Screening of enteropathogenic microorganisms from domestic waste of Jalna city

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Abstract

The most important waterborne enteropathogens are Salmonella species, Shigella species, Vibrio species and Escherichia coli. These microorganisms are released into sewage from various domestic wastes. In present study screening of these microorganisms are carried out by using different media, Sewage sample is collected from different regions of Jalna city and plated on MacConkeys Agar (MA), Salmonella Shigella (SS) agar, Endo Agar (EA) and Nutrient Agar (NA). Variation in number of bacterial colonies from different locations of Jalna was observed. Most commonly E. coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis and Serretia marcescens was isolated from the entire region. Whereas species of true pathogens of Shigella, Salmonella typhi, S. paratyphi A, B and Vibrio are not reported from any region.

Key Words: Enteropathogen, domestic waste, waterborne.

Introduction:

Enteropathogenic microorganisms causes enteric disease for e.g. members of Salmonella cause typhoid and salmonellosis, members of Shigella cause dysenterie and shigellosis, members of Vibrio causes cholera while several strains of Escherichia coli causes varieties of diseases within intestinal tract. Those strains causes enteric infection are generally called diarrhaegenic E. coli pathotypes (DEPs) (Vidal et al, 2005). From developing countries it is estimated that more than 2.5 million death of infant has been reported per year due to the diarrheal disease with an annual mortality rate of 4.9 per 1000 children and incidence of 3.2 episode per child per year among children under 5 year of age (Kosek et al, 2003). DEPs cause diarrhea include
enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Shiga toxin producing *E. coli* (STEC), Diffusely adherent *E. coli* and enteroaggregative *E. coli* (Nataro JP and Kaper JB, 1998).

Salmonella spreads mainly through stools of human, food borne illness among the people and transmission can occur when food and water are contaminated with stool or through direct fecal-oral route. Human stool act as an important reservoir of *Salmonella* serovars. Species isolated from human stool are *S. typhi, S. paratyphi A, S. typhimurium, S. wrothington* and *S. enteritidis* (Kumar et al, 2009). *Shigella* comprises four serogroups, group A (*S. dysenteriae*), group B (*S. flexneri*), group C (*S. boydii*), and group D (*S. sonnei*). Technically DNA relatedness has shown that they are same species as *E. coli* (Henry et al, 1996).

**Materials and methods:**

**Collection of Samples**

Four different samples from four different regions of Jalna city was collected, one from Mantha square, second from Ambad square, third from Aaurangabad square and fourth from Kanhaya nagar. Samples were collected during Dec. 2015 using buffer treated swab and kept in tubes of transport medium i.e. Carry and Blair, (Edward and Ewing, 1972) and is diluted for $10^{-3}$ time.

**Screening of Enteropathogenic Microorganisms**

Isolation and screening of microorganisms was carried from all the samples from all locations. The diluted swab was inoculated on to different media Viz. MacConkeys Agar, Salmonella Shigella (SS) agar, Endo Agar (EA) and Nutrient Agar (NA) plates. The plates were incubated at $37^\circ$C for 18-24 hours.

**Identification of Microorganism**

Isolated colonies of Microorganisms were picked from each plate and characterized biochemically following standard methods (Edward and Ewing, 1972) and confirm by serogrouping using slide agglutination test.
Result and Discussion:

Cultural Characterization: On SS agar (Hi-Media M108) plate after 24 hours initially colonies developed were slight pinkish in color, some were colorless but on incubation at 30°C for next 24 hours some colonies developed were dark black centered and identified as Salmonella serotype enteritidis.

On MacConkeys agar some colony appears pink, some colorless and some appears white with pink center with moist and mucoid appearance. Pinkness of colony is due to the fermentation of lactose present in the medium and mucoidness is due to exopolysaccharide produced by microorganism.

On endoagar plates some colony were detected dark pink in color and some are faint pink in color, very few colonies were colorless.

On nutrient agar plates, colonies detected were colorless, some are in faint bluish color and measures 1 to 2 mm in detention, circular, convex and butyrous to mucoid in nature. On Grams staining they show Gram negative reaction.

Biochemical characterization

Enteropathogenic E. coli and Aerobacter aerogens were distinguished on the basis of IMViC test. E. coli shows indole and Methyl red (MR) test positive, while negative with Voges-Proskauer (VP) and citrate utilization. Whereas Aerobacter aerogens shows negative test with indole production and MR test, while positive with VP and citrate utilization.

Serratia was confirmed by its production of extracellular DNase activity, Lysine decarboxylation and ornithine decarboxylation. This organism also shows presence of dark red pigment when incubated at 30°C on nutrient agar plates.

Proteus was confirmed by its indole production, fermentation of glucose with acid and gas production but no lactose fermentation.

Pseudomonas was confirmed by fermentation of glucose with acid production and no lactose utilization, and bluish green pigmentation on nutrient agar plates.
Identification of *Klebsiella* was done by their motility test using hanging drop technique where they were found non motile and presence of large amount of exopolysaccharide.

### Screening

Total 150 enteropathogenic microorganisms were isolated from all locations out of this 90 *E. coli*, 30 *Pseudomonas* species, 12 *Serratia* species, 9 *Salmonella* serovar *enteritidis*, 7 *Klebsiella* and 2 *Proteus* species were isolated and identified. During study of these enteropathogenic microorganisms high frequency of enteropathogenic *E. coli* has been detected followed by *Pseudomonas aeruginosa*, then *Serratia marcescens*, then *Klebsiella* species, *Salmonella* serovar *enteritidis* and *Proteus* species. This high demonstration of *E. coli* suggest sporadic disease in many developing countries like India such types of finding shown by Edlman and Levine (1983). True pathogen like *Salmonella typhi* and *paratyphi*, species of *Shigella* and *Vibrio* were not detected from all the regions of city. Frequency of microbial viable population shows variation among regions. Highest number of microorganism were reported from Aurangabad square, followed by Kanhaya Nagar, then Mantha square and finaly Ambad square depicted in table No. 1

**Table No. 1. Total viable count of microorganisms using different media from different location**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Location</th>
<th>Average number of total viable count in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MA</td>
</tr>
<tr>
<td>1</td>
<td>Mantha Square</td>
<td>50.3 x 10^3</td>
</tr>
<tr>
<td>2</td>
<td>Ambad Square</td>
<td>48.6 x 10^3</td>
</tr>
<tr>
<td>3</td>
<td>Aurangabad Square</td>
<td>194 x 10^3</td>
</tr>
<tr>
<td>4</td>
<td>Kanhaya Nagar</td>
<td>176 x 10^3</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>117.22 x 10^3</td>
</tr>
</tbody>
</table>

From these observations and result it was concluded that there is more prevalence of enteropathogenic *E. coli* followed by *Klesiella pneumonia*, and *Salmonella enteritidis* while
presence of true pathogens of *Salmonella, Shigella* and *Vibrio* was not reported during investigation.

References:


