



## Original Research Article

## Emergence of metronidazole and clindamycin resistance among anaerobic *Bacteroides fragilis* group - A study from South India

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## Abstract

**Background:** *Bacteroides fragilis* is the most common pathogenic group among anaerobic pathogens, primarily causing infections such as deep visceral abscesses, brain abscesses, diabetic foot ulcers, etc. Recent studies have shown increasing resistance among these bacteria to common antibiotics like metronidazole and clindamycin, which are used for empirical treatment.

**Aim and Objective:** The study aimed to analyze and identify the common pathogenic species of the *Bacteroides fragilis* group isolated from clinical samples and to determine the drug resistance for clindamycin and metronidazole.

**Materials and Methods:** A total of 51 *Bacteroides fragilis* group isolates were obtained from samples received for anaerobic culture from January 2021 to December 2023. The samples were inoculated onto anaerobic blood agar and other essential media. Identification was done using MALDI-TOF MS. Antibiotic susceptibility was performed using E-strip method and interpreted according to CLSI 2023 breakpoints for clindamycin and metronidazole.

**Results:** *Bacteroides fragilis* was found to be the most prevalent among the *Bacteroides fragilis* group isolates, followed by *B. ovatus*. The cultures showed a high incidence of polymicrobial growth (80%, n=41), with *Prevotella* species (n=6) being the most frequent obligate anaerobe grown along with *Bacteroides fragilis* group, while *Escherichia coli* (n=15) was the most common facultative anaerobe. 11 out of 51 isolates showed resistance to metronidazole (21%), while 2 isolates were intermediate (3.9%) and the rest were sensitive. 23 out of 51 isolates (45%) were resistant to clindamycin, 1 was intermediate (1.9%), and 27 isolates were sensitive to clindamycin.

**Conclusion:** This study reiterates that *Bacteroides fragilis* subsp. *fragilis* is the most common *Bacteroides fragilis* group and is known to cause various anaerobic infection. There is an emergence of drug resistance among them towards commonly used antibiotics like metronidazole and clindamycin.

**Keywords:** *Bacteroides fragilis* group, Obligate anaerobe, Antimicrobial resistance, Metronidazole, Clindamycin.

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### 1. Introduction

The *Bacteroides fragilis* group comprises gram-negative non-spore-forming obligate anaerobic bacilli. Similar to Enterobacteriales, they are also part of the indigenous microbiota of the human colon. The *Bacteroides* genus has undergone many alterations since 1980s. Nowadays, the genus *Bacteroides* contains limited members of the *Bacteroides fragilis* group, including *Bacteroides fragilis*, which is the most common, *Bacteroides caccae*, *Bacteroides ovatus*, *Bacteroides eggerthii*, *Bacteroides thetaiotaomicron*, *Bacteroides acidifaciens*, *Bacteroides coprocola*, *Bacteroides coprosuis*, *Bacteroides finegoldii*, etc.<sup>1-3</sup>

They are opportunistic pathogens that primarily cause infection when they enter normal sterile sites in the body and are one of the most commonly isolated anaerobic bacteria in samples of human opportunistic infections.<sup>4</sup> *Bacteroides fragilis* group has been isolated in samples of various infections such as peritoneal abscess, brain abscess, diabetic foot ulcer, aspiration pneumonia, chronic suppurative otitis media, necrotizing fasciitis, pelvic abscess, etc.<sup>5-8</sup> They often cause infection in association with other anaerobic and aerobic bacteria. Isolation of anaerobic bacteria from clinical samples are difficult since they require special media (horse

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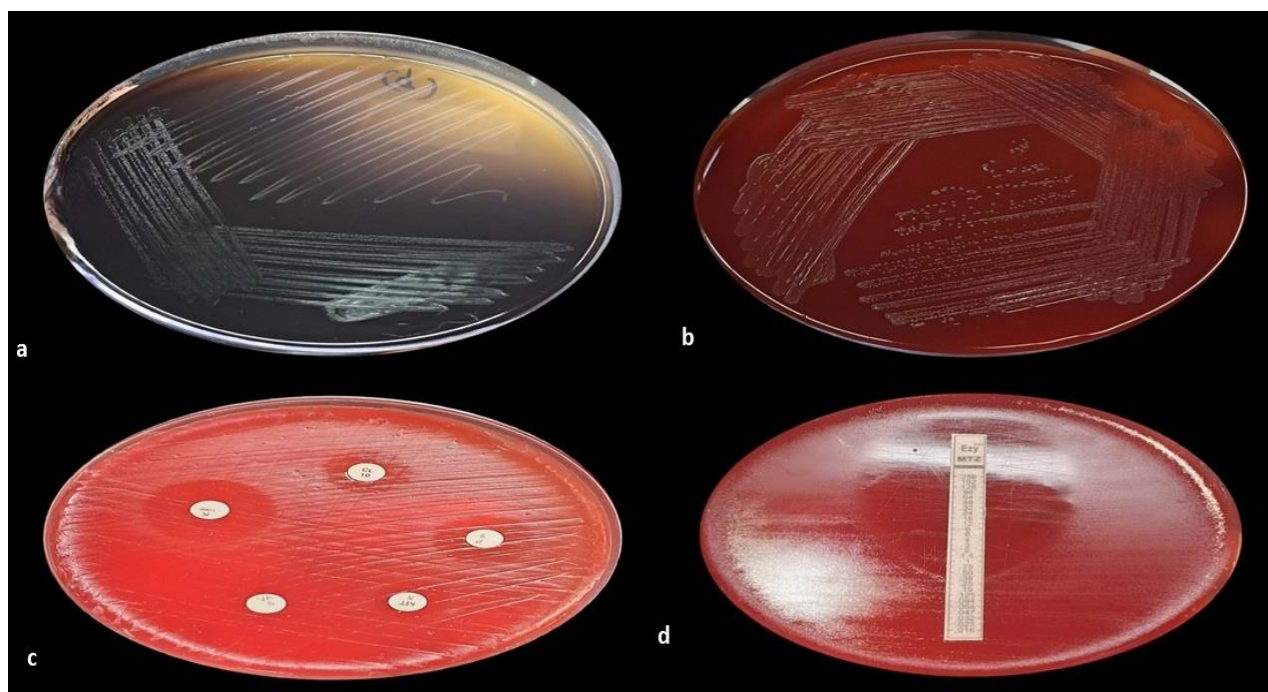
blood agar, thioglycolate broth) along with specialized equipment for maintaining strict anaerobic conditions.<sup>9</sup> Anaerobiosis can be achieved using various techniques like anaerobic chamber, anaerobic jars with Gaspak, anaerobic pouch systems, anoxomat etc.<sup>9</sup> Identification includes use of biochemical tests like *Bacteroides* bile esculin agar, use of special antibiotic disc like kanamycin(1000µg), vancomycin (5 µg) and colistin (10 µg) which are time consuming <sup>9</sup>. Advances in the anaerobic culture techniques and usage of automated identification methods like Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry have greatly improved the isolation and identification of these anaerobes, which was once cumbersome due to long incubation time, the need for special equipment, and high costs. This has helped us to understand the role of anaerobic bacteria like the *Bacteroides fragilis* group in various diseases, often associated with high degrees of morbidity and mortality.<sup>11</sup> Routine antibiotic sensitivity tests are not done for anaerobic isolates in many clinical laboratories because the procedure requires special media and is often difficult and time-consuming.<sup>12</sup> Currently, metronidazole is used as an empirical antibiotic for anaerobic infections and prophylaxis.<sup>11</sup>

Studies conducted in the past two decades have shown an increasing trend of antibiotic resistance among anaerobic bacteria, with the highest incidence among the members of the *Bacteroides fragilis* group.<sup>13,14</sup> The resistance towards

clindamycin has been attributed to presence of *ermF* gene and metronidazole due to *nim* gene.<sup>11,15</sup> The need for routine testing of antibiotic susceptibility patterns has become crucial to mitigate incidences of antibiotic resistance. In this study, we have ascertained the antibiotic susceptibility of the *Bacteroides fragilis* group isolated from the various samples that were received at the microbiology laboratory of St. Johns medical college hospital in southern India.

## 2. Materials and Methods

The study was a descriptive cross-sectional study on *Bacteroides fragilis* group isolated from various samples received for anaerobic culture at the Department of Microbiology of St. Johns medical college hospital in Bangalore during the period from January 2021 to March 2024. Samples such as pus aspirates, body fluids, tissue from various sites suspected of anaerobic infection was cultured on anaerobic blood agar, Robertsons cooked meat broth, neomycin blood agar and brucella blood agar (**Figure 1a**). Additional tests such as growth on Bile esculin agar were also performed for confirmation of the genus *Bacteroides* (**Figure 1b**). The cultures that yielded *Bacteroides fragilis* group isolates were included in the study. Any swabs, like eye swabs, ear swabs, etc., were rejected, and fluid samples like urine samples and stool samples were not included in the study.



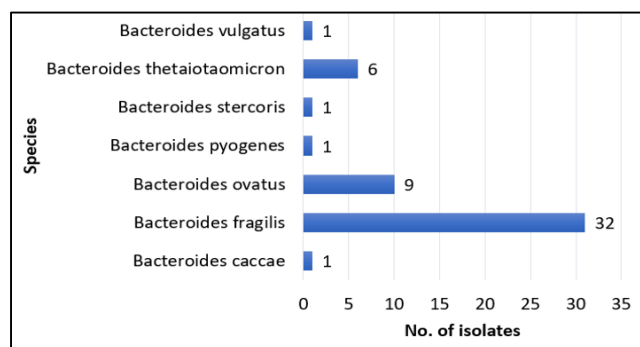
**Figure 1:** a): Bile Esculin agar with kanamycin showing the growth of *Bacteroides fragilis* (BF). b): Brucella blood agar showing the growth of translucent colonies of (BF). c): Antidisc sensitivity pattern of BF. d): E strip sensitivity of metronidazole showing MIC 0.75 which is sensitive

Anaerobiosis was achieved by either the anaerobic jar method with the commercially available gaspak (BD GasPak™) or plastic GasPak pouch system (BD GasPak™ EZ Pouch Systems). Aerotolerance test was put up for each anaerobic isolate. Colonies that resembled the *Bacteroides fragilis* group were gram stained and colonies whose culture smears showed gram-negative bacilli resembling *Bacteroides fragilis* (Figure 1c) were identified using Vitek MS (bioMérieux) which uses Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) technique.

Antibiotic susceptibility for metronidazole and clindamycin was done by Epsilon meter (E strip) method on Brucella Blood agar (Figure 1 d) using HiMedia Ezy MIC strip (EM019) for clindamycin and HiMedia Ezy MIC strip (EM 128) for metronidazole. The MIC was then compared to the CLSI 2023 breakpoints of the specific antibiotic for the *Bacteroides fragilis* group.<sup>16</sup> The isolate was identified as sensitive to Clindamycin if MIC was  $\leq 2\mu\text{g}$ , intermediate if MIC was  $4\mu\text{g}$ , and resistant if MIC  $\geq 8\mu\text{g}$ .<sup>16</sup> The isolate was identified as sensitive to Metronidazole if MIC was  $\leq 4\mu\text{g}$ , intermediate if MIC was  $8\mu\text{g}$ , and resistant if MIC  $\geq 16\mu\text{g}$ .<sup>16</sup> Data was analysed using SPSS v.28. All categorical data were summarized using frequency and percentages, and all continuous data were described using mean and standard deviation or Median based on the distribution.

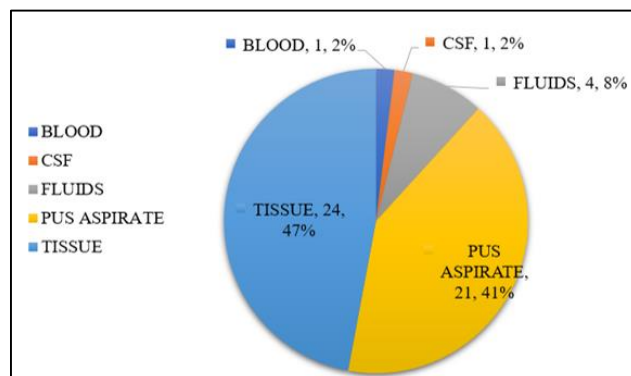
### 3. Results

A total of 51 *Bacteroides fragilis* group isolates were isolated from various patient samples received at the Department of microbiology, St. Johns medical college. The majority of the patients were male (male to female ratio - 2.34:1). 54% of patients were above 45 years, 20 Patients were aged 16-44, 3 patients were less than 15 years of age, including 2 neonates, and the mean age was 46.9 years. *Bacteroides fragilis* (60%) was the common species isolated, followed by *Bacteroides ovatus* (19.6%) and *Bacteroides thetaiotaomicron* (11%) (Figure 2). *Bacteroides pyogenes*, *Bacteroides caccae*, and *Bacteroides stercoris* were the other species.



**Figure 2:** Species diversity of *Bacteroides fragilis* group grown in culture

The most common type of sample was tissue samples (47%), followed by pus aspirates (41%) and body fluids (8%)(Figure 3).



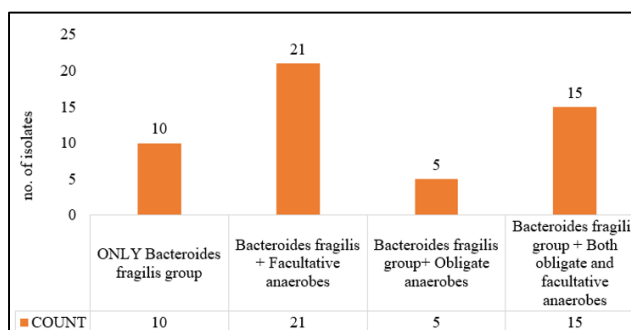
**Figure 3:** Showing the different samples from which *Bacteroides fragilis* group were isolated

In this study, 58% of the patients had co-morbidities, with Diabetes mellitus being the prevalent. Hypertension, thyroid disorders, and cardiac diseases were some of the other comorbidities associated with *Bacteroides fragilis* infection (Table 1).

**Table 1:** Various comorbidities associated with *Bacteroides fragilis* Group infections

Risk Factors (n=29)	Count
Diabetes Mellitus	23
Hypertension	16
Thyroid Disorders	5
Cardiac disease	7
Other Comorbidities (Asthma, COPD, Paraplegia, etc)	5

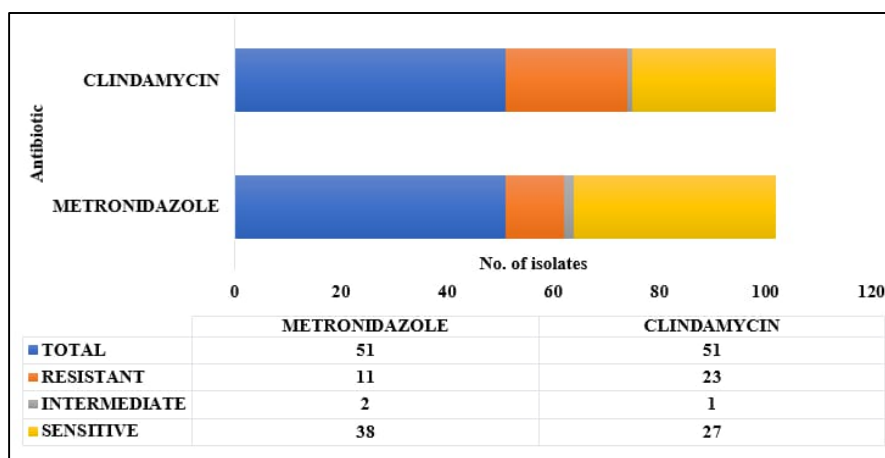
Most of the samples were obtained from the surgical ward / ICU, followed by the medical ward or ICU. The majority of the samples were from the gastrointestinal tract, followed by cutaneous tissue (Table 2). 80% of the samples were polymicrobial in growth (Figure 4), with *Prevotella* (n=6) being the most common obligate anaerobe grown along with the *Bacteroides fragilis* group.



**Figure 4:** Polymicrobial growth among the 51 samples

Other anaerobes include *Peptostreptococcus* species and *Finigoldia* species. The most common facultative anaerobe grown was *E.coli* (n=15) followed by *Streptococcus* species (n=8). Other organisms include *Enterococcus* (n=7) species and *Proteus* (n=3) species. Among Gram positive cocci isolated, *Enterococcus* (n=7) was the predominantly isolated. Most of the *Enterococcus* species (n=4) were Vancomycin

Resistant *Enterococcus*. 11 out of 51 *Bacteroides fragilis* group isolates showed resistance to metronidazole (21%), while 2 isolates were intermediate (3.9%) and the rest sensitive. 23 out of 51 isolates (45%) were resistant, 1 was intermediate (1.9%), and 26 isolates were sensitive to clindamycin. (**Figure 5**)



**Figure 5:** Antibiotic susceptibility of the *Bacteroides fragilis* group isolates

**Table 2:** The various clinical conditions from which *Bacteroides fragilis* group was isolated

S. No.	Systems involved	Conditions	No.
1.	Gastrointestinal tract (22)	Appendicular abscess	3
		Perianal abscess	2
		Abdominal wall abscess	4
		Peritoneal Abscess	4
		Peri tonsillitis	2
		Pancreatitis	2
		Ischiorectal abscess	2
		Hirschsprung's disease	1
		Hepatalithiasis	1
		Esophago-pleural fistula	1
2	Subcutaneous and soft tissue (20)	Gangrene	6
		Fournier's gangrene	3
		Surgical site infection	2
		Bedsore	3
		Diabetic foot & ulcer	5
		Necrotizing fasciitis	1
3	Central nervous system (2)	VP Shunt Infection	1
		Cerebellar abscess	1
4	Genito urinary tract (1)	Fistula in the Genito urinary tract	1
5	Systemic infection (1)	Septic shock	1
6	Orofacial mandibular region (1)	Submandibular abscess	1
7	Other sites		4
	Total		51

#### 4. Discussion

The *Bacteroides fragilis* group is one of the prevalent anaerobic pathogens infecting man and has been found to cause diseases ranging from abscess formation to sepsis. Often, in the case of suspected anaerobic infection, the patients are just given empirical antibiotic therapy with antibiotics like metronidazole and clindamycin. In the recent past, studies show an alarming incidence of drug resistance among the *Bacteroides fragilis* group, which has shown the importance of performing antibiotic susceptibility on anaerobic isolates.

In this study, the male-to-female patient ratio was found to be 2.34:1 among cultures showing growth of the *Bacteroides fragilis* group. Incidences of *Bacteroides fragilis* group infections were thus slightly more in samples of males than females. A similar male predominance was observed in the study conducted by Vishwanath S *et al.* in Manipal.<sup>11</sup> The most common age group affected was 61-75 years (27%) in our study.

Most patients were above the age of 45 years (n= 28, 54%), with 3 patients less than 15 years of age including 2 neonates. The study by Karlowsky *et al.* and others also states that the majority of the patients were also above 45 years of age.<sup>11,17</sup> Among the 51 patients whose samples showed significant growth of the *Bacteroides fragilis* group, 29 patients had one or more comorbidities. The most common comorbidity (**Table 1**) was found to be Type 2 Diabetes mellitus (79%) followed by Hypertension (55%).

The most common sample type (**Figure 3**) were tissue samples (47%), followed by pus aspirates (41%) and fluids (8%) unlike in the studies conducted by Lulu Jhan *et al.*, Vishwanath S *et al.*, and Jung Hyun Byun *et al.*, where the majority of samples were pus aspirates followed by tissue and body fluids.<sup>11,15,18</sup> Seventeen of the samples were aspirates from abscesses (33%), including one cerebellar abscess. This correlated with studies of Vishwanath and Nagy *et al.*<sup>11,14</sup>

Mostly, the samples were from the abdomen (**Table 2**), particularly the Gastrointestinal tract (43%). Samples from Gastrointestinal tract were the most common samples seen in the studies conducted by Lulu Jahan *et al.*, Vishwanath S *et al.*, E. Nagy *et al.*, and Fernández-Canigia *et al.*<sup>11,14,15,19</sup> Other

infections include central nervous system infections (3.9%), gangrene (17.6%), and diabetic foot ulcers (9.8%).

A total of 41 (80%) out of the 51 samples showed polymicrobial growth of aerobic and anaerobic organisms, which is a characteristic of anaerobic infections (**Figure 4**). The studies conducted by Nakamura *et al.*, Elliott D *et al.*, and Tzianabos *et al.* also conclude that most of the infections caused by the *Bacteroides fragilis* group are polymicrobial.<sup>20-22</sup> The common obligate anaerobes grown along with the *Bacteroides fragilis* group include *Prevotella species* (25%), followed by *Peptostreptococcus species* (13%) and *Finnegoldia species* (13%) as seen in the studies conducted by Byun *et al.* and Irina *et al.*<sup>18,23</sup>

*Bacteroides fragilis* was found to be the most prevalent species (62.7%) isolated among the total 51 isolates of *Bacteroides fragilis* group followed by *Bacteroides ovatus* (17.3%) and *Bacteroides thetaiotaomicron* (11%). Other species isolated include *Bacteroides vulgatus*, *Bacteroides pyogenes*, and *Bacteroides caccae* (**Figure 2**). In the study conducted by James A. Karlowsky *et al.*, *Bacteroides fragilis* followed by *Bacteroides ovatus* were the predominant species.<sup>17</sup> Whereas in the study conducted by Viswanath *et al.*, Nagy *et al.*, and Lulu Jahan *et al.*, *Bacteroides fragilis* followed by *Bacteroides thetaiotaomicron* were the commonly isolated species.<sup>11,14,15</sup>

In this study, 11 out of 51 isolates showed resistance to metronidazole (21.5%), while 2 isolates were intermediate (3.9%) and 38 (74.5%) were sensitive to metronidazole. 23 out of 51 isolates (45%) were resistant, 1 intermediate (1.9%), and 27 (50%) isolates were sensitive to clindamycin. In a similar study conducted by Lulu Jahan *et al.* the resistance to metronidazole was found to be 12.5% and in the study by Viswanath S it was 7% of the isolates.<sup>11,14</sup> Metronidazole resistance was found to be 31% among *Bacteroides fragilis* group in a study conducted by Sethi S *et al.* in India.<sup>24</sup> Where as in the studies conducted by Michael E. J. Buhl *et al.*, Viswanath S, Nagy E *et al.*, and James Karlowsky *et al.*, more than 20% *Bacteroides fragilis* group isolates were resistant to clindamycin.<sup>11,14,17,25</sup>

The percentage of isolates showing resistance to metronidazole and clindamycin were significantly higher than previous studies (**Table 3**).

**Table 3:** Different studies on metronidazole and clindamycin resistance among *Bacteroides fragilis* group

Study	Year	Country/ region	% of Isolates resistant to metronidazole	% of Isolates resistant to Clindamycin
Michael E. J. Buhl <i>et al.</i> <sup>25</sup>	2024	Europe	1.8%	20.9%
Lulu Jahan <i>et al.</i> <sup>15</sup>	2023	India	12.5%	-
Viswanath S <i>et al.</i> <sup>11</sup>	2019	India	7%	31%
Sethi S <i>et al.</i> <sup>24</sup>	2019	India	31%	-
Irina Shilnikova <i>et al.</i> <sup>23</sup>	2014	Russia	2.9%	22.4%
Fernández-Canigia <i>et al.</i> <sup>19</sup>	2011	Argentina	0%	26%
Nagy E <i>et al.</i> <sup>14</sup>	2010	Europe	1%	32%
James Karlowsky <i>et al.</i> <sup>17</sup>	2011	Canada	<1%	48%



*Escherichia coli* (44%) and *Streptococcus* species (22%) were the common facultative anaerobes grown along with the *Bacteroides fragilis* group. Similar findings were seen in the study conducted by Elliot *et al.* and Viswanath *et al.*<sup>11,22</sup> About 45% of the facultative anaerobic GNB were resistant to meropenem, with 37% of *Escherichia coli* showing resistance, 50% *Klebsiella* spp and 50% *Proteus* spp showing resistance to meropenem score off in our study. 95% of the GNB were resistant to ampicillin, cefotaxime, ceftazidime and cefuroxime, while almost 90% showed resistance to ciprofloxacin. Among Gram-positive cocci isolated, *Enterococcus* species (n=7) were the most common. Most of the *Enterococcus* species (n=4) were Vancomycin Resistant *Enterococcus*.

## 5. Conclusion

*Bacteroides fragilis* group pathogens are the most prevalent pathogenic anaerobe in clinical samples, but they are often overlooked in routine diagnostics due to the difficulties in isolation. This study shows the trend of an increase in resistance among the *Bacteroides fragilis* group towards metronidazole and clindamycin, which are the common antibiotics used to treat anaerobic infections. Hence, routine screening for the presence of anaerobic pathogens like the *Bacteroides fragilis* group is necessary, and empirical therapy should be followed by appropriate targeted antibiotic therapy for anaerobes after antibiotic susceptibility testing.

## 6. Limitations

No genotypic study was performed to access the presence of resistance genes like *nim* gene and *ermF* gene. The study was not multi centric limiting the findings to only a small population of the country. The resistance of *Bacteroides fragilis* group to other common antibiotics need to be assessed especially carbapenems.

## 7. Source of Funding

Nil.

## 8. Conflict of Interest

The authors declare that there is no conflict of interest.

## 9. Ethics Statement

The study was approved by the Internal ethics committee (IEC Ref no. TH-13/2023) of St. Johns Medical College, Bengaluru.

## 10. Consent and Confidentiality

Separate patient consent was not required as only the laboratory data on anaerobic cultures received as part of routine care was analysed in the study; the identity of the patient was not required or used in the data collection and analysis.

## 11. Authors' Contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. Jacob S was involved in the research, lab work, collection of data, interpretation, statistical analysis and maintenance of records. Gachinmath S was involved in the study topic, study design, data analysis and supervision.

## 12. Acknowledgements

Christobel Sobiya, Dept. of Microbiology. St Johns Medical College. Bengaluru.

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