

# An examination of co-infection in acute gastroenteritis and histo-blood group antigens leading to viral infection susceptibility

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**Abstract.** The aim of the present study was to evaluate co-infection in the gastrointestinal tract in terms of viruses, bacteria and the ABO blood group. We hypothesized that a combination of norovirus (NV) and bacteria in the gastrointestinal tract could affect the likelihood of an individual to contracting NV. Histo-blood group antigens (HBGAs) are considered to act as receptors that can lead to NV susceptibility. In addition to genetics, co-infection in the gastrointestinal tract may be associated with this mechanism. A total of 370 patients with acute gastroenteritis presenting with diarrhea (14-89 years) were recruited. The male/female ratio was 20/17. Single infection (bacteria or virus), co-infection with two viruses, and co-infection with one virus and one bacterium were statistically analyzed. In total, 88 of the 376 subjects (23.4%) were positive for one virus, and 50 (13.3%) were positive for one bacterium. Co-transfection with bacteria and a virus were detected in 46 (47.9%) of the 96 bacterial gastroenteritis cases. Statistical analysis revealed that co-infection of bacteria and NV was not significant in all viral infections ( $P=0.768$ ). In terms of the ABO histo-blood group type and NV infection, the frequency in the O type was not significantly increased ( $P=0.052$ ). Co-infection of bacteria and a virus occurred frequently in the gastrointestinal tract. The ABO blood phenotype expression was not a significant factor in NV infection in the present case series and the results did not suggest an affinity of NV for specific bacteria.

## Introduction

Causative agents of infectious gastroenteritis include viruses, bacteria and *cryptosporidium*. Subsequent to a short incubation

period following oral infection, symptoms such as vomiting, diarrhea, abdominal pain and fever appear (1). As causative viruses, rotavirus (RV), adenovirus (AV) and norovirus (NV) are common, and NV is the most dominant. Differences in the viral epidemic pattern can be observed among countries, regions and climates (2).

Histo-blood group antigens (HBGAs) have been recognized as receptor tissue for NV that is associated with infection of the host. Lewis-positive individuals can have HBGAs on the surface of epithelial cells and NV have an affinity through blood-type substances, which are expressed in intestinal epithelial cells (3). As HBGAs are considered to act as NV receptors and lead to NV susceptibility, we hypothesized that in addition to genetics, co-infection in the gastrointestinal tract could be associated with this mechanism. The present study aimed to investigate the association between bacterial infection and co-infection in acute gastroenteritis and HBGAs, and further studies are also important to evaluate the results.

## Patients and methods

**Baseline characteristics of subjects.** The study is a single-center retrospective cohort study. All the clinical and laboratory data and preoperative backgrounds were reviewed from patient medical records from Toho University School of Medicine, Omori Hospital (Tokyo, Japan). A total of 370 patients with acute gastroenteritis who presented with diarrhea (age, 14-89 years; mean age, 37.0 years) (Table I) were recruited. The male/female ratio was 20/17. Single infection (bacteria or virus), co-infection with two viruses, and co-infection with one virus and one bacterium were statistically analyzed.

**Diagnosis of acute gastroenteritis.** All the patients were diagnosed with acute gastroenteritis according to acute onset and acute diarrhea without any diarrhea-inducing baseline illness. Other symptoms such as nausea, fever or abdominal pain were not considered.

**Examination tools.** Patient stool samples were collected and kits were used to examine for antigens of NV, RV and AV. Stool cultures for pathogenic bacteria were also performed using the same stool samples. The examination tool for NV in Japan is QUICKNAVI-NORO™ (Otsuka Pharmaceutical Co., Tokyo, Japan). The measurement principle is immunochromatography

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and the targets are NV genogroups I and II. The sensitivity and specificity were 81.6 and 96.9%, respectively, compared to the previously used reverse transcription polymerase chain reaction (RT-PCR) method. The examination tool for RV and AV is BD Rota/Adeno Examan Stick™ (BD Biosciences, San Diego, CA, USA). The measurement principle is immunochromatography and the targets are AV B3, B7, F40, F41, RV AG1WA and AG3SA11. Compared to the previously used kit, the sensitivity and specificity were ~95%. Stool cultures for pathogenic bacteria were ordered without any species targeted under routine measurement.

**Statistical analysis.** All the clinical and laboratory data were collected from patient medical records. Continuous variables are expressed as the mean  $\pm$  standard deviation unless otherwise stated and were compared using the Mann-Whitney U test. Categorical variables were compared using the  $\chi^2$  test. Cox proportional hazards regression analysis was used to identify variables that were significant predictors of survival.  $P < 0.05$  was considered to indicate a statistically significant difference. All the statistical analyses were performed using SPSS version 11.0 (SPSS, Inc., Chicago, IL, USA).

**Ethics.** The present study was approved by the Institutional Review Board of Toho University Omori Medical Center (project approval number: 20-106).

## Results

**Viral and bacterial content of each infection.** In total, 88 of the 376 subjects (23.4%) were positive for a virus only, and 50 (13.3%) were positive for bacteria only. The presence of bacteria and a virus was detected in 46 (47.9%) of the 96 bacterial gastroenteritis cases (Table II). Fourteen species of bacteria were detected, including *Campylobacter* [33/96 (36.4%)], enteropathogenic *Escherichia coli* [*E.coli*; 31/96 (34.1%)] and enterotoxigenic *E. coli* [30/96 (33.0%)] (Fig. 1). The mean ages of the infected patients were  $50.0 \pm 24.6$ ,  $36.3 \pm 15.8$  and  $38.6 \pm 18.3$  years, respectively. RV gastroenteritis was detected in 92 patients (24.7%; co-infection 83.7%), AV gastroenteritis in 95 (25.5%; co-infection 86.3%), and NV co-infection gastroenteritis in 93 (24.9%; co-infection 35.4%). Of the co-infection with a virus and bacteria cases, 27.2% had RV, 27.4% had AV and 19.4% had NV. Co-infection with bacteria and NV was not significant in all viral infections ( $P = 0.768$ ) (Fig. 2). The correlation between the serum values of C-reactive protein (CRP) and white blood cells (WBCs) and co-infection was analyzed. Co-infection was evaluated by WBC count not by CRP ( $P = 0.03$ ). In terms of the ABO histo-blood group type and NV infection, the frequency in O-type patients was not significantly increased ( $P = 0.052$ ), although the cumulative number of patients was the largest among the ABO blood groups (Table III).

## Discussion

Causative agents of infectious gastroenteritis include viruses, bacteria and *cryptosporidium*. Subsequent to a short incubation period following oral infection, symptoms such as vomiting, diarrhea, abdominal pain and fever appear (1). As causative viruses, RV, AV and NV are common, and NV is the

Table I. Baseline characteristics of the subjects.

Characteristics	Total, n=370
Male, n	200
Female, n	170
Median age (range), year	37 (14-89)
Norovirus, n	93
Rotavirus, n	92
Adenovirus, n	95
Bacteria, n	96
Virus + virus, n	62
Virus + bacteria, n	46

most dominant. Differences in the viral epidemic pattern can be observed among countries, regions and climates (2).

RV belongs to the *Reoviridae* family and infects the intestinal tract. RVs can be divided into groups A through C, with group B being the most common cause of severe diarrhea in adults. Although rare, the association of RV with diseases outside the intestinal tract, such as the central nervous system, has been suggested. The distribution of viruses in extraintestinal organs has been confirmed in animal experiments (4-6). AV belongs to the *Adenoviridae* family. AV infections often present as conjunctivitis, tonsillitis (which may appear to be identical to strep throat and cannot be distinguished from strep except by throat culture), ear infection or croup. AV types 40 and 41 can also cause gastroenteritis (7). NV is classified as part of the *Caliciviridae* family and infects the intestinal tract. It is broadly classified into genogroups I and II (8,9).

The RT-PCR/enzyme-linked immunosorbent assay method has been approved as a diagnostic kit and has prevailed over the conventional immune electron microscopy/radioimmunoassay method. HBGAs have been recognized as receptor tissue for NV that is associated with infection of the host (3). ABO blood group substances and Lewis blood-type substances are expressed in intestinal epithelial cells in ~84% of Japanese individuals. Lewis-positive individuals can have HBGAs on the surface of epithelial cells. NV is reported to have an affinity to these HBGAs and can recognize them as receptors. It has been reported that the incidence of infection in individuals with blood types A and O, and secretion-type individuals is higher than that of the individuals with blood type B and non-secretion-type individuals (10). In the present study, this significant difference could not be confirmed.

For all infectious diseases, not just viral gastroenteritis, a rapid serological diagnosis is important. The prevention of infectious spread is important, and the accurate differential diagnosis of bacteria or other causes is required. It is essential for prompt measures to be taken to protect against outbreaks in facilities. The basic rapid diagnostic method is the antigen-antibody reaction, in which soluble viral protein antigen and antibody react in the ion chromatography method. The sensitivity and specificity maintain an adequate level (>90%) with the aforementioned kits (i.e., QUICKNAVI-NORO™ and BD Rota/Adeno Examan Stick™), and are adequate rapid diagnostic methods. Rapid

Table II. Viral and bacterial content of each infection.

Content of each infection	Cases, n
NV total	93
Only NV	60
NV + AV	4
NV + RV + AV	11
NV + bacteria	15
NV + AV + bacteria	1
NV + RV + AV + bacteria	2
RV total	92
Only RV	15
RV + AV	41
RV + NV + AV	11
RV + bacteria	5
RV + AV + bacteria	18
RV + NV + AV + bacteria	2
AV total	95
Only AV	13
AV + NV	4
AV + RV	41
AV + NV + RV	11
AV + bacteria	5
AV + NV + bacteria	1
AV + RV + bacteria	18
AV + NV + RV + bacteria	2
Bacteria total	96
Only bacteria	50
Bacteria + NV	15
Bacteria + RV	5
Bacteria + AV	5
Bacteria + NV + AV	1
Bacteria + RV + AV	18
Bacteria + NV + RV + AV	2

NV, norovirus; RV, rotavirus; AV, adenovirus.

Table III. Norovirus infection analysis by the ABO blood type.

Infection	O, n	A, n	B, n	AB, n
Norovirus <sup>+</sup>	28	26	14	1
Norovirus <sup>-</sup>	34 <sup>a</sup>	37	22	17
Total	62	63	36	18

<sup>a</sup>P=0.052

diagnostic methods can contribute to the suppression of infectious spread.

In the developed world, *Campylobacter jejuni* is the primary cause of bacterial gastroenteritis, with half of these cases associated with exposure to poultry (11). *E. coli* and *Salmonella shigella* are other types of source bacteria (12).

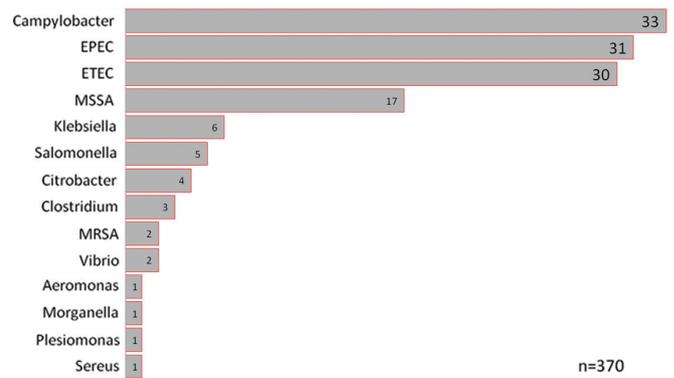


Figure 1. Bacterial species detected in co-infection in acute gastroenteritis. *Campylobacter* species were identified most frequently in this series.

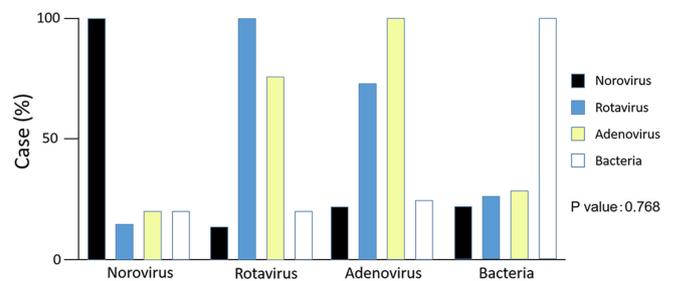


Figure 2. Co-infection in acute gastroenteritis according to each virus and bacteria. Co-infection of bacteria and norovirus was not significant in all virus infections (P=0.768).

When food becomes contaminated with bacteria and remains at room temperature for a period of several hours, the bacteria multiply and increase the risk of infection in those who consume the food. Certain foods commonly associated with illness include raw or undercooked meat, poultry, seafood and eggs; raw sprouts; unpasteurized milk and soft cheeses; and fruit and vegetable juice (13). In the present study, *Campylobacter* and *E. coli* were mainly detected, consistent with the previous study.

The frequency of sporadic NV infection is greater than that of RV and AV. Co-infection of RV and AV occurred frequently. As such, RV and AV may not be able to function well alone and may require a co-worker to trigger an infection. The involvement of HBGAs can be considered to heighten the single infection rate of NV; however, the exact association remains to be elucidated and further research is required. Although it has been thought that NV is specific for co-infection with a bacterial infection compared to RV and AV, this was not demonstrated in the present study. We assume that indigenous bacteria in the intestinal tract and bacterial infection is associated with viral infection. The increasing number of NV infections may be associated with indigenous bacteria in the intestinal tract and bacterial infection. HBGAs are believed to affect the transmission and infection processes of NV and bacteria. Further studies of bacteria and their binding capacity to viruses will provide new insights (14). The presence of blood group-active enteric bacteria, such as *E.coli*, *Salmonella* and *Klebsiella*, has been reported and ~50% of the strains inhibited anti-blood group ABO agglutinins (15).

The blood group activities of enteric bacteria were attributed to the presence of HBGA-like O antigen in lipopolysaccharide (LPS) (16). Bacterial LPS has been shown to have a key role in the infection of mouse mammary tumor virus. The gut microbiota in the human intestine also affects the likelihood of infection with human enteric viruses (17). Elevation in the levels of antigen in the intestine is believed to provide an opportunity for NV infection and could also affect other gastroenteritis virus infections, such as RV (18,19). NV is believed to be specific for co-infection with bacteria; however in the present study, it is suggested that co-infection with bacteria is not confined to NV. The serum values of WBCs and the CRP of co-infection cases (virus and bacteria) are higher than those of single infection (virus). In case of elevated serum values of inflammatory markers, the involvement of bacterial infection should be considered.

The Norwalk virus strain 68, which is the prototype of NV, is absorbed in blood type A and O antigen and secretion-type antigen (20). The blood group antigens are also expressed in intestinal epithelial cells, not just on the surface of human red blood cells. A previous study reported that the villi of the jejunum affected by NV are flattened and atrophied (21). *In vitro* binding assay using virus-like particles (VLPs) has revealed that the incidence of infection in individuals with blood type A and O and secretion-type individuals are higher than that of individuals with blood type B and non-secretion-type individuals (22). Evaluation is difficult as the diagnosis of Lewis blood type was not performed in Omori Hospital. This only pertains to the Norwalk virus strain 68, and it is clear that not all NV strains recognize the blood group antigen in the same manner as the Norwalk virus strain 68.

Infection was shown to occur equally in individuals with A, B and O blood types in the GII/4 genotype, which can be severe and can spread easily (23,24). In the present study, there was no significant difference in the infection rate among ABO blood types in NV infection; the results were similar for RV and AV. It is not clear whether HBGAs are only involved in absorption between cells and NV, or if they invade the intestinal tract. Clarification of the mechanism of NV infection beginning with the adsorption to HBGAs requires elucidation in future studies.

In conclusion, co-infection of bacteria and virus occurred frequently in the gastrointestinal tract. The ABO blood phenotype expression was not significant in the present series of NV infections and the results did not suggest the affinity of NV for specific bacteria. However, it was suggested that there could be another pathway leading to infectious diarrheal disease in addition to the correlation between the genetically determined HBGA expression of an individual and their susceptibility to an enteric virus, particularly NV.

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