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Comparison of clinical features and immunological parameters of patients with dehydrating diarrhoea infected with Inaba or Ogawa serotypes of *Vibrio cholerae* O1

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Abstract

Vibrio cholerae O1, Ogawa and Inaba serotypes, both cause severe cholera. We compared clinical and immunological features in patients in Bangladesh infected with these 2 serotypes. Blood was collected from hospitalized Ogawa ($N=146$) or Inaba ($N=191$) patients at the acute stage (day 2) and 5 and 19 days later. Ogawa patients were younger than Inaba, presented with shorter duration of diarrhoea, and had more frequent abdominal pain, vomiting and need for intravenous fluids ($p < 0.05$). Inaba patients more frequently had dark-field positive stools ($p < 0.01$). Inaba strains were more susceptible to tetracycline and erythromycin than Ogawa strains ($p < 0.001$). Ogawa infection produced higher plasma vibriocidal as well as IgG responses to cholera toxin B subunit, toxin-coregulated pilus subunit and lipopolysaccharide (LPS); higher IgA responses to LPS in ‘antibody in lymphocyte supernatant’ (ALS) specimens were also seen. These results suggest that a cholera vaccine based on the Ogawa serotype needs to be further investigated.

Introduction

Cholera continues to be a major public health problem for developing countries and an important cause of diarrhoea-related morbidity and mortality. *Vibrio cholerae* O1, the primary cause of epidemic cholera, has 2 biotypes, El Tor and classical, and 2 major serotypes, Ogawa and Inaba. The two *V. cholerae* biotypes have distinct phenotypes and differ with respect to the severity of the disease they can cause, the ability to survive outside the human host, and the seasonal pattern of infection [1–3]. The 2 serotypes, on the other hand, differ from one another only with respect to antigenic determinants on the O side chain of the lipopolysaccharide (LPS) antigen. The Inaba serotype has antigenic determinants identified as A and C, while Ogawa organisms have determinants A and B; very rarely, a third serotype, Hikojima, which produces all 3 antigens, has also been identified [4]. Genes that determine the in vitro as well as in vivo interconversions between the Ogawa and Inaba serotypes have been defined [4]. In nature, transition from the Ogawa to the Inaba serotype has been documented, but the reverse is very rare [5].

The Ogawa and Inaba serotypes of *V. cholerae* O1 differ from each other by a 2-O-methyl group that is present in the non-reducing terminal sugar of the Ogawa O-specific polysaccharide (O-SP) of the LPS, but is absent from the Inaba O-SP [6–8]. Although both the Inaba and Ogawa serotypes of *V. cholerae* O1 cause severe illness, the relative preponderance of each type fluctuates from season to season and from one year to another [9]. In areas where cholera is endemic, this fluctuation tends to occur in the intervals between epidemics of the disease [10,11]. Usually, at a given point in time, 1 of the 2 serotypes is responsible for the majority of cases in any geographical area [12,13], but the other serotype may replace the first during a prolonged outbreak [14]. However, the reason for these fluctuations has not been elucidated, and a pattern underlying these fluctuations has not been identified [15].

These changes in serotype may correlate with the immune status of the population, as has been documented in animal models [16]. Epidemiological studies have suggested that the Inaba serotype may be more immunogenic than Ogawa and result in longer-term protection following infection [9]. The Inaba serotype has been used for the development of live oral cholera vaccine candidates, while inactivated whole cell cholera vaccines have mixtures of both serotypes [17]. It is not known if the differences between the 2 serotypes are reflected in clinical illness, in the immunological responses following infection, in the duration of the infectious period, or in the recovery rate of the organisms from the stool.

After natural infection with *V. cholerae*, circulating antibodies can be detected against cholera antigens, including LPS, so it is possible that the antigenic determinants that differ between the Ogawa and Inaba serotypes may play a role in inducing host immunity and in producing different clinical responses. In this longitudinal study in Bangladesh, we compared the clinical and immunological responses in cholera patients following natural infection with the Ogawa or Inaba serotypes of *V. cholerae* O1.

Materials and methods

Study population, enrolment, clinical and immunological data

This study was conducted at the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Patients presenting to the hospital with acute watery diarrhoea and culture-confirmed cholera due to El Tor *V. cholerae* O1 were enrolled in the study between 2001 and 2006, based on previously published inclusion criteria [18]. Patients over the age of 6 months with acute watery diarrhoea, culture-positive for *V. cholerae* O1 Ogawa or Inaba serotype and with no significant comorbid conditions were selected for inclusion in the study. Venous blood was collected from hospitalized Ogawa ($N = 146$) and Inaba ($N = 191$) patients on the second day of hospitalization (day 2), as well as on day 7 and day 21 after onset of illness, during convalescence. This study was reviewed and approved by the research and ethical review committees of the ICDDR, B, as well as by the institutional review board of the Massachusetts General Hospital.

Information regarding clinical features and demographics were collected from patients at enrolment after clinical stabilization. We compared clinical features of cholera caused by Ogawa and Inaba serotypes. Immune responses were measured in sera on days 2, 7 and 21 following illness; key cholera-specific immune responses are those directed to antibacterial components and to the cholera toxin. For the antibacterial responses, vibriocidal antibodies were measured using homologous *V. cholerae* O1, El Tor Ogawa (strain X25049) or Inaba (strain 19479) as target bacteria and guinea pig complement. Antibody responses to recombinant cholera toxin B subunit (rCTB), toxin-coregulated pilus subunit (TcpA), and lipopolysaccharide (LPS) were determined using a kinetic enzyme-linked immunosorbent assay (ELISA), as previously described [19]. Immune responses were also measured using

the ‘antibody in lymphocyte supernatant’ (ALS) assay on specimens recovered from supernatants of cultured peripheral blood mononuclear cells from study days 2 and 7 after onset of disease to determine LPS, CTB and TcpA-specific IgA responses from circulating lymphocytes. We determined baseline zinc and retinol levels in blood in a subsample of patients, as described earlier [20].

For the ELISA assays, in brief, 96-well microtitre plates were coated with either purified homologous LPS serotype (El Tor O1 Ogawa/Inaba; 250 ng/well), sequentially with GM1 ganglioside (100 ng/well) followed by recombinant CTB (50 ng/well), or with recombinant TcpA (150 ng/well). The CTB and TcpA proteins are identical between the Inaba and Ogawa serotypes of El Tor V. cholerae O1. Plates were incubated with diluted patient sera, washed, and horseradish peroxidase-conjugated antihuman IgG or antihuman IgA antibodies were added (Jackson Laboratories, Bar Harbor, ME, USA). Plates were developed using 0.1% orthophenylene diamine (Sigma, St. Louis, MO, USA) in 0.1 M sodium citrate buffer with 0.1% hydrogen peroxide; optical densities were measured kinetically at 450 nm for 5 min at 19-s intervals and values expressed as mAb/ min. Each ELISA plate had a positive control derived from pooled, convalescent sera from patients previously studied with cholera, and this positive control was used to standardize results across different ELISA plates [21].

After admission, severely dehydrated cholera patients at the ICDDR, B hospital are given empirical antimicrobials to which V. cholerae O1 is usually susceptible based on surveillance studies, as well as parenteral fluid rehydration. Data on the antimicrobial susceptibilities of El Tor V. cholerae O1 Ogawa and Inaba strains at the ICDDR, B over the period 2001–2006 were obtained from the surveillance system at the centre [22]. The agar disc diffusion method and commercially available antibiotic discs (Oxoid, Basingstoke, UK) containing tetracycline (30 µg), erythromycin (15 µg), trimethoprim (23.75 µg)/ sulfamethoxazole (1.25 µg), or ciprofloxacin (5 µg) were used. Results of testing were interpreted as recommended by the National Committee for Clinical and Laboratory Standards [23]. We did a separate analysis, deleting the subset of patients who received antimicrobials to which their isolate was shown to be resistant, to eliminate a possible effect of differing susceptibilities between the 2 serotypes on subsequent immune responses.

Data analyses

Data analyses were performed using SPSS for Windows (version 12; SPSS Inc., Chicago, IL, USA) and Epi Info (version 6.0; USD, Stone Mountain, GA, USA). After creating frequency distribution tables, cross tables were generated, and data were summarized for group comparisons. The significance of differences in proportions were evaluated by the Chi-square test, and Fisher’s exact test was applied when appropriate. Means were compared by independent sample *t*-tests. For non-normally distributed data, comparisons were carried out using the Mann–Whitney U-test. A *p*-value of <0.05 was considered statistically significant. Strength of association was determined by calculating relative risks (RR) and their 95% confidence intervals (CI).

Results

Clinical and microbiological features

The relative prevalence of El Tor V. cholerae O1 serotypes Inaba and Ogawa treated at the ICDDR, B varied over the y 2001–2006, as determined from the hospital surveillance system (Figure 1). During this period, both serotypes existed concomitantly, but one serotype usually was more frequent than the other at any particular time; in 2001, 2002,

2004 and 2006, the Inaba serotype predominated, while in 2003 and 2005, the Ogawa serotype predominated.

Patients infected with the Ogawa serotype were younger than those with Inaba infection, presented with a shorter duration of diarrhoeal symptoms, and had more frequent abdominal pain, vomiting and need for intravenous fluids (Table I). On the other hand, patients with Inaba infection had a higher frequency of dark-field positive stools. There was a trend towards more frequent O blood group in patients with Ogawa infection, and significantly more patients with Inaba infection were blood group A.

On examining the antimicrobial susceptibilities of the 2 El Tor V. cholerae O1 serotypes based on surveillance data from the ICDDR, B, Inaba strains were seen to be significantly more susceptible to tetracycline and erythromycin than Ogawa strains (Table II). Neither serotype was commonly susceptible to trimethoprim/sulfamethoxazole, and both were almost always susceptible to ciprofloxacin. This pattern remained unchanged for the 2001–2004 period, but changed in 2005, when increasing resistance to doxycycline and erythromycin was seen [24]. Adult patients at the ICDDR, B were treated with doxycycline in 2001–2004, while children were treated with erythromycin. Based on changes in susceptibility and treatment failures, beginning in 2005, adults were given ciprofloxacin, but this subsequently changed to azithromycin due to poor clinical outcomes with ciprofloxacin [25].

Immune responses

We compared the immune responses after infection with the 2 serotypes of El Tor V. cholerae O1 (Table III). Compared with patients infected with Inaba strains, patients with Ogawa infection had significantly higher serum vibriocidal antibody titres during convalescence, as well as higher serum IgG antibody responses to the 3 cholera antigens evaluated, CTB, TcpA, and LPS. Although there were no significant differences in serum IgA responses to these antigens between patients infected with the 2 serotypes, patients following Ogawa infection also had significantly higher IgA responses to LPS and CTB in the ALS fluids of circulating lymphocytes on day 7 following infection, similar to the vibriocidal and serum IgG responses.

Since there were differences in antimicrobial susceptibilities between the 2 serotypes noted in the surveillance system (Table II), and these differences might have affected the rapidity of response in patients infected with the 2 serotypes to the standard antibiotic therapy they received, we repeated the comparisons of immune responses following cholera with the 2 serotypes, eliminating from the analysis the 22 patients treated with an antimicrobial to which their particular isolate was resistant (Table IV). Patients infected with Ogawa strains in this analysis still had higher vibriocidal responses in convalescence, as well as higher serum IgG to CTB, and IgA responses to LPS and CTB in ALS fluid, compared to those infected with Inaba strains. However using this analysis, we did not find any significant differences in the IgG responses to TcpA or LPS in the 2 groups of cholera patients (Table IV). The differences in these immune responses showed the same trend toward higher responses in patients infected with Ogawa strains, but they did not reach statistical significance with the smaller number of patients analysed.

Discussion

Epidemiological studies over the last 2 decades have been focused mainly on discerning the differences between infection with classical and El Tor biotypes of V. cholerae O1, which may [26,27] or may not result in differences in disease severity or protection from subsequent re-infection [9]. Similar differences between the 2 serotypes of El Tor V.

cholerae O1, Inaba and Ogawa, have not been looked for in depth, and no prospective immunological analysis is available to determine the relative immunogenicity of one serotype over another. Data are conflicting as to whether Inaba or Ogawa strains are more immunogenic and/or more protective [26,28]. Inaba strains possess the C antigen, while the B antigen is present in Ogawa strains; the A antigen is found in both serotypes in equal amounts [29], and this may provide substantial cross-protection between the serotypes [30]. The terminal sugar in the perosamine residue of the Ogawa LPS is a 2-O-methyl group, while in Inaba LPS, a hydroxyl group is attached to the perosamine residue [6]. Each polysaccharide polymer contains 15–18 perosamine residues. In nature, transition from the Ogawa to the Inaba serotype has been documented, but the reverse is very rare [5]. Although the difference between the LPS antigens in the 2 serotypes is the defining difference between the serotypes, differences in phenotypic characteristics based on carbohydrate fermentation have also been reported [31]. Extensive molecular typing of Ogawa and Inaba strains from an epidemic in Kolkata, India showed that the strains were similar in ribotyping, restriction fragment length polymorphism (RFLP) and pulsed-field gel electrophoresis (PFGE) patterns [32,33]. More recently, some differences have been shown by ribotype analyses of the 2 serotypes [5].

In our analysis of data during the study period, we observed biannual peaks of cholera. Of these, peaks in 2 of the y were caused by Ogawa strains, while 4 were caused by Inaba. The first 2 y of this period were dominated by Inaba, followed by alternate increases of one serotype or the other. This pattern of changing serotype may be a survival mechanism of the organism in the presence of host protective mechanisms.

Similar fluctuations of *V. cholerae* O1 serotypes have been seen in a rural setting in Matlab, Bangladesh, as well as in other cholera endemic countries including India, Peru and Malawi [9,13,14]. A mathematical model analysing 40 y (1966–2005) of cholera infections in Bangladesh suggested fluctuation in serotype occurred when cross-immunity between serotypes was high, resulting in immunity at the population level [30]. The analysis of Longini et al. (2002), using data from a 33-y period, suggested that recurrence of Ogawa infection was seen within a 6-month period of a previous outbreak, while recurrence of Inaba predominance took a longer period of time. This suggested that Inaba infection produced longer-term protection. Other studies have also suggested that the Inaba serotype is more protective [28,34]. However, a prospective study of the clinical and immunological responses of hospitalized patients with cholera due to the Inaba and Ogawa serotypes has not previously been carried out.

In our study, we saw differences in disease presentation between patients infected with the 2 *V. cholerae* O1 serotypes. We found that patients infected with the Ogawa serotype were younger in age and had clinical parameters that were more severe, while dark-field positive stools were seen more frequently in Inaba-infected patients. Differences in the clinical symptoms of disease have been reported between the classical and El Tor biotypes of *V. cholerae* O1, but differences between the 2 serotypes have not been previously reported. Abdominal pain, reported previously in severe cholera [20], was more frequent in Ogawa-infected patients.

Clinical stabilization of cholera patients includes rehydration therapy as well as treatment with antimicrobial agents based on the susceptibility pattern of existing *V. cholerae* strains in circulation at the time, and also based on published treatment outcomes [24,35]. The drugs of choice for *V. cholerae* O1 infection until recently were erythromycin for children and doxycycline (long-acting tetracycline) for adults. However, there has been increasing multi-drug resistance in *V. cholerae* O1 starting in 2004 [24]. In our study, Ogawa strains were found to be more frequently resistant to tetracycline (doxycycline) than Inaba strains,

suggesting that such differences may be important when considering treatment options. Previous studies have suggested that resistance to antibiotics emerged first in the Ogawa serotype and later in the Inaba serotype [24]. The changing drug resistance pattern in *V. cholerae* has been studied earlier at the ICDDR, B and the present analyses further verify the findings. The drug of choice for treatment has most recently been changed to azithromycin for the treatment of acute dehydrating diarrhoea. Azithromycin has better in vitro activity against *V. cholerae* O1 than erythromycin, and has produced better treatment outcomes [24,36]. Where possible, antibiotic treatment should be based on antimicrobial susceptibilities.

Our study showed that immune responses to both bacterial surface antigens (LPS, TcpA, and vibriocidal antibodies) and to cholera toxin were higher in patients infected with the Ogawa serotype compared to those infected with the Inaba serotype. It is not certain whether these higher immune responses relate to the differences in the LPS antigens on the cell surface of the 2 serotypes, or may relate to the observed differences in age distribution or severity of infection. Epidemiological and vaccine studies suggest that infection or vaccination with either the classical or El Tor biotype of *V. cholerae* O1, as well as with either the Inaba or Ogawa serotype, provides protection for at least the subsequent 2 to 3 y [37,38]. The oral inactivated killed whole cell cholera vaccine, which consists of a mixture of both serotypes, showed better protection against the Ogawa serotype than Inaba [39]. The live oral Inaba-based cholera vaccine, CVD103HgR, gave short-term protection against challenge by both serotypes [40]. In more recent studies of Inaba-based live oral cholera vaccines, higher immune responses have been seen to the Inaba than to the Ogawa serotype. A higher immune response to the homologous Inaba serotype has also been seen in Peruvian vaccinees given CVD103HgR [41]. In studies of the live oral Inaba-based vaccine candidate, Peru-15, in Bangladeshi participants, vibriocidal immune responses were seen at similar frequency against both the Ogawa and Inaba serotypes in adults and in toddlers; in younger, apparently naive children, the immune responses were satisfactory only against the Inaba serotype [42].

The immunogenicity, B cell epitopes and protective role of the 2 serotype antigens have been studied using synthetic carbohydrates that mimic the Inaba and Ogawa O-specific polysaccharides linked to carrier proteins [43]. The results in mice showed that the Ogawa conjugate resulted in induction of vibriocidal antibodies, while the Inaba synthetic conjugate did not, suggesting that antibody to the Inaba serotype is less effective in a complement-mediated assay [44]. Thus, although immunization with both Ogawa and Inaba LPS resulted in an antibody response, the synthetic conjugate studies suggest differences in the ability to produce functional antibodies. These findings between the 2 serotypes, as well as our data presented here, are at variance with population-based studies that suggest that the Inaba serotype is more effective in providing short-term protection against cholera [9].

We observed that stool of patients infected with the Inaba serotype were more frequently dark-field positive than for patients infected with the Ogawa serotype. Dark-field positivity has been previously shown to reflect the density of bacteria in the stool of cholera patients [45]. However, our recent data on transmission of cholera from patients to household contacts showed no apparent differences in the rates of transmission of Ogawa or Inaba strains in the household setting [46].

This is the first study in which a cohort of hospitalized patients with El Tor *V. cholerae* O1 Inaba or Ogawa infection have been compared for clinical and immunological parameters. The improved immune response following Ogawa infection suggests that a cholera vaccine based on the Ogawa antigen should be further investigated. Although we show that disease caused by *V. cholerae* O1 leads to a more potent immune response, we do not know the

relationship of this to the duration of protection. Future longitudinal field and laboratory analyses will help us to understand this better, and inform the best composition of cholera vaccines to provide optimal protective efficacy.

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Figure 1. Shifting pattern of *Vibrio cholerae* serotypes Inaba and Ogawa during the y 2001–2006, based on the hospital surveillance system at the ICDDR, B.

Table I

Microbiological and clinical features of patients with cholera, stratified by infection with strains of *Vibrio cholerae* O1 Ogawa or Inaba serotype.

Characteristic	Ogawa, <i>n</i> (%) (<i>N</i> = 146)	Inaba, <i>n</i> (%) (<i>N</i> = 191)	RR (95% CI)	<i>p</i> -Value
Median age (months)	202	249		0.18
Age <15 y	71 (48.6)	67 (35.1)	1.37 (1.07–1.74)	0.01
Male	66 (45.2)	84 (44.0)	1.03 (0.80–1.31)	0.91
Dehydration				
Some	13 (8.9)	19 (9.9)	0.93 (0.60–1.44)	0.89
Severe	133 (91.1)	172 (90.1)		
Duration of diarrhoea (h) at presentation, median (25 th , 75 th percentile)	11.0 (6.0, 19.50)	15.0 (8.0, 25.0)		0.01
Clinical features				
Abdominal pain	120 (82.2)	137 (71.7)	1.44 (1.02–2.02)	0.03
Fever (≥ 37.5°C)	30 (20.5)	29 (15.2)	1.22 (0.91–1.62)	0.25
Vomiting	145 (99.3)	182 (95.3)	4.43 (0.69–28.6)	0.04
Received i.v. fluids	143 (97.9)	176 (92.1)	2.69 (0.08–18.02)	0.03
Dark-field positive stool	58 (39.7)	103 (53.9)	0.72 (0.56–0.93)	0.01
Blood group				
O	68 (46.6)	71 (37.2)	1.24 (0.97–1.58)	0.10
A	26 (17.8)	53 (27.7)	0.71 (0.50–1.00)	0.04
B	42 (28.8)	53 (27.7)	1.03 (0.79–1.35)	0.93
AB	10 (6.8)	14 (7.3)	0.96 (0.59–1.57)	0.96
Retinol deficiency (<20 µg/dl)	28 (40.6)	19 (27.1)	1.34 (0.96–1.86)	0.13
Zinc deficiency (<0.60 mg/l)	19 (28.4)	19 (26.8)	1.04 (0.71–1.52)	0.98

RR, relative risk; CI, confidence interval; i.v., intravenous.

Table II

Percentage of *Vibrio cholerae* O1 strains susceptible to different antibiotics during the period 2001–2006, based on data from the hospital surveillance system at the ICDDR, B.

Name of antibiotic	<i>V. cholerae</i>		RR (95% CI)	<i>p</i> -Value
	Ogawa, <i>n</i> (%)	Inaba, <i>n</i> (%)		
Tetracycline	572/1289 (44.4)	1503/1561 (96.3)	0.30 (0.28–0.32)	<0.001
Erythromycin	672/1289 (52.1)	1148/1561 (73.5)	0.62 (0.57–0.67)	<0.001
Trimethoprim/sulfamethoxazole	5/1289 (0.4)	12/1561 (0.8)		NS
Ciprofloxacin	1289/1289 (100)	1561/1561 (100)		NS

RR, relative risk; CI, confidence interval; NS, not significant.

Table III

Comparison of immunological responses in patients with cholera, stratified by infection with El Tor *Vibrio cholerae* O1 Ogawa or Inaba strains.

Responses on different study days	Ogawa, mean $\pm$ SD	Inaba, mean $\pm$ SD	<i>p</i> -Value
Vibriocidal			
Day 2	331 $\pm$ 741	224 $\pm$ 385	0.45
Day 7	9964 $\pm$ 10,507	6481 $\pm$ 7021	<0.001
Day 21	6096 $\pm$ 6213	4460 $\pm$ 4933	0.01
TcpA IgA			
Day 2	9.3 $\pm$ 9.2	7.83 $\pm$ 10.0	0.06
Day 7	37.0 $\pm$ 57.6	34.0 $\pm$ 47.4	0.82
Day 21	19.2 $\pm$ 24.3	19.0 $\pm$ 21.2	0.74
LPS IgA			
Day 2	24.0 $\pm$ 29.1	26.56 $\pm$ 31.5	0.46
Day 7	185.7 $\pm$ 191.3	156.3 $\pm$ 164.5	0.20
Day 21	115.4 $\pm$ 119.8	105.6 $\pm$ 116.7	0.40
CT IgA			
Day 2	27.25 $\pm$ 24.14	32.83 $\pm$ 42.14	0.81
Day 7	162.2 $\pm$ 120.8	141.1 $\pm$ 112.5	0.08
Day 21	102.5 $\pm$ 77.4	86.7 $\pm$ 63.0	0.10
TcpA IgG			
Day 2	65.9 $\pm$ 37.0	42.5 $\pm$ 26.6	<0.001
Day 7	100.3 $\pm$ 78.7	76.06 $\pm$ 61.1	0.01
Day 21	117.0 $\pm$ 78.42	93.32 $\pm$ 78.18	0.01
LPS IgG			
Day 2	92.19 $\pm$ 41.81	87.05 $\pm$ 65.2	0.01
Day 7	249.6 $\pm$ 168.7	220.4 $\pm$ 163.4	0.05
Day 21	246.9 $\pm$ 134.7	225.7 $\pm$ 151.2	0.05
CT IgG			
Day 2	136.9 $\pm$ 85.41	127.1 $\pm$ 111.8	0.04
Day 7	350.8 $\pm$ 163.3	297.6 $\pm$ 167.5	0.001
Day 21	383.1 $\pm$ 166.9	304.6 $\pm$ 145.2	<0.001
TcpA ALS IgA			
Day 2	5.2 $\pm$ 6.7	6.4 $\pm$ 15.6	0.57
Day 7	37.7 $\pm$ 87.8	35.4 $\pm$ 79.2	0.80
LPS ALS IgA			
Day 2	9.6 $\pm$ 25.2	8.7 $\pm$ 21.7	0.82
Day 7	192.0 $\pm$ 242.0	79.84 $\pm$ 123.6	0.01
CT ALS IgA			
Day 2	60.2 $\pm$ 126.0	33.6 $\pm$ 72.1	0.38
Day 7	262.4 $\pm$ 207.7	134.9 $\pm$ 161.7	<0.001

SD, standard deviation; TcpA, toxin-coregulated pilus subunit; LPS, lipopolysaccharide; CT, cholera toxin; ALS, antibody in lymphocyte supernatant.

Table IV

Comparison of immunological responses between serotypes in patients treated with an antibiotic to which their isolate was susceptible.

Responses on different study days	Ogawa, mean $\pm$ SD	Inaba, mean $\pm$ SD	p-Value
Vibriocidal			
Day 2	339 $\pm$ 760	220.6 $\pm$ 379	0.108
Day 7	10,180 $\pm$ 11,029	6497 $\pm$ 7059	0.002
Day 21	6057 $\pm$ 6195	4467 $\pm$ 4960	0.024
TcpA IgA			
Day 2	9.1 $\pm$ 9.3	7.93 $\pm$ 10.1	0.386
Day 7	32.9 $\pm$ 50.5	34.5 $\pm$ 47.7	0.819
Day 21	17.8 $\pm$ 22.4	19.2 $\pm$ 21.3	0.668
LPS IgA			
Day 2	26.2 $\pm$ 30.7	26.8 $\pm$ 31.6	0.872
Day 7	192.6 $\pm$ 109.0	157.9 $\pm$ 164.7	0.097
Day 21	122.5 $\pm$ 125.1	106.8 $\pm$ 116.9	0.284
CT IgA			
Day 2	27.4 $\pm$ 23.5	33.1 $\pm$ 42.3	0.131
Day 7	162.1 $\pm$ 119.6	141.9 $\pm$ 112.8	0.144
Day 21	103.0 $\pm$ 78.9	86.1 $\pm$ 62.9	0.059
TcpA IgG			
Day 2	64.6 $\pm$ 38.1	42.3 $\pm$ 26.6	<0.001
Day 7	92.4 $\pm$ 67.9	76.2 $\pm$ 61.7	0.126
Day 21	110.6 $\pm$ 65.7	92.5 $\pm$ 78.7	0.145
LPS IgG			
Day 2	94.9 $\pm$ 42.9	87.1 $\pm$ 65.6	0.206
Day 7	251.0 $\pm$ 166.6	221.8 $\pm$ 163.8	0.139
Day 21	250.7 $\pm$ 136.7	227.3 $\pm$ 151.3	0.187
CT IgG			
Day 2	136.2 $\pm$ 84.4	127.0 $\pm$ 112.0	0.433
Day 7	348.6 $\pm$ 170.1	295.1 $\pm$ 166.3	0.008
Day 21	378.1 $\pm$ 167.3	302.1 $\pm$ 144.1	<0.001
TcpA ALS IgA			
Day 2	7.00 $\pm$ 7.7	6.59 $\pm$ 15.9	0.901
Day 7	35.7 $\pm$ 84.2	36.44 $\pm$ 80.2	0.965
LPS ALS IgA			
Day 2	11.6 $\pm$ 28.4	7.9 $\pm$ 20.9	0.459
Day 7	231.8 $\pm$ 262.2	80.90 $\pm$ 125.2	0.002
CT ALS IgA			
Day 2	64.8 $\pm$ 129.1	28.62 $\pm$ 60.4	0.122
Day 7	291.2 $\pm$ 197.1	130.8 $\pm$ 162.3	<0.001

SD, standard deviation; TcpA, toxin-coregulated pilus subunit; LPS, lipopolysaccharide; CT, cholera toxin; ALS, antibody in lymphocyte supernatant.