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Infection with bacterial enteropathogens in Swedish travellers to South-East Asia – a prospective study

C. M. ÅHRÉN[1,2]*, M. JERTBORN[1,2], L. HERCLIK[1], B. KAIJSER[3] AND A.-M. SVENNERHOLM[1]

[1] *Departments of Medical Microbiology,* [2] *Infectious Diseases and* [3] *Clinical Bacteriology, University of Göteborg, Göteborg, Sweden*

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SUMMARY

Infection with potential bacterial enteropathogens was studied prospectively in 94 Swedish travellers. Three faecal samples were collected, regardless of diarrhoeal symptoms, after each of three 1-week stays in Singapore, Hong Kong and Japan. The specimens were analysed for salmonella, shigella, yersinia, campylobacter and different enterotoxin-producing bacteria. A potential enteropathogen was identified in 30% (28/94) of the participants, i.e. in 26% of the healthy and in 39% of the travellers with diarrhoea. The most common isolates were enterotoxigenic bacteria of different species (14%), salmonella (11%) and campylobacter (7%). By performing enterotoxin-tests on six different colonies from the primary culture of each specimen enterotoxigenic *Escherichia coli* (ETEC) as well as enterotoxin-producing *Klebsiella* sp., *Citrobacter* sp. and *Morganella morganii* were identified. The latter strains were as prevalent as ETEC. In the 33 participants with diarrhoea enterotoxigenic bacteria (18%) and campylobacter (18%) were the most common isolates. Campylobacter-infected travellers developed symptomatic disease (6/7) significantly ($P < 0{\cdot}02$) more often than those infected with salmonella (3/10) or enterotoxigenic bacteria (6/13; 2 ETEC, 4 other species).

INTRODUCTION

Diarrhoea is by far the most frequent health problem among travellers from industrialized countries to the tropical and subtropical areas of the world. Approximately one-third of these visitors develop diarrhoea during or shortly after their travel [1]. A number of enteropathogens have been associated with travellers' diarrhoea, but so far the most common etiologic agent has been enterotoxin-producing *Escherichia coli* (ETEC), accounting for up to 40–70% of the diarrhoeal episodes [2–4].

However, the isolation rate of enteropathogens was rather low when faecal samples were collected from Scandinavian travellers with diarrhoeal disease within 3 weeks after their return home [5, 6]. This might be due to that shedding of some enteropathogens usually cease within 5–8 days after onset of infection [2]. We, therefore, undertook a prospective study with weekly sampling of stool

* Correspondence and reprint requests to: Christina Åhrén, Department of Medical Microbiology, University of Göteborg, Guldhedsgatan 10, 413 46 Göteborg, Sweden

specimens to determine the incidence of infection with bacterial enteropathogens among a group of Swedish travellers in South-East Asia. The main focus of the study was to identify enterotoxigenic Gram-negative bacteria of different species, including ETEC, by performing enterotoxin analyses on fresh non-subcultured isolates.

Faecal samples from all participants regardless of diarrhoeal symptoms were collected to enable a comparison of the infection rate with bacterial enteropathogens in healthy travellers and in travellers developing diarrhoeal disease.

MATERIALS AND METHODS

Study population

During 3 February to 15 March in 1987, members of the Gothenburg Symphony Orchestra and their relatives conducted a tour in South-East Asia and the USA. Out of these travellers 94 adult persons, 69 men and 25 women, (median age 32 years) gave informed consent to enter the study. During the previous 6 months none of the participants had experienced diarrhoeal disease, and only nine persons had travelled outside Scandinavia. The travel route included 33 days in South-East Asia, i.e. 5 days in Singapore, 9 days in Hong Kong and 19 days in Japan followed by 15 days in the USA. One third of the group went for 1-day excursions to Malaysia, Canton, and China, respectively. All persons stayed in hotels of high standard.

Each participant received a questionnaire in which personal data, diarrhoeal symptoms, medication and eating habits during the tour were to be recorded. If intestinal symptoms were noted, the onset, duration, number of bowel movements, quality of stool and presence of additional symptoms such as fever, vomiting, abdominal cramps and blood in the stool, would be recorded daily during the travel. Intestinal symptoms occurring with an interval of more than 72 h after a previous episode were regarded as due to a different episode. Based on the information from the questionnaire and on the repeated interviews/examinations by two of the investigating physicians who participated in the tour (one part-time) the participants were divided into three groups: travellers with diarrhoea, travellers with loose stools, and healthy persons. Travellers' diarrhoea was defined as the occurrence of three or more watery stools within a 25 h period or any number of watery stools when accompanied by fever, abdominal cramps or vomiting [7]. Persons having either one or two watery stools or two or more unformed stools or one unformed stool with accompanying symptoms per day, were defined as having loose stools.

Seven persons were each given a total of 1–5 capsules of loperamid during 1–3 days while having diarrhoea after consulting one of the physicians. No other antidiarrhoeal therapy was used. No participants were taking antibiotics during the study period.

Bacteriological methods

Faecal specimens were collected, regardless of diarrhoeal symptoms, from almost all the participants 7, 14 and 21 days after departure from Sweden. In addition to these three so-called 'routine'-samples the participants, when possible,

presented a specimen within 24 h after onset of diarrhoea or loose stools. On return to Sweden, the persons who had shed enteropathogens during the tour in Asia presented a fourth sample, i.e. on day 48–53. No pre-travel specimens were collected since previous studies have shown that healthy Scandinavian inhabitants very rarely carry the studied enteropathogens [8, 9]. The specimens were collected as rectal swabs and transported in a slightly modified Stuart's transport medium [10] by airmail express delivery or as accompanying luggage so that specimens arrived in Sweden within 48 h after collection. Immediately on arrival the rectal swabs were processed by standard procedures for salmonella, shigella and yersinia. For isolation of campylobacter, the selective medium of Skirrow was used [11]. Gram-negative rods of other species, i.e. *E. coli*, aeromonas and vibrios were only identified if they were enterotoxin-producing, that is according to the method for isolation of enterotoxigenic bacteria outlined below.

Isolation and identification of enterotoxigenic bacteria

On arrival in Sweden the rectal swabs were also streaked on blood agar and incubated over night at 37 °C. Six representative colonies with the appearance of Gram-negative rods were selected from the blood agar plates and were each analysed for the production of heat-labile enterotoxin (LT) by the GM1-ELISA [12] and for heat-stable enterotoxin (ST) by an ST-GM1-ELISA inhibition method [13]; toxin-positive colonies were subsequently subjected to biochemical typing.

The colonies selected were directly inoculated in individual wells of GM1-coated microtiter plates containing 100 μl of Casamino Acids-yeast extract medium with 45 μg lincomycin/ml and 2·5 mg glucose/ml. After incubation of the microtiter plates at 37 °C overnight with shaking, the culture medium from the individual wells was saved for subsequent ST determination (see below) and the microplates were washed and developed for GM1-bound LT using an LT-specific monocolonal antibody as described [12]. To evaluate production of STa, the culture medium from each well of the originally inoculated plate was transferred to a new GM1-coated microtiter plate to which ST conjugated to cholera B subunit had been bound [13]. The ST content of the culture medium was then determined with an ELISA inhibition method using an STa-specific monocolonal antibody.

After inoculating the GM1-ELISA plate the same individual colony was also streaked on blood agar and colonization factor antigen (CFA) agar and incubated overnight at 37 °C for further analyses. Provided that the colony had been found to produce enterotoxin in either of the GM1-ELISA-tests, the corresponding inoculum on blood agar was subjected to species-analyses according to standard procedures [14]. The non-*E. coli* isolates were also identified by the CCUG (Falsen, E. 1990 Catalogue of strains. 7th ed. Culture Collection of the University of Göteborg, Göteborg, Sweden). The corresponding CFA agar inoculum was used to study the presence of CFA/I, CFA/II and CFA/IV on the bacteria, using mannose-resistant hemagglutination and slide agglutination tests with monoclonal antibodies against CFAs or with CFA-specific polyclonal antisera as described [15].

Culture filtrate from non-*E. coli* strains found to be enterotoxigenic in the GM1-ELISAs, were further analysed for enterotoxicity in the Chinese hamster ovary (CHO) cell test (LT-like toxin) and the infant mouse test (ST-like toxin) as

previously described [16, 17]. The filtrates, including control culture filtrates from toxin negative strains of the same species were concentrated five times against polyethylene glycol prior to analysis.

The CFA agar inoculum of the enterotoxigenic colonies were suspended in TSB with 15% (wt/vol) glycerol and stored at −70 °C without subculturing. After 8 and 30 months storage the frozen colonies were retested for enterotoxin-production.

RESULTS

Bacterial enteropathogens isolated

From almost all of the 94 participants three faecal samples were collected with 1-week intervals, i.e. 75, 91 and 90 persons provided a so-called 'routine' sample regardless of diarrhoeal symptoms after the visit to Singapore, Hong Kong and Japan, respectively. In 28 (30%) of these travellers one or two potential bacterial enteropathogens were recognized; 13 (14%) persons were infected with enterotoxigenic bacteria, 10 (11%) with different salmonella strains, and 7 (7%) with *Campylobacter jejuni*. Three individuals had mixed infection. Altogether 31 different strains were identified. Since 8 of the strains were isolated on 2 (6 strains) or 3 (2 strains) occasions, a potential enteropathogen was isolated in 41 of the 256 routine samples analysed (Table 1). The most common isolates were enterotoxin-producing strains (13/31; 42%) followed by salmonella (11/13; 35%) and campylobacter (7/13; 22%). No shigella or yersinia strains were identified.

Interestingly, more than half (7/13; 54%) of the isolated enterotoxigenic strains were non-*E. coli*, i.e. being of *Klebsiella* sp., *Morganella morganii* or *Citrobacter* sp. Culture filtrates from selected representatives of these enterotoxin-producing non-*E. coli* strains, i.e. an ST-positive klebsiella strain and an LT-positive citrobacter strain, were also found to be enterotoxigenic in the infant mouse test for ST or the CHO-cell test for LT. No enterotoxigenic vibrios or *Aeromonas hydrophilia* strains were found. All of the enterotoxigenic strains produced either ST or LT, and none of them expressed CFA/I, CFA/II or CFA/IV. When retested for enterotoxin production after 8 months of storage at −70 °C and two subcultures after the initial enterotoxin-testing, 3/6 of the *E. coli* and 5/7 of the non-*E. coli* strains still produced enterotoxin. After additional subcultures or storage without further subculturing for up to 30 months after isolation 3/6 of the *E. coli* strains, but only 1/7 of the non-*E. coli* strains, i.e. a citrobacter strain, were still enterotoxigenic.

Most of the isolates (21/31) and especially the enterotoxigenic strains (10/13) were identified already in the first two routine samples after the group had visited Singapore and Hong Kong, respectively (Table 1). Three or four new salmonella strains were identified in each of the routine sample collections whereas no campylobacter strains were isolated until the group had visited Hong Kong. However, one person, later found to be infected with campylobacter, had symptoms already at the first routine sample occasion.

Most (23/31; 70%) of the isolates were detected in only one of the three routine samples from each individual (Table 1). In those instances when a strain was isolated in the first or second routine sample, the same strain was found in the

Table 1. *Bacterial enteropathogens isolated in three weekly faecal samples collected from Swedish travellers regardless of diarrhoeal symptoms*

Isolated strains		Isolates in faecal samples from Singapore (day 7) (75*)	Hong Kong (day 14) (91)	Japan (day 21) (90)	Total no. of isolated strains
Enterotoxigenic *sp*†					
E. coli	ST		3		3
	LT	1		2	3
Klebsiella pneumoniae	ST	2			2
Morganella morganii	ST		1		1
	LT			1	1
Citrobacter freundii	LT	2	1	2 (2)‡	3
Salmonella sp.					
kentucky		2	3 (1)	3 (2)	5
sofia		2	1 (1)	1 (1)	2
blockley			1		1
newport				2	2
derby				1	1
Campylobacter jejuni			4	6 (3)	7
Total		9	14	18	31

* Total number of faecal samples analysed.
† Producing heat-stable (ST) or heat-labile (LT) enterotoxin.
‡ Figure in parenthesis denotes the number of isolates identified also in a previous stool culture from the same individual.

second or third or both, routine samples from this individual; this was seen in 3/3 persons with campylobacter, in 3/7 persons shedding salmonella and in 2/10 participants with enterotoxin-producing bacteria in the stool.

To determine whether any of the isolated strains was excreted for a prolonged period of time a fourth sample was collected on return to Sweden, i.e. after the additional 15 day-tour in USA from all 28 travellers with previous positive stool cultures during the tour in Asia. In six of them a previously identified strain, i.e. two campylobacter strains, one *Salmonella newport* strain, one *E. coli* (LT) strain, one citrobacter (LT) strain, and one *Morganella morganii* (ST) strain could still be isolated. In no instance was a new pathogen identified.

Clinical observations

Out of the 94 individuals participating in the study 33 persons became ill; 18 (19%) persons experienced travellers' diarrhoea and 15 (16%) developed loose stools. As demonstrated in Fig. 1 the majority of the ill participants (22/33; 67%) developed symptoms during the first week in Japan. The disease was generally mild and of short duration, i.e. 73% of the ill individuals presented symptoms for 30 h or less. Abdominal cramps, nausea and a feeling of fever were often reported, whereas vomiting and fever (> 38 °C) were rare. The distribution with regard to sex (24 men, 9 women) and age (median 31 years) among the ill individuals were comparable to that of the whole study group.

Out of the 33 ill participants, 22 presented a faecal specimen while having intestinal symptoms. A bacterial enteropathogen was isolated from 13 (39%) of

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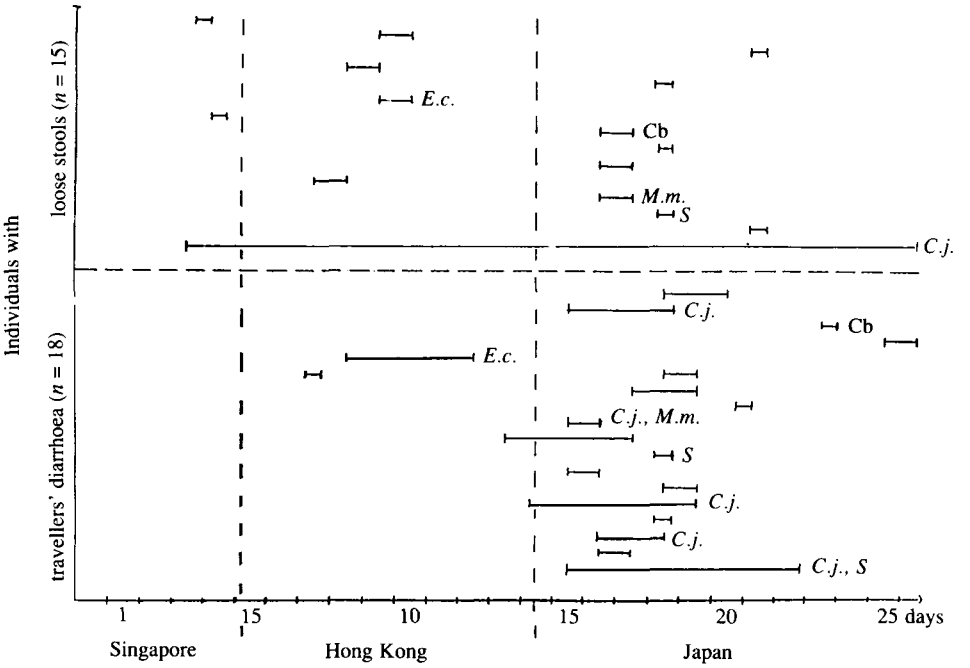

Fig. 1. Duration of symptoms in 33 individuals with travellers' diarrhoea or loose stools. Faecal specimens were collected on days 7, 14 and 21. In those instances when a potential bacterial enteropathogen was identified it is indicated: campylobacter (*C.j.*), citrobacter (Cb), *E. coli* (*E.c.*), *Morganella morganii* (*M.m.*) and salmonella (*S*).

them as indicated in Fig. 1. Three of the 13 participants had mixed infection; in one of them two different *Salmonella* species were found. In 10 of the 13 persons the infecting organism(s) was identified in the most adjacent routine sample(s).

From the 33 persons who developed enteric symptoms enterotoxin-producing bacteria and campylobacter were equally often isolated (18%, Table 2). Campylobacter was, however, the most common isolate in those with travellers' diarrhoea (5/18). As many as 6/7 persons with campylobacter in the stool developed symptomatic infection, which was significantly (χ^2-test; $P < 0{\cdot}02$) more frequent than among travellers infected with other enteropathogens (9/23). Thus, only 30% (3/10) of the salmonella infected individuals and 46% (6/13) of those shedding enterotoxin-producing bacteria developed enteric symptoms.

Overall, there was no significant difference in the isolation rate of potential bacterial enteropathogens between the symptomatic (13/33; 39%) and healthy (15/61; 26%) individuals. However, the infective strain was found significantly (Fisher's exact test; $P < 0{\cdot}05$) more often in two or more of the routine samples collected from the sick as compared to from the healthy participants (9/13 *v.* 3/15).

The participants were also asked to fill in a questionnaire during the tour stating whether they had consumed potentially contaminated food or beverage. Presence of potential enteropathogens in the stool or presence of intestinal symptoms or both, could in no instance be correlated with intake of ice and tap-water or with eating of a particular food item, e.g. green salads, unpeeled fruits, pastry, ice-

Table 2. *Bacterial enteropathogens isolated in the stool in relation to diarrhoeal symptoms from 94 Swedish travellers*

Isolated strains	Travellers with: No intestinal symptoms	Loose stools	Travellers' diarrhoea	Total
Enterotoxigenic *sp**				
E. coli	4 (2)†	1 (0)	1 (1)	6 (3)
non-*E. coli* sp‡	3 (2)	2 (1)	2 (0)§	7 (3)
Salmonella sp.	7	1 ‖	2§	10
Campylobacter sp.	1	1	5§	7
None	46	10	10	66

* Producing either heat-stable (ST) or heat-labile (LT) enterotoxin.
† Figure in parenthesis denotes the number of travellers infected with ST-producing bacteria.
‡ Klebsiella, *morganella morganii*, or citrobacter.
§ One person had mixed infection with *Morganella morganii* and campylobacter and another person with salmonella and campylobacter.
‖ Two different salmonella strains were isolated from the same person.

cream, shell-fish, cold ready-made dishes, non-fully cooked fish or meat or an abundance of these items on one or several occasions.

DISCUSSION

In this prospective study a potential bacterial enteropathogen was isolated in faeces from 30% (28/94) of the travellers, and 46% of the infected individuals developed gastrointestinal symptoms. The overall diarrhoeal attack rate of 35% (33/94) and the relatively mild disease observed was comparable to those found by Steffen and colleagues [1] in their survey of travellers' diarrhoea in European charter tourists and business travellers to various countries in the tropics and subtropics; the attack rate was, however, considerably lower and the disease milder than those reported for travellers to less developed regions [3, 4, 7]. The relatively low attack rate and also comparatively low isolation rate in our study can probably be explained by the fact that the group visited cities in South-East Asia with relatively high hygienic standards. The participants were also accommodated in hotels of high class and had few possibilities for the adventurous style of travelling. Furthermore, enteric viruses, parasites and less frequently occurring enterobacteria were not looked for.

The isolation rates of campylobacter (7%) and salmonella (11%) respectively, were the same as observed in most studies, whereas the relative proportion of ETEC was lower [3, 4]. Nevertheless, enterotoxin-producing bacteria were the most predominant faecal isolates.

Interestingly, approximately half of the enterotoxin-producing organisms represented non-*E. coli* species. Since we analysed all types of colonies with the appearance of Gram-negative rods for enterotoxin-production in the fresh, non-subcultured isolates, we were able to identify a number of enterotoxigenic species

in addition to *E. coli*, namely *Klebsiella* sp., *Citrobacter* sp. and *Morganella morganii*. Enterotoxin-producing aeromonas strains, which have been associated in rather high frequency with travellers' diarrhoea in some Asiatic areas, particularly in Thailand [4, 18] were, however, not detected. Though unconventional, enterotoxigenic aeromonas strains have been identified in stool samples with the applied method in subsequent studies (unpublished observation).

Enterotoxigenic strains other than *E. coli* of the family Enterobacteriaceae, e.g. *Klebsiella pneumoniae* and *Yersinia enterocolitica* have been associated with diarrhoeal disease previously. These strains have, with a few exceptions, been isolated from infants with diarrhoea and not from travellers [5, 8, 19, 20]. The pathogenic significance of enterotoxigenic non-*E. coli* isolates remains to be clarified. In our study the klebsiella strains were only isolated from asymptomatic individuals, whereas both citrobacter and *Morganella morganii* were isolated as the only potential bacterial pathogen in repeated samples from symptomatic individuals. Against this background we suggest that analysis of enterotoxin-production should include also Gram-negative species other than *E. coli*. To minimize loss of enterotoxicity by storage and subculture, analyses of enterotoxin-production should be performed on the fresh isolates, preferably before biochemical typing. Thus, as seen in this and in subsequent studies (unpublished observation) many of the enterotoxigenic non-*E. coli* strains seem to loose enterotoxicity already after two subcultures after primary isolation or after storage for a prolonged period of time.

The relatively high proportion of salmonella strains isolated from the participants may suggest that foodborne transmission was important [3]. But, as in most other studies [21], we failed to correlate diarrhoeal disease with non-restrictive dietary habits.

In conclusion this study shows that enterotoxin-producing bacteria were the most commonly isolated potential bacterial enteropathogens in travellers to South-East Asia. Furthermore it shows that enterotoxin-producing non-*E. coli* species were as prevalent as ETEC. The disease to infection rate was higher in travellers infected with enterotoxigenic bacteria than in those with salmonella, but not as high as in those infected with campylobacter.

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