

Antibiotic Susceptibility Patterns of Gut Microbiota in Inflammatory Bowel Disease

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ABSTRACT

Inflammatory bowel disease (IBD) involves inflammation of the digestive tract and intestines. This inflammation leads to the development of ulcers and causes discomfort during bowel movements. The primary contributors to IBD are poor dietary habits and a lack of physical activity. Inflammation occurs from mouth to anus because of toxic substances released by the bacteria into the system. This study focuses on the antibiotic resistance and susceptibility of the microorganisms that cause IBD. The methodology is based on earlier research related to ASMR and ASPR. Utilizing data till 2019, predictions of IBD incidence for the future in India are provided. The results of the antibiotic sensitivity disc test for the positive samples in the study indicate resistance and susceptibility to specific microorganisms linked to IBD. Statistical analysis shows that India accounts for 8% of global IBD cases annually. More than 37,000 cases have been reported in India. Various regions in newly industrialized countries face an increased risk of developing IBD. Reports from 2019 indicate that males are more frequently affected than females. The prevalence of the disease is higher than the mortality rate among patients, which stands at 20.34%. From this study, in 2025 the males are more affected than females, which stands at 41.25%..

Keywords: Inflammatory bowel disease (IBD), Ulcerative colitis(UC), Crohn's disease(CD), antibiotics, age-standardised mortality rate(ASMR), age-standardised prevalence rate(ASPR), endoscopy, ileoscopy

1. INTRODUCTION

The human body is a reservoir for different microorganisms and their survival. Several factors promote and enhance the growth of microbes. The human gut contains many microorganisms that are colonizers and, in favorable conditions, turn toxic to the body (Sartor, R.B. and Mazmanian, S.K. 2012). Inflammatory Bowel Disease (IBD) is referred to as the "inflammation" that impacts the intestines and digestive system. IBD is a chronic, "recurring and relapsing" disease that attacks human livelihood. Intestinal microbiota plays a crucial role in developing inflammatory bowel disease (Toychiev A. *et.al.* 2021). Inflammatory Bowel Disease (IBD) is generally categorized into two types: ulcerative colitis(UC) and Crohn's disease(CD), both of which are global concerns. Some concerns that are increasing the risk of IBD include diet, stress, irregular sleep patterns, smoking, unhygienic maintenance, use of antibiotics, and various medical impacts (Dolan, K.T. and Chang, E.B. 2017). UC is a recurrent inflammatory and ulcerative disease affecting the colon and rectum, and the condition is clinically characterized by symptoms such as rectal bleeding, cramping, diarrhea, weight loss, abdominal pain, and anorexia (Kirsner, J.B. and Shorter, R.G. 1982). CD shows symptoms like chronic abdominal pain, diarrhea with bloody stools, weight loss, fever, and perianal lesions in susceptible patients (Matsuoka, K. *et.al.* 2018) and decreased appetite (Singh, S. *et.al.* 2011).

The first diagnosis of IBD is made essentially using endoscopy. It is the care and essential method for diagnosing and treating UC and CD (Shergill, A.K. *et.al.* 2015). IBD is a prevalent disease noticed in Europe, affluent Western countries, and newly industrialized countries of Asia, East Africa, and South America, showing reports of a rapid increase in IBD incidence

(Kaplan, G.G. and Ng, S.C. 2017). According to the 2019 survey, about 3.1 million individuals in the US are affected by IBD, which includes CD and UC, to stop the IBD from significantly affecting the quality of life and leading to sustainable utilization of healthcare resources (Click, B. *et.al.* 2021). Furthermore, Innovative diagnostic procedures use pharmacogenetic and serologic markers that help to improve the diagnosis and predict the clinical course of IBD (Nikolaus, S. and Schreiber, S. 2007). Clinical investigations assess the effectiveness of several antibiotics, with ciprofloxacin and metronidazole being the most commonly prescribed combination or alone for CD. Oral metronidazole and ciprofloxacin are preferred therapeutic options for UC (Nitzan, O. *et.al.* 2016). Among the medicated IBD patients, the salicylates used for oral mucosal lesions least affected the symptoms (Laranjeira, N. *et.al.* 2015).

Zhang, M. *et.al.* given that the potential therapeutics include manipulating gut microbiota, which can be achieved by using antibiotics, fecal microbiota transplantation, or probiotics (Zhang, M. *et.al.* 2017). Mengque Xu *et.al.* worked on probiotic supplementation and provided a positive effect on IBD through clinical symptom reduction (Mengque Xu *et.al.* 2025). IBD affects human health significantly negatively, compromises quality of life, causes sick leave, and reduces work involvement (Forss A 2022). According to Ng, S.C., 31,774 new IBD cases were recorded among colleagues' 2019 figures, which accounts for over 8% of the worldwide incidence.. In the 20th and 21st centuries the increase of IBD cases in industrialized areas and, the incidence of IBD has increased (Ng, S.C. *et.al.* 2017) it has been studied that more than 160 risk genes are responsible and are integrated with CD, and most of them affect the microbes present in the bowel, and those are dependent on phenotype (Wehkamp, J. *et.al.* 2016). Chronic granulomatous disease (CGD) is a more congenital immunodeficiency disease that affects 1 in every 250,000 people with genetic mutations in the respiratory burst (Marks, D.J. *et.al.* 2009). In the data provided for 2019, some parts of Western countries reported that 2.4%-5.4% of every 100 children have an increased incidence of IBD. Out of every 100,000 children, 2.56% of CD cases were reported, and 2.14% of UC cases were diagnosed (Seyedian, S.S. *et.al.* 2019). Though it is less than the rate of change of Asian prevalence, the prevalence of IBD in India is more than the worldwide change rate. India is contributing 8% of the incidence to the global rate (Sharma V 2025).

2. MATERIALS AND METHODOLOGY

Site of implementation of work:

DNA Labs- CRIS (Centre for Research and Innovative Studies), of DNA Labs-A Centre for Applied Sciences, located in East Hope Town, Lamipur, Dehradun, Uttarakhand, was the site of all the experiments.

Sample collection and analysis: Endoscopic samples were collected from 40 patients from a nearby hospital. The source of collection of the sample is by a doctor from the intestine by endoscopy and ileoscopy of the colon, rectum, and ileum.

The samples are collected in a sterile sample containers and those are promptly stored at -20C for the microbial examination and analysis.

Materials: The materials used are an endoscopic sample, a biosafety level cabinet 2, Muller-Hinton agar, basic microbiology laboratory instruments (petri plates, swabs, inoculum loop, microscope, BOD incubator), antibiotic discs (gentamicin, amikacin, ciprofloxacin, ampicillin, tetracycline, doxycycline), and caliper scale.

Culture techniques:

To analyze and identify the gut microorganisms, a Muller-Hinton Agar (MHA) plate is prepared with 4.75g of Muller-Hinton agar in 125 mL of distilled water. Mix the contents well in the conical flask and keep it for sterilization in an autoclave at 121°C for 15 minutes. After cooling the media under the sterile conditions of the bio-safety cabinet, pour the media into petri plates with media and let them solidify. To confirm the presence of microbes, the sample is uniformly spread over the media. Keep the plates in the BOD incubator for 24 hours.

To isolate the pure quality of microorganisms, one of the colonies is isolated and streaked over the MHA in the Petri plate. After 24 hours of incubation, pure colonies of microorganisms are isolated.

Morphological examination of microorganisms by a microscope

Generally, microorganisms are categorized into Gram-positive and Gram-negative based on their cell wall composition. The gram-positive bacteria appear in purple by crystal violet stain, gram-negative bacteria appear in pinkish, which upon acquiring color by safranin. The pure culture is obtained to perform slide microscopic analysis by smear preparation which is followed by crystal violet, gram iodine, decolorizer, safranin and each step with washing of slide with distilled water. And observe under the microscope.

Biochemical analysis and characterization

The biochemical tests are carried out, and the tests to confirm the presence of gut bacterial species:

- **Carbohydrate fermentation test (sucrose):** Gas production and the media turn pink color indicating a positive and successful reaction of the test.

- **Methyl Red test:** The media turns red due to fermentation, which results in positivity for the presence of microorganisms.
- **Voges-Proskauer test:** The absence of brick red color in the tube indicates that no fermentation is occurring in the media.
- **Indole test:** The test is successful if a brick-red color ring appears over the medium's top layer.
- **Citrate test:** A blue color colonies are observed over the green citrate slant, indicating attempt of presence of bacterial colonies.
- **Catalase test.** The observance of white bubbles on the slide with bacterial colonies and H₂O₂ because to the catalase enzyme.
- **Coagulase enzyme:** The coagulase enzyme and its activity are performed by plasma and verified.

Antibiotic sensitivity disc test:

125 ml of media is prepared with 4.75g MHA. Along with this, a nutrient broth of 50mL is prepared for reviving of culture. Autoclave the media. Pour the plates with media of each 25mL. Keep the petri plates for incubation. Inoculate the pure colonies of samples into a test tube and keep the tubes for colony growth. After 24 hours, spread the isolated colonies from a revived culture with a swab. Place the antibiotic disc over the media. Incubate the plates for 24 hours. The zone of inhibition indicates that the microorganisms are sensitive, and the growth of colonies is characterized by resistance.

Samples are statistically analyzed for IBD prevalence:

The data is calculated with the positive cases of IBD (33 out of 40 samples). The number of females and males is calculated from the data obtained from the hospital. The patients with IBD positive were interpreted as many positive cases by the total number of cases multiplied by the total population. The percentage is calculated by multiplying the number of positive cases by 100 and dividing the result by the total population.

Prevalence Rate = (Number of Positive Cases / Total Population) × 100,000

Prevalence Percentage = (Number of Cases per Population / total population) × 100.

3. RESULT

The antibiotics are used to destroy the targeted microorganisms and help in the recovery of the patient. Generally, antibiotics penetrate the 'cell wall' of microbes and block the medium of releasing poisonous toxins. We studied the "endoscopic samples" of 40 patients for this paper. Each sample is examined and concluded with the microorganisms. The most common microorganisms that are suspected from the positive samples are *Staphylococcus aureus*, *Klebsiella*, *E. coli*, *Enterobacteria*, and *Pseudomonas aeruginosa*. These are the common microorganisms concluded from the tested positive samples. Each targeted microorganism is treated with specific antibiotics such as imipenem, amikacin, tetracycline, ampicillin, doxycycline, ciprofloxacin, and gentamicin.

The antibiotics gentamicin and imipenem are showing resistance to the *Enterobacter*, *Klebsiella*, and *E. coli*. The majority of antibiotics exhibit "susceptibility" to microorganisms, which can prevent the microorganisms from growing. Figure 1 shows antibiotic susceptibility testing using the disc diffusion method on agar plates. Each Petri plate (labeled A to E) contains a lawn of bacterial growth with antibiotic-impregnated discs placed on the surface. Zones of inhibition (clear rings around the discs) indicate areas where the bacteria could not grow due to the antibiotic's effectiveness. Larger zones suggest higher sensitivity of the bacteria to the antibiotic. Smaller or no zones imply resistance. A to E corresponds to species like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Klebsiella spp.*, and *Escherichia coli*. Multiple antibiotics are tested on each plate, allowing for effectiveness.

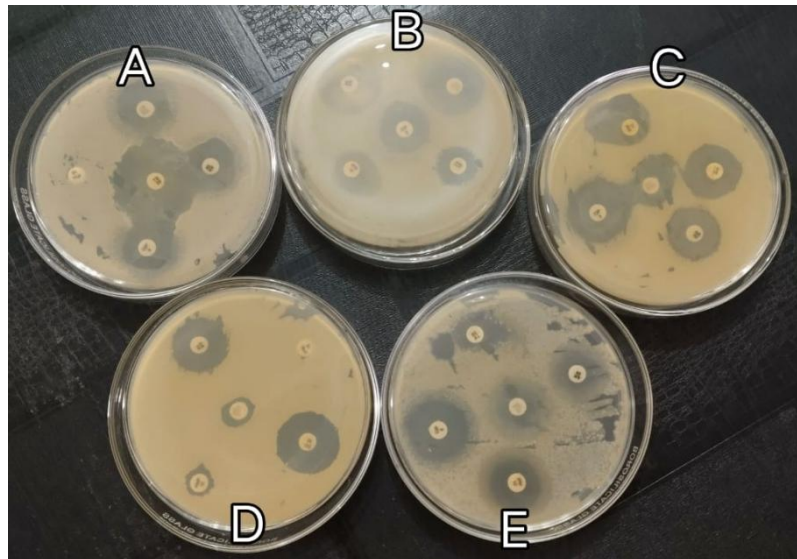


Figure 1 antibiotic susceptibility test of microorganisms of IBD samples A, B, C, D, and E

Table 1: Results of antibiotic resistance and sensitivity tests for selected microorganisms

Name of the antibiotics	Name of the microorganism				
	<i>Staphylococcus aureus</i>	<i>Pseudopmonas aeruginosa</i>	<i>Enterobacter spp</i>	<i>Klebsiella</i>	<i>Escherichia coli</i>
Ampicillin	NA	NA	NA	Resistant	NA
Ciproflaxcin	Sensitive	Sensitive	Sensitive	NA	Sensitive
Amikacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
imipenem	Resistant	Sensitive	Sensitive	Resistant	Sensitive
Gentamicin	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
Tetracycline	Sensitive	Sensitive	Sensitive	NA	Resistant
Doxycycline	NA	NA	NA	Sensitive	Sensitive

4. DISCUSSION

IBD is becoming more prevalent worldwide. The number of patients is more affected by the inflammation of the intestine, and some are chronic. To the years of 2019, the analysis showed that the number of IBD incidences was observed, and the diagnosis of the disease is less prevalent. The recommended course of treatment for IBD, which includes UC and CD, is antibiotics. According to the previous statistical analysis from 1990-2019, the most common cases of IBD are observed and reported in the age group of 15- 35 years. The age group is very prevalent in the occurrence of IBD. The data provided for 2019, some parts of Western countries reported that 2.4%-5.4% of every 100 children have an increased incidence of IBD. Out of every 100,000 children, 2.56% of CD cases were reported, and 2.14% of UC cases were diagnosed. The prevalence of UC and CD varies across areas and their life style. The cases in Iran show that there was a lower number of CD prevalence and an increase in the prevalence of UC cases. This data from different studies by researchers claims that the incidence and prevalence of attacks of IBD vary according to the places and the habitat of people (Seyedian, S.S. *et.al.* 2019).

In India, the occurrence of IBD is more prevalent and significant in males compared to females. The annual rates of IBD change in age-standardized mortality rate (ASMR) for IBD patients with anemia are significantly higher in females compared to males with anemia. The males who are recurrent to the IBD have with 0.44, 95% (per 100000) chance of chronic inflammation. But the annual rate of change in ASMR in females is higher than 0.31, 95% (100000) of chronic inflammation. In India, the age-standardized prevalence rate (ASPR) for IBD was 20.34% in 2019, with 270,718 expected cases. Though it is lower than the rate of change in Asian prevalence, India's IBD prevalence is higher than the global change rate. India is

contributing 8% of the incidence to the global rate (Sharma V 2025).

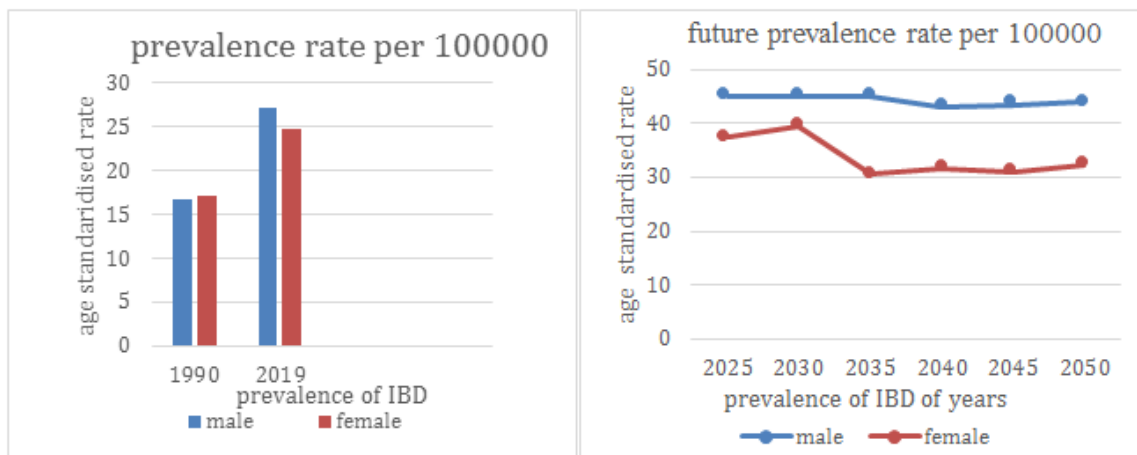


Figure 2 a. prevalence of IBD b. future prevalence of IBD per 100000.

Figure A shows the prevalence of IBD from 1990-2019, and Figure B shows the future prevalence rate of IBD cases per 100000 from 2025-2050.

The number of cases reported according to 2019, the prevalence rate of males (16.8-27.2 per 100000) and females (17.2-24.8 per 100000). The chance of incidence and prevalence rates of patients for females are higher compared to males. From the studies of this paper the substantial changes are predicted in the prevalence of IBD in the future. Based on the endoscopic samples analysed, 33 out of 40 are positive. So prediction of data is in comparison with previous analysis and predicted for the years 2025-2050 per 100000. The future prevalence of IBD in males(44.96-43.96) and females (37.54-32.34) per 100000. The occurrence of IBD is increasing and may decline because of precautions maintained by people, but further, it may increase because of unhealthy and improper diet. These cases can mostly be noted in newly industrialised countries. Timely diagnosis and medication are necessary to overcome the severity of IBD. The antibiotics are most accurately prescribed in treating IBD. This paper deals with the action against the microorganisms associated with IBD and its destruction, and reducing the risk of the severity and cure from inflammation of IBD.

5. CONCLUSION

In conclusion, antibiotics are sensitive to intestinal microorganisms and are more effective at killing them. Among the 33 positive samples, the 5 most common microbes were evaluated with an antibiotic test, and resulted in resistance for 3 microorganisms, and among 7 antibiotics, most of them are sensitive, which are involved in the killing of microorganisms. The present study concludes with a 41.25% prevalence rate of IBD. Based on the cases, the statistical analysis predicts for future is that an increased prevalence of IBD can be observed according to previous cases reported. The future prediction of IBD prevalence is based on the positive cases analysed for the antibiotics test. The present study concludes with a 41.25% prevalence rate of IBD. So there may be a gradual increase in IBD prevalence, and at some point it may decrease and rise in incidence. The chances of an increase in IBD cases are due to industrialization and unhealthy diet intake.

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