Jpn. J. Trop. Med. Hyg., Vol. 23, No. 3 (1995) pp. 159~164

DRUG SENSITIVITY OF *VIBRIO CHOLERAE* AND *SHIGELLA* SPECIES IN THE WORLD

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Received July 20, 1995/Accepted August 16, 1995

Abstract: Recently isolated *Vibrio cholerae* and *Shigella* pecies from 6 countries were examined for their drug sensitivities. The sensitivities of *V. cholerae* were characterized by a narrow inhibitory concentration ranges without any resistant strain. However, the sensitivities of *Shigella* species were variable and mostly resistant to tetracycline and ampicillin. Japanese isolates of *Shigella* species were relatively more sensitive to tetracycline and ampicillin than the isolates from the other countries.

Indonesian isolates of *Shigella* species were relatively more resistant than those of the other countries, even to the new antimicrobials such as ofloxacin and cefdinir, and a highly resistant strain against ofloxacin was found.

INTRODUCTION

Cholera and bacillary dysentery (Shigellosis) are the most notorious illness among diarrheal diseases in the world. It has generally been said that *Shigella* species are mostly resistant to traditional antibiotics such as tetracycline and ampicillin (Carlson *et al.*, 1983),while *Vibrio cholerae* are sensitive to them, although emergence of tetracycline resistant *Vibrio cholerae* has occasionally been reported (Glass *et al.*, 1980; Ramamurthy *et al.*, 1992; Towner *et al.*, 1980; Yamamoto *et al.*, 1995). However, the drug sensitivities of the pathogenic organisms are variable from place to place where they are isolated, and from time to time of isolation. Therefore, the drug sensitivities should intermittently be examined for better understanding of the epidemiological feature. This paper described the drug sensitivities of *V. cholerae* and *Shigalla* species recently isolated from a variety of places in the world.

MATERIALS AND METHODS

Bacterial strains: *V. cholerae* were collected from Argentina, Indonesia, Laos, India, Bangladesh and Thailand in total of 159 strains. The isolates from the former 3 countries are *V. cholerae* O1 El Tor and those from the latter 3 countries are *V. cholerae* O139 synonym Bengal. The strains of *Shigella* species were collected from Bolivia, Dominican Republic, Indonesia, Laos, Kenya and Japan in total of 191 strains. All strains were isolated during the period between 1992 and 1995.

Drugs examined: Ampicillin (ABPC), Tetracycline (TC), Erythromycin (EM), and Ofloxacin (OFLX) were examined for all isolates. Additionally, Polymyxin B (PLB) for *V. cholerae* and Cefdinir (CFDN) for *Shigella* were examined.

Sensitivity tests: Minimum inhibitory concentration (MIC) of the drugs were examined by plate dilution technique. Heart infusion agar plates containing the drug at the serial 2-fold concentrations from 0.025μg/ml to 100μg/ml were prepared. The organisms to be examined were cultured in heart infusion broth over night and the culture fluids were diluted 1 : 10 with normal saline solution for the inoculum (ca. 10^7/ml). They were inoculated by using microplanter (Sakuma Co. MITP#00257) and determination of MIC was made after incubation at 37 C for 24 hours.

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RESULTS

V. cholerae: The drug concentration ranges to inhibit the growth of organisms were 3.13 to 12.5μg/ml of ABPC, 0.2 to 0.4 of TC, 0.4 to 12.5 mostly 3.13 to 6.25 of EM, and less than 0.05 of OFLX. Except PLB, there was no resistant *V. cholerae* strain. The sensitivity pattern was characterized by a narrow inhibitory concentration ranges of each drug. There was no variability depending on the place of isolation (Table 1). *V. cholerae* O139 were resistant to PLB like *V. cholerae* O1 El Tor with the MICs more than 25 μg/ml, mostly 100μg/ml.

Shigella species: Being diffrent from *V. cholerae* most isolates of *Shigella* species were highly resistant to TC and ABPC. However, the sensitivity pattern is different from the place to place of isolation (Table 2). The isolates of Japan were relatively sensitive to these traditional antibiotics. The Indonesian isolates have a tendency to become resistant even against OFLX, and actually there was one strain with the MIC of OFLX at 100 μg/ml. In these sensitivity pattern, a new oral cephem CFDN has a considerably good anti-*Shigella* activity. Visual impression of these different sensitivities is shown in Figure 1.

MIC_{50} and MIC_{90} account for the variety of drug sensitivity of *Shigella* and the resembling sensitivity of *V. cholerae* (Table 3).

DISCUSSION

Results of the present studies revealed that recently isolated cholera vibrios have almost the same drug sensitivity pattern to *V. cholerae* O1 isolated in the past 3 decades (Iwanaga *et al.*, 1982; Iwanaga *et al.*, 1979). Although *V. cholerae* O139, a new cholera vibrio, is known to be resistant to co-trimoxazole (Yam *et al.*, 1994), there must be no difficulties in the antimicrobial therapy for cholera at present. No resistant *V. cholerae* strain against the traditional antibiotics was found in this study. The sensitivity of *V. cholerae* O139 against PLB was different from the previous report by Sarkar et al. in which they described that the MICs were 15 to 50 μg/ml (Sarkar *et al.*, 1993).

On the contrary, *Shigella* species showed a variety of drug sensitivities as being expected. It is interesting that the isolates of Japan are relatively more sensitive to tetracycline and ampicillin than those of the other countries. Japanese isolates were obtained from the epidemics in two separated areas of Okinawa. The cases were indigenous and not imported ones. The consumption rate of tetracycline and ampicillin in Japan supposed to be lower than in the other countries. This may be one of the reasons why Japanese isolates are relatively sensitive to these drugs. The resistant rate of *Shigella* against these traditional antibiotics in the recent may have reached maximum, because this kind of sensitivity pattern has been constantly seen in the past decades (Smollan and Block, 1990; Voogd *et al.*, 1992). We can not account for the reason why Indonesian isolates have a tendency to be resistant to the new antimicrobials such as cefdinir and ofloxacin. Ofloxacin, (a new quinolone) and cefdinir (a new oral cephem) are excellent to inhibit the growth of *Shigella*. but ofloxacin is not preferable to use in the pediatric cases and cefdinir is expensive. Erythromycin (macrolide) is basically not effective to inhibit the growth of enterobacteriaceae. Actually, high MICs against *Shigella* are shown in the present study. However, there were many facts in the past that erythromycin was effective for the treatment of shigellosis regardless the drug sensitivities (Saito *et al.*, 1964; Ukai *et al.*, 1965). Recently, moreover, it is widely recognized that erythromycin is effective to panbronchiolitis infected with *Pseudomonas aerginosa* (Unertl *et al.*, 1986). Therefore, it may be beneficial to use erythromycin for diarrheal diseases due to bacterial enteritis. Erythromycin is the first choice of drug for *Campylobacter* diarrhea and is known to be effective to *Vibrio* diarrhea (Burans *et al.*, 1989; Kobari *et al.*, 1967). If eryhromycin is effective to shigellosis, there is a good possibility that it is also effective to *E.coli* diarrhea. Clinical trials and the study to clarify the mechanism of erythromycin activities are required.

This study was supported by a grant-in aid for scientific research from the Ministry of Education,Science,and Culture of Japan (No. 07922097).

REFERENCES

1) Burans, J.P., Podgore, J., Mansour, M.M., Farah, A.H., Abbas, S., Abu-Elyazeed, R. and Woody, J. N. (1989): Comparative trial of erythromycin and sulphatrimethoprim in the treatment of tetracycline-resistant *Vibrio cholerae* O1, Trans R. Soc. Trop. Med. Hyg., 83, 836-838
2) Carlson, J. R., Thornton, S.A., DuPont, H.L.,West, A.H. and Mathewson, J.J. (1983): Comparative in vitro activities of ten antimicrobial agents against bacterial enteropathogens, Antimicrob. Agents. Chemother., 24,509-13
3) Glass, R.I., Hug, I., Alim, A.R.M.A. and Yunus, M. (1980): Emergence of Multiply Antibiotic-Resistant *Vibrio cholerae* in Bangladesh, J. Infecti. Dis., 142,939-942

Table 1 MICs of 5 antimicrobial agents against *V. cholerae* O1 and O139

V.cholerae O1

Argentina	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	40	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	0	0	0	0
0.4	0	40	0	0	0
0.8	0	0	0	0	0
1.6	0	0	0	0	0
3.13	0	0	13	0	1
6.25	0	0	27	0	39
12.5	0	0	0	0	0
25	0	0	0	0	0
50	0	0	0	0	0
100	0	0	0	0	0
100<	40	0	0	0	0
total	40	40	40	40	40

Indonesia	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	76	0
0.05	0	0	0	2	0
0.1	0	0	0	0	0
0.2	0	15	0	0	0
0.4	0	63	0	0	1
0.8	0	0	0	0	1
1.6	0	0	0	0	0
3.13	0	0	8	0	16
6.25	0	0	61	0	59
12.5	0	0	9	0	1
25	2	0	0	0	0
50	10	0	0	0	0
100	27	0	0	0	0
100<	39	0	0	0	0
total	78	78	78	78	78

Laos	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	41	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	4	0	0	0
0.4	0	37	0	0	0
0.8	0	0	0	0	0
1.6	0	0	0	0	0
3.13	0	0	2	0	8
6.25	1	0	39	0	33
12.5	0	0	0	0	0
25	0	0	0	0	0
50	1	0	0	0	0
100	37	0	0	0	0
100<	2	0	0	0	0
total	41	41	41	41	41

V.cholerae O139

India	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	8	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	8	0	0	0
0.4	0	0	0	0	0
0.8	0	0	0	0	1
1.6	0	0	0	0	0
3.13	0	0	8	0	7
6.25	0	0	0	0	0
12.5	0	0	0	0	0
25	1	0	0	0	0
50	0	0	0	0	0
100	7	0	0	0	0
100<	0	0	0	0	0
total	8	8	8	8	8

Bangladesh	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	13	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	0	12	0	0	0
0.4	0	1	0	0	0
0.8	0	0	0	0	1
1.6	0	0	1	0	4
3.13	0	0	11	0	8
6.25	0	0	1	0	0
12.5	0	0	0	0	0
25	0	0	0	0	0
50	2	0	0	0	0
100	11	0	0	0	0
100<	0	0	0	0	0
total	13	13	13	13	13

Thailand	PLB	TC	ABPC	OFLX	EM
0.025≧	0	0	0	31	0
0.05	0	0	0	0	0
0.1	0	1	0	0	0
0.2	0	29	0	0	1
0.4	0	1	0	0	1
0.8	0	0	0	0	0
1.6	0	0	3	0	7
3.13	0	0	28	0	20
6.25	0	0	0	0	2
12.5	0	0	0	0	0
25	1	0	0	0	0
50	9	0	0	0	0
100	20	0	0	0	0
100<	1	0	0	0	0
total	31	31	31	31	31

Table 2 MICs of 5 antimaicrobial agents against *Shigella* spp.

Shigella spp.

Bolivia	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	2	0
0.05	0	0	0	61	0
0.1	37	0	0	5	0
0.2	24	0	0	0	0
0.4	6	0	0	0	0
0.8	0	12	1	0	0
1.6	0	6	8	0	0
3.13	0	1	7	0	0
6.25	1	2	3	0	4
12.5	0	0	0	0	3
25	0	4	1	0	33
50	0	0	1	0	13
100	0	24	24	0	13
100<	0	19	23	0	2
	68	68	68	68	68

Laos	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	10	0
0.1	3	0	0	3	0
0.2	6	0	0	0	0
0.4	4	0	0	0	0
0.8	0	1	1	0	0
1.6	0	0	0	0	0
3.13	0	0	3	0	0
6.25	0	0	3	0	0
12.5	0	0	0	0	0
25	0	0	0	0	5
50	0	6	0	0	4
100	0	5	2	0	3
100<	0	1	4	0	1
	13	13	13	13	13

Dominica	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	17	0
0.1	8	0	0	4	0
0.2	9	2	0	0	0
0.4	4	5	0	0	0
0.8	0	1	0	0	0
1.6	0	1	0	0	0
3.13	0	0	3	0	1
6.25	0	0	2	0	8
12.5	0	0	1	0	6
25	0	0	0	0	2
50	0	4	0	0	3
100	0	5	0	0	1
100<	0	3	15	0	0
	21	21	21	21	21

Kenya	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	2	0
0.05	0	0	0	8	0
0.1	6	0	0	0	0
0.2	4	0	0	0	0
0.4	0	0	0	0	0
0.8	0	2	1	0	0
1.6	0	0	1	0	0
3.13	0	0	0	0	0
6.25	0	0	3	0	0
12.5	0	0	0	0	0
25	0	0	0	0	8
50	0	1	0	0	1
100	0	6	2	0	1
100<	0	1	3	0	0
	10	10	10	10	10

Indonesia	CFDN	TC	ABPC	OFLX	EM
0.025≧	2	0	0	0	0
0.05	0	0	0	15	0
0.1	18	0	0	9	0
0.2	18	0	0	17	0
0.4	4	2	0	4	1
0.8	2	3	3	0	0
1.6	1	1	7	0	1
3.13	0	0	5	0	0
6.25	1	0	6	0	2
12.5	0	0	0	0	7
25	0	4	0	0	19
50	0	1	0	0	11
100	0	24	12	1	4
100<	0	11	13	0	1
	46	46	46	46	46

Japan	CFDN	TC	ABPC	OFLX	EM
0.025≧	0	0	0	0	0
0.05	0	0	0	20	0
0.1	1	0	0	12	0
0.2	28	0	0	0	0
0.4	3	0	0	1	0
0.8	1	8	0	0	0
1.6	0	25	1	0	0
3.13	0	0	26	0	0
6.25	0	0	6	0	0
12.5	0	0	0	0	0
25	0	0	0	0	1
50	0	0	0	0	8
100	0	0	0	0	24
100<	0	0	0	0	0
	33	33	33	33	33

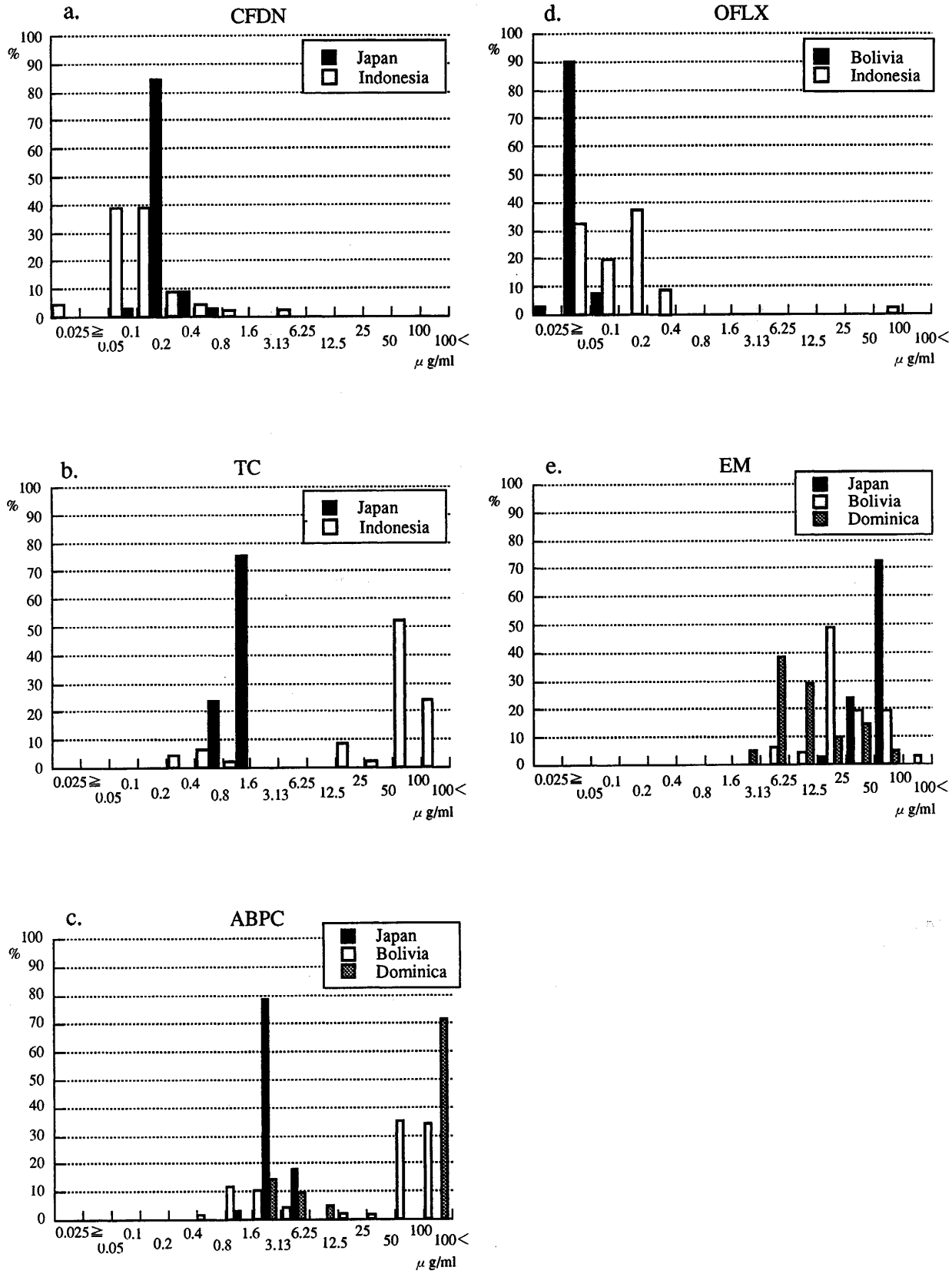

Figure 1 Visual expression of the sensitivities for *Shigella*

Table 3 Variability and Constancy of MIC

Shigella spp.

		Bolivia	Dominica	Indonesia	Laos	Kenya	Japan
CFDN	MIC_{50}	0.1	0.2	0.2	0.2	0.1	0.2
	MIC_{90}	0.2	0.4	0.4	0.4	0.2	0.4
TC	MIC_{50}	100	50	100	50	100	1.6
	MIC_{90}	100<	100<	100<	100	100	1.6
ABPC	MIC_{50}	100	100<	100	6.25	6.25	3.13
	MIC_{90}	100<	100<	100<	100<	100<	6.25
OFLX	MIC_{50}	0.05	0.05	0.1	0.05	0.05	0.05
	MIC_{90}	0.05	0.1	0.4	0.1	0.05	0.1
EM	MIC_{50}	25	12.5	25	50	25	100
	MIC_{90}	100	50	100	100	50	100

V. cholerae O1 & O139

		Argentina	Indonesia	Laos	India	Bangladesh	Thailand
PLB	MIC_{50}	100<	100	100	100	100	100
	MIC_{90}	100<	100	100	100	100	100
TC	MIC_{50}	0.4	0.4	0.4	0.2	0.2	0.2
	MIC_{90}	0.4	0.4	0.4	0.2	0.2	0.2
ABPC	MIC_{50}	6.25	6.25	6.25	3.13	3.13	3.13
	MIC_{90}	6.25	12.5	6.25	3.13	3.13	3.13
OFLX	MIC_{50}	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧
	MIC_{90}	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧	0.025≧
EM	MIC_{50}	6.25	6.25	6.25	3.13	3.13	3.13
	MIC_{90}	6.25	6.25	6.25	3.13	3.13	3.13

4) Iwanaga, M., Mori, K. and Kaviti, J. N. (1982): *Vibrio cholerae* O1 Isolated in Kenya, J. Clin. Microbiol., 16, 742-743

5) Iwanaga, M., Shigeno, H., Hayashi, T., Utsunomiya, A. and Naito, T. (1979): Drug Sensitivity of El Tor Vibrio, Trop. Med., 21, 11-20

6) Kobari, K., Uylangco, C., Vasco, J., Takahira, Y. and Shimizu, N.(1967): Observations on Cholera Treated Orally and Intravenously with Antibiotics, Bull. Wld. Hlth. Org., 37, 751-762

7) Ramamurthy, T., Pal. A., Pal, S.C. and Nair, G.B. (1992): Taxonomical Implications of the Emergence of High Frequency of Occurrence of 2,4-Diamino-6,7-Diisopropylpteridine-Resistant Strains of *Vibrio cholerae* from Clinical Cases of Cholera in Calcutta, India, J. Clin. Microbiol., 30, 742-743

8) Saito, M., Yamaguchi, T. and Sato, H. (1964): Clinical Use of Erythromycin Estolate against Bacillary Dysentery, J. Jpn. A. Inf. Dis., 38, 327-332

9) Sarkar, B. L., De, S.P., Sircar, B.K., Garg, S., Nair, G.B. and Deb, B.C. (1993): Polymyxin B sensitive strains of *Vibrio cholerae* non-O1 from recent epidemic in India, Lancet, 341, 1090.

10) Smollan, G. and Block, C. (1990): Development of antimicrobial drug resistance among Shigellas isolated at an Israeli hospital from 1977 through 1990, Public Health Rev., 18, 319-327

11) Towner, K. J., Pearson, N.J., Mhalu, F.S. and O'Grady, F. (1980): Resistance to antimicrobial agents of *Vibrio cholerae* El Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania, Bull. Wld. Hlth. Org., 58, 747-751

12) Ukai, S., Hori, K., Yanagishita, T., Takayama, Y., Miyaji, H., Ono,Y. and Okajima, S. (1965): Treatment of Bacillary Dysentery with Erythromycin Estolate (Ilosone), J. Jpn. A. Inf. Dis., 39, 330-335

13) Unertl, K., Lenhart, F.P. and Ruckdeschel, G. (1986): Treatment of bronchopulmonary infections in patients during artificial respiration with imipenem/cilastatin, Immun. Infekt., 14, 229-231

14) Voogd, C. E., Schot, C. S., van Leeuwen, W. J. and van Klingeren, B. (1992): Monitoring of antibiotic resistance in shigellae isolated in The Netherlands 1984-1989, Eur. J. Clin. Microbiol. Infect. Dis., 11, 164-167

15) Yam, W. C., Yuen, K. Y., Wong, S.S. and Que, T.L. (1994): *Vibrio cholerae* O139 susceptible to vibriostatic agent O/129 and co-trimoxazole, Lancet, 344, 404-405

16) Yamamoto, T., Nair, G.B., Albert, M. J., Parodi, C. C. and Takeda, Y. (1995): Survey of In Vitro Susceptibilities of *Vibrio cholerae* O1 and O139 to Antimicrobial Agents, Antimicrob. Agents. Chemother., 39, 241-244