Dutta, N., Panse, M. & Jhala, H. (1963) *Brit. med. J.*, **1**, 1200-1203

Ewing, W. H., Davis, B. R. & Martin, W. J. (1966) *Outline of methods for the isolation and identification of V. cholerae*, Atlanta, Ga., US Department of Health, Education, and Welfare

King, E. (1962) *Ann. N. Y. Acad. Sci.*, **98**, 700-711

McIntyre, O. R. & Feeley, J. C. (1965) *Bull. Wld Hlth Org.*, **32**, 627-632

McIntyre, O. R., Feeley, J. C., Greenough, W. B., Benenson, A., Hassan, S. & Saad, A. (1965) *Amer. J. trop. Med. Hyg.*, **14**, 412-418

Mandel, A. & Ellison, R. (1963) *J. Amer. med. Ass.*, **185**, 536-538

Neogy, K. N. & Sanyal, S. N. (1969) *Bull. Wld Hlth Org.*, **40**, 329-330

Sakazaki, R., Iwanami, S. & Fukumi, H. (1963) *Jap. J. med. Sci. Biol.*, **16**, 161-188

Smith, H. (1958) *Bact. Proc.*, p. 69

Drug-sensitivity of *Vibrio parahaemolyticus* isolated in Calcutta during 1969

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Diarrhoea caused by *Vibrio parahaemolyticus*, first recognized in Calcutta in 1968 (Chatterjee, Gorbach & Neogy, 1970), continues to be a major problem there. The pathogen has been characterized and the clinical symptomatology defined. It is well known that the result of *in vitro* testing of the drug-sensitivity of organisms has relevance to the outcome of treatment. The present investigation was therefore planned to evaluate the effectiveness of different antimicrobials on *V. parahaemolyticus* by two standard methods.

Materials and methods

Strains. The organisms used in the present experiment were Gram-negative motile rods; they were oxidase-positive, fermentative, acid-producers without gas in glucose, lysine-decarboxylase-positive but arginine-dihydrolase-negative. Also, the strains were Voges-Proskauer-negative, belonged to Heiberg group VII and conformed to the halophilic property of *V. parahaemolyticus* as described by Chatterjee, Gorbach & Neogy (1970) and Chatterjee, Neogy & Gorbach (1970). All strains were isolated from cases of diarrhoea in Calcutta during 1969.

Agar-diffusion technique, using drug-impregnated discs. Plates of Mueller–Hinton (Difco) medium (without addition of NaCl) were flooded with a 3-hour broth culture of the organism; excess fluid was pipetted off and the surface was dried in an incubator. Multodisks (Oxoid) containing chloramphenicol (10 μg), tetracycline (10 μg), streptomycin (10 μg), kanamycin (5 μg), ampicillin (25 μg), and 50-IU polymyxin B (Difco) discs and furazolidone (100 μg; Smith Kline & French), and colistin (10 μg; BBL) discs were placed on the inoculated medium. Prediffusion for half an hour was allowed in the refrigerator before the plates were incubated overnight at 37°C.

Tube-dilution technique. The sensitivity of 18 strains of *V. parahaemolyticus* to chloramphenicol, (Parke, Davis & Co.), tetracycline hydrochloride (Cynamid India Ltd.) and streptomycin sulfate (Squibb) was studied by the tube-dilution technique with water-soluble powders. Nutrient broth (Difco) with 2.5% additional sodium chloride at pH 7.6 served as the medium in which the respective antibiotics were serially diluted in the following concentrations (μg/ml): 0.1, 0.5, 1.0, 2.5, 5, 6, 7, 8, 9, 10, 15, 20 and 25. The series of tubes with graded concentrations of antibiotic and a control tube containing no antibiotic were inoculated with 1 loopful (3 mm diameter) of young culture. The tubes were shaken well and 1 loopful from the control tube was cultured immediately on nutrient agar. After overnight incubation, the tube having the lowest concentration of antibiotic showing no visible growth represented the minimal inhibitory concentration (MIC). Subcultures were done on to agar plates

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from the tubes showing no visible growth. After overnight incubation of the plates, the lowest concentration of an antibiotic yielding no growth was taken as the bactericidal concentration.

Results

Out of 20 strains of *V. parahaemolyticus* whose drug-sensitivity was determined by the agar-diffusion technique with antimicrobial discs all without exception were sensitive to chloramphenicol, tetracycline, streptomycin, kanamycin, polymyxin B, colistin and furazolidone discs but resistant to ampicillin. The zones of inhibition varied from about 8 mm to 15 mm, except for kanamycin and colistin with which they were a little more than 3 mm.

The minimal inhibitory concentrations of the 18 strains tested by the tube-dilution technique were as follows: chloramphenicol—0.5 μg/ml, 8 strains, 1.0 μg/ml, 9 strains, 2.5 μg/ml, 1 strain; tetracycline hydrochloride—2.5 μg/ml, 3 strains, 5 μg/ml, 14 strains, 6 μg/ml, 1 strain; streptomycin sulfate—15 μg/ml, 15 strains, 20 μg/ml, 2 strains, 25 μg/ml, 1 strain. The range of bactericidal concentrations varied from 1 μg/ml to 2.5 μg/ml for chloramphenicol, from 5 μg/ml to 7 μg/ml for tetracycline hydrochloride, and from 15 μg/ml to 20 μg/ml for streptomycin sulfate.

Discussion

The antimicrobial sensitivity of *V. parahaemolyticus* as evaluated by the two popular methods appears to be consistent with respect to each drug. The results of disc sensitivity tests with chloramphenicol, tetracycline, streptomycin, kanamycin, polymyxin B and ampicillin are the same as those with 13 strains of this pathogen isolated during the year 1968 (Chatterjee, Neogy & Gorbach, 1970).

It is interesting to record that, in the same geographical area, the ranges of minimal inhibitory concentrations of chloramphenicol and tetracycline to *V. parahaemolyticus* are comparable to those to the El Tor vibrio, the organism responsible for cholera in Calcutta during the past few years (Prescott et al., 1968). In the present study, sensitivity to chloramphenicol and streptomycin was assessed by popular methods, since these drugs in combination are available as proprietary preparations which are widely used in Calcutta by clinicians for treating suspected cases of bacillary dysentery, many of which may be due to *V. parahaemolyticus*.

In the light of the present observations, it seems worth while to undertake clinical trials with tetracycline and furazolidone for cases of *V. parahaemolyticus* diarrhoea. However, in paediatric cases furazolidone alone may serve as the drug of choice, in view of the toxicity and side-effects of tetracycline (staining of deciduous teeth and stunting of bony growth) in the age-group involved (Davidson, 1966).

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REFERENCES

Chatterjee, B. D., Gorbach, S. L. & Neogy, K. N. (1970) *Bull. Wld Hlth Org.*, **42**, 460-463

Chatterjee, B. D., Neogy, K. N. & Gorbach, S. L. (1970) *Indian J. med. Res.*, **58**, 235-239.

Davidson, S., ed. (1966) *The practice and principles of medicine*, 8th ed., Edinburgh, Livingstone, p. 83

Prescott, L. M., Datta, A. & Datta, G. C. (1968) *Bull. Wld Hlth Org.*, **39**, 967-970