccharolyticus* in ICUs. *B. fragilis* accounted for between 7% (ICUs) and 21% (surgical wards), while *C. acnes* between 9% (Emergency) and 42% (ICUs). The distribution of the different species by country of detection showed that the 10 most frequent anaerobic isolates accounted for between 50% (Slovenia) and 100% (Romania and Slovak Republic) of the total isolates (Fig. 4). *B. fragilis* accounted for between 5% (Greece) and 57% (Slovak Republic), while *C. acnes* between 2% (Belgium) and more than 70% (Greece and Hungary).

3.3. Burden of antimicrobial resistance in anaerobic species

Detailed susceptibility testing results for MIC and disk diffusion methods were shown in Tables S2 and S3, respectively. Antimicrobial susceptibility profiles according to EUCAST clinical breakpoints v.15.0 of the top 50 anaerobes species tested by MIC methods were summarized in Fig. 5, while those obtained by disk diffusion were reported in Fig. 6. In both figures, species-antibiotic combinations with less than 10 results were not represented. The bubble plots visualized the resistance profiles of bacterial species to various antibiotics and provided an integrative overview of resistance rates and sample coverage. The heatmaps displayed the percentage resistance of bacterial species to various antibiotics, with clustering applied to reveal patterns in resistance profiles. Species that are phylogenetically closer tend to cluster together (i.e. *Bacteroides* species), reflecting shared resistance traits.

An overview of antimicrobial susceptibility profiles in the main anaerobic species was shown in Table 1.

Among *C. acnes* isolates tested by MIC methods, over 10% resistance was shown for benzylpenicillin, ampicillin, clindamycin, and imipenem. Over 90% activity was observed for ertapenem, meropenem, and vancomycin. Among those tested by disk diffusion, over 10% resistance was shown for imipenem. Over 90% activity was observed for benzylpenicillin, amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, ertapenem, meropenem, and vancomycin.

Among *B. fragilis* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, clindamycin, and meropenem. Over 90% activity was observed for ertapenem, imipenem, and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam, clindamycin, ertapenem, and metronidazole. Over 90% activity was observed for amoxicillin/clavulanate, imipenem, and meropenem.

Among *B. thetaiotaomicron* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, and clindamycin. Over 90% activity was observed for imipenem, meropenem, and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam, clindamycin, and metronidazole. Over 90% activity was observed for amoxicillin/clavulanate, imipenem, and meropenem.

Among *Phocaeicola vulgatus* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, clindamycin, and meropenem. Over 90% activity was observed for imipenem and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam and clindamycin. Over 90% activity was observed for imipenem, meropenem, and metronidazole.

Among *Bacteroides ovatus* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, clindamycin, and imipenem. Over 90% activity was observed for meropenem and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam, clindamycin, imipenem, meropenem, and metronidazole.

Among *Bacteroides uniformis* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, piperacillin/tazobactam, and clindamycin. Over 90% activity was observed for imipenem, meropenem, and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam, clindamycin, and imipenem. Over 90% activity was observed for metronidazole.

Among *Parabacteroides distasonis* isolates tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, imipenem, and meropenem. Over 90% activity was observed for metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam, clindamycin, imipenem, and metronidazole.

Among *Bacteroides* isolates for which no precise species identification was provided (*Bacteroides* spp.) and tested by MIC methods, over 10% resistance was observed for amoxicillin/clavulanate, piperacillin/tazobactam, and clindamycin. Over 90% activity was observed for imipenem, meropenem, and metronidazole. Among those tested by disk diffusion, over 10% resistance was observed for piperacillin/tazobactam and clindamycin. Over 90% activity was observed for meropenem and metronidazole.

Among *C. perfringens* isolates tested by MIC methods, over 10% resistance was found for amoxicillin/clavulanate, piperacillin/tazobactam, and clindamycin. Over 90% activity was observed for benzylpenicillin, ampicillin, carbapenems, metronidazole, and vancomycin. Among those tested by disk diffusion, over 10% resistance was shown for clindamycin. Over 90% activity was observed for benzylpenicillin, amoxicillin/clavulanate, piperacillin/tazobactam, imipenem, meropenem, metronidazole, and vancomycin.

Among *F. nucleatum* isolates tested by MIC methods, over 10% resistance was found for benzylpenicillin. Over 90% activity was observed for ampicillin/sulbactam, amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, imipenem, meropenem, and metronidazole. Among those tested by disk diffusion and according to CA-SFM clinical breakpoints, an activity of over 90% was observed for amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, imipenem, and metronidazole.

Among *F. necrophorum* isolates tested by MIC methods, over 10% resistance rate was shown for clindamycin, meropenem, and metronidazole. Over 90% activity was observed for benzylpenicillin, amoxicillin/clavulanate (according to CLSI and CA-SFM clinical breakpoints), piperacillin/tazobactam, and imipenem. Among those tested by disk diffusion, an activity of over 90% was observed for benzylpenicillin, piperacillin/tazobactam, clindamycin, meropenem, and metronidazole, while over 10% resistance was observed for imipenem.

Among *P. micra* isolates tested by MIC methods, over 10% resistance was shown for clindamycin, while over 90% activity was observed for benzylpenicillin, amoxicillin/clavulanate, piperacillin/tazobactam, carbapenems, metronidazole, and vancomycin. Among those tested by disk diffusion and according to CA-SFM clinical breakpoints, an activity of over 90% was observed for all antimicrobials tested.

Among *Eggerthella lenta* isolates tested by MIC methods, over 10% resistance was shown for benzylpenicillin, amoxi-

Fig. 3. Species distribution of the top 50 anaerobic isolates detected. AU, Austria; BE, Belgium; BU, Bulgaria; CRO, Croatia; CZ, Czech Republic; DK, Denmark; FR, France; GE, Germany; GR, Greece; HU, Hungary; ICU, intensive care unit; IR, Ireland; IT, Italy; NED, The Netherlands; NO, Norway; PL, Poland; PT, Portugal; RO, Romania; SK, Slovak Republic; SLO, Slovenia; SP, Spain; SW, Sweden; SWI, Switzerland.

Table 1
Overview of antimicrobial resistance burden in the main anaerobic species according to EUCAST v. 15.0, CLSI M100 ED35:2025, and CA-SFM 2024 v.1.0 guidelines.

Species identification	Antimicrobial drug	MIC methods					Disk diffusion			
		MIC_{50} (mg/L)	MIC_{90} (mg/L)	EUCAST resistance %	CLSI resistance %	CA-SFM resistance %	Inhibition zone diameter, median (mm)	IQR	EUCAST resistance %	CA-SFM resistance %
Cutibacterium acnes	Benzylpenicillin	0.032	0.19	18	3		30	2	2	
	Ampicillin	0.064	0.75	20	6		ND	ND		
	Amoxicillin/clavulanate	0.064	0.25		0	0	38	9	4	0
	Piperacillin/tazobactam	0.25	1		0	1	31	8	3	0
	Clindamycin	0.125	4	39	6	6	29	7	6	3
	Ertapenem	0.032	0.125	8	8	8	39	7	9	
	Imipenem	0.008	0.047	19	1	1	44	7	13	0
	Meropenem	0.032	0.125	9	1	1	32	4	3	
	Vancomycin	0.25	0.5	1		1	23	4	1	0
Bacteroides fragilis	Amoxicillin/clavulanate	0.5	24	24	12	13	32	14	3	1
	Ampicillin/sulbactam	0.25	4	12	4		ND	ND		
	Piperacillin/tazobactam	1	12	22	4	8	29	6	14	2
	Clindamycin	0.5	256	22	21	22	21	18	22	35
	Ertapenem	0.094	0.5	5	5	5	30	6	12	
	Imipenem	0.064	0.5	4	2	3	39	12	4	0
	Meropenem	0.125	3	13	7	7	32	4	9	
	Metronidazole	0.5	1.5	3	2	3	30	5	10	1
Bacteroides thetaiotaomicron	Amoxicillin/clavulanate	1.5	96	42	21	23	33	14	5	6
	Ampicillin/sulbactam	0.75	16	25	9		ND	ND		
	Piperacillin/tazobactam	16	64	93	9	45	22	5	64	12
	Clindamycin	2	256	34	32	34	6	5	67	83
	Imipenem	0.25	0.5	4	1	2	38	9	6	0
	Meropenem	0.25	1	7	2	2	30	2	6	
	Metronidazole	0.5	1.5	3	2	3	30	7	13	0
Phocaeicola vulgatus	Amoxicillin/clavulanate	0.75	48	33	19	22	32	16	ND	13
	Ampicillin/sulbactam	1.5	48	40	10		ND	ND		
	Piperacillin/tazobactam	8	32	75	3	19	24	5	41	5
	Clindamycin	0.5	256	32	31	32	19	23	37	44
	Imipenem	0.19	0.75	2	0	0	37	6	5	0
	Meropenem	0.25	1.5	11	3	3	31	3	5	
	Metronidazole	0.5	1.5	5	4	5	32	5	6	2
Bacteroide ovatus	Amoxicillin/clavulanate	2	128	42	25	28	26	14	0	11
	Ampicillin/sulbactam	0.5	12	29	0		ND	ND		
	Piperacillin/tazobactam	8	64	78	10	21	25	6	35	15
	Clindamycin	1	256	30	30	30	9	16	53	63
	Imipenem	0.25	2	16	2	4	38	8	12	0
	Meropenem	0.25	1	6	0	0	31	5	12	
	Metronidazole	0.5	2	2	2	2	28	7	23	0
Bacteroides uniformis	Amoxicillin/clavulanate	2	24	46	17	20	32	16	ND	5
	Piperacillin/tazobactam	4	16	54	3	8	27	6	21	4
	Clindamycin	1	256	31	30	31	11	10	46	69
	Imipenem	0.25	0.5	0	0	0	40	11	17	0
	Meropenem	0.25	0.75	4	0	0	ND	ND		
	Metronidazole	0.5	2	4	2	4	32	19	6	0
Parabacteroides distasonis	Amoxicillin/clavulanate	4	256	59	34	36	22	8	ND	28
	Piperacillin/tazobactam	8	48	88	6	15	25	9	37	0
	Clindamycin	2	256	32	32	32	6	1	78	96
	Imipenem	0.5	1.5	12	0	2	33	13	17	6
	Meropenem	0.25	1.5	11	0	0	ND	ND		
	Metronidazole	0.5	2	1	1	1	28	6	19	0
Bacteroides spp.	Amoxicillin/clavulanate	1.5	32	41	27	27	ND	ND		
	Piperacillin/tazobactam	8	32	79	4	15	24	4	35	6
	Clindamycin	1	256	13	13	13	14	15	35	59
	Imipenem	0.25	0.5	5	0	0	ND	ND		
	Meropenem	0.25	0.38	5	0	0	32	14	7	
	Metronidazole	0.25	1.5	3	3	3	31	9	0	0
Clostridium perfringens	Benzylpenicillin	0.064	0.25	8	7		23	4	0	
	Ampicillin	0.023	0.19	0	0		ND	ND		
	Amoxicillin/clavulanate	0.016	0.5	11	3	3	31	9	4	0
	Piperacillin/tazobactam	0.032	1	12	0	1	28	5	6	0
	Clindamycin	1	4	69	8	9	20	13	45	35

(*continued on next page*)

Table 1 (*continued*)

Species identification	Antimicrobial drug	MIC methods					Disk diffusion			
		MIC_{50} (mg/L)	MIC_{90} (mg/L)	EUCAST resistance %	CLSI resistance %	CA-SFM resistance %	Inhibition zone diameter, median (mm)	IQR	EUCAST resistance %	CA-SFM resistance %
	Ertapenem	0.008	0.19	0	0	0	ND	ND		
	Imipenem	0.094	0.125	2	0	0	31	11	4	0
	Meropenem	0.008	0.047	4	0	0	30	6	6	
	Metronidazole	1	2	2	1	2	22	4	2	0
	Vancomycin	0.5	0.75	0		0	17	3	0	2
Fusobacterium nucleatum	Benzylpenicillin	0.016	1.5	11	9		ND	ND		
	Ampicillin/sulbactam	0.016	0.064	7	3		ND	ND		
	Amoxicillin/clavulanate	0.016	0.25	7	5	5	40	10		0
	Piperacillin/tazobactam	0.032	0.125	1	0	1	40	6		0
	Clindamycin	0.032	0.25	8	5	5	35	9		4
	Imipenem	0.032	0.064	0	0	0	40	8		0
	Meropenem	0.008	0.047	2	1	1	ND	ND		
	Metronidazole	0.016	0.25	3	1	3	45	11		0
Fusobacterium necrophorum	Benzylpenicillin	0.016	0.064	8	5		32	7	0	
	Ampicillin/sulbactam	ND	ND				ND	ND		
	Amoxicillin/clavulanate	0.032	0.75		9	9	40	8		0
	Piperacillin/tazobactam	0.016	0.064	4	0	0	40	9	9	0
	Clindamycin	0.032	0.5	12	8	8	36	6	9	0
	Imipenem	0.032	0.064	8	0	0	40	7	24	0
	Meropenem	0.008	0.064	16	0	0	40	2	0	
	Metronidazole	0.094	0.75	11	7	7	40	11	2	0
Parvimonas micra	Benzylpenicillin	0.016	0.064	4	2		ND	ND		
	Amoxicillin/clavulanate	0.016	0.19	2	1	1	38	8		0
	Piperacillin/tazobactam	0.032	0.125	2	0	1	39	8		1
	Clindamycin	0.125	1.5	13	9	10	24	10		4
	Imipenem	0.016	0.032	0	0	0	38	11		0
	Meropenem	0.016	0.064	0	0	0	ND	ND		
	Metronidazole	0.125	1	4	3	4	40	9		0
	Vancomycin	0.38	1	2		2	28	10		3
Eggerthella lenta	Benzylpenicillin	1	2	61	20		ND	ND		
	Amoxicillin/clavulanate	0.5	2	47	1	1	35	9		0
	Clindamycin	0.19	4	18	10	10	27	12		14
	Imipenem	0.38	0.75	2	2	2	39	6		0
	Meropenem	0.25	0.5	0	0	0	ND	ND		
	Metronidazole	0.25	2	7	6	7	32	9		0
	Vancomycin	1	2	2		2	29	13		10
Staphylococcus saccharolyticus	Benzylpenicillin	0.008	0.125	0	0		ND	ND		
	Ampicillin/sulbactam	0.016	0.016	0	0		ND	ND		
	Amoxicillin/clavulanate	0.064	0.125	0	0	0	ND	ND		
	Piperacillin/tazobactam	0.047	0.38	0	0	0	ND	ND		
	Clindamycin	0.125	0.38	6	1	1	28	3		0
	Imipenem	ND	ND				ND	ND		
	Meropenem	0.016	0.032	0	0	0	ND	ND		
	Vancomycin	0.75	1	2		2	ND	ND		

Grey shading highlights resistance rate ≥10%. MIC_{50}, MIC_{90}, and IQR of inhibition zone diameter only reported if obtained from ≥10 values. EUCAST clinical breakpoints for resistance: *Bacteroides* spp.: ampicillin/sulbactam >2 mg/L; amoxicillin/clavulanate >2 mg/L; piperacillin/tazobactam >2 mg/L; ertapenem >2 mg/L; meropenem >1 mg/L; imipenem >1 mg/L; clindamycin >4 mg/L; metronidazole >4 mg/L. *Prevotella* spp.: benzylpenicillin >0.5 mg/L; ampicillin >0.5 mg/L; amoxicillin >0.25 mg/L; ertapenem >0.5 mg/L; imipenem >0.125 mg/L; meropenem >0.25 mg/L; clindamycin >0.25 mg/L; metronidazole >4 mg/L. *Fusobacterium necrophorum*: benzylpenicillin >0.125 mg/L; ampicillin >0.5 mg/L; ampicillin/sulbactam >0.5 mg/L; amoxicillin >0.5 mg/L; amoxicillin/clavulanate >0.5 mg/L; piperacillin/tazobactam >0.5 mg/L; ertapenem >0.06 mg/L; imipenem >0.125 mg/L; meropenem >0.03 mg/L; clindamycin >0.25 mg/L; metronidazole >0.5 mg/L. *Clostridium perfringens*: benzylpenicillin >0.5 mg/L; ampicillin >0.25 mg/L; ampicillin/sulbactam >0.25 mg/L; amoxicillin >0.25 mg/L; amoxicillin/clavulanate >0.25 mg/L; piperacillin/tazobactam >0.5 mg/L; ertapenem >0.5 mg/L; imipenem >0.5 mg/L; meropenem >0.125 mg/L; vancomycin >2 mg/L; clindamycin >0.25 mg/L; metronidazole >4 mg/L. *Cutibacterium acnes*: benzylpenicillin >0.06 mg/L; ampicillin >0.25 mg/L; amoxicillin >0.25 mg/L; ceftriaxone >0.06 mg/L; ertapenem >0.25 mg/L; imipenem >0.03 mg/L; meropenem >0.125 mg/L; vancomycin >2 mg/L; clindamycin >0.25 mg/L; linezolid >2 mg/L. *Clostridioides difficile*: vancomycin >2 mg/L; fidaxomicin >0.5 mg/L; metronidazole >2 mg/L. For all the other species: EUCAST guidance on 'Where there are no breakpoints in breakpoint tables?' – MIC values above which therapy with the agent should be discouraged: benzylpenicillin >0.5 mg/L; amoxicillin >0.5 mg/L; amoxicillin/clavulanate >0.5 mg/L; ampicillin/sulbactam >0.5 mg/L; piperacillin/tazobactam >2 mg/L; meropenem >1 mg/L; imipenem >1 mg/L; ertapenem >0.25 mg/L; clindamycin >0.5 mg/L; metronidazole >4 mg/L; vancomycin >2 mg/L (only for Gram-positives); rifampicin >0.125 mg/L (only for Gram-positive). CLSI clinical breakpoints for resistance: benzylpenicillin ≥2 mg/L; ampicillin ≥2 mg/L; amoxicillin/clavulanate ≥16 mg/L; ampicillin/sulbactam ≥32 mg/L; piperacillin/tazobactam ≥128 mg/L; imipenem/relebactam ≥16 mg/L; ticarcillin/clavulanate ≥128 mg/L; cefoxitin ≥64 mg/L; ceftriaxone ≥64 mg/L; cefotaxime ≥64 mg/L; ertapenem ≥16 mg/L; imipenem ≥16 mg/L; meropenem ≥16 mg/L; tetracycline ≥16 mg/L; moxifloxacin ≥8 mg/L; clindamycin ≥8 mg/L; chloramphenicol ≥32 mg/L; metronidazole ≥32 mg/L. CA-SFM clinical breakpoints for resistance: amoxicillin >8 mg/L (only for Gram-positives); amoxicillin >2 mg/L (only for Gram-negatives, except *Prevotella* and similar, and *Bacteroides fragilis* group); amoxicillin >0.25 mg/L (only for *Prevotella* and similar); amoxicillin/clavulanate >8 mg/L; piperacillin/tazobactam >16 mg/L; ertapenem >0.5 mg/L; imipenem >4 mg/L; meropenem >8 mg/L; chloramphenicol >8 mg/L; clindamycin >4 mg/L; linezolid >4 mg/L; metronidazole >4 mg/L; moxifloxacin >2 mg/L; rifampicin >4 mg/L; tigecycline >8 mg/L; vancomycin >2 mg/L (only for Gram-positives).
IQR, interquartile range; ND, no data or <10 values; spp., species.

Species	AU	BE	BU	SWI	CZ	DK	FR	GE	GR	CRO	HU	IR	NED	NO	PL	PT	RO	SK	SLO	SP	SW
Actinomyces oris	2%				1%	3%				4%											
Actinomyces spp		6%	3%																		
Actinomyces viscosus			5%									2%									
Actinotignum schaalii														2%							
Anaerococcus spp																					5%
Bacteroides faecis																			2%		
Bacteroides fragilis	8%	17%	22%	25%	8%	9%	20%	15%	5%	17%	6%	9%	20%	22%	13%	13%	10%	57%	18%	18%	17%
Bacteroides ovatus		2%										2%		2%	1%				2%		
Bacteroides spp		7%							2%				5%								
Bacteroides thetaiotaomicron	3%	5%	5%			2%	6%	4%	3%	3%	1%	3%	7%	6%	3%	3%			4%	5%	6%
Bacteroides uniformis							2%														
Bifidobacterium spp												2%									
Clostridioides difficile			3%																		
Clostridium butyricum																	10%				
Clostridium innocuum													2%								
Clostridium paraputrificum			5%						1%						1%	1%					
Clostridium perfringens	3%	5%		3%	5%	3%	4%	3%	2%	6%	2%	13%	7%	5%	7%	9%	10%	14%	6%	11%	4%
Clostridium septicum												2%									
Clostridium spp		6%							2%	2%											
Clostridium subterminale			3%																		
Clostridium tertium															1%	1%					
Cutibacterium acnes	42%	2%	41%	3%	35%	32%	10%	18%	73%	29%	71%	25%	13%	6%	37%	41%			6%	8%	5%
Cutibacterium avidum	2%										1%					2%					
Cutibacterium spp	6%							2%		2%							10%	14%			4%
Eggerthella lenta				2%			2%		2%		1%	2%		2%	3%	3%				3%	
Finegoldia magna					2%	2%		2%													
Fusobacterium necrophorum			3%	2%			2%														
Fusobacterium nucleatum		7%		5%	3%	3%	5%	4%	2%	2%	2%		5%	3%		1%			5%	5%	3%
Fusobacterium spp				2%									3%								
Gemella haemolysans																	10%				
Gemella morbillorum			3%		3%																
Lacticaseibacillus casei												3%									
Lacticaseibacillus paracasei						6%															
Lactobacillus gasseri					2%																
Lactobacillus jensenii										2%											
Lacticaseibacillus rhamnosus				3%	3%	6%		3%		5%					3%						
Lactobacillus spp																	10%	14%		2%	4%
Limosilactobacillus mucosae																	10%				
Parabacteroides distasonis				3%																	
Parvimonas micra	2%	5%		6%	2%		4%	5%	1%		1%		2%	2%	3%	2%			4%	4%	5%
Peptoniphilus spp																					3%
Phocaeicola vulgatus	2%													3%					2%	2%	
Schaalia odontolytica						5%	2%				1%						20%				
Staphylococcus saccharolyticus								7%			4%										
Thomasclavelia ramosa	2%												2%							2%	
Veillonella parvula																			2%		
Winkia neuii																	10%				
TOP 10 SUM	71%	63%	92%	53%	65%	71%	55%	62%	91%	70%	89%	64%	66%	54%	73%	77%	100%	100%	50%	59%	55%

Abbreviations: ICU: Intensive Care Unit; SW: Sweden; FR: France; GE: Germany; NO: Norway; SP: Spain; PT: Portugal; SLO: Slovenia; PL: Poland; NED: The Netherlands; HU: Hungary; IT: Italy; SWI: Switzerland; AU: Austria; IR: Ireland; BE: Belgium; GR: Greece; CRO: Croatia; DK: Denmark; CZ: Czech Republic; BU: Bulgaria; RO: Romania; SK: Slovak Republic.

Fig. 4. The top 10 anaerobic isolates by country of detection.

cillin/clavulanate, and clindamycin. An activity of over 90% was observed for imipenem, meropenem, metronidazole, and vancomycin. Among those tested by disk diffusion, over 10% resistance was observed for clindamycin and vancomycin according to CA-SFM clinical breakpoints.

Among *S. saccharolyticus* isolates tested by MIC methods, an activity of over 90% was observed for benzylpenicillin, ampicillin/sulbactam, amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, imipenem, meropenem, and vancomycin. Among those tested by disk diffusion and according to CA-SFM clinical breakpoints, an activity of over 90% was observed for clindamycin.

Overall, among *Prevotella* species isolates tested by MIC methods, over 10% resistance was observed for benzylpenicillin and clindamycin. An activity of over 90% for metronidazole (except in *Prevotella bivia* and *Prevotella melaninogenica*) and carbapenems was observed. Among those tested by disk diffusion, resistance to piperacillin/tazobactam, clindamycin, and metronidazole of more than 10% was observed in *P. bivia*. Among *Veillonella* species isolates tested by MIC methods, over 10% resistance was observed for benzylpenicillin, amoxicillin/clavulanate (*Veillonella atypica* and *Veillonella parvula*), piperacillin/tazobactam (V. *atypica* and V. *parvula*), meropenem (V. *parvula*), and metronidazole (V. *atypica* and V. *parvula*). An activity of over 90% was observed for clindamycin.

4. Discussion

This *real-world lab* study illustrated current practices on anaerobes diagnostics from blood culture and their close connection to surveillance, antibiotic consumption, and antimicrobial resistance in Europe. Its findings might serve as a foundation for future comparative analyses and provide support for the development of diagnostic and antimicrobial stewardship strategies, as well as the optimization of current antibiotic treatments. This snapshot of anaerobic diagnostics highlighted the participating centres' awareness of their role in the underdiagnosis of anaerobic infections, both due to the technical limitations of the equipment and the shortcomings of the facilities, such as the presence of a dedicated sector with trained staff. MALDI-TOF coupled to mass spectrometry was the most widely used tool for species identification. Approximately 40% of the laboratories had multiplex rapid molecular tests applicable to positive blood cultures, capable of detecting only one or a few anaerobic species (mainly *B. fragilis*). Antimicrobial susceptibility testing was performed in the vast majority of centres, using mostly EUCAST guidelines, although sometimes they seemed to be adapted to local contexts, laboratory needs, or clinical considerations (pathogen vs. contaminant). The most commonly used methods were gradient diffusion strip and disk diffusion and no centre performed agar dilution as recommended by the main guidelines. The main mechanisms of resistance were rarely investigated. Together with *C. acnes*, the *B. fragilis* group, and *C. perfringens*, two other species (*F. nucleatum* and *P. micra*) emerged as particularly frequent. *C. acnes* was also the anaerobe most frequently not tested for antimicrobial susceptibility, as it was considered a contaminant by microbiological and clinical criteria. Antimicrobial resistance profiles showed great diversity depending on the susceptibility testing method and clinical breakpoints used, as well as depending on species identification. Overall and according to current EUCAST guidelines, clindamycin and piperacillin/tazobactam showed the lowest activity towards the most frequently detected species. *B. fragilis* showed high resistance to amoxicillin/clavulanate, piperacillin/tazobactam,

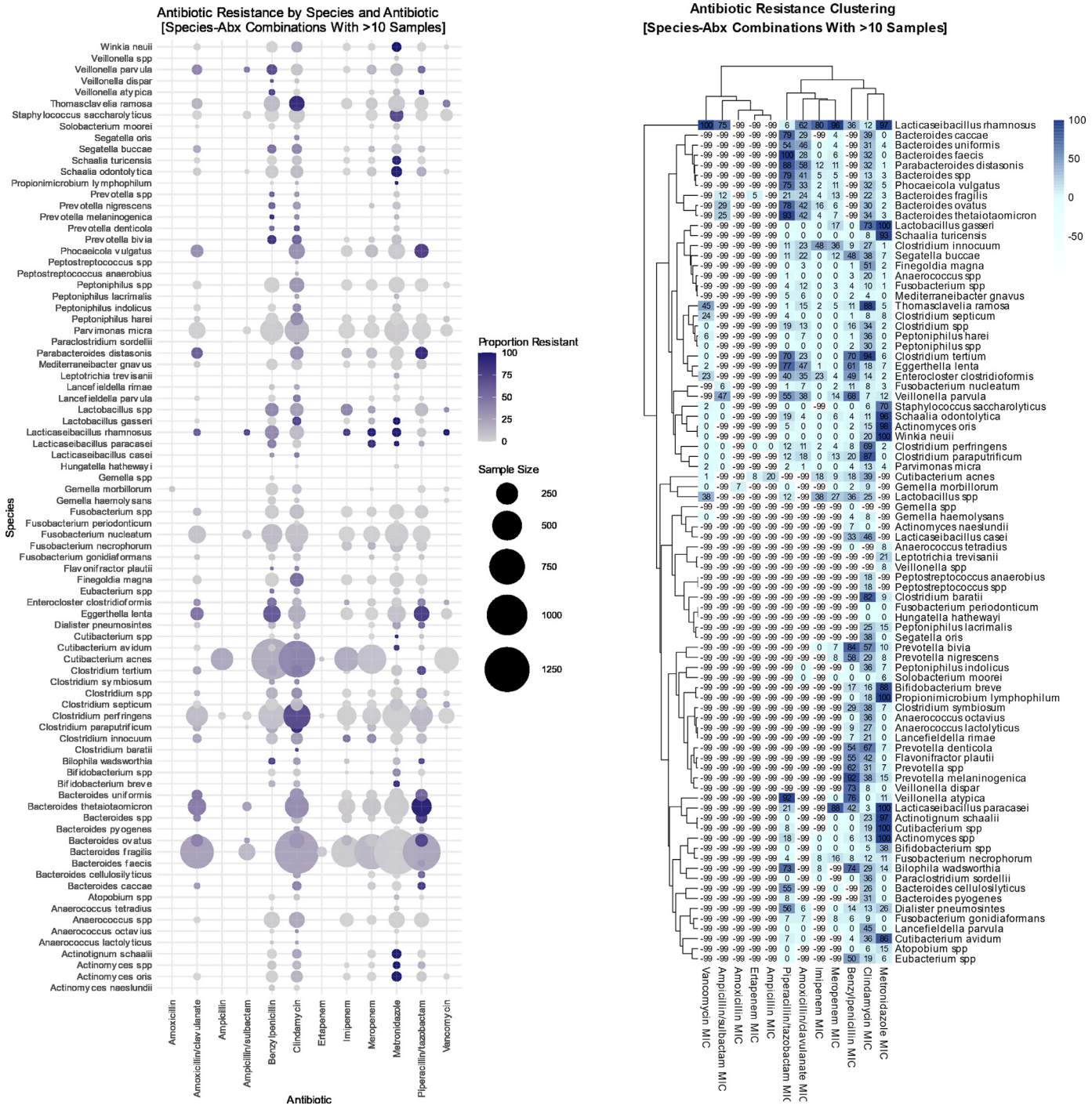


Fig. 5. Antimicrobial susceptibility profiles by MIC methods of the top 50 anaerobes according EUCAST clinical breakpoints v.15.0. The bubble plot visualizes the resistance profiles of bacterial species (*y*-axis) to various antibiotics (*x*-axis). The size of each bubble represents the sample size for the species-antibiotic combination, while the colour intensity, ranging from light blue to dark blue, corresponds to the percentage resistance. White spaces indicate missing data, where no resistance data was available for the respective species-antibiotic combination. The plot reveals patterns in antibiotic resistance, with larger and darker bubbles highlighting species-antibiotic pairs of concern due to higher sample sizes and elevated resistance rates. Phylogenetically related species tend to show similar resistance profiles, though these patterns may not be fully consistent due to uneven data availability and sampling limitations. The heatmap displays the percentage resistance of bacterial species (rows) to various antibiotics (columns), with clustering applied to both rows and columns to reveal patterns in resistance profiles. The dendrograms illustrate hierarchical clustering based on Euclidean distances and complete linkage, grouping species and antibiotics with similar resistance patterns. Shades of blue represent resistance rates, ranging from light blue (low resistance) to midnight blue (high resistance), as per the colour gradient. Data calculated on less than 10 species-antibiotic combinations, replaced with the placeholder value of −99, is depicted in grey for clarity. The displayed values correspond to resistance percentages derived from experimental data, highlighting key trends and gaps in coverage. Species that are phylogenetically closer tend to cluster together (i.e. *Bacteroides* species), reflecting shared resistance traits. However, this clustering is not perfect due to the variability in data availability and potential biases introduced by incomplete or missing data.

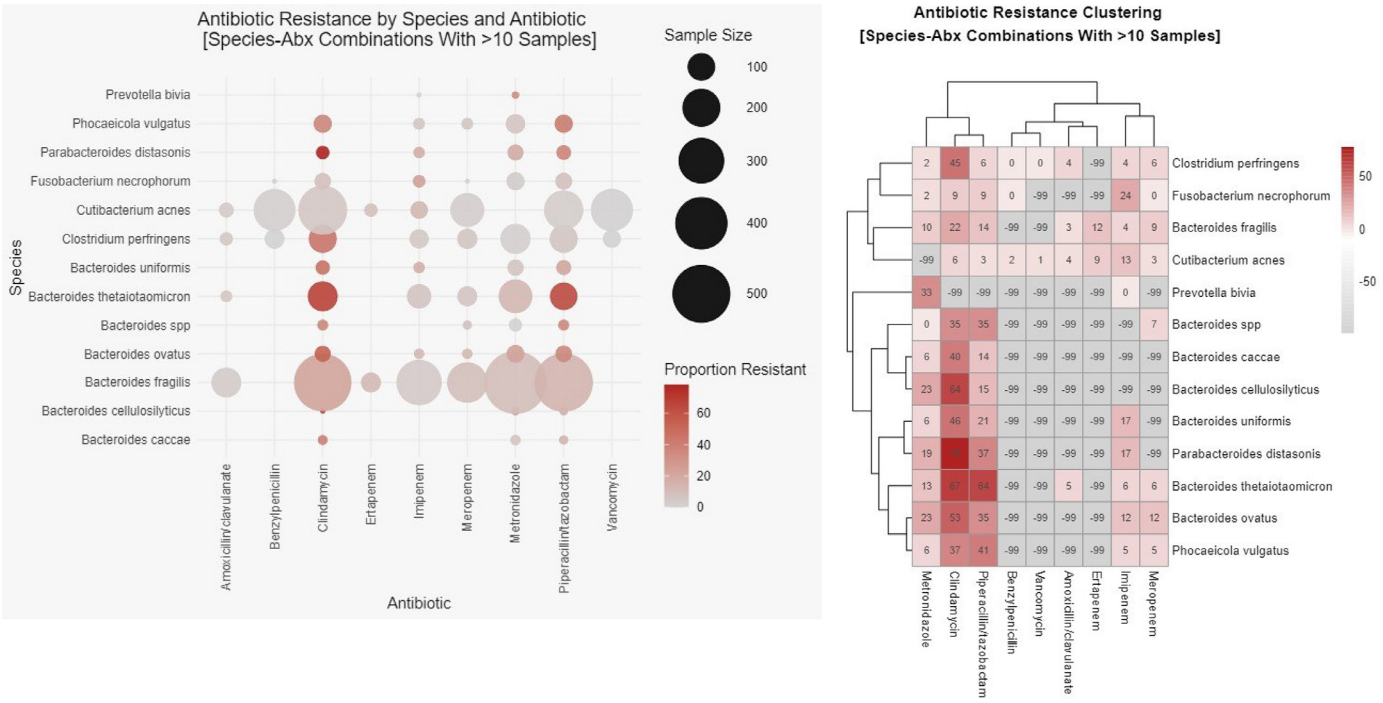


Fig. 6. Antimicrobial susceptibility profiles by disk diffusion of the top 50 anaerobes tested according EUCAST clinical breakpoints v.15.0. The bubble plot visualizes the resistance profiles of bacterial species (*y*-axis) to various antibiotics (*x*-axis). The size of each bubble represents the sample size for the species-antibiotic combination, while the colour intensity, ranging from light red to dark red, corresponds to the percentage resistance. White spaces indicate missing data, where no resistance data was available for the respective species-antibiotic combination. The plot reveals patterns in antibiotic resistance, with larger and darker bubbles highlighting species-antibiotic pairs of concern due to higher sample sizes and elevated resistance rates. Phylogenetically related species tend to show similar resistance profiles, though these patterns may not be fully consistent due to uneven data availability and sampling limitations. This visualization provides an integrative overview of resistance rates and sample coverage. The heatmap displays the percentage resistance of bacterial species (rows) to various antibiotics (columns), with clustering applied to both rows and columns to reveal patterns in resistance profiles. The dendrograms illustrate hierarchical clustering based on Euclidean distances and complete linkage, grouping species and antibiotics with similar resistance patterns. Shades of red represent resistance rates, ranging from light rose (low resistance) to dark red (high resistance), as per the colour gradient. Data calculated on less than 10 species-antibiotic combinations, replaced with the placeholder value of −99, is depicted in grey for clarity. The displayed values correspond to resistance percentages derived from experimental data, highlighting key trends and gaps in coverage. Species that are phylogenetically closer tend to cluster together (i.e. *Bacteroides* species), reflecting shared resistance traits. However, this clustering is not perfect due to the variability in data availability and potential biases introduced by incomplete or missing data.

clindamycin, meropenem, and metronidazole (only by disk diffusion). A similar resistance pattern was observed in *B. thetaiotaomicron, B. ovatus*, and *P. distasonis. C. perfringens* showed high resistance to clindamycin, while benzylpenicillin and metronidazole maintained high activity. *F. nucleatum* showed high resistance to benzylpenicillin, while *F. necrophorum* showed alarming rates of resistance to clindamycin, meropenem, and metronidazole. *P. micra* showed high resistance to clindamycin.

Over the years, the diagnostic landscape of anaerobes has alternated between phases of stagnation and phases of acceleration. There were periods when identification and antimicrobial susceptibility testing of isolates were performed in a haphazard manner [35–37] and more recent periods characterized by the widespread use of MALDI-TOF coupled to mass spectrometry and when resistance profiles are no longer considered predictable from species identification alone [4,38]. However, some fundamental flaws persist. Despite being considered the reference method for conducting antibiotic susceptibility testing, agar dilution was not carried out at any centre probably because it was considered unsuitable for routine laboratory workflows. The gradient diffusion strip method for MIC testing continues to be the most widely used despite its performance being considered only acceptable [32] and presented several discrepancies, especially in relation to clindamycin [39].

Although the EUCAST guidelines were the most widely followed, the use in Europe of other guidelines such as those of the CA-SFM (exclusively in France) and CLSI highlighted the strengths and weaknesses of each. The EUCAST guidelines provide clinical breakpoints for the interpretation of MIC and inhibition zone diameter values for six of the most frequent anaerobic species (*Bacteroides* spp., *C. acnes, F. necrophorum, Prevotella* spp., *C. perfringens*, and *C. difficile*) [34]. This is supplemented by a document reporting only MIC values (and not on inhibition zone diameters) to guide the use of antimicrobials against anaerobic bacteria for which there are no breakpoints in the EUCAST standard tables [31]. CA-SFM and CLSI present clinical breakpoints that are not species-specific and, in many cases differ from those provided by EUCAST, leading to very different data on resistance rates. Furthermore, CLSI does not provide clinical breakpoints for disk diffusion, while CA-SFM provides ATU values for disk diffusion that are absent in EUCAST guidelines. The heterogeneity of guidelines and sometimes the need to make them flexible to the local context therefore seem to be the most significant obstacles in the path towards understanding the burden of antimicrobial resistance in anaerobes. This study showed that the use of MALDI-TOF coupled with mass spectrometry for anaerobes diagnostics is now systematised in Europe. This could form the basis of a strategy to establish specific breakpoints for all anaerobic species for both MIC and disk diffusion methods. This may be even more urgent for *F. nucleatum* and *P. micra* in particular, which this study showed to be epidemiologically relevant.

Our study also identified relevant antimicrobial susceptibility findings. Although it is often considered a contaminant and its role in orthopaedic post-surgical infections is still debated [40], our findings showed that *C. acnes* displayed a much more complex

antimicrobial profile than in the past [3], reporting similar rates of resistance to benzylpenicillin and clindamycin as in Kuwait in a previous study [3] together with the non-negligible emergence of resistance to imipenem detected by both MIC and disk diffusion methods, which further studies will have to confirm. With regard to *Bacteroides* species, our data consolidated those on clindamycin resistance reported in an ATLAS programme study [5] and extended them in relation to other antibiotics. Indeed, our study highlighted worrisome resistance rates to amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, meropenem, and metronidazole in *B. fragilis, B. thetaiotaomicron, B. ovatus*, and *P. distasonis*. Our data were only partially consistent with the results of previous European multicentre studies on *Bacteroides*/*Parabacteroides* species [3] and *B. fragilis* [5] and attest to a general increase in resistance rates. In particular, in comparison with the results of the European multicentre study ReSuBacFrag on *B. fragilis* isolates detected by blood culture in 2022, our study confirmed relevant resistance rates to piperacillin/tazobactam, clindamycin, meropenem and reported the emergence of resistance to metronidazole tested by disk diffusion. Moreover, our data revealed that *C. perfringens* displayed high resistance rates to clindamycin, previously only documented in Belgium and the Netherlands [3], and showed over 10% resistance to amoxicillin/clavulanate and piperacillin/tazobactam with MIC methods. Similarly, our data revealed a worrying reduction in benzylpenicillin activity against *F. nucleatum* [3], and highlighted the emergence of significant resistance to clindamycin, meropenem, and metronidazole in *F. necrophorum*. As for the most frequently isolated species, the antimicrobial multi-susceptibility profile of *P. micra* was consistent with those reported in the literature [3,41], although the data on clindamycin deserve further monitoring.

The strength of the present study was to collect data from a large, multicentre surveillance study, attempting to treat anaerobic bacteria with a synthetic perspective and on a par with other bacterial species, filling critical gaps in European epidemiological knowledge and trying to identify aspects to be developed and improved. However, some limitations should be acknowledged. Firstly, neither the different species nor the different countries were equally represented, so there is an important bias that is worth recognising. Secondly, susceptibility testing was performed by various methods as well as there may have been selection bias in the antibiotics tested at the first line and afterwards, which could have affected the integrity of the data. Thirdly, the study faced challenges in reinterpreting MIC values and inhibition zone diameters according to both EUCAST, CLSI and CA-SFM breakpoints given some commercial microdilution methods provided results within a limited range and the discrepancies in guidelines for disk antibiotic concentration, except for piperacillin/tazobactam, ertapenem, imipenem, meropenem, clindamycin, and metronidazole. Finally, the limited number of isolates identified restricts the generalizability of results, particularly concerning the occurrence of more rare species and their contribution to antimicrobial resistance.

In summary, this study revealed that most participating European centres would like to improve their accuracy in diagnosing anaerobes while maintaining the need to adapt diagnostics to the routine workflow. No centre used agar dilution, while gradient diffusion strip and disk diffusion were the preferred methods for antibiotic susceptibility testing. *F. nucleatum* and *P. micra* were among the most frequently isolated anaerobes, but do not currently have species-specific EUCAST breakpoints. Overall, the most frequently isolated species showed high resistance to clindamycin and piperacillin/tazobactam, strengthening the evidence warning against their empirical use for the treatment of infections with suspected aetiology of these organisms. Species belonging to the *B. fragilis* group showed different resistance profiles, with the most alarming being amoxicillin/clavulanate, piperacillin/tazobactam, clindamycin, meropenem, and metronidazole detected in *B. fragilis, B. thetaiotaomicron, B. ovatus*, and *P. distasonis*. Benzylpenicillin maintained high activity against *C. perfringens* but not against *F. nucleatum*. Shared guidelines, consistent with laboratory workflows, with species-specific breakpoints for both MIC and disk diffusion methods, and their strict adherence seem to be a must in order to fully understand the burden of antimicrobial resistance associated with anaerobes in Europe.

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Ethical approval: This study was conducted in accordance with the Declaration of Helsinki. Formal ethical approval was obtained by the institutional review board of the coordinating Centre (Città della Salute e della Scienza di Torino, Protocol No. 0048443).

Sequence information: Not applicable.

Data availability: The authors confirm that the data supporting the findings of this study are available within the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2025.107478.

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