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Minimum inhibitory concentration (MIC) of some antibiotics against *Vibrio cholerae* O139 isolates from Pondicherry

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SUMMARY

The antibiotic susceptibility pattern of *Vibrio cholerae* O139, Bengal, an emerging intestinal pathogen has been determined by the Kirby Bauer technique and the MIC values of some antibiotics against these strains by agar dilution technique. All the strains were susceptible to tetracycline, norfloxacin, ciprofloxacin and a majority was susceptible to gentamicin (95·7%) and nalidixic acid (82·9%). Only 51% were susceptible to cefotaxime and most strains were resistant to furazolidone (95·7%), ampicillin (87·3%) and co-trimoxazole (91·5%). The study shows the importance of judicious use of antibiotics in cholera cases and the need for monitoring the susceptibility status of these strains particularly because of their ability to cause extra-intestinal infections like septicaemia.

INTRODUCTION

Vibrio cholerae O1 was responsible for epidemics and pandemics of cholera for many years. In October 1992, a new serogroup, now designated as *V. cholerae* O139 Bengal, emerged causing outbreaks of cholera in many Asian countries [1–6]. Apart from causing acute gastroenteritis, this organism also has the potential to cause extra-intestinal infections like septicaemia similar to other non O1 *Vibrio* strains [7–8]. Keeping this in view and also the indiscriminate widespread use of antibiotics in various situations particularly in developing countries, we have determined the drug susceptibility pattern of some *V. cholerae* O139 strains and also the minimum inhibitory concentration (MIC) of some antibiotics against these isolates.

MATERIALS AND METHODS

V. cholerae O139 strains isolated from cases of acute gastroenteritis attending the JIPMER hospital, Pondicherry were utilized for the study. A total of 47 *V. cholerae* O139 strains were tested for susceptibility to the following antibiotics by the disk diffusion technique of Kirby and Bauer [9]. MIC of some antibiotics against 24 isolates were determined by the agar dilution method [10].

The following antibiotics were used in determination of MIC: ampicillin, ciprofloxacin, furazolidone, norfloxacin, tetracycline, trimethoprim, sulphamethoxazole and gentamicin. For the susceptibility test using the Kirby Bauer technique, in addition to the above antibiotics (used for MIC study), cefotaxime and nalidixic acid were used. In the latter test, co-trimoxazole disks were used instead of sulphamethoxazole and trimethoprim.

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RESULTS

Susceptibility status

The antibiotic susceptibility pattern of 47 *V. cholerae* O139 isolates by the Kirby Bauer technique is shown in Table 1. All the strains were susceptible to tetracycline, ciprofloxacin and norfloxacin while more

Table 1. *Antibiotic susceptibility pattern of 47 isolates of* V. cholerae *O139 by the Kirby Bauer technique*

Antibiotics	Sensitive *n* (%)*	Intermediate *n* (%)	Resistant *n* (%)
Ampicillin	4 (8·5)	2 (4·3)	41 (87·3)
Ciprofloxacin	47 (100·0)	0 (0·0)	0 (0·0)
Cefotaxime	24 (51·1)	22 (47·0)	1 (2·1)
Co-trimoxazole	4 (8·5)	0 (0·0)	43 (91·5)
Furazolidone	2 (4·3)	0 (0·0)	45 (95·7)
Gentamicin	45 (95·7)	0 (0·0)	2 (4·3)
Nalidixic acid	39 (82·9)	2 (4·3)	6 (12·8)
Norfloxacin	47 (100·0)	0 (0·0)	0 (0·0)
Tetracycline	47 (100·0)	0 (0·0)	0 (0·0)

* (%) of 47 tested.

than 80% of the strains were resistant to ampicillin, furazolidone and co-trimoxazole.

MIC values

The MIC values of the antibiotics tested against 24 isolates of *V. cholerae* O139 is shown in Figure 1. The MIC value of tetracycline was 0·5 μg/ml and those of ciprofloxacin and norfloxacin were in the ranges $< 0{\cdot}0005$–$0{\cdot}002$ μg/ml and $< 0{\cdot}004$–1 μg/ml respectively. The MIC values of ampicillin, furazolidone, trimethoprim and sulphamethoxazole were in the ranges 32–128 μg/ml, $< 0{\cdot}5$–> 16 μg/ml, < 4–128 μg/ml and < 1–16 μg/ml respectively. The MIC value of gentamicin was in the range 1–16 μg/ml. Determination of resistance as per the MIC values is shown in Table 2. All the strains were resistant to ampicillin, while 91·6 and 83·3% of strains were resistant to trimethoprim and sulphamethoxazole respectively.

Multiple drug resistance

The patterns of multiple drug resistance by the two techniques are shown in Tables 3 and 4. About 75% of the strains were resistant to three or more drugs by either of the techniques.

DISCUSSION

In the treatment of cholera, apart from rehydration, appropriate antibiotic therapy has been found effective in reducing stool volume, duration of diarrhoea and excretion of vibrio. This reduces the intravenous fluid requirement enabling maintenance on oral rehydration and also shortening the hospital stay [11]. In the current study, all strains of *V. cholerae* O139 were susceptible to tetracycline, the drug of choice in the treatment of cholera. However, it cannot be prescribed to certain groups of patients like pregnant women and young children, even if the organisms are susceptible. Earlier studies reported a high degree of resistance to co-trimoxazole, furazolidone and streptomycin by *V. cholerae* O139 [1, 2, 12]. However, in the present study, apart from resistance to co-trimoxazole and furazolidone, a high degree of resistance to ampicillin was also observed. Ampicillin resistance, although reported earlier, was only at a very low level [12]. Ciprofloxacin and norfloxacin are effective but should be utilized with care, particularly because the MIC of norfloxacin is slowly on the increase. This was evidenced from the MIC value for the isolates in the year 1995 being 1 μg/ml as compared to $< 0{\cdot}004$ μg/ml for the isolates in the year 1992–3. Besides the proportion (%) of the isolates showing multiple drug resistance (three or more) also increased in the year 1995 compared to 1992–3, indicating the increased tendency to acquire multiple drug resistance with time due to antibiotic pressure. Moreover, the drug susceptibility pattern of *V. cholerae* O139 has a bearing on *V. cholerae* O1 isolates as shown in a study from India. It was shown that *V. cholerae* O1 isolated after the epidemic of *V. cholerae* O139 had an expanding R type with resistance to variety of drugs as compared to the O1 strains isolated before the advent of *V. cholerae* O139 [13].

Thus the study shows the importance of monitoring drug susceptibility status of strains isolated in endemic localities. This is useful for epidemiological surveillance and for the management of cases with systemic infection.

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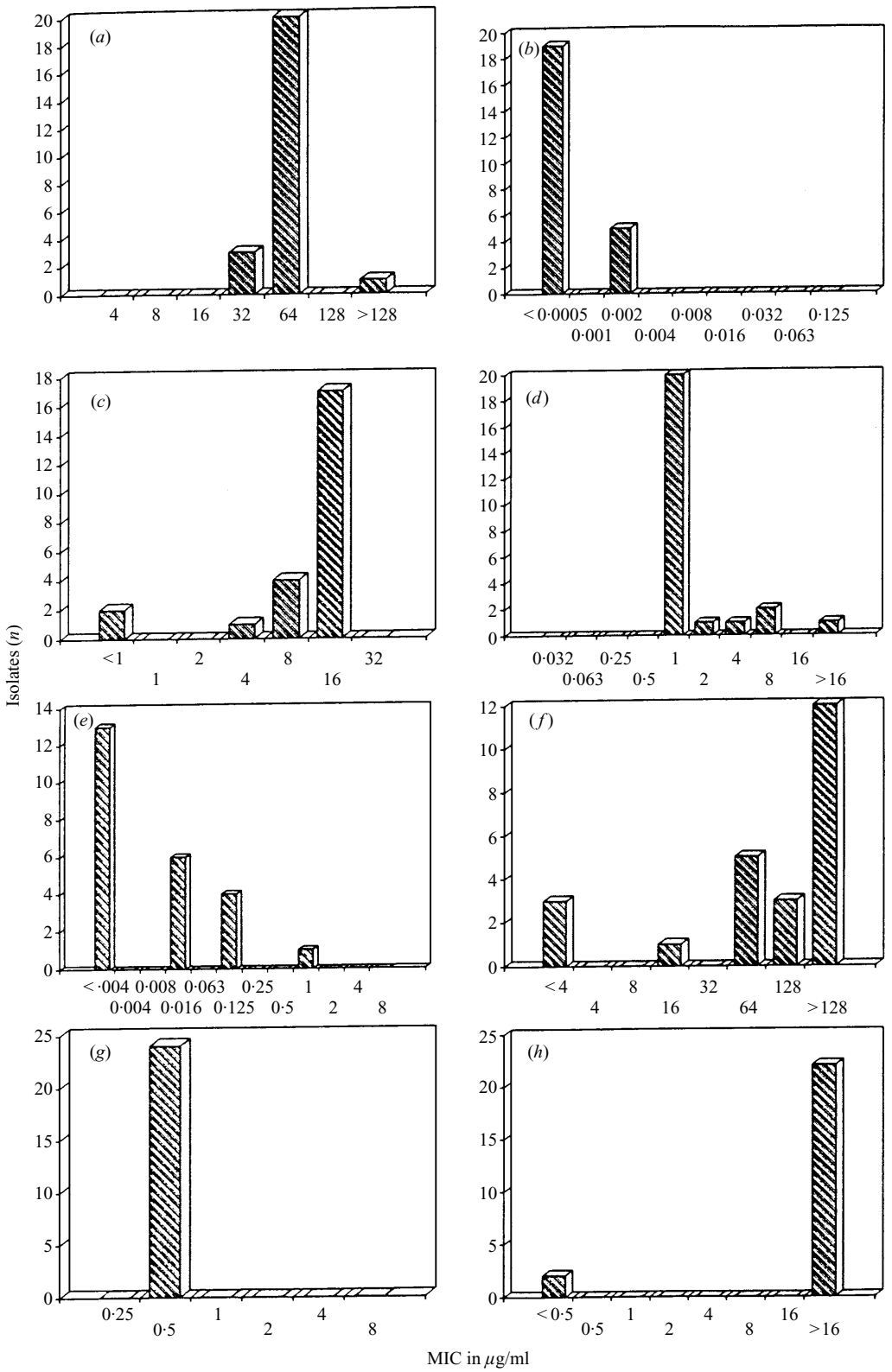

Fig. 1. Number of isolates (*x* axis) with different MIC values of the antibiotics (*y* axis) in alphabetical order. (*a*) Ampicillin (PSC = 10 μg/ml); (*b*) ciprofloxacin (PSC = 4 μg/ml); (*c*) furazolidone; (*d*) gentamicin (PSC = 8 μg/ml); (*e*) norfloxacin (PSC = 6 μg/ml); (*f*) sulphamethoxazole (PSC = 40 μg/ml); (*g*) tetracycline (PSC = 4 μg/ml); (*h*) trimethoprim (PSC = 2 μg/ml).

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Table 2. *Number* (%) *of* V. cholerae *O139 strains showing resistance to different antibiotics as per the MIC values in comparison to peak serum concentration* (*PSC*)

Antibiotics	PSC*	Strains (*n*) MIC < PSC	MIC > PSC	Resistance (%)
Ampicillin	10	0	24	100·0
Ciprofloxacin	4	24	0	0·0
Gentamicin	8	23	1	4·0
Norfloxacin	6	24	0	0·0
Tetracycline	4	24	0	0·0
Trimethoprim	2	2	22	91·6
Sulphamethoxazole	40	4	20	83·3

* PSC, peak serum concentration levels.

Table 3. *Number of strains showing resistance to one or more drugs by the Kirby Bauer technique*

Antibiotics*	Number ($n = 47$)	Percentage (of *n*)
CO	2	4·3
CO, FX	4	8·5
A, FX	4	8·5
A, CO	2	4·3
A, CO, FX	28	59·6
A, CO, FX, G	2	4·3
A, CO, FX, NA	4	8·5
A, CO, FX, NA, CE	1	2·1

* Co, co-trimoxazole; FX, furazolidone; A, ampicillin; G, gentamicin; NA, nalidixic acid; CE, cefotaxime.

Table 4. *Number of strains showing resistance to one or more drugs as per agar dilution technique*

Antibiotics*	Number ($n = 24$)	Percentage (of *n*)
A	2	8·33
A, T	2	8·33
A, T, S	19	79·16
A, T, S, G	1	4·16

* A, ampicillin; T, trimethoprim; S, sulphamethoxazole; G, gentamicin.

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