

An Eleven-Year Retrospective Survey of Anaerobic Bloodstream Infection in Adults in a General Hospital

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Objective: During conditions accompanied by the disruption of normal mucosal barriers, anaerobic bacteria, which a part of normal human mucosal microflora, may cause various infections. In this study, clinical features of anaerobic bloodstream infections (BSI) in a general hospital in China were investigated.

Methods: Patients with anaerobic BSI were retrospectively enrolled between 2012 and 2022. Demographic data, clinical manifestations, antibiotic treatments, and disease outcomes were analyzed.

Results: In total, 391 anaerobic bacterial strains were isolated from 381 patients aged older than 11 years of age. Given that medical records of 47 patients were missing, 334 patients were included in the clinical investigation. Patients with anaerobic BSI included in the study were predominantly older than 50 years of age. Intra-abdominal infections were the most common source of anaerobic BSI (59%), followed by those of the female genital tract (10.2%) and lower respiratory tract (7.2%). Among the isolates, *Bacteroides* and *Clostridium* spp. were the most frequently isolated anaerobes. Most of the patients received antibiotic therapy. The crude mortality was 4.5%.

Conclusion: The detection rate of anaerobic BSI in Ningxia, China, remained relatively stable from 2012 to 2022. These results provide a reference for the diagnosis and empirical treatment of anaerobic BSI in this region of China. Continuous multicenter studies should be conducted to monitor the incidence of anaerobic BSI and drug resistance of anaerobic isolates to improve the treatment outcomes of patients.

Keywords: anaerobes, bloodstream infections, blood cultures, clinical features

Introduction

Anaerobic bacteria are a major component of the normal human mucosal membrane microbiota in the oral cavity, gastrointestinal tract, and female reproductive tract. However, when normal mucosal barriers break down, these anaerobes cause a wide array of manifestations, ranging from mild mixed infections to life-threatening invasive conditions such as bloodstream infections (BSI).^{1,2} Anaerobes accounted for 20–30% of isolates from blood cultures before 1970, but their incidence decreased to approximately 0.5–12% of all cases of bacteremia in the 1990s.³ Recent studies showed that the detection rate of anaerobes in blood cultures remained low, comprising approximately 0.5–11% in all bacteremia episodes.⁴ Despite their relatively low prevalence, the mortality rate of anaerobic BSI varies between 14 and 25% and is even higher in patients with characteristic underlying conditions.⁵ Furthermore, isolation of anaerobic bacteria requires appropriate methods of the collection and transportation of specimens as well as a fastidious cultivation environment. However, many facilities do not have suitable equipment for the isolation of anaerobic microorganisms.⁶ Anaerobic bacterial infections are often neglected, as they are difficult to diagnose. It has been reported that the identity of anaerobic isolates varies depending on the geographical location, hospital patient demographics, and patient conditions. Therefore, more clinical information is required to guide the selection of empirical antibiotics for the treatment of anaerobic BSI. In

this study, we aimed to investigate the clinical role of anaerobic BSI in the microbiomes of adult patients admitted to a teaching hospital in the Ningxia Hui Autonomous Region.

Materials and Methods

Data Acquisition

The clinical data of patients included in this study were retrospectively reviewed from the electronic medical and clinical microbiology records at the General Hospital of Ningxia Medical University, a 3500-bed tertiary care establishment, from January 2012 to December 2022. Data regarding age, sex, underlying diseases, isolate identity, antimicrobial treatment, and outcome were collected. Anaerobic bloodstream infection was diagnosed when the patient had one or more blood cultures that were positive for anaerobes.

Cultures, Isolation, and Identification of Strains

For blood culture, 5–10 mL of blood was inoculated into parallel paired aerobic and anaerobic bottles. All blood culture bottles were placed into a BACT/ALERT 3D automatic blood culture system (bioMérieux, France) or BacT/ALERT VIRTUO blood culture system (bioMérieux, France) at 37 °C and incubated for 5 days, according to the manufacturer's instructions. Positive bottles were examined using standard laboratory methods. Briefly, blood in the positive bottles was smeared directly and investigated using microscopy of gram-stained bacteria. Further, blood samples were transferred to a blood agar plate and a MacConkey agar plate, and cultured in an aerobic environment at 35 °C. If no bacterial growth was observed in the aerobic culture, another blood agar plate was cultured in an anaerobic bag at 35 °C or incubated in an atmosphere of 90% N₂, 5% H₂, and 5% CO₂ in an anaerobic chamber (Bactron SHEL LAB, USA) for at least 2 days at 35 °C. The bacteria were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, VITEK2 compact Anaerobe and *Corynebacterium* cards, or an API 20A kit (all from bioMérieux, France).

Statistics

Categorical variables are presented as the number of cases and percentages. Continuous variables that did not follow a normal distribution were described as medians with the 25th and 75th percentiles. Figures were generated using GraphPad Prism software version 8 (GraphPad Software Inc., San Jose, CA, USA), and organism distribution was analyzed using Whonet 5.6 software.

Results

Clinical Characteristics

During the 11-year study period, 175,570 sets of blood cultures were obtained, of which 18,345 were positive for clinically relevant isolates. The data on the number of isolates obtained from blood cultures per year are shown in [Figure 1](#). Among the isolated microorganisms, 551 (3%) were anaerobes from 381 patients. The median age of the patients was 58 years (interquartile range: 44.5–69 years), and the male-to-female ratio was 1.28 (214 males). Anaerobic BSI was more likely to occur in 63.5% (242/381) of patients over 50 years of age, and the incidence of anaerobic BSI peaked in the 61–70 yearsold group (23.9%; 91/381). The patients in this study came mainly from the emergency department (28.3%; 108/381), intensive care unit (ICU) (16.8%; 64/381), gastrointestinal surgery department (9.2%; 35/381), and oncology department (6.6%; 25/381) ([Figure 2](#)).

After reviewing the medical records, 334 patients were enrolled for the analysis of clinical characteristics. Among these 334 patients, 95.5% had at least one underlying disease. The primary underlying diseases were malignancy (35.6%, 119/334), hypertension (21.3%, 71/334), diabetes mellitus (13.2%, 44/334), trauma (10.2%, 34/334), and peritonitis (8.4%, 28/334). Fever and abdominal pain were the most frequent symptoms and appeared in 48.5% and 39.8% of the patients, followed by nausea or vomiting (16.2%, 54/334), bloating (9.6%, 32/334), cutaneous mucous membrane swelling pain (8.1%, 27/334), hematochezia (6.3%, 21/334), tight chest, shortness of breath (4.5%, 15/334), vaginal

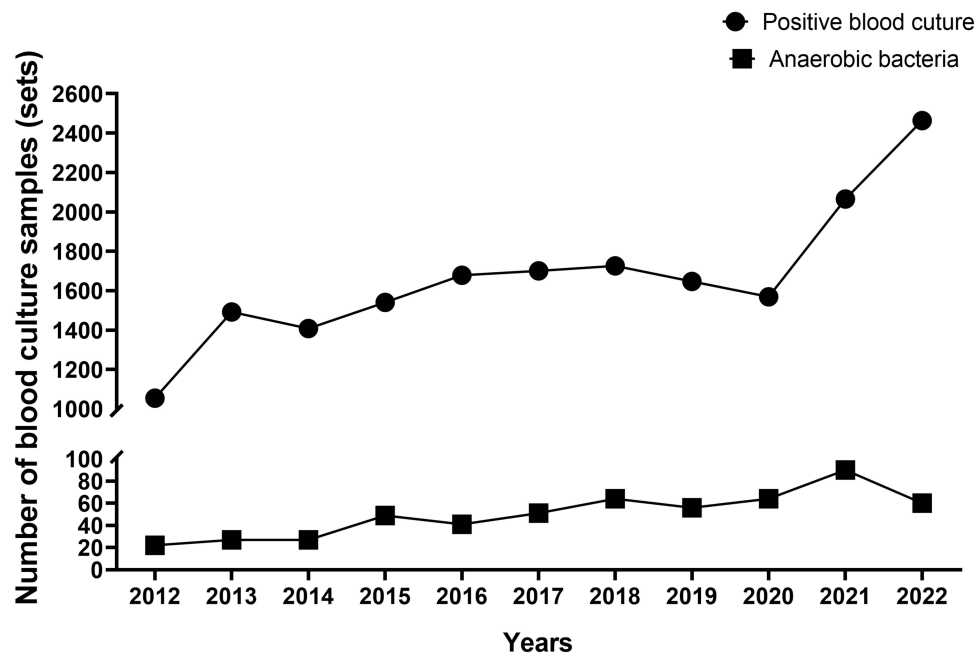


Figure 1 Number of blood culture samples (sets) each year.

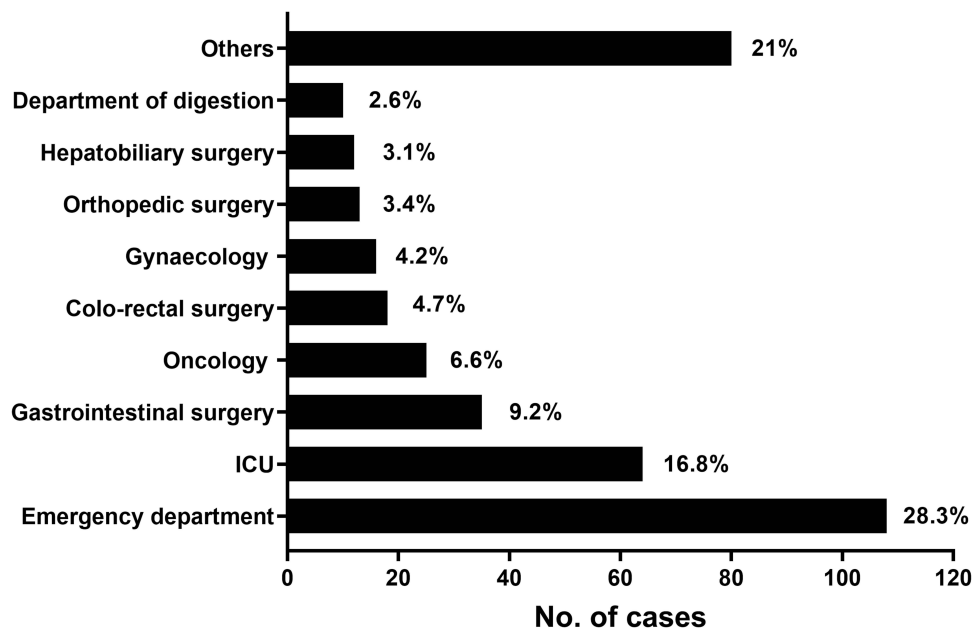


Figure 2 Departments distribution of patients with anaerobic BSI.

bleeding (4.5%, 15/334), diarrhea (4.2%, 14/334), cough (3.6%, 12/334), confusion (3.3%, 11/334), melena (3.3%, 11/334), and other symptoms, including chest pain, sore throat, et al.

Overall, the lower gastrointestinal tract was the most common port of entry (59% of cases), being associated with a broad spectrum of intra-abdominal clinical conditions, such as peritonitis, appendicitis, cholecystitis, and oncological complications. More than half of the patients underwent abdominal surgery. The female genital tract (10.2%, 34/341) was the second most common site of origin. The lower respiratory tract as well as skin and soft tissues were considered ports of entry in 7.2% and 6.9% of the patients, respectively. The eyes, ears, nose, mouth, and throat were considered ports of entry in 2.1% of the patients (7/334), including two with tonsillitis, one with periodontitis, one with swollen gums, one

with pharyngeal space infection, and one with pus discharge from both ears. In eight and four patients, the sources of BSI were multiple injuries and urinary system pathologies respectively. The primary focus of infection was not identified in 8.1% (27/334) of the patients. Among the 341 enrolled patients, 122 were co-infected with other pathogens and 43 were co-infected with more than one pathogen. *Escherichia coli* was the most common bacterial species isolated (55/122, 45.1%), followed by *Acinetobacter baumannii* (20/122, 16.4%). The demographic and clinical characteristics of the patients with anaerobic BSI are shown in Table 1.

Table 1 Demographic and Clinical Characteristics of Patients with Anaerobic BSI

	No. Cases (n/%)
Sex (N=381)	
Male	214/56.2
Female	167/43.8
Age (year) (N=381)	
≤30	33/8.7
31–40	39/10.2
41–50	67/17.6
51–60	70/18.4
61–70	91/23.9
>70	81/21.2
Underlying conditions (N=334)	
None	15/4.5
Malignancy	119/35.6
Hypertension	71/21.3
Diabetes mellitus	44/13.2
Trauma	34/10.2
Peritonitis	28/8.4
Intestinal obstruction	20/6.0
Gallbladder disease	19/5.7
Skin infection	19/5.7
Coronary heart disease	17/5.1
Pulmonary infection	15/4.5
Liver abscess	15/4.5
Appendicitis	11/3.3
Others	69/20.7
Symptoms (N=334)	
Fever	162/48.5
Abdominal pain	133/39.8
Nausea or vomiting	54/16.2
Bloating	32/9.6
Cutaneous mucous membrane swelling pain	27/8.1
Hematochezia	21/6.3
Tight chest, shortness of breath	15/4.5
Vaginal bleeding	15/4.5
Diarrhea	14/4.2
Cough	12/3.6
Confusion	11/3.3
Melena	11/3.3
Others	52/15.6

(Continued)

Table I (Continued).

	No. Cases (n/%)
Portal of entry / focus, (N=334)	
Not found	27/8.1
Intra-abdominal	197/59.0
Female genital tract	34/10.2
Lower respiratory tract	24/7.2
Skin and soft-tissue	23/6.9
Multiple injury	14/4.2
Eye, ear, nose, mouth and throat	7/2.1
Urinary system	4/1.2
Others	4/1.2
Co-infected pathogens (N=122)	
<i>Escherichia coli</i>	55 /45.1
<i>Acinetobacter baumannii</i>	20/16.4
<i>Enterococcus faecium</i>	19/15.6
<i>Pseudomonas aeruginosa</i>	15/12.3
<i>Klebsiella pneumoniae</i>	14/11.5
Others	54/44.3

Isolates Identification

After removing the duplicated strains from the same patient, 391 anaerobes were analyzed. Blood cultures from eight patients yielded mixed growth of two different organisms, and one patient yielded mixed growth of three different organisms. Overall, gram-negative anaerobic rods and gram-positive spore-forming anaerobic rods accounted for 86.7% of all reported cases of anaerobic BSI. The three most common anaerobic species were *Bacteroides fragilis* (38.1%), *Bacteroides thetaiotaomicron* (8.4%), and *Eggerthella lenta* (6.6%). The detailed species distribution of anaerobic isolates in clinically relevant anaerobic BSI cases each year is presented in Table 2. *Prevotella bivia* was detected in 12 patients, and ten of them were pregnant.

Antibiotic Treatment and Outcomes

Overall, 60.8% (203/334) of the patients received broad-spectrum antibiotics as empirical therapy. Most patients (94.9%) received antibiotic treatment based on the blood culture results. The most common antibiotics were β -lactams. Of the 334 patients, 233 (69.8%) were cured and 59 (17.7%) deteriorated. The main reason for the deterioration of the disease was that the patients discontinued the treatment. Fifteen patients were transferred to another hospital. Fifteen patients died during hospitalization (crude mortality rate, 4.5%). The outcomes of twelve patients were unknown.

Discussion

Anaerobic microorganisms are important human pathogens that cause BSI and are associated with high morbidity and mortality rates. To the best of our knowledge, there were few studies in the Ningxia Hui Autonomous Region that addressed the epidemiology and clinical characteristics of patients with anaerobic BSI. In this study, we investigated clinical characteristics of patients with anaerobic BSI at a teaching hospital in the Ningxia Hui Autonomous Region of China over an 11-year period.

Previous studies reported isolation rates of anaerobes in 1–17% of positive blood cultures.⁷ The average annual proportion of anaerobic isolates in our study was 2.9% (ranging from 1.8% to 4.4%), which is in line with that reported by Blair et al⁸ and higher than 0.6% reported by Zahar et al.⁹ Currently, the prevalence of anaerobic BSI is much lower than earlier historical rates reported in the literature^{10,11} owing to the prophylactic use of broad-spectrum antibiotic therapy and preoperative treatments before bowel surgery. However, in this 11-year study, there was a slight upward trend in the occurrence of anaerobic bacterial BSI in our hospital, which may be because several measures were taken to

Table 2 Anaerobic Microorganisms Isolated from Bloodstream Infections

Isolates	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Total (%)
Gram-negative anaerobic rods	16	15	20	19	22	26	30	32	35	41	24	280 (71.6)
<i>Bacteroides fragilis</i>	8	10	8	5	8	17	18	23	21	21	10	149
<i>Bacteroides thetaiotaomicron</i>	3		4	3	5	2	2	4	6	3	1	33
<i>Bacteroides uniformis</i>	1	2	1	2	1					1		8
<i>Bacteroides vulgatus</i>	1	1	3	2	3	2	1		1	2	2	18
<i>Bacteroides ovatus</i>				1			2		3	3	1	10
<i>Bacteroides stercoris</i>				1	2	1		1				5
<i>Bacteroides caccae</i>							2			1		3
<i>Bacteroides capillosus</i>								1				1
<i>Fusobacterium nucleatum</i>				1	1	1	2	2	1	4	4	16
<i>Fusobacterium varium</i>		1				1				1	1	4
<i>Fusobacterium periodonticum</i>								1		1		2
<i>Fusobacterium necrophorum</i>									1		1	2
<i>Fusobacterium gonidiaformans</i>											1	1
<i>Prevotella bivia</i>	2	1	1	3			3			2		12
<i>Prevotella buccae</i>						1			2			3
<i>Prevotella intermedia</i>			1	1	1							3
<i>Prevotella melaninogenica</i>			1								1	2
<i>Prevotella nigrescens</i>											1	1
<i>Prevotella oralis</i>					1	1						2
<i>Prevotella buccalis</i>												0
<i>Prevotella oris</i>										1		1
<i>Prevotella denticola</i>										1	1	2
<i>Dialister pneumosintes</i>											1	1
<i>Porphyromonas asaccharolytica</i>	1		1									2
Gram-positive, spore-forming anaerobic rods		3	1	10	4	4	12	11	7	6	1	59 (15.1)
<i>Clostridium clostridioforme</i>		1		5	1	1		2				10
<i>Clostridium ramosum</i>		2		1	1		2	1	3			10
<i>Clostridium sordellii</i>			1									1
<i>Clostridium barati</i>				1			4	1	2			8
<i>Clostridium bifermentans</i>				1								1
<i>Clostridium butyricum</i>				1					2			3
<i>Clostridium innocuum</i>					1	1	2	2				6
<i>Clostridium limosum</i>					1			1				2
<i>Clostridium cadaveris</i>						1				1		2

<i>Clostridium paraputrificum</i>						1	1			1		3
<i>Clostridium symbiosum</i>										1		1
<i>Clostridium perfringens</i>										2		2
<i>Clostridium tertium</i>				1			1	2				4
<i>Clostridium septicum</i>							1	1			1	3
<i>Clostridium difficile</i>							1	1				2
Gram-positive, non-spore-forming anaerobic rods	2	3	1	1		1	3			10	8	29 (7.4)
<i>Eggerthella lenta</i>	2		1	1		1	3			10	8	26
<i>Bifidobacterium bifidum</i>		3										3
Gram-positive anaerobic cocci				3	5	3		1		3	6	21 (5.4)
<i>Parvimonas micra</i>				1	2					2	6	11
<i>Peptostreptococcus asaccharolyticus</i>					3			1		1		5
<i>Peptostreptococcus anaerobius</i>				2		3						5
Gram-negative anaerobic cocci							1				1	2 (0.5)
<i>Veillonella parvula</i>							1				1	2

improve the detection rate of blood cultures, such as the collection of multiple sets of blood cultures using two bottles, including sampling before antibiotic administration. Furthermore, we used MALDI-TOF MS that provided reliable identification of anaerobic isolates, allowing clinicians to select appropriate antibiotic therapy.^{2,12}

In our patient population, males had anaerobic BSI more frequently than females (56.2% vs 43.8%). The median age was 58 years, and the incidence was the highest in the age group of 61–70 years (23.9%). This result shows that patients with anaerobic BSI were mostly elderly, which is consistent with the results of previous studies.^{13,14} This may be because older individuals tend to have more health conditions.

Underlying diseases reported as risk factors for anaerobic BSI include polymicrobial infections as well as heart, renal, and liver diseases.⁶ In this study, most patients (95.5%) had one or more underlying disease at the time of BSI detection. The most common underlying condition was malignancy (35.6%), which is consistent with the report by Cobo et al⁴ (39.7%). This may be because surgery for tumors often results in the entry of bacteria into the bloodstream in the intestine, and/or because cancer chemotherapy causes both profound granulocytopenia and the disruption of physical barriers. Other underlying diseases included hypertension (21.3%) and diabetes mellitus (13.2%). Patients with diabetes are susceptible to infections caused by impaired innate and adaptive immune responses.¹⁵ Among the patients with diabetes in this study, three developed diabetic foot condition.

Intra-abdominal abscesses and the female genital tract were the two most frequent sources of anaerobic BSI. Consistent with many previous studies,¹³ intra-abdominal infection was the most common source of BSI in this study (59%), followed by BSI incidence in the female genital and lower respiratory tracts. Watanabe et al² reported that intra-abdominal infection was the source of 36.7% of anaerobic BSI cases. Lombardi et al¹⁶ reported that the gastrointestinal tract was the source of BSI in two-thirds of patients and was clearly implicated in 80% of fatal anaerobic bacteremia cases. In the present study, 6.9% of BSI were associated with skin and soft tissue infections. Intra-abdominal and soft-tissue infections were the most common sources of BSI in another recent study, where they accounted for 39% and 33% of all bacteremia cases, respectively.¹³

According to previous studies, gram-negative anaerobic rods were the predominant isolates.¹⁷ In the present study, gram-negative anaerobic rods accounted for 71.6% of BSI cases. *Bacteroides* spp. isolates were the most common, accounting for over a half (58.1%) of all BSI cases and 81.1% of gram-negative anaerobic rod infections, which is consistent with results of previous studies.^{2,13,18} *Bacteroides fragilis* was the most abundant anaerobic species isolated from blood cultures in the present study, followed by *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, *Fusobacterium nucleatum*, *Prevotella bivia*, and others. Previous studies showed that *B. fragilis* isolates were relatively resistant to antibiotics.^{19,20} Therefore, antimicrobial susceptibility testing was performed for *B. fragilis* routinely to guide treatment. *Prevotella bivia* is a dominant opportunistic pathogen causing bacterial vaginosis, which invades the human cervix⁸ and causes intrauterine infections.²¹ In the present study, we identified 12 cases of *Prevotella bivia* BSI. Except for one patient with an unknown infection source, eight cases of BSI were associated with the female genital tract. Six pregnant women had intrauterine infection. It was also reported that *Prevotella bivia* causes other infections, such as scrotal, chest wall, and subcutaneous shoulder abscesses.^{22–24} In line with the results of previous studies, gram-positive non-spore-forming anaerobic rods and gram-positive bacteria accounted for a minority of anaerobic isolates.²⁵ *Eggerthella lenta* is the most common species of non-spore-forming anaerobic rods that constitutes part of the normal human intestinal microbiota and has been recognized as an important cause of anaerobic BSI associated with intra-abdominal infections.²⁶ A total of 26 *Eggerthella lenta* strains were isolated in this study, excluding two patients without records, and the medical records of 24 patients were analyzed. Eighteen cases of BSI were associated with intra-abdominal infections during the 11 years in this study. Gram-negative anaerobic cocci isolated from anaerobic BSI were uncommon. Only two *Veillonella parvula* strains were isolated from two patients with multiple traumas.

Most patients received broad-spectrum antimicrobial therapy. Fifteen patients died during hospitalization, and the crude mortality rate of the patients in our series was 4.5%, which is lower than that reported in other reported studies.⁸ This may be because 17.7% of the patients in this study deteriorated and most of these patients discontinued treatment. However, the outcomes of these patients were unknown. Consistent with our results, a previous study reported that the most commonly used empirical antibiotic therapies were combinations of a beta-lactam and a beta-lactamase inhibitor.¹³ Antimicrobial susceptibility testing of anaerobic bacteria is not routinely performed in most clinical laboratories. The

prevalence of antibiotic resistance among anaerobes is increasing. High overall resistance rates to penicillin, clindamycin, and cefoxitin were observed, especially for gram-positive anaerobic cocci and *Bacteroides spp.*^{4,27} Therefore, antimicrobial susceptibility testing of anaerobic bacteria is necessary.

Our study has some limitations. This was a retrospective, single-center study. In addition, antibiotic therapy against anaerobes was typically empirical.

Conclusion

In summary, the detection rate of anaerobic BSI in Ningxia, China, remained relatively stable from 2012 to 2022. Anaerobic BSI in our patient population were predominantly associated with intra-abdominal infections. The most common anaerobic bacteria were *Bacteroides spp.* and *Clostridium spp.* Further multicenter studies should be conducted to monitor the incidence of anaerobic BSI and drug resistance in anaerobic isolates to improve treatment outcomes.

Statement Covering Patient Data Confidentiality

To protect patients' personal information and maintain the security of General Hospital of Ningxia Medical University's patient information, we are committed to fulfilling our obligation to keep patients' personal information confidential.

Data Sharing Statement

The data in this study are available from the corresponding author upon reasonable request.

Ethics Approval

In accordance with the Declaration of Helsinki, this retrospective study was permitted by the ethics committee of the General Hospital of Ningxia Medical University, and the requirement to obtain informed written consent was waived.

Funding

This work was supported by the Natural Science Foundation of Ningxia Hui Autonomous Region (No. 2022AAC03542) and the Key Research and Development Project of Ningxia Hui Autonomous Region (No. 2023BEG03046).

Disclosure

The authors declare no conflicts of interest in this work.

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