Detection of CTXB Gene by PCR

K S N Ravi Teja

Osmania Medical College

E-mail: ravitejaksn@gmail.com

Objectives

The culture based techniques in isolation of Vibrio cholera have disadvantage like requirement of upto 72hrs for completion ,failure to detect viable but non-culturable (VNBC) cells and some difficulties arising where Vibrio cholerae are outcompeted by other bacteria during enrichment and subculturing steps. Thus trials for detection of ctxb gene using molecular technique like Polymerase Chain Reaction (PCR) were done by us which was rapid, sensitive, highly selective and did not require extensive hands on time. As a preliminary step, we wanted to check wether clinical isolates produced from culture of stool amplified the toxin gene in PCR. We also studied the antibiotic susceptibility patterns of the isolates to check for the emergence of any resistant strain.

Methods

Confirmed cases of Vibrio cholera (using biochemicals) in the months of July and August last year were analysed for the subtype, biotype and serotype they belonged to, their antibiotic susceptibility patterns and for the presence of the ctxb gene in them using PCR .

Results

Fourty cases were reported with Cholera in that period last year and all the isolated strains belonged to the O1 subtype and El tor biotype. Only one strain belonged to the Inaba serotype and all the others were of the Ogawa serotype. There was no new resistance pattern isolated in any strain. All the strains showed uniform pattern of antibiotic susceptibility to doxycycline, tetracycline, ofloxacin, ciprofloxacin, norfloxacin, cefotaxime, ceftriaxone, amikacin, gentamycin, azithromycin and resistance to cotrimoxazole, Polymyxin B, Nitrofurantoin, Furoxone. All the clinical samples yielded positive results for ctxb gene after 35 cycles of amplification. Chromosomal DNA extracted from Vibrio cholera strains was used as the template for PCR & it was found that DNA extracts containing lesser concentration of DNA yield clear bright bands.

Conclusion

The observations in our study imply that all the O1 El tor strains of Vibrio cholera which are most commonly seen possess the cholera enterotoxin. As dilute samples produce more accurate results, standardisation of procedures to extract it directly from stool and from the water bodies (for screening) could be used for early diagnosis and screening of vibrio cholera.