VARIATION IN POLYMYXIN SENSITIVITY AMONG COLONIES IN PRIMARY PLATE CULTURES OF VIBRIO ELTOR

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Resistance to polymyxin B and cholera group-IV phage, and the ability to agglutinate chicken RBC, are important characteristics employed in differentiating the Vibrio cholerae biotype El Tor (V. eltor) from classical V. cholerae (Felsenfeld, 1966; Barua, 1970). A large number of polymyxin-sensitive variants of El Tor vibrios were isolated at this laboratory during 1971–72 (Pal et al., 1973). In the present study, an attempt has been made to investigate the variation in polymyxin sensitivity and other important characters among colonies in primary plate cultures of V. eltor isolated from stool samples of cases of cholera in Delhi during 1971–72.

MATERIALS AND METHODS

Twenty colonies from each of 27 randomly selected primary plate cultures of cholera vibrios on bile-salt agar were purified, tested for agglutination with polyvalent and monospecific cholera antisera, and studied for sensitivity to polymyxin B and cholera group-IV phage and ability to agglutinate chicken RBC. Polymyxin sensitivity was tested by three methods: (i) the disk-diffusion method (Han and Khie, 1963), each disk containing 50 units of polymyxin B; (ii) the spot culture technique (Roy, Mridha and Mukerjee, 1965) with nutrient-agar plates containing 15 μg per ml; and (iii) the standard turbidimetric method with nutrient broth containing 15 μg per ml of the antibiotic. Sensitivity to group-IV phage was determined by the method of Mukerjee (1963) with phage at the routine test dilution. For the chicken-RBC agglutination test, the method of Finkelstein and Mukerjee (1963) was followed. Authentic strains of classical V. cholerae and V. eltor were included as controls in each test series.

RESULTS

The 27 primary cultures of cholera vibrios on bile-salt agar randomly selected for the study originated from stool samples of clinically accepted cases of cholera admitted to the Infectious Diseases Hospital, Delhi during 1971–72. Twenty individual colonies picked from each of these primary cultures contained cholera vibrios that were agglutinated by polyvalent antiserum and monospecific Ogawa antiserum and were uniformly positive for chicken-RBC agglutination and resistance to cholera group-IV phage. These vibrios would be labelled as V. eltor but they varied in their sensitivity to polymyxin. In 10 of the primary cultures, all the colonies contained vibrios resistant to polymyxin B, and in another nine cultures all the colonies proved to be of organisms sensitive to the antibiotic. The remaining eight primary cultures were found to be mixtures of sensitive, partially sensitive and resistant colonies; in all of these cultures except one, the sensitive colonies predominated. The results of polymyxin-sensitivity tests were uniformly reproducible with all of the three methods used, namely disk diffusion, spot culture and turbidimetric procedures (see Methods). Individual colonies showing sensitivity to polymyxin B were found to retain this characteristic even after repeated subculture. When partially resistant colonies originating from different primary cultures were individually plated and 20 colonies from each of the subcultures were examined, polymyxin-sensitive colonies again predominated.

An attempt was made to compare the level of sensitivity to polymyxin B of six typical

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strains of *V. eltor* and of 14 polymyxin-sensitive variants of El Tor vibrios isolated from Delhi during 1971–72. Four strains of classical *V. cholerae* isolated from Madhya Pradesh in 1970 and six of *V. eltor* from Delhi during 1968–69 were included as controls. The minimum inhibitory concentration (MIC) for 11 of 14 polymyxin-sensitive variants of El Tor vibrios was found to be the same as that for the strains of classical *V. cholerae*, i.e., 2.5 μg per ml. The MIC for the remaining three such strains was only slightly higher, i.e., 3.5 μg per ml. The MIC for six of the typical El Tor strains isolated from Delhi during 1971–72 as well as that for six typical El Tor vibrios from Delhi during 1968–69 was in the range of 45–60 μg per ml. For one strain from each of these two El Tor groups a lower MIC value of 30 μg per ml was obtained.

**DISCUSSION**

It is generally held that *V. eltor* is resistant to polymyxin and that *V. cholerae* is sensitive. The results of the present study have demonstrated heterogeneity with regard to polymyxin sensitivity among colonies in some primary cultures of cholera vibrios otherwise typical of *V. eltor*. In some other such cultures the entire population of vibrios appears to be polymyxin sensitive. Consistency in the polymyxin sensitivity of successive subcultures of individual colonies indicated that the variation was heritable and stable. The MIC of the antibiotic for these polymyxin-sensitive variants of the El Tor biotype was the same as or only slightly higher than that for strains of classical cholera vibrios. The fact that all the individual colonies in the primary cultures examined were found positive for haemagglutination and resistance to cholera group-IV phage irrespective of their pattern of polymyxin sensitivity indicates that these two characters are not linked with resistance to polymyxin B.

**SUMMARY**

Twenty colonies from each of 27 randomly selected primary cultures of cholera vibrios on bile-salt agar were investigated. All of the individual colonies comprised cholera vibrios that would be labelled as *Vibrio eltor* but they varied in their sensitivity to polymyxin. In 10 of the primary cultures, all of the colonies were of vibrios resistant to polymyxin B, and in another nine cultures all the colonies proved to be of organisms sensitive to the antibiotic. The remaining eight primary cultures were found to be mixtures of sensitive, partially sensitive and resistant colonies. The fact that all the individual colonies in the primary cultures examined were found positive for haemagglutination and resistance to cholera group-IV phage irrespective of their pattern of polymyxin sensitivity indicates that these two characters are not linked with resistance to polymyxin B.

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**REFERENCES**


