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

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Of the 110 consecutive isolates of *Vibrio cholerae* recovered from cholera patients admitted to the Infectious Diseases Hospital, Calcutta, India, between July 1989 and October 1990, 90 and 82.7% were resistant to 10 and 150 μg of 2,4-diamino-6,7-diisopropylpteridine (O/129), respectively. Additionally, all O/129-resistant strains of *V. cholerae* were multiply resistant to antimicrobial agents. Except in the cases of four strains, resistance to O/129 was invariably linked with resistance to co-trimoxazole. Although O/129 susceptibility is still a useful test for *Vibrio* identification, resistance of *V. cholerae* to this compound in local areas might occasionally pose a problem.

Susceptibility of vibrios to the vibriostatic agent 2,4-diamino-6,7-diisopropylpteridine (O/129) has been used as an important taxonomical trait to differentiate between vibrios and other gram-negative bacilli, particularly aeromonads; also, the degree of susceptibility of vibrios to this compound has been useful for intraspecific identification. The usefulness of O/129 susceptibility was originally proposed by Shewan et al. (9); Bain and Shewan later concluded that all O/129-sensitive gram-negative bacilli belonged to *Vibrio* spp. (2). The importance of this trait in intergeneric and intraspecific differentiation of members belonging to the family *Vibrionaceae* has been emphasized by several workers. In fact, *Bergey’s Manual of Systematic Bacteriology* (3) lists inhibition by 10 μg and by 150 μg of O/129 as 2 of the 22 important traits that differentiate *Vibrio* species and allied genera likely to be encountered in clinical laboratories.

This paper was presented by one of the authors [G.B.N.] at the closed meeting of the ICSB Subcommittee on the Taxonomy of *Vibionaceae*, 18 September 1990, Osaka, Japan.

Sundaram and Murthy (11) first documented that 1.8% of the human isolates of *Vibrio cholerae* were resistant to O/129 and that all these strains showed resistance to one or more commonly used antibiotics. Subsequently, Matsushita et al. (8) reported that resistance to O/129 was transferable, and mating experiments with O/129-resistant strains of *V. cholerae* O1 revealed that other drug markers, especially trimethoprim, transferred jointly. The cross-resistance to trimethoprim and O/129 was explained as being due to their common chemical structure, namely, the diaminopyrimidine moiety (8). O/129-resistant strains of *V. cholerae* (4) and *Vibrio parahaemolyticus* (6) have now been isolated in several other countries.

Against this background, the present study was conducted to determine the extent and prevalence of O/129-resistant strains of *V. cholerae* isolated from recent clinical cases in Calcutta, India, where cholera is still endemic. A total of 98 consecutive isolates of *V. cholerae* O1 biotype El Tor (49 strains each of Ogawa and Inaba) and 12 strains of *V. cholerae* non-O1 isolated from cholera patients admitted to the Infectious Diseases Hospital, Calcutta, India, between July 1989 and October 1990 were included in this study. Susceptibility to water-soluble O/129 compound (Sigma Chemical Co. [lot 46F-0586]) was determined by the procedure described by West and Colwell (12), which advocates spot inoculation of nutrient agar (Difco) plates containing 10 and 150 μg of the vibriostatic agent per ml. Nutrient agar plates without the test compound served as the medium control. Classical *V. cholerae* O1 (strain 569B) and *Aeromonas hydrophila* were included as the susceptible and resistant controls with every batch of test performed. In addition, the susceptibilities of the test strains to a variety of commonly used antimicrobial agents were examined by the dry-disc diffusion technique (7) with the commercially available discs (Span Diagnostics, Baroda, India) listed in Table 1.

Of the 110 strains of *V. cholerae* examined, 90% were resistant to 10 μg and 82.7% were resistant to both 10 and 150 μg of O/129 compound (Table 1). All of the O/129-resistant strains of *V. cholerae* were multiply resistant to antimicrobial agents (Table 1). The most common resistance pattern of O/129-resistant strains of *V. cholerae* was resistance to ampicillin, chloramphenicol, co-trimoxazole, furazolidone, neomycin, streptomycin, and tetracycline (31.3%), while the common resistance pattern of O/129-susceptible strains of *V. cholerae* was resistance to ampicillin, furazolidone, neomycin, and tetracycline (36.4%). Except in the cases of one strain of *V. cholerae* O1 serotype Ogawa and three strains of *V. cholerae* non-O1, O/129 resistance was always associated with resistance to co-trimoxazole (trimethoprim, 1.25 μg; sulfamethoxazole, 23.75 μg). Intriguingly, one strain of O/129-susceptible *V. cholerae* O1 serotype Ogawa showed resistance to co-trimoxazole. A majority of the strains of *V. cholerae* O1 and non-O1 were also resistant to tetracycline, which is the drug of choice for treatment of cholera.

The percentages of isolation of O/129-resistant strains of *V. cholerae* and *V. parahaemolyticus* reported previously (6, 8, 11) were very low, ranging between 0.02 and 1.8%, and therefore did not warrant attention with regard to the value...
of this trait in the identification of vibrios and allied genera. However, in this study, a majority of the recent isolates of *V. cholerae* demonstrated resistance to O129. Acquired resistance to O129 may occasionally complicate an identification but should not be a particular problem for most species and in most areas of the world. We have reason to believe that O129 resistance among vibrios is increasing in other parts of India also. An interesting report from southern India indicates that within a span of 4 months, resistance of *V. cholerae* O1 El Tor (Ogawa) to co-trimoxazole increased from 4.5% in July 1987 to 81.5% in October 1987 and continued to increase thereafter (5). Although O129 resistance of *V. cholerae* was not monitored in the study mentioned above, it is clear that most of the co-trimoxazole-resistant strains would also be resistant to O129. Trimethoprim, in association with sulfonamides, is one of the most commonly used antibiotics because of its broad spectrum of activity, marked synergic effect on both sulfonamide-susceptible and sulfonamide-resistant strains, excellent clinical results, and very low price (1). The indiscriminate usage of co-trimoxazole has, however, led to the emergence of bacterial resistance.

Studies of the genetic basis of resistance to trimethoprim and O129 in clinical isolates of *Vibrio* has shown that both plasmids and transpositions contribute to the dissemination of this type of resistance (4). It is unlikely that resistance to O129 would revert in the case of transposon-mediated resistance to this compound, because transposons which integrate into the host chromosome are stably inherited. In view of the present finding, we would like to caution investigators attempting to interpret data on the susceptibility of vibrios to the vibriostatic agent O129. It should, however, be mentioned that O129 is not frequently used in diagnostic laboratories. Outside the foci of cholera endemicity, this test is not of sufficient importance to warrant keeping the chemical on hand. Neither is O129 much used for identification of *V. cholerae* per se in areas of endemicity, since colonial and microscopic morphology, oxidase testing, and antibody reactivity are used for identification. Thus, while information regarding O129 susceptibility is of importance for taxonomy and for general information, O129 resistance is not likely to interfere with clinical diagnosis. Further, this study was addressed only to the El Tor biotype and non-O1 *V. cholerae*, which are presently prevalent in Calcutta. It would be interesting to see whether the multiple-drug-resistant strains, including those resistant to co-trimoxazole (98.1% of strains were resistant), of the classical biotype of *V. cholerae* O1 currently being isolated from southern Bangladesh (10) are also resistant to O129.

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**REFERENCES**


<table>
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<th><em>V. cholerae</em> serotype</th>
<th>No. of strains tested</th>
<th>O129 (μg/ml)</th>
<th>A (10)</th>
<th>C (30)</th>
<th>Ct (25)</th>
<th>Fz (50)</th>
<th>G (10)</th>
<th>N (30)</th>
<th>Na (30)</th>
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* A, ampicillin; C, chloramphenicol; Ct, co-trimoxazole; Fz, furazolidone; G, gentamicin; N, neomycin; Na, nalidixic acid; S, streptomycin; T, tetracycline. Numbers in parentheses are disc drug amounts in micrograms.