Original Article

# Isolation and Identification of the Types of Bacteria that Cause Irritable Bowel Syndrome

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## Abstract

**Background:** Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal (GI) disorder with an estimated prevalence of 10% around the globe. Human body possesses about 39 trillion of microbial cells, the majority of which inhabit the GI tract. The recent realization is that small intestinal bacterial overgrowth may be associated with symptoms of IBS. **Aim:** The current work aimed at the isolation and identification of different bacteria strains from IBS patients. **Materials and Methods:** One hundred and thirty patients of different sex and ages were diagnosed with IBS (which was previously diagnosed by a specialist doctor) in the time period from November 2021 to May 2022. **Results:** The current results showed that there is a correlation between the number and percentage of isolates for each type of bacteria with a group of study factors, where in terms of sex, females were more. Concerning the age group, the most common age group was 36–55 years. On the other hand, the percentage of uneducated people was higher in the current study. It also showed the other factor, which is the possession of domestic animals; the majority of the study population was not owning animals. Regarding the prevalent weight, the body mass index (BMI) of 24–29.9 is the highest. The percentage of nonsmokers was also the highest, and the highest percentage of isolates was for *Escherichia coli* in all. **Conclusions:** This study proved that *E. coli* is the most common type of bacteria that has an effect on IBS patients and that *Helicobacter pylori* has the least effect.

**Keywords:** Bacteria, irritable bowel syndrome (IBS), microbiota

## Introduction

The condition called irritable bowel syndrome (IBS) is a prolonged effective digestive system disorder that had a global widespread presence of 10%; IBS is a common condition distinguished by recurring epigastric pain or pain related to a change in the bowel habit.[1] Also in the patients with irritable bowel disease and inflammatory bowel disease, vitamin D deficiency is a common disease.[2] A new work indicates that the gut microbiota can influence the motility of intestine, the exudation of intestine, visceral oversensitivity, the activation of the mucosal immune, and the permeability of intestine.[3]

Chamomile has long been used to treat gastrointestinal issues such as digestive disorders, "spasm" or colic, stomach pain, flatulence (gas), gastritis, IBS, and gastrointestinal irritation.[4] Matricaria chamomilla contains phenolic compounds as flavonoids such as flavon glycoside, a glycogen apigenin, and luteolin. Phenols have an important role against intestinal parasite.[5] In the pomegranate peel, active phenolic substances were detected and their effectiveness against bacteria isolated from the stomach and intestines, including *Escherichia coli* and *Salmonella*, which may cause most IBS and gastrointestinal diseases.[6] The phenolic compounds have a potential role against intestinal parasite.[7] Under typical circumstances, the mucus epithelium barrier restricts commensal microbiota to either the surface of the epithelium or the intestinal lumen. It is in these locations that homeostatic immune cells are induced in order to maintain the integrity of the barrier and tolerance among mutualistic bacteria. Because of this, it is possible for microorganisms to permanently

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colonize the gut and engage in symbiotic activities. However, as soon as the barrier is broken by the influx of mediators of immune system, pathogens materials, or any factors that lead to severe reactions, intense inflammation takes place, which alters the environment of the intestines and alters the composition of the gut microbiota. This results in a number of negative health outcomes.[8]

There are approximately 39 trillion microbial cells in the human body; microbiota is the name given to the dynamic ecological environment that is formed by the majority of the organisms that are found in the digestive system tract.[9] Up to 500 different transitory and indigenous strains of bacteria, viruses, and fungus can be found in the microbiota, together with up to 20 million different genes.[10] Recent research has shown that an imbalance in communities of gut bacteria, or "dysbiosis," may contribute to the pathophysiology of IBS, a condition characterized by a decrease/loss of microbial diversity and richness as a result of the shift from microbes to pathogens in the human intestine; an overgrowth of the intestinal bacteria is known to affect a subset of IBS patients.[11] The recent discovery that the overgrowth of the intestinal bacteria may be related to IBS symptoms has resulted in a paradigm shift in recognizing the situation pathogenesis, which was previously thought to be primarily related to factors of the psychology,[12] which has been linked to *Helicobacter pylori* infection,[13] and also reported that *E. coli*, *Enterococcus* species, and *Klebsiella* were the most common species in patients with IBS who had small intestinal bacterial overgrowth.[14] Only a few studies have attempted to categorize the bacteria in IBS patients and discovered an increase in *Klebsiella* and *Escherichia*/*Shigella*.[15] Some prospective studies have also discovered a link between acute gastroenteritis and subsequent IBS. Following a salmonellosis outbreak, patients invented different IBS symptoms that persisted 1 year after infection.[16] Aside from these positive effects, a new research has shown that changes in microbiota balance (dysbiosis) play a role in certain intestinal disorders such as enteric infections, colon cancer, inflammatory bowel, IBS, and even overweight.[17]

## Materials and Methods

### Sample collection

One hundred and thirty patients of different sex and ages were diagnosed with IBS (which was previously diagnosed by a specialist doctor)[18] in the time period from November 2021 to May 2022.

### Irritable bowel syndrome diagnosis

All patients in the study and control groups were evaluated by a gastroenterologist for the presence of IBS symptoms after an organic cause was ruled out; every patient was questioned using a specialized questionnaire to determine whether or not they experienced any recurrent pain in abdominal region, infrequent intestinal movements at times of elevated or reduced activity, pain with defecation, and the symptoms onset with differences in feces frequency. In addition to the Rome II and new Rome III criteria, which were developed by the British Society of Gastroenterology, these are the symptoms that are most frequently considered when making a diagnosis of IBS.[19]

### Samples culturing

It was necessary to collect a sample of the internal organs, so a length of 1 cm was cut from the intestinal tract, and a little portion of liver that weighed 10 g was cut off. This sample included the liver and the colon. Following a 24-h incubation period at 37°C, individual growth models were submerged in a nutrient broth. There was a loop inoculation of bacterial growth that was implanted and cultivated on the culture medium provided by the HiMedia India firm. The culture media consisted of MacConkey agar and Salmonella Shigella agar. After being in the incubator for 24 h at a temperature of 370°C, the petri dishes were removed. Pure and single colonies were selected and cultured on a selective medium such as eosin-methylene blue agar. This was done so that the information on the staining reactions of Gram's stain could be obtained.

### Microscopic examinations

After the bacteria had been subcultured on a nutrient broth medium at 370°C for 4 h, microscopic examinations were carried out on the isolates to investigate their structure, arrangement, and the reaction of the staining. These examinations included Gram's stain, capsule stain, and a motility test.[20]

### Biochemical tests

On bacterial isolates, tests for catalase, oxidase production, indole test, methyl red test, Voges–Proskauer test, hydrogen sulphide generation, urease test, and sugar fermentation, including glucose, xylose, rhamnose, and mannitol, were carried out.[21]

### Statistical analysis

For statistical analysis, software (SPSS, USA) was used. The data were presented as mean and standard deviation and analyzed using the t-test or rank sum test. The X2 test was used to analyze categorical data, which were expressed as frequency and percentage. $P < 0.05$ was considered a statistically significant difference.

### Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee

according to the document number 42437 (including the number and the date in 9/12/2021) to get this approval.

## Results

Table 1 shows that there is a correlation between the number and percentage of isolates for each type of bacteria with a group of study factors, where in terms of sex, females were more, and the highest percentage of isolates was for *E. coli*, which amounted to 36 (43.4%) isolates. Concerning the age group, the most age group was 36–55 years, and the highest percentage of isolate is for *E. coli* bacteria, reaching 31 (51.7%) isolates. On the other hand, the percentage of uneducated people was higher in the current study, and the highest percentage of isolates is for *E. coli*, which reached 41 (51.9%) isolates. It also showed the other factor, which is the possession of domestic animals; the majority of the study population was not owning animals, and the highest isolation rate was for *E. coli* bacteria, which amounted to 39 (39.0%) isolation. Regarding the prevalent weight, the body mass index (BMI) of 24–29.9 is the highest, and the highest isolate percentage is for *E. coli*, which reached 26 (37.1%) isolates. The percentage of nonsmokers was also the highest, and the highest percentage of isolate is for *E. coli*, which reached 49 (41.5%) isolates.

## Discussion

IBS is a functional bowel disorder characterized by recurrent episodes of abdominal pain and altered bowel habits. According to the study, patients with IBS have a higher number of bacteria in the intestine.[22] Several microbes of zoonotic origin have been linked to acute gastrointestinal symptoms caused by bacterial infections caused by contact with animal feces.[23] The current study showed that there is a difference in the rate of infection between males and females, so the infection rate was higher for males with *E. coli* bacteria than for females, so the rate for males was 44.7%, and for females, it was 43.4%, and the lowest percentage of infection is with mixed bacteria; although there are gender differences in the growth and the demonstration of different diseases, the underlying mechanism is unknown.[24] Sex differences in the intestinal microbiota may play a prominent role in the sexual dimorphism of gut microbiota. It is widely acknowledged that males and females have different immune systems. Males are more vulnerable to infection in general.[25] The study showed the incident of bacterial infection with the highest incidence rate was for the age group 36–55 years, followed by age group 15–35 years, and then followed by older age group (56–75 years). Age-related changes in the intestinal microbiota are linked to physiological changes in the gastrointestinal tract as well as dietary patterns, as well as a decline in immune system function, which may contribute to the elevated disease risk and frailty.[26] The gut microbiota of the elderly people has a lowered bacterial diversity.[26] According to the findings of the study, the prevalence of bacterial infections was higher among individuals with lower levels of education (51.9% vs. 29.4%), despite the fact that education is often linked with an improved physical health. Investigations on the effects that a person's level of education has on their physical well-being are producing an increasing body of work. Adults with lesser educational attainment have been found to have poorer health when compared with other demographics, according to a recent research. This tendency is linked to significant health inequalities brought about by differences in education. Therefore, having a solid grasp of the health advantages that may be gained from education is potentially the most important factor in eliminating health disparities and working toward improving the overall health of future generations. Despite a growing interest, the education-health field of the study does not yet have the ability to give real solutions to some of the most significant concerns. One of the reasons for this is that the two occurrences are related throughout hundreds of years of populations as well as across life lengths.[27] According to the study, the incidence of microorganism's infection in people who have animals is higher (56.7%) than in people who do not have animals (39.0%). Nearly two-thirds of pathogens and three-quarters of emerging pathogens are highly infectious in origin. Several zoonotic pathogens have been linked to acute gastrointestinal symptoms caused by contact with animal feces. Poorly managed animal feces can expose humans to pathogens, especially in societies where animals live closest to humans. Humans are more likely to be exposed to animal feces in developing countries where domesticated animals and their stool are not properly contained or separated from home environments.[28-30] The study showed that people with a BMI between 18 and 23.9, which was 53.8%, are more likely to be infected, followed by 30–36, which accounted for 47.1%, and then 24–29.9, which represented 37.1%. BMI has been identified as a predictor of nutritional problems, and malnutrition and condition of overnutrition can result in underweight condition, in both.[29] Although the relationship between BMI and the incidence of infectious disease has been studied in several populaces at a large observational research, the results have been inconsistent and vary depending on the profile of the sample.[30] According to the findings of the study, smokers have a 58.3% greater chance of becoming infected with germs than nonsmokers do (41.5% vs. 58.3%, respectively). The possible methods through which cigarette smoke raises an individual's risk of contracting a systemic illness. One of these may be a change in the structural, functional, or immunologic characteristics of the host defences. In addition, smoking cigarettes has a dual function in the progression of irritable bowel syndrome.[31] This study confirms that people who drink untreated water are more susceptible to infection, and their percentage is 45.8% while the percentage of people who drink sterile water is 30.4%. Water is essential

**Table 1: The type of bacteria in the patients of IBS**

| Factors | *Escherichia coli pathogen* | | *Escherichia coli normal* | | *Helicobacter pylori* | | *Salmonella spp* | | *Shigella spp* | | *Klebsiella spp* | | *Escherichia coli pathogen, Salmonella* | | *Shigella, Escherichia coli pathogen* | | *Escherichia coli pathogen, Klebsiella spp* | | *Escherichia coli normal, Shigella* | | *Escherichia coli normal, Salmonella, Shigella* | | Chi-square *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 10.361 |
| Male | 21 | 44.7 | 6 | 12.8 | 2 | 4.3 | 6 | 12.8 | 3 | 6.4 | 2 | 4.3 | 3 | 6.4 | 4 | 8.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 |
| Female | 36 | 43.4 | 15 | 18.1 | 3 | 3.6 | 6 | 7.2 | 3 | 3.6 | 11 | 13.3 | 5 | 6.0 | 1 | 1.2 | 1 | 1.2 | 1 | 1.2 | 1 | 1.2 | 21 |
| Age (years) | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 20.479 |
| 15–35 | 17 | 40.5 | 6 | 16.7 | 3 | 7.1 | 4 | 9.5 | 2 | 4.8 | 4 | 9.5 | 4 | 9.5 | 1 | 2.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0005 |
| 36–55 | 31 | 51.7 | 10 | 16.7 | 1 | 1.7 | 6 | 10.0 | 2 | 3.3 | 3 | 5.0 | 3 | 5.0 | 2 | 3.3 | 0 | 0 | 1 | 1.7 | 1 | 1.7 | |
| 56–75 | 8 | 28.6 | 3 | 10.7 | 1 | 3.6 | 2 | 7.1 | 2 | 7.1 | 6 | 21.4 | 2 | 7.1 | 3 | 10.7 | 1 | 3.6 | 0 | 1.7 | 0 | 0 | |
| Education* | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 14.693 |
| Yes | 15 | 29.4 | 10 | 19.6 | 1 | 2.0 | 6 | 11.8 | 3 | 5.9 | 9 | 17.6 | 3 | 5.9 | 2 | 3.9 | 0 | 0 | 1 | 2.0 | 1 | 2.0 | 0.003 |
| No | 41 | 51.9 | 11 | 13.9 | 4 | 5.1 | 6 | 7.6 | 3 | 3.8 | 4 | 5.1 | 6 | 7.6 | 3 | 3.8 | 1 | 1.3 | 0 | 0 | 0 | 0 | |
| Keeping animals | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 27.107 |
| Yes | 17 | 56.7 | 17 | 23.3 | 0 | 0 | 1 | 3.3 | 3 | 10.0 | 1 | 3.3 | 0 | 0 | 0 | 0 | 1 | 3.3 | 0 | 0 | 0 | 0 | 0.0008 |
| No | 39 | 39.0 | 14 | 14.0 | 5 | 5.0 | 11 | 11.0 | 3 | 3.0 | 12 | 12.0 | 9 | 9.0 | 5 | 5.0 | 0 | 0 | 1 | 1.0 | 1 | 1.0 | |
| BMI | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 26.354 |
| 18–23.9 | 14 | 53.8 | 1 | 3.8 | 1 | 3.8 | 1 | 3.8 | 4 | 15.4 | 2 | 7.7 | 3 | 11.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0007 |
| 24–29.9 | 26 | 37.1 | 17 | 24.3 | 3 | 4.3 | 8 | 11.4 | 2 | 2.9 | 6 | 8.6 | 3 | 4.3 | 2 | 2.9 | 1 | 1.4 | 1 | 1.4 | 1 | 1.4 | |
| 30–36 | 16 | 47.1 | 3 | 8.8 | 1 | 2.9 | 3 | 8.8 | 0 | 0 | 5 | 14.7 | 3 | 8.8 | 3 | 8.8 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Smoking* | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 15.410 |
| Yes | 7 | 58.3 | 2 | 16.7 | 0 | 0 | 2 | 16.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8.3 | 0 | 0 | 0 | 0 | |
| | | | | | | | | | | | | | | | | | 0 | 0 | 1 | 0.8 | 1 | 0.8 | 0.00 |
| No | 49 | 41.5 | 19 | 16.1 | 5 | 4.2 | 10 | 8.5 | 6 | 11.1 | 13 | 11.0 | 9 | 7.6 | 5 | 4.2 | | | | | | | 6 |
| Water supply | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 20.538 |
| Normal | 49 | 45.8 | 18 | 16.8 | 5 | 4.7 | 8 | 7.5 | 3 | 2.8 | 8 | 7.5 | 9 | 8.4 | 5 | 4.7 | 0 | 0 | 1 | 0.9 | 1 | 0.9 | 0.0006 |
| | | | | | | | | | | | | | | | | | | | | | | 0 | |
| Sterile | 7 | 30.4 | 3 | 13.0 | 0 | 0 | 4 | 17.4 | 3 | 13.0 | 5 | 21.7 | 0 | 0 | 0 | 0 | 1 | 4.3 | 0 | 0 | 0 | | |
| Other disease* | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | No | % | 5.527 |
| Yes | 15 | 41.7 | 5 | 13.9 | 2 | 5.6 | 4 | 11.1 | 1 | 2.8 | 5 | 13.9 | 2 | 5.6 | 1 | 2.8 | 1 | 2.8 | 0 | 0 | 0 | 0 | 0.038 |
| No | 41 | 43.6 | 16 | 17.0 | 3 | 3.2 | 8 | 8.5 | 5 | 5.3 | 8 | 8.5 | 7 | 7.4 | 4 | 4.3 | 0 | 0 | 1 | 1.1 | 1 | 1.1 | |

*Note 1: Yes = present, No = absent

to life, but many people do not have access to clean and safe drinking water, and many die of waterborne bacterial infections. Pathogenic *E. coli* strains and other arising pathogens in having a drink waterborne diseases. Everyone needs an adequate, safe, and readily available supply. Enhancing access to safe tap water has the potential to significantly improve health. Every effort is being made to achieve the highest possible level of drinking water safety.[32] According to this study's findings, those who did not have any other diseases had a significantly greater prevalence of harmful bacteria (43.6%), as compared to people who did have other diseases (41.7%); however the proportion was still lower than that. Since its discovery, a great number of research have shed light on the importance of bacteria in both health and illness, and it has been found that the gut microbial balance is strongly connected to both human diseases and health. When compared with other parts of the body, the digestive system of a human being houses a particularly robust microbial population that is home to up to 100 trillion different species of bacteria.[33] The presence of microorganisms, species of genus agents (*E. coli*, *Salmonella*, *Shigella*, *H. pylori*, and *Klebsiella*), may contribute to the onset, development, and progression of IBS. Subjects with IBS had higher levels of *E. coli* and higher levels of *Klebsiella* spp., which correlated strenuously with quantitative culture data.[34] The presence of *E. coli* has been linked to IBS symptoms as well as bacterial overgrowth.[35] Recent studies have revealed an increase in *E. coli* in IBS patients, which supports the findings of our study.[36] Clinical responses of IBS to antibiotics developed to target *E. coli* associated with travelers' diarrhea provide additional evidence linking the intestinal microbiota to the pathogenesis of IBS, such as fecal transplantation. The nonpathogenic coli may also be present in the intestine, so its presence is normal.[37] Following a Salmonella outbreak, the patients invented different IBS symptoms that persisted 1 year later. This higher frequency may indicate a more serious disease.[38] The incidence of digestive infectious disease caused by *Salmonella* and *Shigella* was calculated from national incidence rates on food-borne disease. Significant overlaps exist between IBS and dyspepsia patients, implying a possible pathogenic role for *H. pylori* in IBS. *H. pylori* transmission is unknown, but it is thought to be fecal-oral.[39]

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## Conflicts of interest
There are no conflicts of interest.

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