Age-specific patterns of enteropathogenic infections and co-infections among patients with different severity of acute diarrhea in China from 2009 to 2020

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Abstract

Background

Acute diarrhea contributes to a significant global burden of disease. However, the infection or co-infection patterns of enteropathogens, along with their age dependence and clinical effects, remain ambiguous.

Methods

A nationwide sentinel surveillance was conducted in all-age patients with acute diarrhea in China from 2009 to 2020. The clinical severity was assessed using a modified Vesikari score method, which categorized cases into moderate-to-severe diarrhea (MSD) or mild diarrhea. The association between clinical severity and age-specific patterns of enteropathogenic infections and co-infections was analyzed through a binary logistic regression model.

Results

A total of 195,988 individuals were enrolled and tested for 17 enteropathogens, among whom 27,358 (13.96%) patients developed MSD. In comparison to patients with mild diarrhea, MSD patients showed significantly higher rates of viral-bacterial co-infection in adults and older adults, as well as higher rates of viral-viral co-infection across almost all age groups except children aged < 6 months. The multivariate analyses revealed a significantly distinct pattern in the infections and co-infections of viral and bacterial enteropathogens associated with MSD between children and adults.

Conclusion

These findings highlight the age-specific patterns of enteropathogen infection among cases of MSD and mild diarrhea and underscore the necessity for age-related strategies in vaccine schedules, clinical diagnosis and treatment.

Introduction

Acute diarrhea resulted in approximately 99.00 million cases and 1.53 million fatalities worldwide in 2019[1]. Despite significant advancements in water and sanitation that have greatly reduced the risk of acute diarrhea among children in low- and middle-income countries, it is important to note that diarrhea continues to be one of the leading causes of disease burden and death among children aged 5–9 years globally in 2019[2, 3].

The high number of deaths caused by acute diarrhea and the potential impact of moderate-to-severe diarrhea (MSD) on stunted growth faltering in children necessitate a comprehensive understanding of the causative pathogens or host-related factors contributing to severe diarrhea[4, 5]. Previous studies have indicated that the severity of diarrhea varies with the infection of the enteric pathogens in patients with diarrhea[6–8]. A study conducted in Guatemala has suggested that rotavirus infection tends to be more severe than norovirus infection in children[8]. Furthermore, the case-control study involving African children aged 0–59 months revealed that rotavirus was the predominant cause of MSD, while norovirus and adenovirus infections presented similar levels of disease severity[9]. The findings of a Canada cohort study demonstrated that rotavirus, norovirus, adenovirus, *Salmonella* and co-infection of rotavirus and adenovirus all caused more severe diarrhea among children under the age of 18 years. Notably, rotavirus infection showed greater severity compared to norovirus infection[6–8]. The implementation of non-pharmacological interventions during the COVID-19 pandemic in China has been found to significantly reduce infection rates of enteric pathogens among patients with acute diarrhea. Relaxation of these interventions is likely to result in a resurgence of enteropathogen infections[9], emphasizing the importance of investigating the characteristics and dynamics of such infections and their association with MSD.

To date, the majority of studies on diarrhea severity have primarily focused on pediatric populations, with limited research investigating etiological characteristics across all age groups[6, 10, 11]. By conducting a retrospective observational analysis using nationwide etiological surveillance data of acute diarrhea spanning a 12-year period, we explore the age-specific etiological and epidemiological characteristics associated with MSD among patients with acute diarrhea, aiming to facilitate targeted therapy for individuals of different ages affected by MSD and refine evidence-based strategies to reduce the incidence and mortality of MSD.

Methods

Study design and case definition

Between January 2009 and December 2020, a nationwide etiological surveillance project on acute diarrhea was conducted throughout all 31 provinces (autonomous regions or municipalities) of the Chinese mainland under the leadership of the China Center for Disease Control and Prevention (China CDC). A case of acute diarrhea is defined as the occurrence of ≥ 3 passages of watery, loose, mucus, or bloody-stools within a 24-h period. Patients who were referred from other hospitals, not initially diagnosed at sentinel hospitals or those having non-infectious diseases, were excluded from this study.

The participating hospitals and laboratories all implemented a standardized protocol developed by China CDC, which was publicly published[12], encompassing guidelines for patient enrollment, specimen collection, laboratory testing, data management and other procedural aspects. All the sentinel hospitals had undergone training to be qualified for recruiting patients, sample collection, and testing following the SOP, before the surveillance started. Pre-study training, whole-procedure supervision, monthly enrollment

reports, data audits, and annual study-site visits were conducted to ensure uniform procedures were followed as guided among the study sites and remain unchanged across the surveillance years[13, 14]. In this study, diarrheal cases were classified into patients with MSD or mild diarrhea based on a modified Vesikari score method that followed pre-defined criteria[15]. Briefly, the Vesikari score was calculated using seven variables including duration of diarrhea and vomiting, average number of episodes per day before admission for diarrheal stools and vomiting, body temperature upon admission, healthcare provider visits, and status of treatment received. MSD was defined as an overall Vesikari score ≥ 9, while mild diarrhea was defined as an overall score between 0–8 (details in Supplementary Table 1).

Data extraction and management

The data, including demographic information, timeline of symptom onset and hospital admission, clinical presentations, microbiological test results, and clinical outcomes, were collected through a thorough review of medical records and entered into a standardized database by trained clinicians. Stool specimens for each enrolled patient were collected and tested for seventeen enteric pathogens. Rotavirus A was tested using enzyme-linked immunosorbent assay (ELISA). Norovirus, adenovirus, astrovirus, sapovirus, rotavirus B, and rotavirus C were detected through polymerase chain reaction (PCR) or reverse transcription polymerase chain reaction (RT-PCR). Ten bacterial pathogens were tested by isolation and culture with or without enrichment procedures in the first step. For *Yersinia enterocolitica* (*Y. enterocolitica*), diarrheagenic *Escherichia coli* (DEC), *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), isolation was subsequently tested by PCR, and for *nontyphoidal Salmonella* (NTS), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Vibrio cholerae* (*V. cholerae*), *Aeromonas hydrophila* (*A. hydrophila*), *Plesiomonas shigelloides* (*P. shigelloides*) and *Shigella*, the isolation was subsequently confirmed through biochemical and serological assays. The testing methods for each of the seventeen enteric pathogens are detailed in our previous study[14]. The sample collection or testing strategies were consistent for patients with MSD and mild diarrhea. Positive results underwent thorough examination and evaluation by at least one infectious disease physician. In cases where multiple types of enteropathogens tested positive, a co-infection was identified.

Statistical analysis

The descriptive statistics included frequencies (proportions) for categorical variables, and medians with interquartile ranges (IQR) for continuous variables. The Chi-squared test or Fisher's exact test was applied for inter-group comparisons of categorical variables. A binary logistic regression model was applied to examine the variables associated with MSD, including sex, age, residence, season of infection, and positive detection of pathogens as explanatory factors. Multivariate analysis was performed by including all variables with a p-value < 0.10 from the univariate analysis as covariates. The OR and 95% CI were estimated using the maximum likelihood method. The percentage change in the positive rate between the average of pre-pandemic years and the pandemic year was calculated as: [(PRt1(k) − PRt0(k)]/PRt0(k) × 100%, where the k indicated the phase of pre-pandemic years or the pandemic year, the PRt0(k) indicated the average positive rate during phase k in 2012 − 2019, and PRt1(k) indicated that

of 2020[9]. All the statistical analyses were performed using R version 4.2.0. P-value of < 0.05 was considered statistically significant.

Results

Among the 195,988 patients with acute diarrhea enrolled in this study between January 2009 and December 2020, a total of 27,358 cases (13.96%) were classified as MSD patients with 68 deaths. Out of all the patients, 79,313 cases were tested for all seven viral pathogens (rotavirus A, norovirus, adenovirus, astrovirus, sapovirus, rotavirus B, and rotavirus C), while 106,103 cases were tested for all ten bacterial pathogens including *Yersinia enterocolitica* (*Y. enterocolitica*), diarrheagenic *Escherichia coli* (DEC), *Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*), *nontyphoidal Salmonella* (NTS), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Vibrio cholerae* (*V. cholerae*), *Aeromonas hydrophila* (*A. hydrophila*), *Plesiomonas shigelloides* (*P. shigelloides*) and *Shigella*; additionally, 45,860 cases were tested for all 17 viral and bacterial pathogens mentioned above. Females accounted for a higher proportion of MSD than that of mild diarrhea (41.59% vs 40.77%, p = 0.0106). For different age groups, a significantly higher proportion of MSD was shown in the children aged 6—11 months (21.46% vs 13.41%) and 1—4 years old (34.60% vs 21.62%) (p < 0.0001). Regarding ecological regions, Inner Mongolia-Xinjiang (10.49% vs 5.96%), Qinghai-Tibet (1.47% vs 0.62%) and Southwest China (6.61% vs 3.65%) presented a higher proportion of MSD than that of mild diarrhea (p < 0.0001). This pattern is also shown in rural areas (29.68% vs 20.21%), in the cold season (47.66% vs 37.11%), and among inpatients (8.05% vs 6.94%) (all p < 0.0001) (Table 1). The fatal outcome was recorded in 0.03% (63/195,988) of the patients, with no significant differences observed among sex, age, ecological region, urban/rural residence, and season of infection except for the outpatient/inpatient group (Supplementary Table 2).

Table 1

Demographic characteristics of patients with acute diarrhea in the mainland of China, 2009 – 2020.

	Total (n = 195,988)	Mild diarrhea (n = 168,630)	MSD (n = 27,358)	P value*
Sex, n (%)				0.0106
Female	80,126 (40.88)	68,748 (40.77)	11,378 (41.59)	
Male	115,862 (59.12)	99,882 (59.23)	15,980 (58.41)	
Age group, n (%)				< 0.0001
< 6 month	22,199 (11.33)	19,322 (11.46)	2,877 (10.52)	
6 – 11 month	28,487 (14.54)	22,616 (13.41)	5,871 (21.46)	
1 – 4 years	45,927 (23.43)	36,462 (21.62)	9,465 (34.60)	
5 – 17 years	12,804 (6.53)	11,218 (6.65)	1,586 (5.80)	
18 – 59 years	65,027 (33.18)	59,301 (35.17)	5,726 (20.93)	
≥ 60 years	21,544 (10.99)	19,711 (11.69)	1,833 (6.70)	
Ecological regions, n (%)				< 0.0001
North China	45,714 (23.32)	39,276 (23.29)	6,438 (23.53)	
Northeast China	9,211 (4.70)	8,329 (4.94)	882 (3.22)	
South China	24,642 (12.57)	20,698 (12.27)	3,944 (14.42)	
Inner Mongolia-Xinjiang	12,914 (6.59)	10,045 (5.96)	2,869 (10.49)	
Qinghai-Tibet	1,443 (0.74)	1,042 (0.62)	401 (1.47)	
Southwest China	7,958 (4.06)	6,151 (3.65)	1,807 (6.61)	
Central China	94,106 (48.02)	83,089 (49.27)	11,017 (40.27)	
Residence				< 0.0001
Urban	153,786 (78.47)	134,548 (79.79)	19,238 (70.32)	
Rural	42,202 (21.53)	34,082 (20.21)	8,120 (29.68)	
Season of infection, n				< 0.0001

Data are presented as n (%). MSD = moderate-to-severe diarrhea. *p value was compared between mild diarrhea group and MSD group based on chi-square test or Fisher's exact test. †For each patient with acute diarrhea, the onset date was divided into cold season or warm season based on the climate characteristics of each sentinel city, that is, the 6 months with the highest monthly average temperature were included in the warm season, and the other months were included in the cold season.

	Total (n = 195,988)	Mild diarrhea (n = 168,630)	MSD (n = 27,358)	P value*
(%)				
Cold	75,614 (38.58)	62,574 (37.11)	13,040 (47.66)	
Warm	120,374 (61.42)	106,056 (62.89)	14,318 (52.34)	
Case type, n (%)				< 0.0001
Outpatient	182,079 (92.90)	156,924 (93.06)	25,155 (91.95)	
Inpatient	13,909 (7.10)	11,706 (6.94)	2,203 (8.05)	

Data are presented as n (%). MSD = moderate-to-severe diarrhea. *p value was compared between mild diarrhea group and MSD group based on chi-square test or Fisher's exact test. †For each patient with acute diarrhea, the onset date was divided into cold season or warm season based on the climate characteristics of each sentinel city, that is, the 6 months with the highest monthly average temperature were included in the warm season, and the other months were included in the cold season.

A total of 79,313 patients were tested for all the seven viral pathogens, among which 12,384 (15.61%) were MSD patients, and 46.45% (5,752) of the MSD patients were tested positive for at least one virus. The highest virial positive rate of the MSD patients was observed in children aged 6 − 11 months (57.63%, 1,593/2,764) and 1—4 years (57.38%, 2,699/4,704), followed by 42.87% (538/1,255) in children aged < 6 months, 30.89% (211/683) in adolescents (5—17 years old), 24.39% (180/738) in older adults (aged ≥ 60 years), and 23.71% (531/2,240) in adults (aged 18—59 years). The overall viral positive rates were significantly higher in patients with MSD compared to mild diarrhea patients across all age groups: children < 6 months (42.87% vs 29.24%), children aged 6 − 11 months (57.63% vs 41.41%), children of 1—4 years old (57.38% vs 41.37%), adolescents (30.89% vs 23.16%), adults (23.71% vs 17.76%), and older adults (24.39% vs 15.89%) (all p < 0.0001). This pattern was also observed for both sexes, in both urban and rural areas, as well as during both cold and warm seasons (Supplementary Table 3). Among all-age patients with MSD, rotavirus A had the highest positive rate (28.09%), followed by norovirus (15.38%), adenovirus (3.93%), astrovirus (3.02%), sapovirus (1.72%), rotavirus C (0.38%) and rotavirus B (0.37%), and significantly higher rates of these viruses were seen in MSD patients than those with mild diarrhea, except for rotavirus B. The rankings for viral positive rates in MSD patients varied among different age groups, i.e., rotavirus A > norovirus > adenovirus for children aged < 6 months, children aged 6 − 11 months and children aged 1—4 years, norovirus > rotavirus A > adenovirus for both adolescents and older adults, and norovirus > rotavirus A > astrovirus for adults (Fig. 1).

A total of 106,103 acute diarrhea patients were tested for all ten bacterial pathogens, among which 14,352 (13.53%) were MSD patients, and 17.76% (2,549) of the MSD patients were tested positive for at least one bacterium. The highest bacterial positive rate of the MSD patients was observed in adults (22.55%, 931/4,128) and adolescents (21.57%, 187/867), followed by 18.11% (237/1,309) in older adults, 17.70% (737/4,165) in children aged 1 − 4 years, 13.39% (338/2,524) in children aged 6 − 11 months, and

8.76% (119/1,359) in children aged < 6 months. The overall viral positive rates were significantly higher in patients with MSD compared to those with mild diarrhea among adolescents (21.57% vs 18.10%, p = 0.0142) and older adults (18.11% vs 15.90%, p = 0.0383), but no significant differences were observed among the other age groups. This pattern was also observed for females, in rural areas, and during the warm season (Supplementary Table 3). Among all-age patients with MSD, NTS had the highest positive rate (6.97%), followed by DEC (5.76%), *V. parahaemolyticus* (2.26%), *Shigella* (1.83%), *A. hydrophila* (0.60%), *C.jejuni* (0.56%), *P. shigelloides* (0.34%), *C. coli* (0.11%), *V. cholerae* (0.09%), and *Y. enterocolitica* (0.08%), and significantly higher rates of NTS (6.97% vs 4.52%) and *Shigella* (1.83% vs 1.52%) were seen in MSD patients than those with mild diarrhea, while DEC (5.76% vs 6.94%), *C. jejunni* (0.56% vs 0.98%), *C. coli* (0.11% vs 0.20%) and *V. cholerae* (0.09% vs 0.22%) presented a reversed pattern (all p < 0.05) (Supplementary Table 3). The most prevalent bacterial pathogens in MSD patients varied across different age groups: DEC > NTS > *Shigella* for children aged < 6 month, NTS > DEC > *Shigella* for both children groups aged 6 – 11 months and aged 1 – 4 years, *Shigella* > NTS > DEC for adolescents, DEC > *Shigella* > NTS for adults, DEC > NTS > *V. parahaemolyticus* for older adults (Fig. 1).

A total of 45,860 patients were tested for all the 17 enteropathogens, among which 6,492 (14.20%) were MSD cases. Patients with MSD had a higher overall co-infection rate of ≥ 2 pathogens compared to those with mild diarrhea (8.78% vs 5.25%, p < 0.0001). Furthermore, both viral-viral (4.87% vs 2.13%, p < 0.0001) and viral-bacterial (3.25% vs 2.30%, p < 0.0001) co-infection rates were significantly higher in patients with MSD than in those with mild diarrhea, while there were no significant differences in bacterial-bacterial co-infection rates between the two groups. The coinfection rates in MSD patients were higher than those in mild diarrhea patients across almost all age groups (all p < 0.05), except for children aged < 6 months. Specifically, higher viral-viral coinfection rates were observed in MSD patients among children aged 6 – 11 months (7.05% vs 4.29%, p < 0.0001), children aged 1 – 4 years (7.27% vs 4.45%, p < 0.0001), adolescents (3.61% vs 1.61%, p = 0.0210), adults (1.69% vs 0.87%, p = 0.0012) and older adults (1.94% vs 0.73%, p = 0.0061). A significantly higher overall co-infection rate was also observed in MSD patients compared to patients with mild diarrhea during both cold (10.01% vs 5.54%, p < 0.0001) and warm (7.65% vs 5.07%, p < 0.0001) seasons, and the same pattern was also seen for viral-viral coinfection rates. Higher viral-bacterial coinfection rates were observed in MSD patients for adults (3.66% vs 2.32%, p < 0.0001) and older adults (3.36% vs 1.52%, p = 0.0021). No significant differences of bacterial-bacterial coinfection rates between the two groups were observed across any age groups or during any seasons of infection (Fig. 2A, Supplementary Table 4).

The most common viral-viral co-infections observed were rotavirus A-norovirus, norovirus-adenovirus, and rotavirus A-adenovirus, all of which had significantly higher rates among MSD moderate-to-severe than those with mild diarrhea (2.16% vs 0.75%, 0.96% vs 0.32%, and 0.82% vs 0.33%, all p < 0.0001). Viral-bacterial co-infection primarily occurred in norovirus-DEC, rotavirus A-DEC, and norovirus-NTS, all of which had significantly higher rates in MSD moderate-to-severe than those with mild diarrhea (1.00% vs 0.72%, p = 0.0187; 0.74% vs 0.32%, p < 0.0001; 0.45% vs 0.26%, p = 0.0160). Bacterial-bacterial co-infection primarily occurred in DEC-NTS, DEC-*C. jejuni*, and DEC-*V. parahaemolyticus*, with higher co-infection rates of DEC-*C. jejuni* and DEC-*V. parahaemolyticus* in patients with mild diarrhea than those

with MSD (Fig. 2B). The top co-infection in patients with MSD varied among different age groups, i.e., rotavirus A-norovirus in children and adolescents, norovirus-DEC in adults and older adults (Supplementary Table 5).

The association between MSD and the etiological and epidemiological characteristics of acute diarrhea was explored for children and adolescents (aged < 18 years). Multivariate logistic regression analysis showed that the occurrence of MSD was associated with positive detection for rotavirus A-DEC (OR: 2.96, 95% CI: 1.90–4.61), rotavirus A (OR: 2.77, 95% CI: 2.52–3.05), rotavirus A-norovirus (OR: 2.60, 95% CI: 2.08–3.26), norovirus-adenovirus (OR: 2.51, 95% CI: 1.69–3.71), norovirus (OR: 2.21, 95% CI: 1.97–2.48), NTS (OR: 2.09, 95% CI: 1.73–2.52), rotavirus A-astrovirus (OR: 1.99, 95% CI: 1.24–3.18), norovirus-astrovirus (OR: 1.93, 95% CI: 1.13–3.29), rotavirus A-adenovirus (OR: 1.92, 95% CI: 1.28–2.88), and norovirus-DEC (OR: 1.64, 95% CI: 1.05–2.57), living in rural areas (OR: 1.49, 95% CI: 1.38–1.61), and disease onset in the cold season (OR: 1.52, 95% CI: 1.41–1.64) (Fig. 3A, Supplementary Table 6). For adults ≥ 18 years, significant association was observed for positive detection of rotavirus A-DEC (OR: 2.68, 95% CI: 1.42–5.04), *V. parahaemolyticus* (OR: 2.44, 95% CI: 1.97–3.03), norovirus-DEC (OR: 2.42, 95% CI: 1.69–3.49), adenovirus (OR: 2.11, 95% CI: 1.32–3.38), NTS (OR: 2.09, 95% CI: 1.69–2.58), norovirus (OR: 1.93, 95% CI: 1.68–2.22) and rotavirus A (OR: 1.83, 95% CI: 1.46–2.30). In addition, there was a significantly negative association between MSD and adult males (OR: 0.76, 95% CI: 0.70–0.83) (Fig. 3B, Supplementary Table 6).

Positive rates of enteropathogens were compared between the pre-pandemic (2009–2019) and the pandemic year (2020). In patients with MSD, there was a significant decrease in the annual cumulative positive rates of adenovirus, astrovirus, norovirus and rotavirus. Among these pathogens, adenovirus showed the largest drop in positive rate (-79.25%), followed by astrovirus (-58.31%), norovirus (-53.14%), and rotavirus A (-51.26%) (Fig. 4A, 4C). The positive rate of *V. parahaemolyticus* in patients with MSD presented a significant decrease (-51.50%). Conversely, there were notable increase in the positive rates of *C. jejuni* (142.31%), DEC (130.00%) and NTS (59.73%) (Fig. 4B, 4D). In patients with mild diarrhea, there was a significant decrease in the positive rates of five viral pathogens. The most substantial decline in positive rate was observed for sapovirus (-55.33%), followed by rotavirus A (-48.30%), astrovirus (-39.67%), adenovirus (-39.43%), and norovirus (-31.01%) (Fig. 4E, 4G). The positive rates of three bacterial pathogens in patients with mild diarrhea presented a significant decrease. Among them, the most substantial decline was observed for *Shigella* (-82.28%), followed by *A. hydrophila* (-60.87%), and *V. parahaemolyticus* (-38.21%). However, there were notable increase in the positive rates for *C. coli* (255.56%), DEC (81.45%) and NTS (51.36%) (Fig. 4F, 4H). The detailed comparison of the proportion of MSD patients among different age groups in each season between pre-pandemic years (2009–2019) and the COVID-19 pandemic year (2020) is given in supplementary Fig. 1.

Discussion

Based on the nationwide etiological surveillance of acute diarrhea in China during 2009–2020, we investigated the association between MSD and the infection/coinfection patterns of enteropathogens

among patients with acute diarrhea across different age groups, sexes, regions, and seasons. Additionally, we examined the impact introduced by non-pharmacological interventions implemented during the COVID-19 pandemic in China. Our findings revealed that rotavirus A was the predominant virus causing acute diarrhea and MSD in children under 4 years old, whereas norovirus dominated in MSD cases among adolescents (aged 5—17 years), adults (aged 18—59 years), and older adults (aged ≥ 60 years). Several studies have demonstrated a high severity of acute diarrhea caused by rotavirus[16, 17], whereas a systematic review indicated an equivalent level of severity between noroviruses and rotaviruses[18]. In addition, adults and older adults also experienced higher levels of severity caused by noroviruses and rotaviruses[11]. NTS dominated in the bacterial MSD subgroup for both children aged < 6 months and 1 − 4 years old, while DEC dominated in the bacterial MSD subgroup for both children aged 6 − 11 months and older adults. *Shigella* played a predominant role in the bacterial MSD subgroups for adolescents. One study showed that younger age was identified as a risk factor for bacteremia associated with NTS gastroenteritis[19]. A case-control study conducted in Asia and Africa demonstrated that DEC infection significantly increased the risk of severe outcomes among infants aged 0 to 11 months[20]. Similarly, a study carried out in the United States revealed that adolescents aged 13—17 years presented the highest rates of severe diarrhea following *Shigella* infection, which aligns with our findings[21].

A review has focused on the confounding impact of co-infections of enteropathogens in children with MSD[22]. In this study, we demonstrated that adults and older adults with MSD exhibit higher rates of viral-bacterial co-infections than those with mild diarrhea. Furthermore, higher rates of viral-viral co-infection were observed across various age groups, including infants aged 6 − 11 months, children aged 1 − 4 years, adolescents, adults, and older adults. This finding suggests a potential role of virus-related co-infection in the development of MSD, supporting the hypothesis that virus-related co-infection may exert a synergistic effect resulting in more severe clinical manifestations[23, 24]. No significant differences were observed in bacterial-bacterial co-infection between patients with MSD and mild diarrhea among any age groups. This could be attributed to the mechanisms such as metabolic competition between bacteria, production of antibacterial compounds, and interference with pathogen virulence during the process of bacterium-related co-infection[25].

We also demonstrated a clear age difference in the co-infection patterns of enteropathogens. Children and adolescents with MSD presented a higher co-infection rates of rotavirus A-norovirus, while adults and older adults with MSD showed higher co-infection rates of norovirus-DEC. MSD was found to be significantly associated with multiple viral-viral co-infections of rotavirus A-norovirus, norovirus-adenovirus, rotavirus A-astrovirus, norovirus-astrovirus and rotavirus A-adenovirus, as well as viral-bacterial co-infections of rotavirus A-DEC and norovirus-DEC in children and adolescents (< 18years). Previous studies have demonstrated that co-infections of specific viruses significantly exacerbated diarrhea symptoms in children, potentially due to interplay between enteric pathogens[26, 27]. High susceptibility to MSD was primarily observed among children and adolescents in rural areas, and this age group also exhibited a higher susceptibility to MSD during the cold season. Several studies have indicated that the higher prevalence of diarrheal pathogens in rural areas is associated with inadequate

sanitation, unsafe drinking water, and limited access to medical resources [14, 28, 29], which may contribute to the heightened severity of diarrhea living in those areas. Viral-bacterial coinfections of Rotavirus A-DEC and Norovirus-DEC have been found to be associated with MSD in adults aged ≥ 18 years. Low susceptibility to MSD in males was observed among adults aged ≥ 18 years, which aligns with the finding of a study conducted in India that reported a higher prevalence of severe diarrheal disease among women compared to men[30]. In addition, the positive detection rate for most viruses decreased in patients with MSD during the COVID-19 pandemic. A recent study demonstrated that non-pharmaceutical interventions implemented during the COVID-19 pandemic resulted in a reduction in the positive rate of these enteropathogens among patients with acute diarrhea[9]. It's suggested that enhanced sanitation and personal hygiene measures prove effective in mitigating both the incidence and severity of diarrhea[29].

The study has certain limitations. First, some variables in the modified Vesikari scoring system were self-reported by patients or the family members, such as the duration of diarrhea and vomiting, the average number of episodes of diarrheas and vomiting, etc. This introduces a potential recall bias when assessing the severity of diarrhea. Second, it is challenging to determine whether the pattern of pathogen infections is a cause or consequence of MSD. Factors like underlying disease and body mass index (BMI) evidently influence disease severity, particularly among older adult populations; therefore, these factors need to be analyzed in conjunction with causal pathogens.

Conclusions

Our study reveals an age-specific pattern of enteropathogenic infection in patients with MSD, which can help to identify predominant pathogens potentially responsible for MSD across different age groups and serve as a reference for the treatment, prevention and control of MSD. The investigation into infection patterns of patients with MSD in this study can assist clinicians in developing more precise diagnostic and therapeutic measures. Furthermore, these findings may contribute to enhancing our understanding and awareness regarding the severity of diarrhea, thereby providing an epidemiological foundation for mitigating the severity of diarrhea in China.

Declarations

Ethical Approval and Consent to participate

The National Health Commission of the People's Republic of China decided that since data from patients with diarrhea was part of continuing public health surveillance and implemented national surveillance guidelines; parents/guardians of participants in this study were only required to provide brief verbal consent during their enrollment, which was recorded in each questionnaire by their physicians. This project and the above procedure for obtaining consent were approved by the ethical review committee of China CDC (2015-025).

Consent for publication

Not applicable.

Availability of data and materials

Relevant data that support the findings of this study and model results generated as part of this study are publicly available within the paper and its Supplementary Appendix. Raw data are not publicly available due to restrictions by the data provider, which were used under license for the current study, but are available upon reasonable request to the corresponding author and with permission from the data provider (Li-Ping Wang and Wei Liu).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Z.J.L., G.F.G., W.Z.Y., L.P. W., F.H., S.I.H., L.Q.F. and W.L. conceived, designed, and supervised the study. Z.J.L., G.F.G., W.Z.Y., L.P.W. and W.L. formulated the protocols, guidelines, and SOP of the active sentinel pathogenic surveillance. T.T.L., L.P.W., Q.X., Y.N.L., C.L.L., B.G.J., HL and G.L.W. collected, cleaned, and analyzed the data. S.I.H., L.Q.F. and W.L. wrote the drafts of the manuscript. Z.J.L., G.F.G., W.Z.Y., L.P.W., F.H., S.I.H., L.Q.F. and W.L. interpreted the findings. Z.J.L., G.F.G., S.I.H., W.Z.Y. and F.H. commented on and revised drafts of the manuscript. All authors read and approved the final report.

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Figures

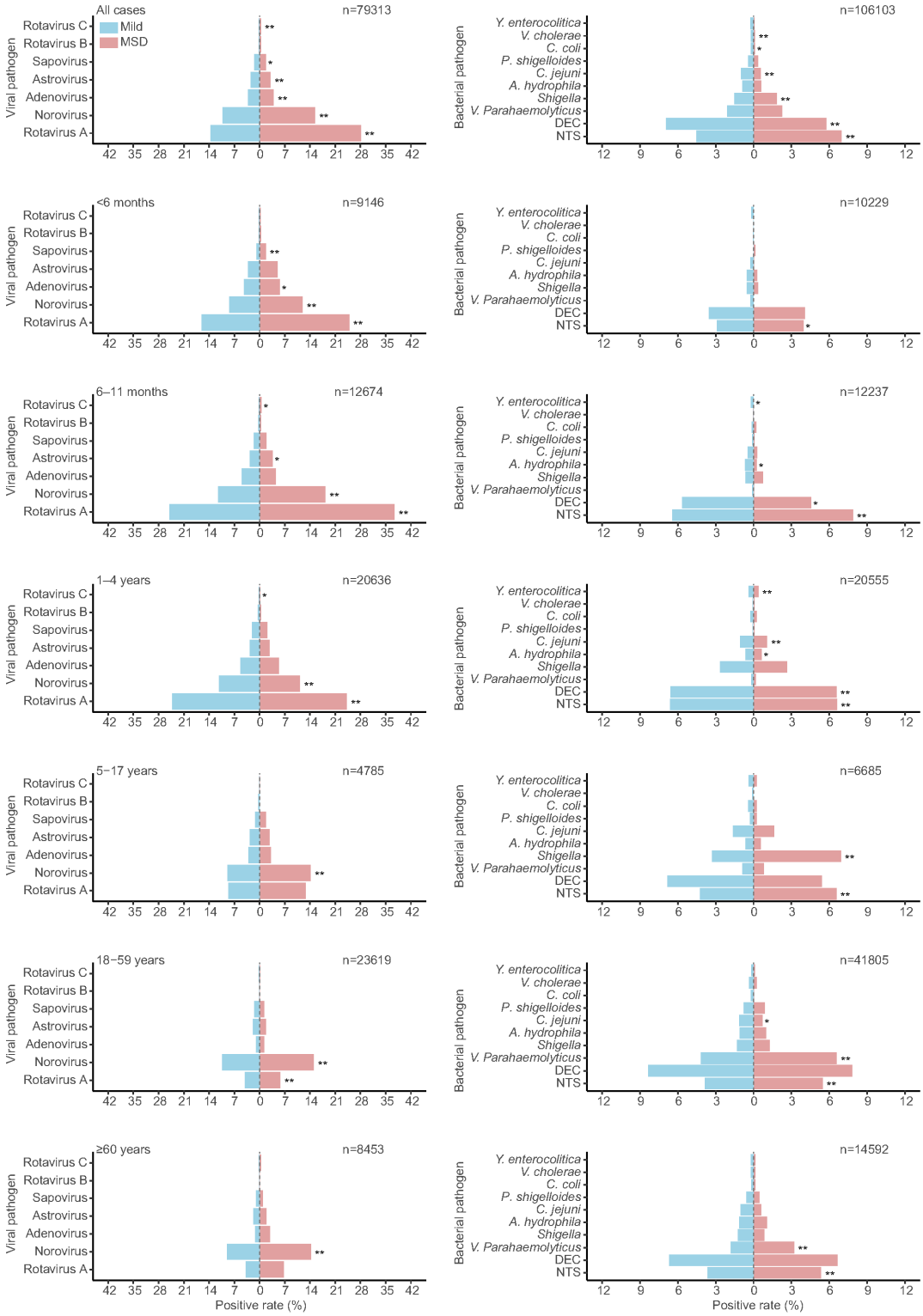

Figure 1

Comparison of positive rates of seven viral and ten bacterial pathogens in patients with MSD and mild diarrhea in the mainland of China, 2009–2020. The positive rate of each pathogen among 79,313 patients with diarrhea (12,384 MSD and 66,929 mild diarrhea) tested for all the seven viral pathogens and among 106,103 patients with diarrhea (14,352 MSD and 91,751 mild diarrhea) tested for all the ten bacterial pathogens was compared for different age groups. The length of the red bar indicates the positive rate of MSD, and the length of the blue bar indicates the positive rate of mild diarrhea. The positive rate was calculated by taking the number of positive tests for each pathogen as the numerator and the number of diarrhea patients tested as the denominator. The significant differences in positive rates (chi-square test or Fisher's exact test) are indicated. DEC=diarrheagenic *Escherichia coli*; NTS=nontyphoidal *Salmonella.**p<0.05. **p<0.01.

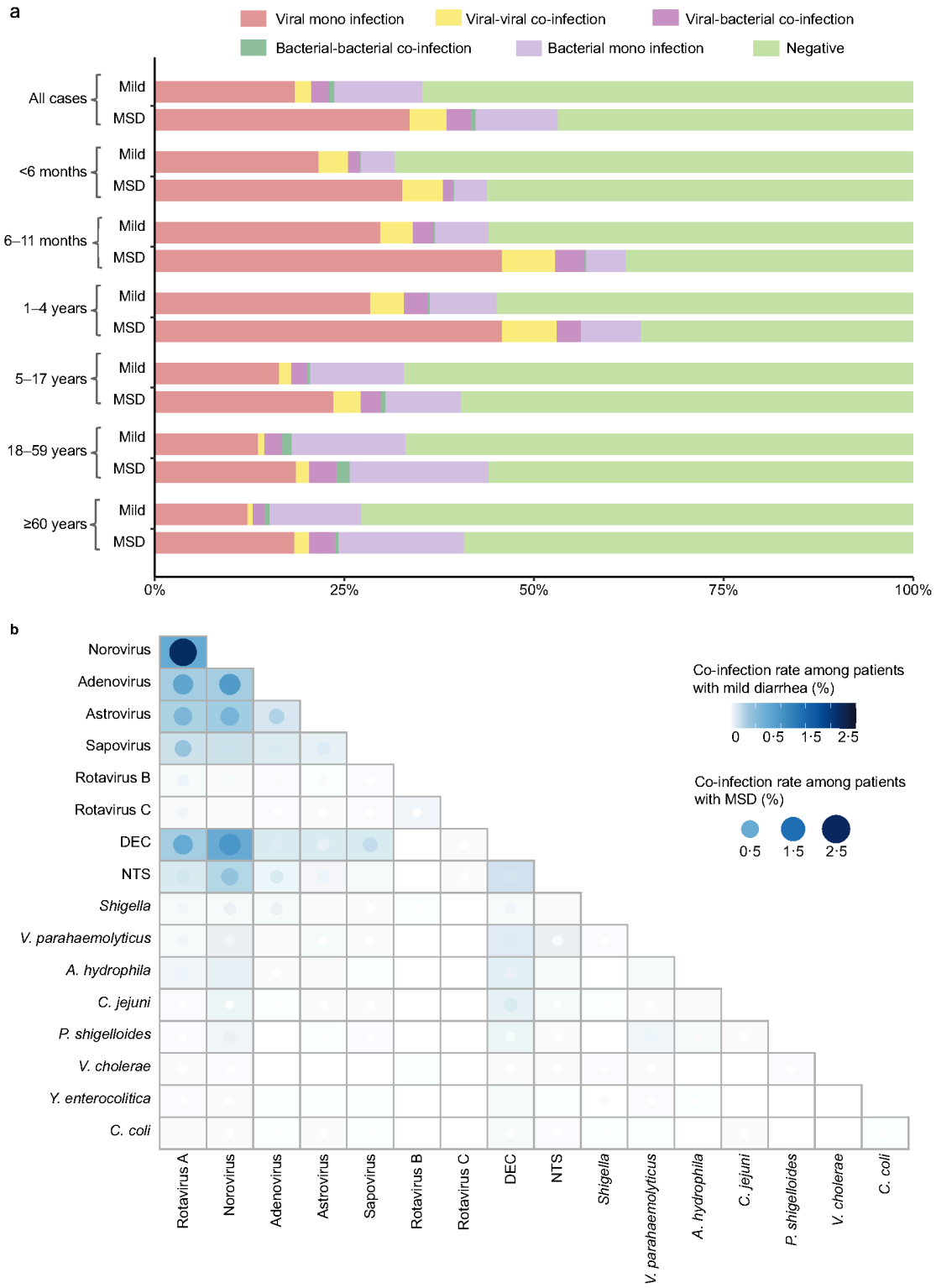

Figure 2

Prevalence of pathogens in mono infection and co-infection in patients with MSD and mild diarrhea in the mainland of China, 2009–2020 (A) Positive proportions of viruses, viral–viral co-infections, viral–bacteria co-infections, bacterial–bacterial co-infections, and bacteria in different age groups. (B) Heatmap of the co-infection rate of diarrhea pathogens. The grid color indicates the co-infection rate of diarrheal pathogens among patients with mild diarrhea, and the dot color indicates the co-infection rate

of diarrheal pathogens among patients with MSD. The larger size and darker color of the circles indicate a higher co-infection rate between the pair of pathogens. DEC=diarrheagenic *Escherichia coli*; NTS=nontyphoidal *Salmonella.*

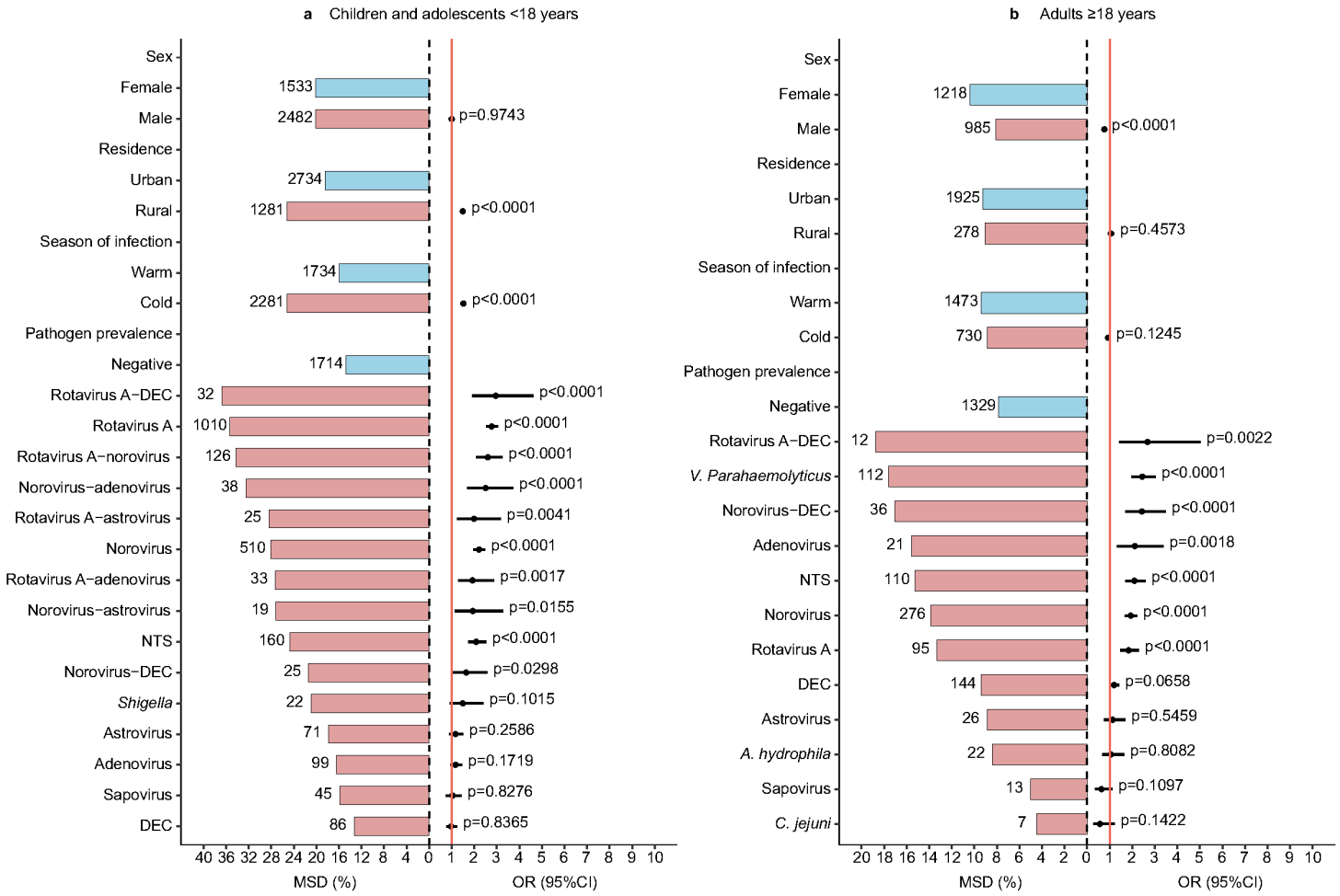

Figure 3

The association between MSD and etiological and demographic factors examined using the multivariate logistic regression model. (A) Incidence and adjusted OR for MSD in children and adolescents younger than 18 years. (B) Incidence and adjusted OR for MSD in adults aged 18 years or older. The numbers next to the bars indicate the number of patients with MSD in each group. The length of the bars indicates the incidence rate of MSD, with blue bars representing the reference group. The black dots are the adjusted ORs for the MSD and the black error bars are the 95% CIs. DEC=diarrheagenic *Escherichia coli*; NTS=nontyphoidal *Salmonella.*

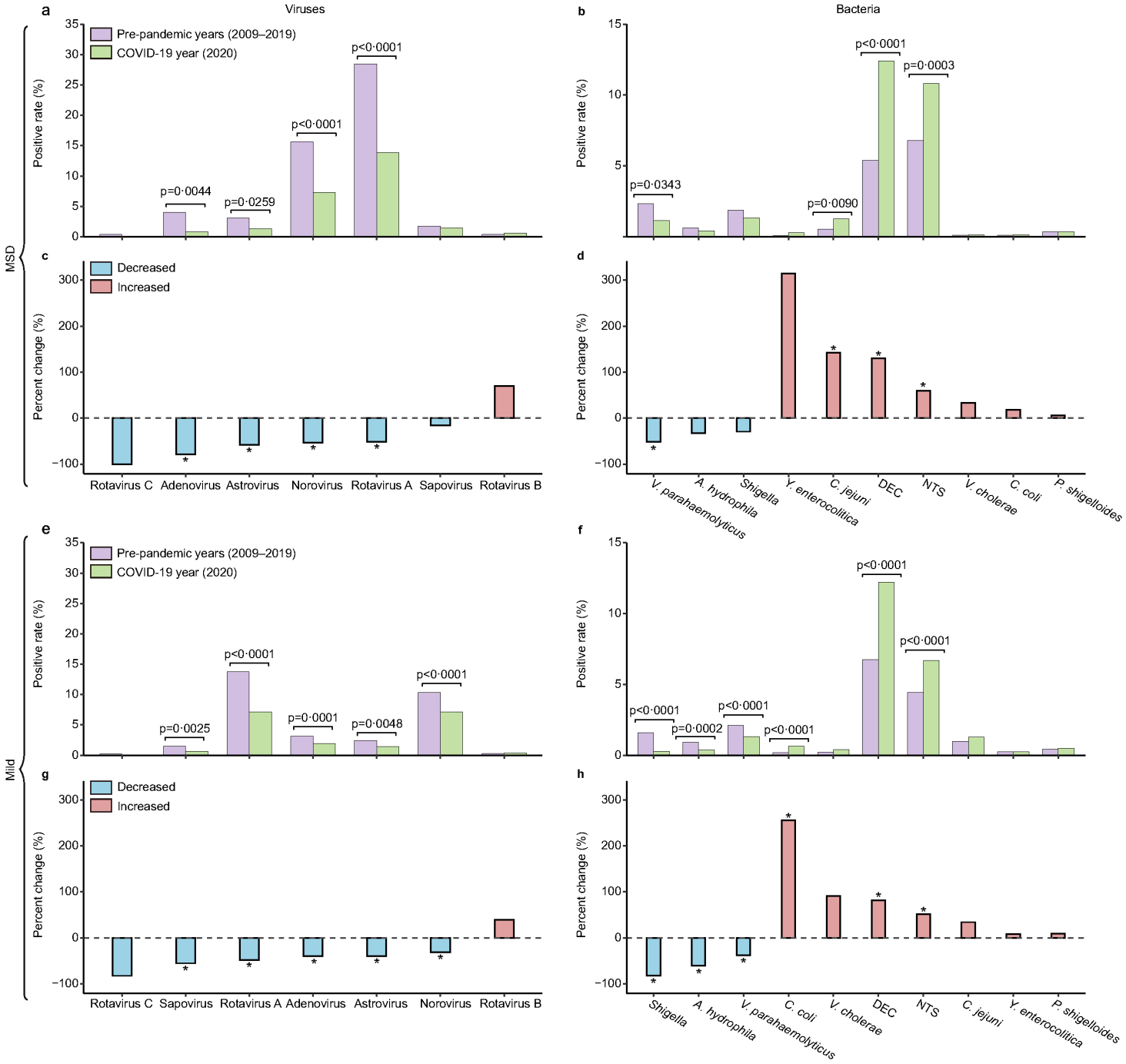

Figure 4

Comparison of test positive rates of pathogens and their percent changes between the pre-pandemic years (2009–2019) and the first COVID-19 pandemic year (2020). (A) Positive rates of seven viruses detected in patients with MSD during pre-pandemic years and the first COVID-19 pandemic year in the mainland of China. (B) Positive rates of ten bacteria detected in patients with MSD during pre-pandemic years and the first COVID-19 pandemic year. (C) Percent change of test positive rates of seven viruses detected in patients with MSD. (D) Percent changes of test positive rate of ten bacteria detected in patients with MSD. (E) Positive rates of seven viruses detected in patients with mild diarrhea during pre-pandemic years and the first COVID-19 pandemic year in the mainland of China. (F) Positive rates of ten

bacteria detected in patients with mild diarrhea during pre-pandemic years and the first COVID-19 pandemic year. (G) Percent change of test positive rates of seven viruses detected in patients with mild diarrhea. (H) Percent change of test positive rate of ten bacteria detected in patients with mild diarrhea. Statistical significance is based on chi-square test or Fisher's exact test. DEC=diarrheagenic *Escherichia coli*; NTS=nontyphoidal *Salmonella*. *p<0.05.

Supplementary Files

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