

REVIEW OF RESEARCH

IMPACT FACTOR : 5.7631(UIF)

ISSN: 2249-894X

VOLUME - 1 | ISSUE - 2 | MARCH - 2019

COMPARATIVE STUDIES ON ANTIBIOTIC RESISTIVITY OF ISOLATED ESCHERICHIA COLI FROMCLINICAL & NON-CLINICAL SEWAGE WATER SOURCES OFGUHAGAR REGION, RATNAGIRI DISTRICT

Nilkanth S. Bhalerao¹ and Santosh V. Jadhav² Department of Microbiology, Khare-Dhere-Bhosale College, Guhagar, Dist-Ratnagiri-415703, Maharashtra India. *bhalerao_nils@yahoo.co.in*

ABSTRACT :

Scientific studies on clinical and non-clinical Sewage waste water for the purpose of isolation of antibiotics resistance bacterial species especially E.coli strain. We have used important antibiotics like Tetracycline, streptomycin, ampicillin, Penicillin, Nitrofurantoin etc as per NCCLS criteria. During our lab work and we have analyzed that the sample from clinical sewage source shows resistance to most of the antibiotics whilesample from non-clinical sewage sources shows susceptibility to most of the antibiotics. By this study we can easily highlighted the current scenario of an **antibiotic resistivity** because Antibiotic resistivity of E.coli is one of the today's major problem.

KEYWORDS : Clinical and Non clinical Sewage Samples, Antibiotics, E.coli.

INTRODUCTION

Antibiotic resistance in bacteria is a growing problem among humans and wildlife in terrestrial or aquatic environments. In this respect, the spread and contamination of the environment, especially through "hot spots" such as hospital wastewater and untreated urban waste water is a growing and serious public health problem. Antibiotics have been polluting the environment since their introduction through human waste(medication, farming), animals and the pharmaceutical industry.Due to exposure of bacteria to the antibiotic waste, in many bacteria there is introduction of antibiotic-resistance. *Escherichia coli* is a member of the family enterobacteriaceae. It is abbreviated as *Escherichia coli* is named after its discovery, Theodor Escherich, a German pediatrician and bacteriologist. *Escherichia coli* is residual microorganism present in colon region of intestine and has an important role in the proper digestion of food as well as in immunity system. *Escherichia coli* is a gram negative short bacillus 1-3 um long and 0.42-0.7 um broad, motile and spore forming.

As bacteria replicate quickly, the resistant bacteria that enter the environment replicate their resistance genes as they continue to divide. In addition, bacteria carrying resistance genes have the ability to spread those genes to other species via horizontal gene transfer. Therefore, even if the specific antibiotic is no longer introduced into the environment, antibiotic-resistance genes will persist through the bacteria that have since replicated without continuous exposure. The present work is oriented with the Comparative studies on antibiotic resistivity Of *Escherichia coli* from Clinical & Non-clinical Sewage water sources of Guhagar region, Ratnagiri district.

Review of Research

MATERIALS AND METHODS:

• Antibiotics:

1) Tetracycline 2) Streptomycin 3) Ampicillin 4) Penicillin 5) Nitrofurantoin 6) Carbenicillin 7) Amoxicillin 8) Amikacin 9) Vankomycin 10) Ciprofloxacin 11) Cefpodoxime

(Concentrations of antibiotics are as per NCCLS for disc diffusion methods)

- Sewage water sample –
 1) Clinical (Rural hospital Guhagar, Dist-Ratnagiri, Maharashtra)
 2) Non-clinical (Shivaji Chowk, Guhagar, Dist-Ratnagiri, Maharashtra)
- Media: Selective and Differential Media 1) Mac.Conkeys Agar 2) Endo agar 3) Nutrient agar
- IMViC test Kit
- Urease test Kit
- Catalase test Kit
- Amylase test Kit.
- Sugar Utilization Test Kit.
- Lysine Decarboxylase Test kit.

In Methodology following steps are taken:

- 1) Sample collection
- 2) Processing of Sample
- 3) Isolation of Microorganisms
- 4) Identification of isolates
- 5) Antimicrobial susceptibility and resistivity

Isolation of organisms:

Sample (clinical, Non-clinical)

\downarrow

0.1 ml of each sample was spread on Mac Conkeys agar plate

V

Kept for incubation at 37^oC for 24hrs

J

Pink colour colonies were observed

\downarrow

Grams staining of suspected colonies were performed

\downarrow

Pink coloured short rods were observed

(Gram Negative)

Screening of Organism / Sub culturing :

Suspected colonies from Mac Conkeys agar plate were streaked on endo agar plate

Kept for incubation at 37 $^{\circ}$ C for 24hrs

,... ↓

Pink coloured colonies showing metallic sheen were observed

$$\checkmark$$

Gram staining was performed

\downarrow

Pink coloured short rods were observed

"Advances in Fisheries, Biological and Allied Research"

 \checkmark

This colony showing above result was again streaked on Endo agar plate

 \checkmark

Kept for incubation at 37^oC for 24hrs

The plate showed the pink coloured colonies having metallic sheen

J

Gram staining was performed.(Gram Negative)

Morphological Properties:

Gram Reaction	: Negative	
Arrangement	: Single	
Shape	: Short Rod	
Motility	: Positive	
MacConkey Agar	: Pink colour Clonies	
Endo Agar	: Pink Coloured Metallic Sheen	

Confirmatory test/Biochemical Properties

1. IMViC Test

Indole- Cherry red coloured ring- positive Methyl Red – dark red colour – positive Citrate- No turbidity – Negative Voges- Proskauer- Negative

2. Urease test

Loopful of E.coli culture – Urease Broth – No pink colour – Negative

- 3. Catalase test-Loopful of E.coli culture 3% H₂O₂ No formation of air bubble Negative
- 4. Amylase test Loopful of *E.coli* culture Iodine No zone of clearance Negative
- 5. Sugar utilization test- Glucose-lactose-Mannose-Maltose-Xylose-Sucrose-Acid & Gas Production.
- 6. Lysine Decarboxylase Test Positive

To check antibiotic resistance of the organism of different water sample (Clinical, Non-clinical)-

0.1ml of isolated culture of *E.coli* spread on nutrient agar plate

 \downarrow

Aseptically place the disc of appropriate concentrations of antibiotics on Nutrient agar plates

Kept the plates for incubation at 37° C for 24 hrs

 \checkmark

Observe the zone of inhibition/clearance of both antibiotics

\downarrow

Clear zone indicates susceptibility while no zone indicates resistance

Results- Antibiotic activity on *E.coli* isolated from sewage water sample Non clinical:



Resistivity and Susceptibility of *E.coli* to various antibiotics: (Diameter measured in cm)

Sr. No.	Antibiotics	Sample & Zone of Clearance	
		Clinical (cm)	Non-Clinical (cm)
1.	Tetracycline	1.4	2.3
2.	Streptomycin	0.9	2.0
3.	Ampicillin	1.0	2.2
4.	Penicillin	0.0	0.0
5.	Nitrofurantoin	1.1	2.1
6.	Carbenicillin	1.7	2.8
7.	Amoxicillin	1.7	2.4
8.	Amikacin	2.2 (S)	2.6
9.	Vankomycin	0.0	0.0
10	Ciprofloxacin	1.2	4.0
11	Cefpodoxime	1.9 (S)	2.5



Graphical Representation: Effect of antibiotic on *E.coli* isolated from different sewage water sample (Clinical, Non-clinical)

CONCLUSION:

E.coli isolated from clinical waste water sample showed resistance to most of the antibioticsexcept Amicacin and cefpodoxime while compared to the *E.coli* isolated from the sewage waste of Non-clinical sample which shows susceptibility to all used antibiotics. This resistance to the antibiotics may be due to the repeated exposure of *E.coli* from clinical waste water to different antibiotics.

"Advances in Fisheries, Biological and Allied Research"

Review of Research

REFERENCES:

- 1. Stamm W E & Nobby S R (2001).Urinary tract infections: disease panorama and challenges J.infect.Dis.183(1):S1-4.
- 2. Orskav, Orskav *Escherichia coli* in extra-intestinal infections. Journal of Hygiene (London) 1985;95:551-575.
- 3. Ramirez Castillo F.Y., Avelar Gonzalez FJ, Garneau P, Marquez Diaz F, Guerrero Barrera AL., Harel J. Presence of multi drug resistant pathogenic Escherichia coli in the San Pedro river located in the state of Aguascalientes, Mexico. Front Microbial. 2013; 4:147.
- 4. Abe CM, Salvador FA, Falsetti IN, Vieria MA, Blanco J, Blanco M, Machado AM, Elias WP, Hernandes RT, et al., Uropathogenic Escherichia coli (UPEC) strains may carry virulence properties of diarrhoeagenic E.coli FEMS Immunol Med Microbiol. 2008; 52(3):397-406
- 5. Hirai, K., Aoyama, H., Suzue, S., Irikura, T., Iyobe, S. & Mitsuhashi, S. (1986). Isolation and characterization of norfloxacin resistant mutants of Escherichia coli K-12. Antimicrobial Agents and Chemotherapy 30, 248-53.
- 6. Malik, A., 2004. Metal bioremediation through growing cells. Environ. Int., 30, 261–278.
- 7. Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K. Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. *Rev Inst Med Trop Sao Paulo*. 2014;56(4):341-6.
- 8. Chattaway MA, Day M, Mtwale J, et al. Clonality, virulence and antimicrobial resistance of enteroaggregative Escherichia coli from Mirzapur, Bangladesh. *J Med Microbiol*. 2017;66(10):1429-1435.
- 9. Mellata M. Human and avian extraintestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathog Dis*. 2013;10(11):916-32.
- 10. Zhang A, Li Y, Guan Z, et al. Characterization of Resistance Patterns and Detection of Apramycin Resistance Genes in *Escherichia coli* Isolated from Chicken Feces and Houseflies after Apramycin Administration. *Front Microbiol*. 2018;9:328. Published 2018 Feb 27. doi:10.3389/fmicb.2018.00328.
- 11. Barrow G, Feltham R. Cowan and Steel Manual for the identification of medical bacteria. Cambridge University Press; 1993. p. 331.
- 12. Emody L, Kerenyi M, Nagy G. Virulence factors of uropathogenic *Escherichia coli*. Int J Antimicrob Agents. 2003;22:529–533.
- 13. Ron EZ. Distribution and evolution of virulence factors in septicemic *Escherichia coli*. Int J Med Microbiol. 2010;300:367–70 10.1016/j.ijmm.2010.04.009
- 14. Toval F, Köhler CD, Wagenlehner F, Mellmann A, Fruth A, Schmidt MA, et al. Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. J. Clin. Microbiol. 2014;52(2):407–18.