



Studies on Drug Resistant pattern of *E-coli* isolated from Godavari River

¹D. U. Bhusare, ²Jinturkar Sarita, ³Kulkarni.S.S, ³Bhate M.A.

¹Department of Microbiology, Assistant Professor, DB, ACS College, Bhokar (MS) India.

²Research Scholar, N.S.B. College Nanded (MS) India.

³Department of Microbiology, Associate Professor, Shri Shivaji College, Parbhani (MS)India.

Abstract:

Nowadays drinking water sources especially, the rivers become mode of transfer of drug resistant pathogens to human and animal. Severe infectious disease caused by drug resistant pathogen has become more difficult to treat. The human and animal excreta like urine and feces commonly consist of enteric pathogens like *E. coli* is one of them. The present study was carried out to investigate the presence of drug resistant *E. coli* from Godavari river dist. Nanded. River water sample were streak on MacConkey agar plate and incubate at 37°C for 24 hours. After incubation colonies were observed and proceed for biochemical and morphological characteristics. The isolates were tested for resistance to eight commonly used antibiotics by well diffusion method. The resistance observed to cefixime, while the susceptibility was shown to remaining seven drugs. The resistant isolate is become an enough alert to save drinking water resources in future.

Key words: *E. coli*, Drug resistant, Godavari River.

Introduction:

The water of river can acts as great source of multiple type of drug resistant strain which may cause serious health issues in human. Microbial assessment of drinking water carried out by using presence of fecal coliform bacteria specially *E. coli*. They were present in human gut which considered as reservoirs of genes resistant to antibiotic might be transferred to other bacteria. These bacteria acquired resistant gene become more pathogenic to human health in fecal contamination of water (Richa Bhardwaj *et al* 2015). Due to low water quality increase the chances of disease caused by polluted water in many developing countries. water pollution caused by discharge of industrial waste and sewage in water sources and the quality of water determined by determine many factors like biochemical oxygen demand(BOD), chemical oxygen demand(COD), total dissolved solids(TSS), dissolved oxygen (DO), pH, color, odour, test and presence of faecal coliform bacteria (Olukosiet *al* 2016). *E. coli* commonly found in human and animal feces. To examine the water quality *E. coli* acts as indicator organism. In drinking water bodies form a biofilm by indigenous microorganisms. It also contains opportunistic microflora which cause water born diseases in human. Iron corrosion product are responsible for increase growth of free living bacteria and biofilm formation (T.Ishaq and B.Ali2018).

Environmental Protection Agency (EPA) in USA used *E. coli* as an indicator organism to check fecal contamination. *E. coli* naturally present in human intestine microbiome. The human, agricultural animal and wild animal become a major source of fecal contamination in waterways. (Antony D. Kappell *et al* 2015).

The objective of present study was to isolate antibiotic resistant *E. coli* in Godavari river dist. Nanded. Isolated water sample were streak on MacConkeys agar plates and after incubation proceed for morphological and biochemical characteristics for identification. Commonly used antibiotic like ciprofloxacin, ampicillin, gentamycin, amikacin, amoxicillin, ofloxacin, vancomycin, cefixime were used to check antibiotic suitability by using agar well diffusion method zone of inhibition was observed.

Material and Methods:

Collection of Sample

The sample was collected from Godavari river Nanded in sterilized screw capped bottles and transported to the laboratory in cold temperature.

Screening and identification of *E. coli*

The sample was streak on MacConkey's agar plate and incubate at 37°C for 24-48 hours. After incubation pink colored colonies were observed on plates. The isolated colonies were identified on the basis of morphological characteristics and biochemical test (Anita , Amit Kumar *et al* 2014)

Determination of antibiotic susceptibility of *E. coli*

The isolated sample was proceeding to check the antibiotic susceptibility by well diffusion method using Muller Hinton agar. Isolated culture was enriched by inoculating loopfull of isolated *E. coli* culture in 5ml of nutrient broth at 37°C for 24 hours. After overnight incubation the 0.1 ml of bacterial suspension was mix with Mueller Hinton agar and plates were prepare by pour plate technique. About 80µl antibiotic concentration were added in the well by using micropipette. The plates were kept in refrigerator for 20ml for diffusion of antibiotics into the agar and incubated at 37°C for 24 hours. After 24 hours observed the zone of inhibition.

(Practica Microbiology by Dr.R.C. Dubey , Dr. D.K.Maheshwari ,S.chand Publications.)

Result and discussion:

Isolation of *E.coli*

E.coli was isolated from Godavari river water sample by observing pink colored colonies on MacConkey's agar plates and further identified on the basis of morphological and biochemical characteristics . After incubation pink colored colonies occurred due to fermentation of lactose .Colonies were further confirmed by Gram's staining and motility by hanging drop method. In biochemical test the isolate show Indole (I), Methyl Red (MR), catalase positive and Voges proskauer (VP), Citrate utilization (C) negative. The isolate also ferment the sugars maltose, mannitol, lactose, inositol, glucose which confirmed typical characteristics of *E.coli*.

Table 1: Colony characters of the isolates:

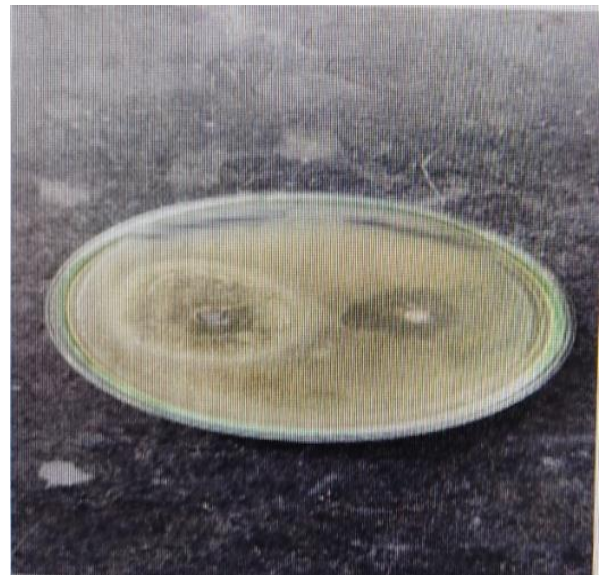
Morphological character	<i>Escherichia coli</i>
Size	Small
Shape	Rod
Margin	Entire
Elevation	Convex
Opacity	Opaque
Consistency	Viscous
Gram's nature	Negative
Motility	Motile
Colour	Pink
Surface	Smooth, Rough

Table 2: Biochemical Test of the isolated bacterial strain

Test	Result
Indole	Positive
Catalase	Positive
Oxidase	Negative
Citrate utilization	Negative
Gelatinase	Negative
Methyl Red	Positive
Voges-Proskaur	Negative
Triple sugar iron agar	Positive

Antibiotic susceptibility:

Many studies have been confirmed that highly resistant *E.coli* isolated from drinking water bodies specially river water. There was multidrug resistant *E.coli* strains were isolated from river water (Richa Bharadwaj 2015). In Urban water sources also antibiotic resistant gene were found in *E.coli* (Anthony D.Kappell *et al* 2015). In present study the isolated strain of *E.coli* show resistant to cefixime antibiotic which is also supported by Ayallahi J MD *et al* 2013 and Mohammad Al-Tamimi *et al* 2022. The isolate show resistant against ciprofloxacin, ampicillin, gentamycin, amikacin, amoxicillin, ofloxacin, vancomycin also observed by Achhada Ujalkaur Avast singh *et al* 2023 and Olukosi O.M *et al* 2016.

**Fig .1. Zone of inhibition****Fig.2. Resistance to Cefixime****Fig.3.Biochemical Tests Media****Fig.4. Triple Sugar Iron Test****Fig. 5. Gelatinase Test****Fig.6. Catalase Test**

Conclusion:

The results of the current study indicate that the Godavari River District in Nanded, Maharashtra, has low-quality drinking water samples. The isolate strain of *E.coli* was resistant to the drug cefixime. Therefore, it is advised to provide a sufficient basic treatment before drinking water.

References

1. Adzitey, F., N. Sumaila and C.S.K. Saba, 2015a. Isolation of *E. Coli* from drinking watersources for humans and farm animals in Nyankpala Community of Ghana. Res. J. Microbiol.
2. Achhada Ujalkaur Avatsingh, Shilpa Sharma, 2023 Prevalence of antibiotic- resistant Gram-negative bacteria having extended- spectrum β - lactamase phenotypes in polluted irrigation-purpose wastewaters from Indian agro-ecosystems.
3. Anita, A. Kumar, A.K. Verma, M.K. Gupta and A. Rahal, 2014. Multidrug resistant pathogenic *Escherichia coli* status in water sources and Yamuna River in and around Mathura, India. Pak. J.Biol. Sci., 17: 540-544.
4. Ayatollahi J MD, Shahcheraghi S H MSc, Akhondi R Bs , Soluti SS MSc April 2013, Antibiotic Resistance Patterns Of *Escherichia coli* Isolated from Children in Shahid Sadoughi Hospital of Yazd.
5. Azam, M.; Jan, A.T.; Haq, Q.M. Blactx-m-152, a novel variant of cox-m-group-25, identifiedina study performed on the prevalence of multidrug resistance among natural inhabitants of River Yamuna, India. Front. Microbiol. 2016, 7, 1-13.
6. B. C. Sharma and B. Rai, "Incidence of multi-drug resistance in *Escherichia coli* strains isolated from three lakes of tourist attraction (Mirik lake, Jorepokhari lake and Nakhapani lake) of Darjeeling hills, India," Indian Journal of Fundamental and Applied Life Sciences, vol. 2, pp.108-114, 2012.
7. B. Rowe, L. R. Ward, and E. J. Threlfall, "Multidrug-resistant *Salmonella typhi*: a worldwide epidemic," Clinical Infectious Diseases, vol. 24, no. 1, pp. S106-S109, 1997.
8. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. PLoS Med.2014;11 (5):e1001644. DOI:10.1371/journal.pmed.1001644 PMID:24800926 PMCID:PMC4011876
9. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
10. Carattoli, A. Resistance plasmid families in enterobacteriaceae. Antimicrob. Agents Chemother. 2009, 53, 2227-2238.
11. Carnot, A., J.S. Guerra, T.S. Souza and L.C. Carneiro, 2014. Antimicrobial resistance and plasmid characterization of *Escherichia coli* isolated in natural water. Am. J. Drug Discov .Dev., 4: 80-84.
12. Chen Z, Yu D, He S, Ye H, Zhang L, Wen Y, Zhang W, Shu L, Chen S. Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in Hangzhou city. Frontiers in microbiology. 2017; 8:1133. DOI: 10.3389/fmicb.2017.01133 PMID: 28670309 PMCID.
13. Dr. R.C. Dubey,,Dr.D.K Maheshwari, Practical Microbiology ,S .Chand Publications.
14. Elvis Kichana *et.al* Prevalence of multidrug-resistant *Escherichia coli* in household drinking water in rural Ghana 2022.
15. Frederick, A., 2011. *Escherichia coli*, it prevalence and antibiotic resistant in Malaysia.
16. Gould IM, The epidemiology of antibiotic resistance, International Journal of Antimicrobial Agents, 32 Supplementary Issue, 2008.S2-S9.

17. Iqbal MK , Patel IK, Susceptibility patterns of *Escherichia coli*: Prevalence of multidrug-resistant isolates and extended spectrum beta-lactamase phenotype, Journal of Pakistan Medical Association, 2002,52,407-417
18. Machado A, Bordalo AA. Prevalence of antibiotic resistance in bacteria isolated from drinking well water available in Guinea-Bissau (West Africa). Ecotoxicology and environmental safety.2014;106:188-194. DOI: 10.1016/j.ecoenv.2014.04.037PMID: 24846754 mini review. Microbial. J., 1: 47-531: 47-53MC5472731
19. Mohammad Al-Tamini , Hadeel Albalawi , Farah Alhaj ,22 May 2022, Cefixime and cefixime – clavulanate for screening and confirmation of extended-spectrum beta-lactamases in *Escherichia coli*
20. Richa Bhardwaj, Anshu Gupta, Jai K Garg, Prevalence of multidrug resistance in *Escherichia-coli* strains isolated from river Yamuna, Delhi.
21. Tambekar DH, Wankahde SJ, Yadav SD, Tambekar SD, Correlation of antibiotics resistance profiling of E.coli and source of fecal pollution in water, Pollution Research, 2008,27,507-510.