PLASMID MEDIATED DRUG RESISTANCE IN VIBRIO CHOLERAE 0139 BENGAL

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ABSTRACT

Sixteen strains of *Vibrio cholerae* were isolated from cases of diarrhoea. Out of these, 12 (75%) were identified as *Vibrio cholerae* 0139 synonym Bengal and 4 (25%) as *Vibrio cholerae* El Tor by standard biochemical and serological tests. Modified CAMP reaction in sheep blood agar showed that 0139 produced moderate hemolysis, El Tor produced wider zone of hemolysis whereas Classical *Vibrio cholerae* produced no zone of hemolysis (CAMP negative). Break point minimum inhibitory concentration (MIC) by agar dilution method showed that all 0139 strains were resistant to ampicillin 8 mg/L, streptomycin 1 mg/L, chloramphenicol 8 mg/L, sulphamethoxazole 32 mg/L and trimethoprim 0.3-128 mg/L, 58.3% were sensitive to gentamicin 1 mg/L, and all were sensitive to norfloxacin 1 mg/L and cefotaxime 1 mg/L. Resistance to trimethoprim, sulphamethoxazole, ampicillin and gentamicin in 5 strains could be transferred to *E coli* K-12 by conjugation experiment at a rate of 5×10^{-6} to 4×10^{-3} . Distinct plasmid bands of 35.8 mega daltons could be seen in agarose gel electrophoresis.

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Key words: CAMP test; Drug resistance; Plasmid; *Vibrio cholerae* 0139.

Introduction

V*ibrio cholerae* sero group 0139 emerged in the Indian subcontinent in October 1992 to become the first non-01 *V cholerae* serogroup slated to cause the eighth pandemic of cholera. The 0139 epidemic has been occurring in populations assumed to be largely immune to *V cholerae* 01 and has rapidly spread to many countries in Asia, Europe and the United States [1]. This antigenically novel clone has been designated serogroup 0139 synonym Bengal. Molecular analysis of virulence determinants in clinical isolates suggests that 0139 strains are highly related to El Tor 01 strains [2]. It can cause severe diarrhoea typical of cholera and gastroenteritis [3], and does response similar to that seen with *V cholerae* El Tor. The *V cholerae* 0139 isolates were characterised by production of larger amount of cholera toxin, hemagglutination, weak hemolytic properties, resistance to polymyxin B and resistance to both classical and El Tor specific phages [4]. Unlike other non 01 strains, 0139 strains carry multiple copies of the cholera toxin genetic element and also genes for toxin co-regulated pilus [5]. The virulence factor include presence of pili, lipopolysaccharide (LPS) and O antigen, capsule polysaccharide which are highly antigenic and involved in intestinal colonization process [6,7].

Though most of the studies have been directed at

characterisation of various genetic and virulence factors of 0139 Bengal there is paucity of literature on their drug resistance pattern and plasmid mediated transferability. This study has been undertaken to find the resistance of 0139 Bengal to various drugs and their transferability to susceptible strains which might play a vital role in its epidemiology.

Material and Method

V cholerae were isolated from 16 cases of diarrhoea. They were identified by their standard morphological, cultural and biochemical characters and confirmed by serological methods [8]. Of these 12 were identified as 0139 Bengal and 4 as *V cholerae* El Tor. The antisera were raised in rabbit in our institute by inoculating killed standard 0139 strains [9].

Modified CAMP test : Modified CAMP test [10] was carried out on 5% sheep blood agar against beta toxin producing *Staph aureus*. *V cholerae* and *Staph aureus* were streak inoculated perpendicular to each other at a distance of 3-5 mm without touching. The plates were incubated at 37C under 5% CO₂. After 24 hours, hemolysis was observed. The zone of hemolysis was wider for El Tor than for 0139. There was no hemolysis by classical strains (Fig 1).

Antibiotic resistance : Break point MIC was carried out by agar dilution method on Mueller Hinton agar (MHA) with standard concentration of antibiotics: ampicillin 8 mg/L, norfloxacin 1 mg/L, sulphamethoxazole 32 mg/L and trimethoprim full range 0.3 mg/L to 128 mg/L. The last two antibiotics were tested on MHA with 4% lysed horse blood [11].

Transfer of drug resistance : Transfer of drug resistance was attempted to standard *E coli* K-12 strains. 0139 strains were used

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Fig. 1 : CAMP reaction by *V cholerae* 0139

as donors. Depending on their sensitivity to nalidixic acid 50 mg/L or rifampicin 50 mg/L, the recipients were selected. Transfer was done by conjugation experiment. The trans-conjugates were checked for their antibiotic resistance pattern in the same manner as described above. The frequency of transfer per donor is calculated as follows [12].

$N \times 10^4 / 6 \times 10^7$, where N is number of trans-conjugates grown from 0.1 ml of 1:100 of conjugant mixture.

Agarose gel electrophoresis : The overnight cultures of *V cholerae* 0139 and trans-conjugates were treated with alkaline sodium dodecyl sulfate (SDS) for extraction of plasmid and electrophoresis was carried out in 0.7% agarose gel when distinct bands of plasmid could be seen under the ultra violet light. They were stained by ethidium bromide and photographed [13,14]. A standard *E coli* V517 obtained from IMTECH, Chandigarh was used for comparison of plasmid bands.

Results

The antibiotic resistance pattern showed that all the strains were resistant to ampicillin, chloramphenicol, sulphamethoxazole and trimethoprim. Seven strains were sensitive to gentamicin whereas all were sensitive to norfloxacin and cefotaxime.

In the conjugation experiment the rate of transfer varied from 5×10^{-6} to 4×10^{-5} /donor. The trans-conjugates were checked for their resistance pattern. They were uniformly resistance to sulphamethoxazole 32 mg/L and trimethoprim 128 mg/L and ampicillin 8 mg/L. Only 5 out of 12 strains were resistant to gentamicin 1 mg/L. This indicated that 7 strains transferred drug resistance to three antibiotics and 5 strains transferred drug resistance to all the four antibiotics by means of plasmids. None of the trans-conjugates grew in cefotaxime 1 mg/L, norfloxacin 1 mg/L and chloramphenicol 8 mg/L. Distinct plasmid bands of 35.8 mega daltons seen in agarose gel electrophoresis both in donors and trans-conjugates [Fig 2].

Discussion

The patients were suffering from diarrhoea due to *V cholerae* 0139 strains and *V cholerae* El Tor strains. The presentation of clinical problems were similar. The cluster of cases which occurred at the same time and the fact that *V cholerae* 0139 can survive in fresh and salt water which can be carried by migrant population shows that it has an epidemic potential [1,2,3].

Fig. 2 : Plasmid bands of *V cholerae* 0139.

The morphological and biochemical similarities of *V cholerae* 0139 with classical *V cholerae* poses a lot of diagnostic problems. Without serology it is very difficult to differentiate between the two strains in a standard clinical laboratory. The different identification kits which are available include SMART [6,15], coagglutination [16] etc, depend mainly on serological identification. However in our laboratory, the antisera has been raised in the rabbit by a standard 0139 strain and slide agglutination can be carried out on the isolate without much difficulty.

Modified CAMP reaction is one additional identifying feature, which can be used to advantage for differentiation between classical, El Tor and 0139. If standardized in the laboratory, it is quite helpful.

All the 0139 strains were resistant to trimethoprim and sulphamethoxazole and ampicillin. Five strains were resistant to gentamicin in addition which is a disturbing trend. Plasmid mediated resistance can be another virulence factor in addition to presence of pili, capsular polysaccharide and toxigenicity. Strains with plasmid encoded multidrug resistance including tetracycline appeared in Tanzania in 1977, Dacca in 1981. Though drug resistance is rare in other vibrios, plasmid encoding tetracycline, chloramphenicol and sulphonamide resistance have been found in *V anguillarum* and could be transferred readily to *V cholerae* [17]. Algal blooms where cholera organisms reside, are in fact "giant floating gene pools" in which antibi-

otic resistance, virulence genes and plasmids move about freely between viruses and bacteria [18]. Thus other vibrios may provide a reservoir of plasmids transmissible to human pathogens which share the same aquatic environment.

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