Journal of Infection 89 (2024) 106212

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Bacteria and Bacterial Diseases

Colorectal cancer and association with anaerobic bacteraemia: A Danish nationwide population-based cohort study

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ARTICLE INFO

Article history:
Accepted 21 June 2024
Available online 2 July 2024

Keywords:
Colorectal cancer
Bacteraemia
Anaerobic bacteria
Gut microbiota
Cohort study

SUMMARY

Objectives: We aimed to identify specific anaerobic bacteria causing bacteraemia and a subsequent diagnosis of colorectal cancer.

Methods: A nationwide population-based cohort study, which included all episodes of defined specific anaerobic bacteraemia from 2010 (5,534,738 inhabitants) through 2020 (5,822,763 inhabitants) and all cases of colorectal cancer diagnosed from 2010 through 2021 in Denmark. We calculated the incidence and risk of colorectal cancer after bacteraemia with specific anaerobic bacteria using *Escherichia coli* bacteraemia as reference.

Results: Nationwide data on colorectal cancer and specific anaerobic bacteraemia (100% complete, representing 11,124 episodes). The frequencies of colorectal cancer within one year following anaerobic bacteraemia were higher for species, which almost exclusively reside in the colon, such as *Phocaeicola vulgatus/dorei* (5.5%), *Clostridium septicum* (24.2%), and *Ruminococcus gnavus* (4.6%) compared to 0.6% in 50,650 *E. coli* bacteraemia episodes. *Bacteroides* spp. had a subhazard ratio for colorectal cancer of 3.9 (95% confidence interval [CI], 3.0 to 5.1) and for *Clostridium* spp. it was 8.9 (95% CI, 6.7 to 11.8, with *C. septicum* 50.0 [95% CI, 36.0 to 69.5]) compared to *E. coli* (reference).

Conclusion: This nationwide study identified specific colorectal cancer-associated anaerobic bacteria, which almost exclusively reside in the colon. Bacteraemia with these bacteria could be an indicator of colorectal cancer.

Introduction

Colorectal cancer is one of the most common types of cancer in the world and is associated with serious illness and death.[1,2] Its cause is thought to be multi-faceted, including environmental (diet, alcohol, smoking, obesity, inactivity) and genetic elements.[2] People

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https://doi.org/10.1016/j.jinf.2024.106212

with pre-existing conditions, such as inflammatory bowel disease, are more likely to develop colorectal cancer.[3] More recently, microorganisms have been identified as potential contributors to the development of colorectal cancer.[1]

Metagenomic association studies on the gut microbiome of colorectal cancer patients have focused on the distribution of bacterial DNA.[4,5] The distributions are often presented as a summary of species clustered in families, orders or even classes of bacteria, which can mask the association between specific species and diseases. As an example, some strains of the anaerobic species *Bacteroides fragilis* produce the potentially carcinogenic *B. fragilis* toxin which has been associated with the development of colorectal cancer. A study by Boleij et al. found toxin-producing *B. fragilis* by culture and PCR on mucosal samples from late-stage colorectal cancer.[6] Such specific associations can be lost in metagenomic analyses of a faecal sample.

Colorectal cancer can lead to bacterial translocation that allows intestinal bacteria to enter the bloodstream.[7] Earlier studies have suggested an association between bacteraemia caused by bovis group streptococci and colorectal cancer.[8] However, due to improved laboratory diagnostic methods, only *Streptococcus gallolyticus* subsp. *gallolyticus* seems to be regularly associated with colorectal cancer or advanced adenomas (≈60%).[9] Bacteraemia caused by the anaerobic bacterium *Clostridium septicum* has also been found to be highly associated with colorectal cancer (≈20–30%).[10] Other anaerobic bacteria have also been reported to be associated with colorectal cancer in other studies but to a lesser degree.[11–13] A study from Hong Kong revealed that bacteraemia with *Bacteroides* species (2074 cases) was associated with the highest number of cases of colorectal cancer (n = 65; ≈3.1%) compared to other bacteria.[11] In our own pilot study, we identified specific *Bacteroides* species, such as *B. ovatus* (6.7%), which were associated with colorectal cancer.[12] Interestingly, there is a disproportionate relationship between the bacteria from bacteraemia, which are found to be highly associated with colorectal cancer, and their numerical presence in the colon (e.g. *C. septicum*, which is not part of the normal gut microbiota).[14] This supports the hypothesis of a potential role of specific bacteria in the development of colorectal cancer, but it is important to remember that epidemiological studies can only demonstrate association and the relationship between microorganisms and cancer has yet to be completely understood.[1,15] Another explanation could also be that the presence of colorectal cancer is a driver for intestinal bacteria to enter the blood stream and that some bacteria are more predisposed than others.

To systematically define all potential colorectal cancer-associated bacteria, which can be used as indicators of colorectal cancer and to guide physicians in identifying patients, who should be examined further, large-scale nationwide studies are needed. With the introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of bacteria around 2009, identification to the species level of most bacteria has become routine.[16] This makes association studies between bacteria and specific diseases possible and more reliable, than studies performed in the pre-MALDI-TOF MS era.

This nationwide population-based cohort study was designed to explore the potential association between specific anaerobic bacteria causing bacteraemia and the risk of a subsequent diagnosis of colorectal cancer in Denmark 2010–2021.

The focus of the study was on anaerobic bacteria, which usually reside in the colon, such as *Bacteroides* and *Clostridium* species. However, other anaerobic species, as defined per-protocol, such as the Gram-positive anaerobic cocci (*Anaerococcus, Finegoldia, Parvimonas, Peptoniphilus* and *Peptostreptococcus*), which can be found in the colon but also at other sites, and species like *Cutibacterium acnes*, which are not found in the colon, were also included for comparison.[11,12]

Materials and methods

Study design

Nationwide population-based cohort study that included all episodes of specific anaerobic bacteraemia as defined in the study protocol (Supplementary Material 1) from January 1, 2010, through December 31, 2020, and all cases of colorectal cancer diagnosed from January 1, 2010, through December 31, 2021. Reporting was done according to the STROBE statement.[17] The study was registered at the central register for research projects in the Region of Southern Denmark, record no.: 21/20262 and approved by the Region of Southern Denmark, record no. 21/21964. By Danish law, this non-interventional study did not need ethical committee approval.

Setting

In Denmark, the public health system is funded by taxes and accessible to all residents without charge. In the case of acute illness, individuals are admitted to a public hospital and receive all necessary medical treatments.[18] This includes cancer diagnostics and treatment. Every Danish inhabitant has a unique civil personal registration (CPR) number, which serves as an identifier in all health-related contacts and allows for linking between national health registers.[19] The total Danish population was 5534,738 inhabitants in 2010, increasing to 5822,763 inhabitants in 2020. The total number of blood cultures in Denmark was 402,059 in 114,001 unique patients in 2010 increasing to 711,792 in 174,751 unique patients in 2020, corresponding to 2059 blood cultures per 100,000 inhabitants in 2010 and 3001 blood cultures per 100,000 inhabitants in 2020.[20]

Study population

Data on positive blood cultures with the specific anaerobic bacteria and *Escherichia coli* were extracted from the Danish Microbiology Database (MiBa), which receives all final blood culture reports electronically from all departments of clinical microbiology in Denmark.[21] The database includes all blood cultures performed in Denmark since 2010. *E. coli*, the most frequent species found in blood cultures globally, was included as a reference for comparison. Bacteraemia with *E. coli* usually originates from the urinary tract and is not typically suspected to be associated with colorectal cancer, although it is an intestinal bacterium.[12] Bacteraemia episodes were defined using accepted algorithms for retrospectively derived data (Supplementary Material 1).[22,23] The study involved patients with one or more episodes of bacteraemia but only species with 25 or more episodes were included for further analyses. The following specific anaerobic bacteria (genera including species within the genera and single species) were included: *Actinomyces, Anaerococcus, Bacteroides, Bifidobacterium, Bilophila, Clostridioides, Clostridium, Cutibacterium, Dialister, Eggerthella, Eubacterium, Finegoldia magna, Fusobacterium, Lactobacillus, Mobiluncus, Parabacteroides, Parvimonas micra, Porphyromonas, Peptonihpilus, Peptostreptococcus, Prevotella, Propionibacterium, Ruminococcus gnavus* and *Staphylococcus saccharolyticus*. The selection of the included specific anaerobic bacteria was based on the assumption from our pilot study that these were most likely to have 25 or more episodes of bacteraemia.[12]

Data on colorectal cancer was obtained from the Danish national registry for colorectal cancer – the Danish Colorectal Cancer Group (DCCG) database.[24] This database encompasses 99% of all Danish citizens of 18 years or above diagnosed with primary colorectal cancer in Denmark since 2010 according to the most recent report from 2016.[24] The high level of completeness was achieved by parallel searches in several databases such as the Danish Cancer Registry, the National Patient Registry, and the Danish Pathology Registry using

Table 1
Gender and age distribution according to bacteraemia[a] with the different genera or species of anaerobic bacteria and *E. coli*.

Bacteria	CRC	No.	Female (%)	Male (%)	Median age (25%–75% percentile)	Missing age and gender
Bacteroides	0	3982	47.6	52.4	72.3 (61.6-81.5)	6
	+	100	44.7	55.3	76.9 (68.6-84.2)	
- *B. fragilis*	0	2219	47.1	52.9	72.4 (61.9-81.7)	
	+	40	40.5	59.5	76.1 (70.1-83.5)	
- *B. thetaiotaomicron*	0	600	47.7	52.3	71.6 (61.2-80.7)	
	+	20	40.0	60.0	76.8 (64.1-83.2)	
Clostridium	0	1652	47.5	52.5	73 (63.2-82.6)	5
	+	82	58.0	42.0	77 (68.3-85.3)	
- *C. septicum*	0	140	49.3	50.7	73.7 (65.4-83.2)	
	+	46	52.2	47.8	78.3 (67.9-85.3)	
Fusobacterium	0	585	45.6	54.4	62.6 (37.2-75.6)	3
	+	13	15.4	84.6	68.7 (65.9-81.6)	
Remaining bacteria	0	4006	42.7	57.3	67.3 (51.5-77.5)	18
	+	44	52.4	47.6	72.9 (68.5-82.8)	
All anaerobic bacteria	0	10,875	45.6	54.4	70.1 (56.6-80.0)	
	+	249	49.4	50.6	76.5 (68.0-83.4)	
E. coli	0	50,063	50.5	49.5	74.3 (64.2-82.9)	113
	+	587	39.9	60.1	76.3 (69.4-82.5)	
All bacteria	0	60,938	49.7	50.3	73.7 (63.1-82.4)	
	+	836	43.3	56.7	76.4 (68.8-82.7)	

CRC: colorectal cancer.
[a] Genera and species are only included if 10 cases or more of colorectal cancer (*Cutibacterium acnes* is not included as this species is not normally residing in the colon and not associated with colorectal cancer, Supplementary Table 1).

the CPR number. Data from DCCG were updated as of December 31, 2021.

Data were also linked to the Danish Civil Registration System to obtain information on vital status, including death or emigration. Patients diagnosed with colorectal cancer before or on the date of bacteraemia were excluded as were those who emigrated prior to that date.

Linkage procedures

The bacteraemia data from MiBa were combined with the colorectal cancer data from the DCCG database to determine the incidence of colorectal cancer after bacteraemia and to the Danish Civil Registration System based on the CPR number through the central research server of Statistics Denmark.

Statistical analysis

A detailed description of the statistical analyses is presented in the study protocol (Supplementary Material 1). We reported the incidence of colorectal cancer for the specific anaerobic bacteraemia bacteria and *E. coli* both within a one-year limitation and to the end of the observation period. For the same genera and species (using *E. coli* as the reference) we then applied competing risk regression analyses, up to one year and to the end of the observation period, with death as the competing event,[25] after assessing graphically that the proportional hazard assumption was met (Supplementary Material 1). From these analyses we reported subhazard ratios with 95% confidence intervals if there were 10 or more cases of colorectal cancer, adjusting for age, sex, and type of bacteraemia episode (monomicrobial versus polymicrobial). Based on these multivariable analyses, we plotted the cumulative incidence function curves, within and beyond one year. We reiterated all analyses by only including each patient's incident (first-time) bacteraemia episode. The program Stata, version 16.1 (Stata Corp., College St., Texas, USA) was used for all analyses.

Role of funding source

The study sponsor had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Results

This study presents nationwide data on colorectal cancer and specific anaerobic bacteraemia.

Patients

The median age of the bacteraemia patients was in the seventies except for patients with *Fusobacterium* species bacteraemia (late sixties) (Table 1). Patients with colorectal cancer diagnosed after bacteraemia were approximately 4–6 years older. The total number of colorectal cancer cases was distributed with a slight preponderance of males compared to females.

Bacteraemia

From MiBa 11,124 episodes of anaerobic bacteraemia and 50,650 episodes of *E. coli* bacteraemia (100% complete) were included (Table 2 and Supplementary Table 1). The total follow-up time for all bacteraemia episodes was 69,277,377 days, with an average of 1123 days (≈3.1 years) per episode.

Colorectal cancer frequencies

Of the 11,124 episodes of anaerobic bacteraemia, 196 patients (1.8%) were subsequently diagnosed with colorectal cancer within one year compared to 288 patients (0.6%) in 50,650 episodes of *E. coli* bacteraemia. Table 2 shows the frequencies of colorectal cancer following bacteraemia with specific anaerobic bacteria and *E. coli*. The top 10 anaerobic bacterial species with the highest frequencies of colorectal cancer after bacteraemia in descending order were: *C. septicum* (24.7%), *Phocaeicola vulgatus/dorei* (5.5%), *C. tertium* (4.9%),

Table 2
Bacteraemia episodes with anaerobic bacteria and *E. coli* and relation to colorectal cancer.

Bacteria (no. of bacteraemia episodes)	No. (%) of colorectal cancer cases within 1 year	No. (%) of colorectal cancer cases to the end of the observation period	Median no. of days from bacteraemia to colorectal cancer diagnosis
Bacteroides spp. (4082)	81 (2.0)	100 (2.4)	11 (3-97)[a]
- *B. caccae* (103)	3 (2.9)	3 (2.9)	1
- *B. fragilis* (2259)	29 (1.3)	40 (1.8)	27 (6-507)
- *B. ovatus/ xylanisolvens* (163)	4 (2.5)	5 (3.1)	1
- *B. thetaiotaomicron/ faecis* (620)	17 (2.7)	20 (3.2)	7 (2-28)
- *B. uniformis* (128)	3 (2.3)	3 (2.3)	8
- *Phocaeicola vulgatus/ dorei*[b] (170)	8 (4.7)	9 (5.3)	9
Parabacteroides spp. (119)	1 (0.8)	5 (4.2)	689
- *P. distasonis* (107)	0	4 (3.7)	765
Bifidobacterium spp. (152)	2 (1.3)	5 (3.3)	493
Bilophila wadsworthia (33)	0	0	
Clostridium spp. (1734)	76 (4.4)	82 (4.7)	10 (2-42)
- *C. cadaveris* (36)	0	0	
- *C. clostridioforme* (62)	2 (3.2)	2 (3.2)	3
- *C. innocuum* (84)	2 (2.4)	2 (2.4)	10
- *C. paraputrificum* (52)	2 (3.9)	2 (3.9)	59
- *C. perfringens* (632)	7 (1.1)	9 (1.4)	20
- *C. ramosum* (195)	6 (3.1)	6 (3.1)	24
- *C. septicum* (186)	45 (24.2)	46 (24.7)	7 (1-18)
- *C. sordellii* (28)	1 (3.6)	1 (3.6)	9
- *C. symbiosum* (32)	0	0	
- *C. tertium* (81)	4 (4.9)	4 (4.9)	86
Eggerthella lenta (243)	2 (0.8)	3 (1.2)	62
Fusobacterium spp. (598)	5 (0.8)	13 (2.2)	566 (54-1102)
- *F. necrophorum* (151)	1 (0.7)	3 (2.0)	672
- *F. nucleatum* (287)	3 (1.1)	6 (2.1)	216
GPAC (657)	7 (1.1)	8 (1.2)	50
Anaerococcus spp. (34)	0	0	
Finegoldia magna (29)	0	0	
Parvimonas micra (128)	2 (1.6)	2 (1.6)	67
Peptoniphilus spp. (176)	2 (1.1)	3 (1.7)	56
- *P. harei* (110)	2 (1.8)	3 (2.7)	50
Peptostreptococcus spp. (290)	1 (0.3)	3 (1.0)	1273
Prevotella spp. (329)	6 (1.8)	9 (2.7)	15
- *P. bivia* (30)	0	1 (3.3)	1912
- *P. buccae* (145)	2 (1.4)	2 (1.4)	4
- *P. denticola* (25)	0	0	
Ruminococcus gnavus (44)	2 (4.6)	2 (4.6)	4
Escherichia coli (50,650)	288 (0.6)	587 (1.2)	412 (37-1165)

GPAC: Gram-positive anaerobic cocci (*Anaerococcus, Finegoldia, Parvimonas, Peptoniphilus* and *Peptostreptococcus*).
[a] Interquartile range is included if 10 cases or more of colorectal cancer.
[b] *P. vulgatus*/*dorei* was previously named *Bacteroides vulgatus*/*dorei*.

Ruminococcus gnavus (4.6%), *C. paraputrificum* (3.9%), *Parabacteroides distasonis* (3.7%), *C. sordellii* (3.6%), *Prevotella bivia* (3.3%), *B. thetaiotaomicron/faecis* (3.2%), and *C. clostridioforme* (3.2%), which almost exclusively reside in the colon compared to species, which usually do not reside in the colon, such as *Prevotella denticola* (0%). In Supplementary Table 1, frequencies of anaerobic species are shown, which normally do not reside in the colon and are not associated with colorectal cancer.

Competing risk regression analyses

The regression analyses demonstrated a similar higher risk of colorectal cancer for the anaerobic genera and species compared to *E. coli*, both within the one-year limitation and to the end of the observation period (Table 3). With *E. coli* as the reference, *Bacteroides* spp. had a subhazard ratio for colorectal cancer of 3.9 (95% confidence interval [CI], 3.0 to 5.1) within the one-year limit and for *Clostridium* spp. it was 8.9 (95% CI, 6.7 to 11.8, with *C. septicum* 50.0 [95% CI, 36.0 to 69.5]). The same analyses were performed with incident bacteraemia episodes and resulted in almost the same subhazard ratios (Supplementary Table 2). In Fig. 1, the cumulative incidences of colorectal cancer are shown for the anaerobic genera with 10 or more cases of colorectal cancer, i.e. *Bacteroides*, *Clostridium*, and *Fusobacterium*, together with *E. coli* and the remaining bacteria. Beyond one year, the number of new colorectal cancer cases is almost the same for all groups except for *Fusobacterium*, which has a higher incidence. In Supplementary Table 3 the number of colorectal cancer cases distributed within the first year and after one year of the bacteraemia episode is shown.

Discussion

Bacteraemia is a serious life-threatening event, and physicians need to be aware that if specific anaerobic bacteria, which almost exclusively reside in the colon, are detected in the blood, there is a risk of colorectal pathology including colorectal cancer. This study shows that bacteraemia with specific anaerobic bacteria can be used as an indicator of possible colorectal cancer.

Stool-based tests are often used for colorectal cancer screening with differences between countries around the world. In the Minnesota Colon Cancer Control Study from 2000 by Mandel et al., the positive predictive value for colorectal cancer of a single positive test for faecal occult blood was as low as < 1%.[26] In a 2007 Cochrane review with data from four countries, including data from the Minnesota study, the positive predictive value of faecal occult blood ranged from 0.9% to 18.7%.[27] In Denmark, the positive predictive value is around 4%.[28] If this positive predictive value of 4% is used as a cut-off for colorectal examination after anaerobic bacteraemia in a Danish setting, many species should be considered as possible indicators for colorectal cancer.[10–13] This includes new species such as *P. vulgatus/dorei* (previously named *Bacteroides vulgatus/dorei*), *Parabacteroides* species, *C. tertium*, and *R. gnavus*, (although the number of bacteraemia episodes for some of the species was low, and resulted in wide CIs for some groups and species shown in Table 3). However, the cut-off is one of the very important questions that need to be addressed before these findings can be implemented in clinical practice. Defining a cut-off percentage for the examination of patients for colorectal cancer will depend on many factors, such as the clinical status of the patient, local circumstances, national guidelines, and pre-existing screening protocols.[29,30] There were no apparent differences between the unadjusted and adjusted analyses, and gender or age could not be used to refine decision-making. Again, numbers were low for some of the groups and species, which would impact statistical precision but would certainly be a possibility to explore further if larger datasets become available.

There is also the question of bacteraemia as the inclusion criteria for this study. A diagnosis of bacteraemia, would usually suggest that the patient had symptoms, which would result in blood cultures. It is possible that asymptomatic bacteraemia occurs, and it has been shown that circulating bacterial DNA can be detected in the blood of colorectal cancer or adenoma patients. One recent study

Table 3
Subhazard ratios[a] with 95% confidence intervals, with *E. coli* bacteraemia as the reference.

Bacteria[b]	Within 1 year	To the end of the observation period
Bacteroides spp.	3.9 (3.0-5.1)	2.4 (1.9-2.9)
Bacteroides spp. without *B. fragilis* & *B. thetaiotaomicron/faecis*	5.3 (3.7-7.8)	3.0 (2.1-4.2)
- *B. fragilis*	2.3 (1.6-3.4)	1.6 (1.1-2.2)
- *B. thetaiotaomicron/faecis*	5.1 (3.1-8.5)	3.0 (1.9-4.7)
Clostridium spp.	8.9 (6.7-11.8)	4.7 (3.6-6.1)
Clostridium spp. without *C. septicum*	3.6 (2.4-5.3)	2.1 (1.5-3.0)
C. septicum	50.0 (36.0-69.5)	25.8 (18.7-35.8)
Fusobacterium spp.	2.0 (0.84-4.9)	2.5 (1.4-4.4)
Remaining bacteria	1.5 (1.0-2.1)	1.2 (0.88-1.5)
Escherichia coli (reference)	1	1

[a] Ratios are adjusted for age, sex and episode type (mono- versus polymicrobial bacteraemia) and taking competing risk from death or emigration into account.
[b] Genera and species are only included if 10 cases or more of colorectal cancer (*Cutibacterium acnes* is not included as this species is not normally residing in the colon and not associated with colorectal cancer, Supplementary Table 1).

demonstrated differences in circulating bacterial DNA in blood between colorectal cancer and adenoma patients and healthy controls and concluded that this could potentially be used as a biomarker.[31]

Another interesting aspect is the possible role of specific anaerobic bacteria in the development of colorectal cancer. Culture, identification, and molecular genetic characterization of the specific anaerobic bacteria from bacteraemia patients with colorectal cancer is of great importance going forward. Within the anaerobic genera *Bacteroides*, there is a considerable difference phenotypically between *B. fragilis* (genome size ≈5.2 Mb) and *B. thetaiotaomicron* (genome size ≈6.2 Mb), although they are phylogenetically closely related. Some of these phenotypic differences, which are based on genetic differences, might explain why the frequency of colorectal cancer is almost twice as high following *B. thetaiotaomicron* bacteraemia compared to *B. fragilis* bacteraemia. It is also likely that differences within the species could explain why some strains are more likely to be associated with colorectal cancer than other strains (one example such as toxin-producing *B. fragilis* has been described above). The prospect of using special biomarkers from colorectal cancer-associated bacteria for screening is very attractive if this could increase diagnostic sensitivity and specificity or to identify possible specific causative or contributing factors to the development of colorectal cancer.

There are several shortcomings of our study.

One of the shortcomings is the limited amount of background information and clinical data from the patients; only age, gender, bacteraemia and colorectal cancer status. Especially information on colorectal cancer stage and comorbidity would add to the evaluation of the benefit for patients of using bacteria as an indicator of colorectal cancer.

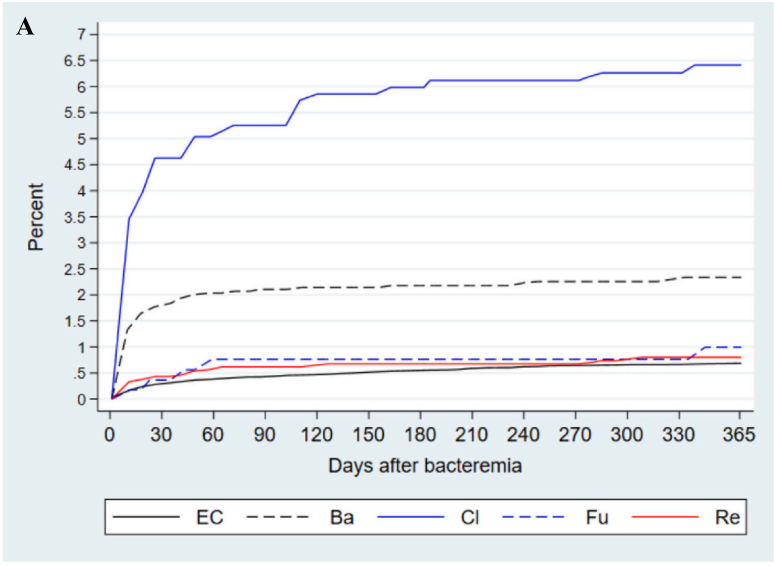
Another shortcoming is the observation period for colorectal cancer, which ended one year after the last episodes of bacteraemia were included in the study. For most of the bacteria, which could be described as colorectal cancer-associated, the time from bacteraemia to colorectal cancer diagnosis was much shorter than one year. However, for *Parabacteroides* species, this was not the case (median 689 days) and the frequency of colorectal cancer might have been higher with a longer observation period. This also means that *Parabacteroides* species could be the exemplary case where bacteraemia can be used to predict colorectal cancer and benefit the patient with an early diagnosis. For other of the colorectal cancer-associated bacteria, the time from bacteraemia to the diagnosis of colorectal cancer is very short (a few weeks), which would indicate that the benefit for the patient of knowing that bacteraemia was caused by colorectal cancer-associated bacteria could be limited (Table 2). To expand the usefulness of anaerobic bacteraemia as a biomarker it would have been ideal to have information from different types of adenomas and bacteraemia. The question is if bacteraemia with specific anaerobic bacteria could be used to find precancerous adenomas to increase the probability of patient benefit. Unfortunately we do not have the same rigorous registration of adenomas in Denmark compared to colorectal cancer, but it would be an interesting aspect for future research.

The fact that some polymicrobial bacteraemia episodes were recorded in the present data as monomicrobial if other bacteria than included in this study (e.g. *Staphylococcus aureus*) were detected within two days is also a shortcoming. However, in our previous study including all bacteria, this did not change the results.[12]

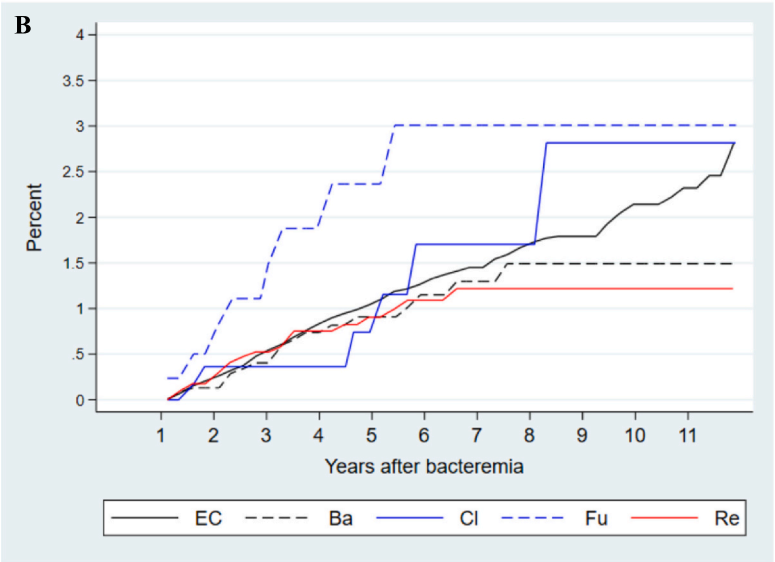
The question of whether our results can be applied to other parts of the world is also important. Two other smaller studies from Queensland Australia,[13] and Hong Kong[11] support this with similar findings, but other anaerobic species may be involved in other parts of the world. It is also possible that other non-anaerobic species, such as the bovis group streptococci (not included in this study), could play a role. Both studies from Australia and Hong Kong report *Gemella morbillorum*, a streptococcus-like species, as colorectal cancer-associated (7.9% in both studies).[11,13]

Finally, we chose bacteraemia with *E. coli* as the reference because this is a common clinical entity. Patients with *E. coli* bacteraemia were around the same age as our patients with colorectal cancer-associated anaerobic bacteraemia (Table 1). As anaerobic bacteraemia was the key to be included in this study it was reasonable to choose a control group with bacteraemia. The frequency of colorectal cancer to the end of the observation period within this large control group (50,650 *E. coli* bacteraemia episodes) was 1.2%. This is the same finding as the study from Australia, supporting it as a robust reference, but another control group might have been more suitable.[13] It is possible that *E. coli* could also use mucosal damage from colorectal cancer as a way to enter the blood stream, but *E. coli* bacteraemia would not be a useful indicator, as most cases would originate from the urinary tract.

The strength of our study is the almost 100% complete data set including anaerobic bacteraemia and colorectal cancer on a nationwide scale and a long follow-up period of 12 years.[21,24]



Bacteria	Day 0	Day 100	Day 200	Day 300	Day 365
Escherichia coli	50,611	39,506	36,894	35,077	34,099
Bacteroides spp.	4078	2799	2638	2523	2464
Clostridium spp.	1702	852	753	674	654
Fusobacterium spp.	597	480	450	435	427
Remaining bacteria	4703	3554	3318	3151	3058



Bacteria	Year 2	Year 4	Year 6	Year 8	Year 10
Escherichia coli	26,547	16,124	9416	4919	2037
Bacteroides spp.	1977	1286	773	435	199
Clostridium spp.	500	311	172	98	39
Fusobacterium spp.	328	213	127	71	33
Remaining bacteria	2424	1532	924	453	189

Fig. 1. Cumulative incidence curves of colorectal cancer in patients with bacteraemia within one year (A) and to the end of the observation period (B). *E. coli* (EC), and the three anaerobic genera *Bacteroides* (Ba), *Clostridium* (Cl), and *Fusobacterium* (Fu), and the remaining bacteria (Re) with numbers at risk at the different time points: within 1 year (A) and beyond 1 year (B).

Conclusion

We found high frequencies of colorectal cancer following bacteraemia with specific anaerobic bacteria. This can be used as a general warning sign of colorectal cancer and be a guide to who should be examined further. However, for some patients the time from bacteraemia to the diagnosis of colorectal cancer was very short (a few weeks), which would indicate that the benefit for the patient could be limited. Overall, the species with the highest frequencies were, *Bacteroides* spp., *Clostridium* spp. (up to 24.2% *with C. septicum* within the first year), and *Ruminococcus gnavus*. All the top 10 species with the highest frequencies mainly reside in the colon (except for *P. bivia*, which is normally associated with the oral cavity and the female genital tract). The "within 1-year" frequencies could be compared to the incidence of colorectal cancer, which is 260–460 per 100,000 person-years in the Danish population with an age ≥70 years. Further studies are needed to evaluate the findings from this study in a clinical setting.

Funding

This work was supported by an unrestricted grant from the NEYE Foundation. The study sponsor had no role in the conduct of the research and/or preparation of the article; in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Author contribution statement

USJ and KOG had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. USJ, MBE, NQ, and KOG designed the study. All authors participated in the acquisition, analysis, or interpretation of data. USJ and KOG drafted the first manuscript. All authors performed critical review of the manuscript for important intellectual content. MI and KOG performed the statistical analyses. USJ obtained funding for the study. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Data availability

Data cannot be shared. All data analysis was performed through the central research server of Statistics Denmark using encryptions of the unique civil personal registration number, which serves as an identifier of Danish inhabitants. According to Danish law and regulations, it is prohibited to disclose microdata, with or without encryption of the civil personal registration number.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The Danish Colorectal Cancer Group Database (DCCG) is acknowledged for its valuable contribution to the study and we thank the Open Patient Data Explorative Network, Odense University Hospital, Region of Southern Denmark (http://www.sdu.dk/ki/open), for the help with data management and storage on the research server of Statistics Denmark.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106212.

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