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Resistance to ceftriaxone in *Vibrio cholerae*

Sir,

Cholera characterized by rice water stools is more common in developing countries due to poor sanitation and improperly protected water supply[1]. As with most enteric pathogens cholera can be controlled by rehydration alone but antimicrobials are indicated in severe cases and also to reduce the course of disease. Spread of cholera epidemics has been associated with emergence of drug resistant strains[2]. The current therapy for cholera as indicated by the World Health Organization[3] primarily involves tetracycline and ciprofloxacin. Drug resistance in bacterial pathogens has increased at an alarming rate over the last few decades. Progressive drug resistance in *Vibrio cholerae* has been noted as evident from the available literature[4-8]. Though the use of a third generation cephalosporin is not yet recommended for the treatment of cholera, but the appearance of resistance to this group of antimicrobials is quite alarming[9,10]. Here, we report the resistance to ceftriaxone, a third generation cephalosporin, in *V. cholerae* isolates from three paediatrics cases.

The three cases (age range two boys of 1 and 4 yr and one 2 yr girl) had reported to the department of Pediatrics between the months of June and August 2010 at Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry, with history of passing loose, watery stools (>9-10 time in a day) and were provisionally diagnosed to be suffering from cholera. The cases reported to the hospital on different months, independent of each other. Also, they did not belong to the same locality and were unrelated.

The stool samples were inoculated onto MacConkey agar and thiosulphate citrate bile-salt sucrose (TCBS) agar (Hi-media, Mumbai). Enrichment was provided with alkaline peptone water, subculture from which was done after 6 h of incubation at 37°C onto MacConkey agar and TCBS agar. Suspected colonies were identified as *V. cholerae* by the standard biochemical tests[11,12], the serogroup was confirmed by agglutination with specific antiserum (BD Difco™, Becton Dickinson, USA). Antibiotic susceptibility was determined by the Kirby-Bauer method as per Clinical Laboratory Standards Institute guidelines (CLSI)[13], which showed that this isolate was resistant to ampicillin (10 µg), ceftriaxone (30 µg), co-trimoxazole (25 µg) and sensitive only to ciprofloxacin (5 µg) and tetracycline (30 µg). All the three children were treated with ciprofloxacin.

The three isolates were subjected to the agar dilution test and E test (BioMérieux, Sweden) for the determination of the minimum inhibitory concentration (MIC) of ceftriaxone. For the agar dilution method, ceftriaxone-sodium salt (Hi-media, Mumbai, India) and Mueller-Hinton (M-H) agar (Hi-media, Mumbai, India) were used. The drug was reconstituted in sterile distilled water (as described by the manufacturer), was stored at 4°C and used within 2 days of reconstitution. Different dilutions of the drug were used starting from 0.5 to 128 µg/ml. For growth controls, plates containing drug free agar were also prepared. The MIC was performed as per recommendations[13]. ATCC *Escherichia coli* 25922 was inoculated on each plate as growth control. To check for viability of each test isolate and also as an added check for purity, control plates that did not contain drugs were inoculated last. Inoculated plates were allowed to stand for several minutes until the inoculum drops had been completely absorbed by the medium; then the plates were inverted and incubated at 37°C for 20 h before the results were read. The E-test was carried out as per the manufacturer's instructions. The strips containing the antibiotics were applied with sterile forceps and incubated at 37°C overnight. Tear drop shaped zone of inhibition was observed in all

three agar plates. MIC was read as the value on the E strip where the two arms of the ellipse met. According to the CLSI[13], the breakpoints for MIC of cefrtiaxone are sensitive (<1 µg/ml), intermediate resistant (2 µg/ml) and resistant (>4 µg/ml). The MIC of the three *V. cholerae* isolates was 16 µg/ml, by the agar dilution method as well as the E test method, which indicated resistance to ceftriaxone. The double disk synergy test was performed as described earlier[14] which was negative. As the genetic analysis of these isolates could not be done, therefore the mechanisms mediating the reduced susceptibility of these isolates to ceftriaxone is not yet clear.

Increase in antimicrobial resistance in enteric pathogens is especially important in developing countries where diarrhoea is common. Globally, many strains of *V. cholerae* have shown resistance to commonly used antibiotics including tetracycline, co-trimoxazole, ampicllin, chloramphenicol and nalidixic acid[10] but have largely remained susceptible to the third generation cephalosporins. Resistance to cephalosporins was first documented in Argentina[10] where the MIC of ceftazidime was determined to be 64 µg/ml.

The detection of resistance to ceftriaxone in *V. cholerae* is a matter of concern, considering the epidemic potential of the organism. Excessive use and misuse of antimicrobials can create an effective selection pressure on microbes which in turn can develop mechanisms to render these antimicrobials ineffective. There is a need to monitor the resistance pattern of this organism and also the use of antimicrobials in its control.

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