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Studies on Microbial Status and Characteristic features from Polluted Coastal Habits at Visakhapatnam, India

K. Ramesh Babu[a*], V. HimaSailaja[a], K. V. Siva Reddy[a], K. G. Anand[a], G.VijayaPratap[a] and **M. RatnaRaju[b]**

[*, a, b] Department of Marine Living Resources, College of Science and Technology, Andhra University, Visakhapatnam- 530 003
[b]Department of Zoology, Andhra University, Visakhapatnam-530 003.

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Abstract

The current study focused on the microbial status and culture characteristic features from three different polluted sites at coastal area of Visakhapatnam. The eight different harmful pathogens were identified as Escherichia coli Enterococcus species, Shigella species, Salmonella species, Proteus/Klebsiella species, Vibrio species, Faecal Streptococci, and Pseudomonas species. For enumeration of bacterial strains spread plate method was employed. IMViC biochemical tests (Indole, Methyl red, Voges-Proskauer and Citrate tests) were used to assess the characteristic features of bacterial strains. The greater distributions of enteric pathogens were reported from the Visakhapatnam fishing harbour (Station-I) because of indiscriminate discharge of the sewage, industrial effluents and fishing activities. Moderate occurrence was reported at Hindustan shipyard (Station-II), this might be aided by the acidic pH conditions will have profound impacts on the bacterial proliferation. Relatively low enteric pathogens load at offshore station (Bhimili-Station-III) this may be attributed due to variations in the salinity of marine water at offshore regions compared to nearshore waters and also marginal stress from the saline water.

Key words: *Coastal waters, Enteric pathogens, Vibrio cholera and Escherichia coli*

1. Introduction

Microorganisms are cosmopolitan in distribution and may cause greater number of diseases because from ancient period, humans mainly depend on marine resources as their livelihood. Microorganisms play significant biochemical and ecological role in the marine environment by regulating the transformation of major bioactive elements (i.e. carbon, nitrogen, phosphorus, oxygen and sulphur and by affecting the degradability of organic matter [1]. Human health risk is due to contamination of the coastal water bodies by urban waste discharges [2]. Pathogen richness at coastal waters is aided by the faecal matter and vegetable wastes [3, 4]. Pathogens inhabiting in contamination site frequently cause a lot of diseases and sometimes a great threat to the human life [5]. The pathogens generated by contaminated sites can also cause severe diseases in major marine organisms like corals [6, 7] and in fishes [8]. Continuous monitoring of the coastal habits is a major research sector because majority of the peoples depends on coastal environments in different regions of the world. The present study was carried in three different polluted sites at coastal waters of Visakhapatnam for the analysis of microbial status and culture characteristics of bacteria.

2. Materials and methods

2.1Study area and sampling

Visakhapatnam lies on the east coast of India and is midway between Calcutta and Madras latitude 17°38'N and 17°45'N longitude 83°16'50" and 83°21'31''E. It is bordered by Rishikonda and Yarada hills in north and south places respectively. The coast runs roughly north east to south west.

Sampling for the current study was carried out during one year period of time from 2012 to 2013, The three different stations includes, Visakhapatnam fishing harbour (Station-I), Hindustan shipyard (Station-II) and Bhimili (Station-III) along the east coast of Visakhapatnam, India.

Surface water samples were collected from three different stations in 50ml sterile screw capped bottles for bacteriological assessment. Immediately, all the samples were brought to the laboratory of Marine Living Resources Department in Andhra University, upon arrival inoculations were made in specific media for the establishment of the pure cultures of the bacterial strains.

2.2Bacteriological assessment

For enumeration of the different bacterial species the spread plate method was adopted with marine agar medium, after inoculation the plates were incubated in an inverted position at a temperature of 37^0Cfor 24 hours to 48 hours. The pure and good quality bacterial colonies were identified and picked up from inoculated Petri dishes and re-streaked on agar plates for further bacteriological parameters analysis.

Specific media used for the for the growth of major bacteria were TCBS agar for *Vibrio* species (M870S, Hi-media), MacConkey agar (M008) for *Escherichia coli* and M-FC (M1122, Hi-media) agar for *Coliforms*, XLD (031 Hi-media) agar for *Salmonella*, *Shigella* and *Klebsiella species*, M-enterococcus agar for *Enterococcus species* and Cetrimide (MM024, Hi-media) agar for *Pseudomonas species* respectively. The resulted values were represented as colony forming units (CFU/ml). IMViC tests (Indole, Methyl red, Voges-Proskauer and Citrate tests) were performed by following the method previously reported by [9] and the strains were analyzed by following the Bergey's manual of bacteriology reported by [10].

3. Results

3.1Identification of Bacterial cultures

3.1.1Escherichia coli

3.1.2Morphology

It is a Gram negative; rod shaped measuring 1-3x0.4-0.7µm arranged singly or in a pair. It is motile by peritrichate flagella though non motile capsules and fimbriae are found in some strains.

3.1.3 Culture characteristics

It is aerobic and facultative anaerobic. The temperature range is 10°- 40°C. The optimum temperature is 37°C. They grow well on ordinary media; colonies are large thick, greyish, white, moist, smooth, opaque or translucent disks. The description applies to the smooth form seen on fresh isolation which is easily emulsifiable in saline many pathogenic isolated strains have polysaccharide capsules. Some strains may occur in the mucoid form.

3.1.4Biochemical test

It shows positive reaction with Indole& methyl red and negative reaction with-Voges-Proskauer and citrate test.

3.2 Klebsiella species

3.2.1Morphology

It is a gram negative, rod shaped, short plump, straight rod, capsule, non motile, measured about 1.2x0.8 µm.

3.2.2 Culture characteristics

It is aerobic and facultative anaerobic grow well on ordinary media forming large dome shaped mucoid colonies of varying degree of stickness. On MacConkey agar it forms pink color colonies due to lactose fermentation.

3.2.3 Biochemical test

It shows positive reaction with Voges-Proskauer and citrate test and negative reaction with Indole and Methyl red.

3.3 Pseudomonas species

3.3.1Morphology

It is a gram negative bacillus measures 1-5x3-0.5 µm, actively motile by polar flagellums. It is non capsulated but many strains have a mucoid slime layer.

3.3.2Culture characteristics

It is obligate aerobe but can grow anaerobically if nitrate is available. Growth occurs in a wide range of temperature between 6-42° C, the optimum growth occurs at 37° C. They grow well on ordinary media producing large opaque, irregular colonies with a distinctive, musty, and mawkish with earthy smell. Iridescent patch with a metallic sheet are seen in culture on nutrient agar. On MacConkey agar colorless colonies are observed due to non lactose fermentation.

3.3.3Biochemical test

It shows positive reaction with citrate and negative reaction with Indole, methyl red and Voges- Proskauer.

3.4Salmonella species

3.4.1Morphology

It is a gram negative, rod shaped about 1-3 um x 0.5 µm in size. They do not form capsules or spores.

3.4.2Culture characteristics

It is aerobic and facultative anaerobic growing readily on simple media over a range of pH6-8 and temperature 15-41° C, optimum temperature for the growth of bacteria is 37 ° C. Colonies are large 2-3 mm in diameter, circular

low convex and smooth. On MacConkey ager colonies are colourless due the absence of lactose fermentation.

3.4.3Biochemical test

It shows positive reaction with methyl red and citrate test and negative reaction with Indole and Voges-Proskauer.

3.5 Shigella species

3.5.1 Morphology

It is a gram negative, rod shape about 0.5umx1-3 µm in size. They are non motile, none sporing and non capsulated.

3.5.2 Culture characteristics

It is aerobic and facultative anaerobes with a growth temperature range of 10-40° C and optimum temperature is 37 °C and pH 7.4. They grow well on ordinary media but less readily than other enterobacteria. After overnight incubation colonies are small about 2mm in diameter, circular convex, smooth and translucent. On MacConkey agar colorless colonies are observed due to the absences of lactose fermentation.

3.5.3Biochemical test

They show positive reaction with methyl red and negative reaction with Indole, citrate and Voges-Proskauer.

3.6Vibrio species

Morphology

It is a gram negative , rigid curved rod that are actively motile by means of a polar flagellum, *Vibrios* are present in marine environments and surface waters worldwide.

3.6.1 Culture characteristics

It is aerobic, growth being scantly and slow anaerobically. It grow with in a temperature range of 16-40° C, optimum temperature for the growth is 37°C Growth is increases by increasing 0.5 – 1% of NaCl and inhibited by increasing the concentration of 6%. On nutrient agar after overnight incubation colonies are moist, translucent, round disks. On MacConkey agar the colonies are colorless at first but become reddish on prolong incubation due to late lactose fermentation. In peptone water growth occur in about six hours as a fine surface pellicle. On TCBS agar produce large yellow convex colonies which may become green on continued incubation.

3.6.2Biochemical test

It shows positive reaction with Indole and citrate, negative reaction with methyl red and Voges- Proskauer

3.7 Faecal streptococcus

3.7.1 Morphology

It is a gram positive cocci arranged in a chains and in pairs.

3.7.2 Culture characteristics

It is aerobic and facultative anaerobic. They grow well on 5% horse blood agar on pour plate culture and do not show haemolysis. On MacConkey agar better growth is obtain when 5% Nacl is added to the media. They optimum temperature for the growth is 37°C.

3.7.3Biochemical test

They show positive reaction with methyl red, Voges-Proskaeur and citrate test and negative reaction with Indole.

3.8 Proteus species

3.8.1Morphology

It is a gram negative bacillus and actively motile. They are widely distributed in nature as saprophytes. They have a characteristic putrefactive odour described as fishy or seminal.

3.8.2 Culture characteristics

These are aerobic and facultative anaerobic growth will occur at a temperature of 10 – 40 °C The optimum temperature for the growth is 37°C. On solid agar media discrete colonies are seen in young culture but thereafter actively motile cells spread on the surface of the plate. On MacConkey agar swarming does not occur on which smooth colorless colonies are formed.

3.8.3Biochemical test

Fig. 1IMViC test for Identified bacterial strains

They show positive reaction with methyl red and negative reaction with Indole, Voges-Proskaeur and citrate.

4. Discussion

For marine water quality total *coliform*, *Faecal coliform* &*Faecal streptococci* can be used as pollution indicators from contaminated sites. The *Total coliforms* were the better indicators than faecal streptococci.

The findings of the current study revealed that, the occurrence of 8 different harmful pathogenic microorganisms along the coastal waters of Visakhapatnam. The bacterial strains include the number of *Total coliform* (TC), *Faecal coliform* (FC), *Faecal Streptococcus* (FS), *Salmonella species* (SA), *Shigella species* (SA), *Total Vibrio species* (TV), *Proteus/Klebisella species* (K/P) and *Pseudomonas species* (PA).

For the current study the water samples were collected from three different stations includes Visakhapatnam fishing harbour, Hindustan Shipyard and Bhimili to know the status of microbial pollution and colony counts for the selected sites.

As per the guidelines of U.S.EPA the permissible *E .coli* count was 200 CFU/100ml.[11]. The study reports were recorded as The total viable count (TVC) ranged from 120 to 812 CFU/ml, The total count (TC) ranged from 15 to 92 CFU/ml, The number of *faecal coliform* varied from 7 to 39 CFU/ml, The number of *Enterococcus species* varied from 5 to 28 CFU/ml, The *Shigella species* colony count varied from 10 to 23 CFU/ml, The *Salmonella species* colony count varied from 5 to 30 CFU/ml, The *Proteus/Klebsiella species* varied from 12 to 50 CFU/ml, The total *Vibrio species* count varied from13 to 55 CFU/ml, The faecal*Streptococcus species* count varied from 5 to 30 CFU/ml, The *Pseudomonas species* count varied from 3 to 28 CFU/ml.

Differential distribution of faecal *coliforms*, *Escherichia coli*, *Shigella*s pp. and *Salmonella* spp. was found to be more in the nearshore waters than offshore waters, and survival rate of microorganisms in marine habits depends on salinity variations and marginal stress from the saline water. The occurrence of harmful pathogenic bacteria was mostly seen in near shore water compared to the offshore regions. According to the observations of [12, 13] the interaction of microorganisms with sediments may enhance their survival by reducing exposure to stressors such as infrared radiation and predation or by increasing the availability of nutrients. The percentage occurrence of faecal *coliforms* in the coastal waters indicates that the faecal contamination is from the human and animal source.

The dominated species reported from coastal contaminated waters was *Shigell spp*. followed by *Vibrio cholerae*, *Vibrio para-haemolyticus*and the least one for *Salmonella* spp. There were quite good number of studies reported on the distribution of *Vibrio spp* in the marine contaminated waters.[14, 15]. Survival rate of salmonella species was very less compared to the other pathogens. The maximum densities of 3860 CFU/ml *Shigellaspp*. were enumerated at Cochin near-shore [16].

Visakhapatnam fishing harbour was highly polluted because of the sewage discharge, industrial effluent, fishing activities etc. These activities will help to the transfer of pathogenic bacteria to the humans consumed the fish and other marine sources as their livelihood. According to the studies of [17], microorganisms in seawater which are able to produce diseases such as diarrhoea and cholera and make serious threat to human health finally lead to dread full diseases like diarrhoea. The next polluted site is Hindustan shipyard it is less polluted than the fishing harbour, the pollution was due to the transfer of shipping activities through Visakhapatnam port, Naval base activities, dumping the waste, discharge the waste from cities. The very less polluted station is Bhimili because there were no industries, less number of population when compare to the Visakhapatnam.

5. Conclusion

The current investigation revealed that the greater distribution of enteric pathogens were reported from the Visakhapatnam fishing harbour (Station-I), moderate occurrence was reported at Hindustan shipyard (Station-II). Relatively low enteric pathogens load at offshore station (Bhimili-Station-III). The colony count values from all the three stations were exceeded than permissible limits as per guide lines of U.S. EPA (1986). So the study concluded that the water from all the three stations was not suitable for human use.

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