

Carriage of Pathogenic Organisms in Gastrointestinal Tract Among Chandigarh Population

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How to cite this article: Varsha Gupta Anku Goel, Meenakshi Singh et. al. Carriage of Pathogenic Organisms in Gastrointestinal Tract Among Chandigarh Population. Indian Journal of Public Health Research and Development / Vol. 17 No. 2, April-June 2026.

Abstract

Objectives: To understand the epidemiology of various pathogens, it is imperative to understand the carriage of these pathogens in asymptomatic individuals. This helps in understanding the transmission of disease and thus the control measures. This study was carried out to detect the carriage of various pathogenic bacteria, parasites, and Rotaviruses in stool samples of the healthy population of Chandigarh.

Materials and Methods: 500 non-duplicate fecal samples from healthy subjects were collected and processed for isolation of pathogenic bacteria by microbiological culture. An enzyme-linked immunosorbent Assay was performed for *Clostridium difficile* toxins and Rotavirus antigen detection. Intestinal parasites were detected by wet mount and iodine mount directly, and after the formol-ether concentration technique for parasites. The data were analyzed descriptively using Microsoft Excel, and the results were summarized as percentages to provide a clear understanding of the distribution and trends in the dataset.

Results: Carriage of intestinal pathogens was found to be 44.4% and 55.6% in children and adults, respectively. Carriage of *Shigella* species, *Salmonella* species, and *Vibrio cholerae* was nil in our study, with 2.6% carriage of *Clostridium difficile*. Rotavirus carriage was 3.6% in children. The carriage of parasites was more in adults than in children.

Conclusions: The two main concerns in our study were *C.difficile* carriage and intestinal parasite carriage in adults. Educating the masses regarding hand hygiene is the most important step in this regard. Regarding parasitic infections, a single dose albendazole in school-going children has resulted in lower carriage in children than adults. The same approach, along with education regarding hygienic practices, can be started in the adult population also.

Keywords: Carriage, Stool, Community, Pathogenic, Gastrointestinal tract

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Submission date: September 27, 2025

Revision date: Nov 12, 2025

Published date: April 14, 2026

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Introduction

The human gastrointestinal tract is home to a variety of bacteria, parasites, fungi, and viruses. Infectious causes of acute diarrhea in symptomatic patients, including viruses, bacteria, and parasites, can lead to high morbidity and mortality, particularly in children¹. However, the carriage of pathogens among asymptomatic patients in a community is not known in our region. Asymptomatic patients are those who do not possess signs and symptoms of gastrointestinal illness, but their gut harbors pathogens². This type of surveillance is of utmost importance due to shedding of pathogens in the environment by healthy asymptomatic populations that may potentially lead to dissemination of infection in the community and transmission to the susceptible host. Food handlers working in the food service establishments can also act as carriers of different enteropathogens, possibly causing fecal contamination of foods by their hands, which may be the cause for the transmission of infections to the local community³. A carriage rate of as high as 41% has also been reported⁴. There is currently limited epidemiological data to provide an accurate assessment of the burden of enteropathogens in the asymptomatic population of developing countries. This study was carried out with the aim of knowing the prevalence of various pathogenic bacteria, parasites, and Rotavirus in stool samples of the healthy population of Chandigarh. To our knowledge, this is the first study to look for all the major pathogens in a healthy population in our region.

Materials and Methods

This prospective, observational study was carried out at the Department of Microbiology, Government Medical College and Hospital, Chandigarh, and involved 500 healthy individuals from Chandigarh who gave informed consent. This study protocol was approved by Institute of Ethics Committee of Government Medical College and Hospital, Chandigarh on 26th November 2020 (GMCH/IEC/2020/356/120). These participants were divided into two groups: pediatric and adult. The pediatric group included individuals under 14 years old, while those over 14 years of age formed the adult group. Demographic details such as age,

sex, and address were recorded on a proforma. A comprehensive laboratory analysis was performed on non-duplicate fecal samples, including bacterial culture, Enzyme-Linked Immunosorbent Assay for *Clostridioides difficile* toxins and Rotavirus antigen, and microscopic examination for intestinal parasites using wet mount, iodine mount, and the formol-ether concentration technique. Stool samples were cultured on MacConkey agar, Xylose Lysine Deoxycholate agar, and Thioglycollate Citrate Bile Salt Sucrose agar, with enrichment in Selenite F broth and Alkaline Peptone Water to isolate pathogenic bacteria.

Results

A total of 500 participants were observed, comprising 222 [44.4%] children and 278 [55.6%] adults. Among them, females accounted for 60% (n = 300) and males represented 40% (n = 200). This distribution indicates a higher representation of females in the cohort, with a female-to-male ratio of 1.5:1. Distribution of age and sex is shown in **Table 1**.

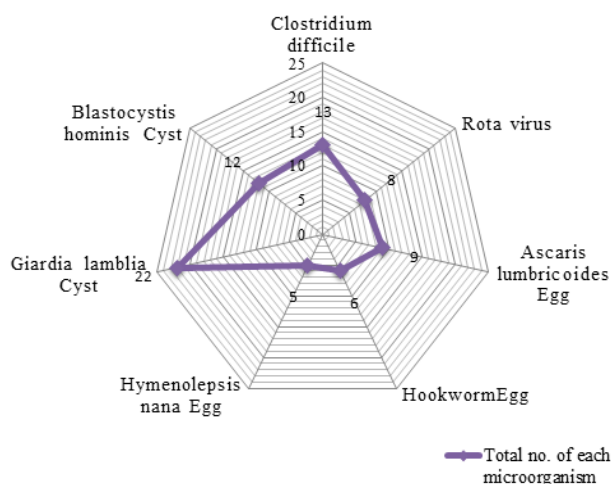
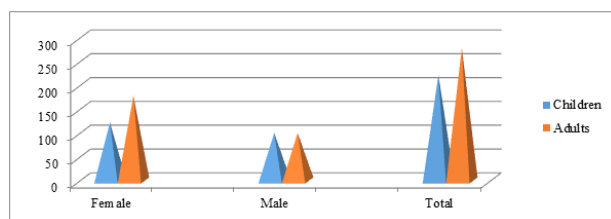
Table 1: Age and sex distribution of the stool samples collected from community

	Female		Male		Total
	N	%	N	%	
Children	122	54.9 %	100	45 %	222
Adults	178	64 %	100	35.9 %	278

The study found no carriage of *Shigella* species, *Salmonella* species, or *Vibrio cholerae* among the participants. However, *C. difficile* carriage was detected in 2.6% (n = 13) of the study population, with a higher prevalence in adults (3.9%, n = 11) compared to children (0.9%, n = 2). Five carriers among these gave the history of animal handling, with two members from the same family. Rotavirus carriage was 1.6% (n=8) and was seen only in children, making it 3.6% in the children population (**Figure 1, Table 2**). The carriage of parasites was more in adults than children, as shown in **Figure 2**. A notable proportion (48%, n = 240) of the study participants reported a lack of adherence to handwashing practices before meals.

Table 2: Carriage of various microorganisms in the stool samples collected from community

	Method used	Total		Adult		Children	
		N [500]	%	N [278]	% in Adult	N [222]	% in Children
Bacteria							
<i>Clostridium difficile</i>	ELISA for toxins A & B	13	2.6%	11	3.9%	2/222	0.9%
<i>Shigella spp.</i>	Culture	Nil					
<i>Salmonella spp.</i>	Culture	Nil					
<i>Vibrio cholera</i>	Culture	Nil					
Virus							
Rota virus	ELISA for Antigen	8	1.6%	Nil		8/222	3.6%
Parasites							
<i>Ascaris lumbricoides</i> Egg	Saline & Iodine Mount	9	1.8%	7	2.5%	2/222	0.9%
HookwormEgg	Saline & Iodine Mount	6	1.2%	4	1.4%	2	0.9%
<i>Hymenolepis nana</i> Egg	Saline & Iodine Mount	5	1.0%	5	1.7%	Nil	
<i>Giardia lamblia</i> Cyst	Saline & Iodine Mount	22	4.4%	14	5.0%	8	3.6%
<i>Blastocystis hominis</i> Cyst	Saline & Iodine Mount	12	2.4%	8	2.8%	4	1.8%

**Figure 1: Carriage of various microorganisms in the stool samples collected from the community****Figure 2: Age and sex distribution of the stool samples collected from the community**

Discussion

Clostridium difficile infection (CDI) is an important healthcare-associated infection (HAI) seen mostly

in Intensive Care Unit (ICU) settings⁵. *Clostridium difficile* carriage in our community came out to be 2.6% (13), with 3.9% (11) in adults and 0.9% (2) in children. It is important to know the carriage of *C. difficile* in the community as these carriers act as reservoirs for transmission of the microorganism and are also at risk of developing CDI⁶. Baron *et al* have reported that 38.1% of the asymptomatic carriers progressed to symptomatic CDI versus 2% in non-carriers, and this association was found to be statistically significant⁶. Children colonized with *C. difficile* were significantly more likely to develop CDI within 90 days compared to non-colonized children (25% vs 0%, $p = 0.002$)⁷. *C. difficile* carriage thus poses the carriers at risk of developing symptomatic CDI. Recent hospitalization and livestock proximity have been observed as important risk factors for asymptomatic carriage⁸.

Poor hand hygiene after animal contact is a contributing factor, as shown in published literature⁹. In our community, 5 out of 13 people had a history of animal contact, including two members from same family. Hands and the environment easily get contaminated with *C. difficile* from cases and carriers and act as the source of transmission of this microorganism. Gilboa *et al* found that the rooms of *C. difficile* carrier patients were as contaminated as rooms of symptomatic CDI patients, emphasizing the role of carriers also in spreading the infection¹⁰. In a

study on environmental sampling of microbiology laboratory dealing with *C.difficile* samples, 11.8 of % hands of laboratory workers were contaminated with *C.difficile*¹¹. Hand hygiene becomes a very important factor in limiting the spread of this microorganism. We also educated the carriers in our study regarding the importance of hand hygiene, especially after animal handling.

Other studies on carriage of *C.difficile* have reported 10% carriage in Iranian children less than 5 years age at day zero of hospitalization¹², 10.4% in US hospitalized patients⁸, 7.4% asymptomatic carriers in ICU settings⁵ and 2.3% in veterinary healthcare workers in Netherlands⁹. A Chinese study followed 29 children from birth till one year of age for *C.difficile* carriage and found that 20 out of 29 (68.9%) carried *C.difficile* by one year of age¹³. Antibiotic exposure has been known to be the biggest risk factor for CDI. Over the counter antibiotic intake could be a factor in the carriage of *C.difficile* in our community.

Shigella, Salmonella, and *V.cholerae* were not isolated in our study. Subclinical carriage of *Shigella* is known, which can persist for months and also account for the spread of the disease, especially because a low infective dose is required for infection but it is a hardier organism to isolate^{14, 15}. Carriage rate for Nontyphoidal Salmonella and *Vibrio cholerae* has been reported as quite low^{16, 17}.

Rotavirus is known to shed in feces of children without diarrhoea¹⁸. For the same reason recovery of rotavirus is of little diagnostic value in a diarrhoea patient because it cannot be attributed as the cause in all the cases. A study showed that among 83 children shedding Rotavirus in stool, 40 [48%] did not have diarrhea¹⁸. In our study, Rotavirus was detected in 1.6% (8/500) of the overall study population and in 3.6% (8/222) of the pediatric subgroup.

Soil-transmitted helminths (STH) is a common public health problem of all age groups. India has achieved one of the largest deworming programs in children by giving single dose albendazole twice yearly to school-going children¹⁹. Even though single dose albendazole is very effective, children tend to get re-infected from their community. Also, these programs do not target the adult population who could be acting as a source of infection. The

prevalence of parasitic carriage in our study was less in children than in adults, which is mostly due to the albendazole given to children at their school under the national programme. The parasites found in our study are *Ascarislumbricoides* 2.5% in adults vs 0.9% in children; hookworm (1.2% vs 0.9%); *H.nana* (1% vs nil); *Giardia lamblia* (5% vs 3.6%), and *Blastocystishominis* (2.4% vs 1.8%). A study from South India has shown a prevalence of 21% for hookworm and <1% for Ascaris in adults¹⁹. They showed that increasing age and higher vegetation was associated with higher prevalence while high socioeconomic status, higher education and improved sanitation with low prevalence. A few of our individuals gave a history of no hand washing before meals and were advised for do so. An Ethiopian study has shown prevalence of 12.5% for Ascaris and 7.5% for hookworm in a community-based study on adults and adolescents²⁰. For adults, education on hygiene and sanitation and albendazole treatment can help to tackle the problem.

Protozoa are also an important cause of diarrhea, but detection of *Giardia* cannot be associated with diarrhoea in all times²¹. *Giardia* carriage in children has been reported by various studies, ranging from 1.3% to 37%^{22, 23, 24} with 3.6% in our study. Adult population showed carriage of 5% for *Giardia*, while a study from Tanzania reported 53%²⁵. An interesting observation was made in a study on Israeli children that faltering growth was observed after *Giardia lamblia* infection, rather than predisposing it, but this observation needs further studies²¹. *Blastocystishominis* carriage was also reported in the same study to be 61% which was 2.4% in our study, which is much higher than ours. These variations could be geographically determined. A study from Zambia done on the pediatric population with children 5-18 years, showed a carriage rate of 37.9% for *Blastocystis* sp., 30.9% for *Giardiaintestinalis*, 13.3% for *Entamoebadispar*, and 4.3% for *Cryptosporidium* spp. No *E.histolytica* was detected in their study. Molecular methods were used for detection in this study, which are more sensitive, and hence the higher detection rates²⁶.

Conclusion

In conclusion, pathogenic bacterial carriage for most bacteria was nil in our study, which, in all

probability, is due to the safe water supply in our city, but *C.difficile* carriage is a concern for which educating the masses is important regarding hand hygiene, especially in animal handlers. Regarding parasitic infections, a single dose of albendazole in school-going children has resulted in lower carriage in children than adults. The same approach, along with education regarding hygienic practices, can be started in the adult population also.

Funding: Department of Science and Technology, Chandigarh, India

Conflicts of interest: The authors have declared no conflicts of interest.

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