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STUDY OF *VIBRIO CHOLERAE*, ITS TYPES OGAWA, INABA SEROTYPE & *E.COLI* ANTIMICROBIAL SUSCEPTIBILITY IN DELHI REGION, M.V.I.D. HOSPITAL

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ABSTRACT

The study illustrates the role of *Vibrio cholera*, *Escherichia coli* in causing cholera tested from stool samples obtained from hospital and urinary tract infections. Further *V. cholera* was studied on the basis of respective serotype Ogawa or Inaba. Presumptive identification using O1antisera was done. *V. cholera* O1 E1 tor Ogawa serotype remained dominant in Delhi region. Antimicrobial susceptibility for E. coli was done and eight different antibiotics were used for isolates for routine checkup of urinary tract infections. 80-85% urinary tract infections were found to be caused by *E. coli*..

Key words: *Vibrio cholera*, El tor biotype o1, Ogawa & Inaba serotype, E.coli, antimicrobial susceptibility.

INTRODUCTION

Cholera is an acute diarrheal disease caused by *V.cholerae o*1. It is commonly due to the El Tor biotype. Cases range from symptomless to severe infections. Typical cases are characterized by then sudden onset of profuse, effortless, watery diarrhea followed by vomiting, rapid dehydration, muscular cramps, and suppression of urine. Unless there is rapid replacement of fluid and electrolytes, the case fatality may be as high as 30 % -40%.*V.cholerae* colonizes the small intestine and produces an enterotoxin cholera toxin (CT).

Currently, the larger endemic foci of cholera are found in Maharashtra, Tamilnadu, Karnataka, Delhi and Kerala. The disease is known to be highly seasonal starting from April and lasting upto November every year.

In Delhi, different government and private hospitals refer all the suspected cholera cases to M.V.I.D. HOSPITAL for isolation and treatment round the year. Here it is found that the dominant serotype is Ogawa. Emergence of *V. cholerae* O1 serotype Inaba has been reported in many parts of the country.

Epidemiological features- Cholera is both epidemic and endemic disease. The epidemicity and endemicity of a disease will depend on the characteristics of the agent, and those of the system (environment). Global experience has shown that the introduction of cholera can't be prevented, but cholera can create a problem only in areas where sanitation is defective and scarcity of safe drinking water.

Agent factors-

1. Agent- The organism that causes cholera is labeled as *V.cholerae* O1. The term "epidemic strain" has also been used for

these vibrios. NCV/NAG vibrios i.e. non-cholera vibrios(NCV) & non – agglutinating vibrios(NAG) included some species that are pathogenic for humans (e.g., *Vibrio parahaemolytics*) is now recognized to be causing outbreaks of cholera-like diarrhea. Within the O-group 1, two biotypes classical and El tor biotype have been differentiated. Cholera is mostly caused by El tor biotype. It is further classified into 3 Serotypes namely Inaba, Ogawa, and Hikojima.

2. Toxin production- The vibrios multiply in the lumen of small intestine of human & produce an exotoxin. This toxin produces diarrhea through its effect on the adenylate cyclase–cyclic AMP system of mucosal cells of the small intestine. The exotoxin has no effect on any other tissues except the intestinal epithelial cells.
3. Infective material- The immediate sources of infection are the stools and vomit of cases and carriers. Large numbers of vibrios(about 10^{10} vibrios per ml of fluid) are present in the watery stools of cholera patients & an average patient excretes 10-20 liters of fliud.

MATERIALS AND METHODS

Vibrio cholerae in cholera cases – laboratory diagnosis is simple procedures for a trained person in a minimally equipped laboratory as *V. cholerae* is present in large numbers in the stools of such cases, whether mild or severe.

Collection of specimens:

The keystone of success in the laboratory investigation is the proper collection of the sample according to the availability of the transportation and laboratory facilities.

Stool samples which are collected early in the course of the disease especially before the administration of the antimicrobial drug are the most rewarding material. Rectal swabs were collected at the time of admission from all the suspected cholera or acute gastroenteritis patients referred to MVIDH, DELHI in alkaline peptone water.

1. Enrichment in alkaline peptone water:

Vibrio species grow very rapidly in alkaline peptone water and within 6 -8 hours will be present in greater numbers than non-vibrio organisms. Alkaline peptone water can be inoculated with liquid stool, fecal suspension, or a rectal swab. The stool inoculums should not exceed 10 % of the volume of the broth. Incubate the tube with cap loosened at 35 to 37 ^{0} c for 6-8 hours. Subculture one to two lapfuls of alkaline peptone water to thiosulfate citrate bile salt sucrose (TCBS) medium (Figure-1).

2. Inoculation and isolation from TCBS selective agar:
   Inoculate the TCBS plates by streaking. After 18-24 hours of incubation at 35- 37^{0} c, the amount of growth on the TCBS plate is recorded on the data sheets. Colonies suspicious of cholera appeared on TCBS agar as yellow, shiny colonies 2-4 mm in diameter. Using an inoculating needle, lightly touch only the centre of the colony incubated it for 24 hours.

Figure-1. Colonies of *V.cholerae* growing on TCBS media of sample no. 31250 dated 18th June, 2012.

The typical colonies appearing on BSA were confirmed by the standard biochemical and serological procedures with the commercial antisera kits containing polyvalent and monovalent antisera manufactured by Difco

laboratories, USA and denka-seiken co. limited, japan.

Transportation of specimens:
As Vibrio cholera is not likely to survive from more than few hours in stool the specimens are preserved in a holding medium and kept at room temperature.

Cary-Blair medium:
it is a semi solid media for the preserve of specimen for the isolation of salmonella vibrio cholera.

Alkaline peptone water:
The alkaline peptone water is used extensively as a holding as well as enrichment for vibrio cholera. The ph is adjusted to about 8.4. 20gm of powder is suspended in 1 ltr of sterile demineralised water the medium is suspended in 8-10 quantities in screw caped vials or test tubes and autoclave for 15 min at 121 degree Celsius.

TCBS:
It is a selective media for vibrio cholerae.

ABSA (alkaline bile salt agar):
It is widely as non selective growth medium for *V.cholerae.*

Serological identification of v. cholerae O1:
Slide agglutination procedure: agglutination test for vibrio cholerae somatic O antigen is carried out on a clean glass slide and inoculating loop is used to remove the growth from the surface of ABSA. The growth is mixed with the two drops of saline. A small drop of anti serum is added to one of the suspension. A bent inoculating loop may be used to despense small amounts of anti sera if the slide back and forth to observe for agglutination. If the reaction is positive, clumping will appear in 30 sec.

Antimicrobial Susceptibility Of E.Coli::
Normally, urine is sterile. It is usually free of bacteria, viruses and fungi. An infection occurs when tiny organism, usually bacteria from the digestive tract, cling to the opening of urethra and begin to multiply. Most infection arises from one type of bacteria, E.coli which normally live in the colon.

E.coli is gram negative, facultative anaerobic and nonsporulating. Sales are typically rod shaped and are about 2 micrometre long and 0.5 micrometre diameter. It can live on a wide variety of substrate. E.coli use as mixed acid fermentation and anaerobic condition producing lactate, succinate, ethanol and carbon dioxide

Isolation of *E.coli:*
Samples collected in a casualty or as well as in the lab where used for plating on MAC & CLED agar plates and plates where incubated at 37 degree Celsius for 18 to 24 hrs. MAC meium colonies were pink due to lactose fermentation and colonies were circular, moist, and smooth with entire margin and non-Mucoid.

Biochemical test for *E.coli*

Wet mount (hanging drop) preparation:
Test strength was grown in a peptone water for 3 hrs at 37 degree Celsius. A drop of culture was placed on a clean cover slip, which was inverted on a cavity slide. Preparation was prepared was observed under 40x. test strength preparation were found either motile or non motile.

Anti-microbial susceptibility test for E.coli:
The disk method of Kirby-bauer was used for testing of anti-microbial susceptibility of 128 isolated E.coli strengths as per standards laid down by clinical labourity standard institute (CLSI) against 10 anti-microbial agents. This culture was purified on CLED agar medium.

Plates are incubated at 37 degree Celsius for 18 hours. Well-isolated 4-5 colonies were selected and transferred to tryptis soya broth. Bacterial suspension was compared to the 0.5 Mcfarland's turbidity standard. After adjusting the turbidity of the test strain, the suspension was streaked by using sterile cotton swab over the surface of Mueller-Hinton agar plate. The working supply of anti-microbial dish were removed from the refrigerator and applied on the charged surface of mh agar plate. Plates were incubated at 35 degree celcius for 16-18 hrs.

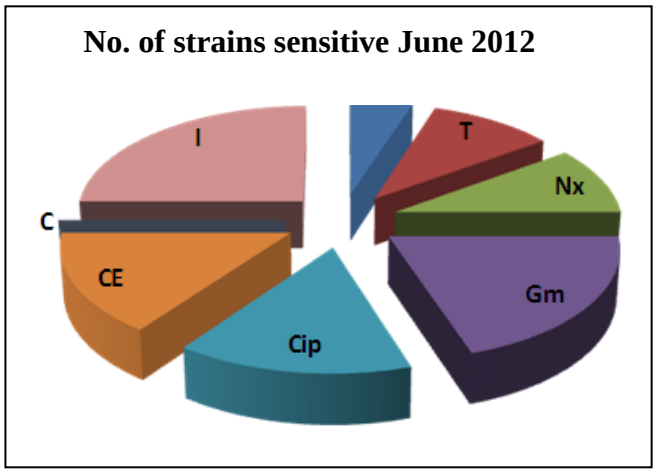
The diameter of the zone of complete inhibition (including the diameter of the disk in mm. records were recorded as susceptible, intermediate or resistant on the basis of zone of inhibition in comparison to CLSI standard.

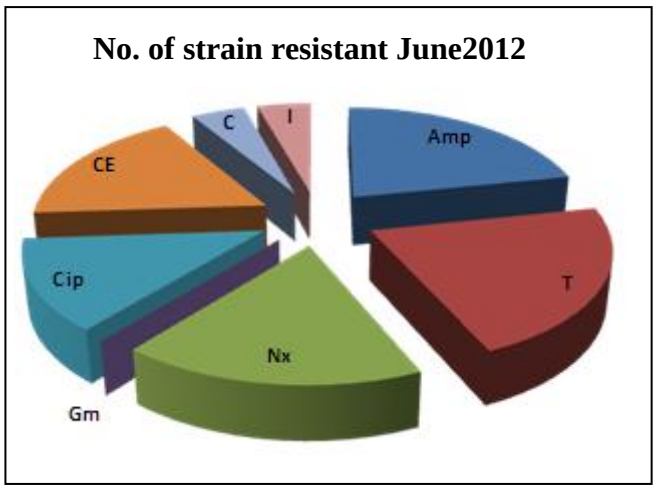
RESULTS AND DISCUSSIONS

V. cholerae:

Presumptive identification using O1 antisera was performed. Also slide agglutination procedure was carried out on glass slide using antisera.positive clumping was appeared for many cases.

Figure-2. Antimicrobial sensitivity of 7 *E.coli* isolates in June, 2012





Note: Amp-Ampicillin; T-Tetracycline; Nx-Norfloxacin; Gm-Gentamycin; Cip-Ciprofloxacin; CE-Cefotaxim; C-Chloramphenicol; I-Imipenem.

V.cholerae O1 El tor serotype Ogawa remained dominant in Delhi. IN month of June 2012, overall detection rate of *V. cholerae* is 29.52%. out of 210 samples, 62 tested positive. Ogawa remained dominant and present as 61 samples, inaba have to be found in only 1 sample. Apart from that a rough stain was obtained. Sample no. 31705 dated 26th June, 2012 was Inaba (Figure-2).

E.coli:

Antimicrobial susceptibility was performed and list of patients were made A/C to their resistance and sensitive nature towards respective antibacterial drug.7 patients were tested positive in M.V.I.D.H. in the month of June, 2012. So further they were given respective doses of antibacterial doses. 80-85% UTIs mainly caused by *E.coli*.

Based on these reports acute cholera stool sample cases admitted in infectious hospitals in Delhi it is being referred to NCDC for their examination by microbiology division for *V. cholera* and daily reports are prepared. On the basis of these daily reports, the cell prepares zone wise weekly reports and forwards them to MCD Delhi and various public awareness programs are conducted for the people where there is maximum chance of this disease.

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Figure-3. Year wise graphical distribution of *V.cholerae* cases in Delhi in MVID hospital