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ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF ANTIBIOTICS RESISTANCE *E. COLI* FROM INDUSTRIAL EFFLUENT

Microbiology

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ABSTRACT

Waste water originated from domestic and industrial discharge. External contact and ingestion of bacteria from fecal contamination can cause disturbance in Health. *E.coli* is one of most common bacteria in human intestinal tract. It is known as fecal contaminant in water and food. The bacterium *E.coli* is one of the best free living microorganisms. ***E.coli*** is not a pathogenic but occasionally it causes traveler's diarrhea, food born disease and urinary tract infection. Due to various hazardous effluents mixed in the water this common bacterium is showing multidrug resistance property which is great risk for human health, in this context we isolates **25** ***E. coli*** bacterial isolates and identified it as drug resistance bacteria against six antibiotics.

KEYWORDS

E.coli, antibiotics, MIC, waste water

INTRODUCTION

Water is essential for known life. Water is important component of cell and is essential solvent on earth to survive life. Living system composed of cells and cells contain cytoplasm. Cytoplasm contains **80%** of water. A normal body contains 42 liters of water. The above facts indicate that the water is essential for living systems. Water is universal solvent, which dissolve organic, inorganic compound and gases which participates in metabolic process of cells. Water in the cells stabilized the plasma membrane, thermoregulation, homeostasis, nutrient transport **(Sawka *et al*., 2005)**. Scientific reports suggest that the agriculture and veterinary application of antimicrobial compound is one of the sources of antimicrobial resistance of bacteria in the environment. (**Gellin *et al*., 1989, Molbak, K. 2004).**

The appearance of antibiotic resistance microbes in the environment basically from the waste water have a great risk to incorporate these organism in the food chain (**Van den Bogaard *et al.,* 2000).** Genetic elements like Transposons, plasmid, integrons, and bacteriophage play an important role in antibiotic resistance. All contributes the spreading of antibiotic resistance in different environments **(Caratolli A. 2009; Molbak, K. 2004; Stokes *et al.,* 1989).** Resistance isolates are more common in long term care residents than in the general. Antibiotic resistance in bacterial isolates has forced to scientific community to established some alternative approaches to control this problem , in this regards bacteriophage is one of the good alternative strategies which couse lysis or death of the host cells is different from mechanism of antibiotics **(Jamala M *et al.,* 2015)**.

E.coli strains are of biological significance for humans. On the basis of genetic and clinical criteria ***E. coli*** can be broadly classified in to three major groups: commensal *E.coli*, intestinal pathogenic diarrhea genic *E.coli,* and extra intestinal pathogenic *E.coli* (ExPEC).

Contaminated drinking water invites various water borne diseases. The contamination of drinking water can be considered as presences of *E. coli* which indicate the fecal contamination **(Orskov and orskov, 1992).** *Escherichia coli* (*E. coli*) was first described by a pediatrician "Theodor Escherich" later it was renamed as *E. coli* in his honor **(Kaper *et al.*, 2004).** *E.coli* belongs to Enterobacteriaceae that are anaerobic, facultative and gram-negative rods that live in the intestinal tracts of animals and humans. Gram negative bacteria possess an extra cytoplasmic outer membrane that consists of a lipid bilayer, lipoproteins, and capsule of lipopolysaccharide **(LPS)**. The outer membrane interfaces with the bacterial and host environment. A variety of components of the outer membrane are critical determinants in antimicrobial resistance and pathogenesis **(Russo and Johnson, 2005).** Drug-resistance ***Escherichia coli*** isolates may constitute a significant reservoir of antibiotic resistance determinants which can spread to those bacteria pathogenic for animals and/or humans. For this reason, the reported emergence of resistance to new drugs such as extended-spectrum β-lactam and Fluoroquinolones (FQs) is of particular concern to both animal and public health alike. The potential for transmission of ***E. coli*** clones between different animal hosts and humans has been documented by various workers **(Chopra et al., 2001**: **Kummerer, 2004).**

METHODOLOGY

SAMPLES COLLECTION

Samples were collected applying the following protocol. Total five water sample were collected from different sites of industrial area of Rampur. Industrial waste water was collected in sterile container from the six inches beneath the surface of water from industrial area, Rampur at different sites. Before the collection of water samples we ensured the Ph and salinity of water samples. Other fine particles present in water samples wait to be settled down before the serial dilution process. The water was then placed at -20 ºC.

ISOLATION OF DRUG RESISTANCE BACTERIAL ISOLATES

Isolation of bacteria were done by serial dilution agar plate method by the following methods One ml of each water samples were dissolved in nine ml of sterile water and mixed thoroughly. The supernatant of this suspension was used further. 1ml of suspension was transferred to 9ml of sterile distilled water to get serially diluted $10^{-1}$to 10^{-6}. Then 0.1 ml of suspension was spreaded on the surface of EMB agar (10^{-3}) with the help of spreader. Nutrient agar plates were incubated at 37 ºC for 28-32 hours. Individually each isolates treated with antibiotics for detection of MDR property as method described by **(Pandey et al., 2011)**.

PURE CULTURE MAINTENANCE BY STREAK PLATE TECHNIQUE

The single colonies of pure culture were maintained by Streak plate methods by the following process. Inoculating loop was sterilized by putting it in flame till red hot After cooling down, it was dipped into 95% ethyl alcohol and further heated for proper sterilization. Distinguished colonies from spread plate were further streaked over EMB Agar plate surface by sterilized inoculating loop. These were the incubated for 28-32 hours at 32ºC.

BIOCHEMICAL CHARACTERIZATION

Biochemical characteristics of bacterial isolates were studied by performing:- Indole production test , Methyl-Red and Vogues-Proskauer (MRVP) test Citrate utilization test

MINIMUM INHIBITORY CONCENTRATION (MIC) OF ANTIBIOTICS

The lowest concentration of antibiotics which inhibit the growth of

bacteria as well as other organism is known as Minimum Inhibitory Concentration. Minimum inhibitory concentration of antibiotics was identified by the spot inoculation methods. Antibiotics plates were prepare at varying concentration of 1 to 0.3125 mg/30ml. Spot inoculations were performed with the help of sterilized loop of different multidrug resistance isolates as described by **Shafiani and Malik, 2003.** Commercial grade of antibiotics (Tetracycline, Amoxicillin, Erythromycin Ciprofloxacin, Cefadroxil, Doxycycline, Azithromycin, Cephalexin, Ampicillin) were used. Concentration of the antibiotics was calculated on the basis of dilution methods. Stock solutions of the antibiotics were prepared in distilled water and then supplemented in Nutrient Agar in such manner where concentration of antibiotics varying 1 to 0.3125 mg/30ml in a plates. After spot inoculation each plate were incubated at 32ºC for 28-32 hours.

RESULLT AND DISCUSSION

1) Total five water sample were collected from different sites of industrial area of Rampur. 25 pure colonies of ***E. coli*** were selected for antibiotic resistance and cultured on **EMB** agar plates. The Texture (surface) was smooth and the appearance of ***E.coli*** was shiny.
2) The single colonies of pure culture were maintained by Streak plate methods. A total 25 ***(E.coli)*** bacterial isolates were showed rod shaped and gram negative in morphology. Identification was based on the morphological and biochemical characterization using conventional technique according to Bergey's manual of determinative Bacteriology (Table 1& 2)**.**
3) All isolates showing green metallic sheen on EMB plates and positive for Indole and methyl red. Isololates was identified as an *E.coli* based on comparison of these characters with standard description in Bergey's manual of determinative bacteriology.
4) Minimum inhibitory concentration of antibiotics against different multidrug resistance bacterial isolates was done by spot inoculation methods in which we prepared different concentration of antibiotics plates varies from 1 to 0.3125 mg/30ml. Spot inoculations were performed with the help of sterilized loop.
5) Commercial grade of antibiotics (Tetracycline, Amoxicillin, Erythromycin Ciprofloxacin, Cefadroxil, Doxycycline, Azithromycin, Cephalexin and Ampicillin) were used for MIC determination.

Concentration of the antibiotics was calculated on the basis of dilution methods. We used varied concentration of antibiotics (1mg \ml, 0.5mg\ml,0.25mg\ml,0.125mg\ml, 0.0625mg\ml, 0.03125mg\ml) for MIC determination of *E.coli*. For Tetracycline we found all isolates were inhibited at1 mg\ml concentration of antibiotics. In Ampicillin only 28% of isolates were showing 1mg\ml MIC. Doxycycline showed the MIC pattern similar with Tetracycline. Cephalexin showed the Maximum MIC (32%) at 1 mg \ml. Cefadroxil also showed the similar pattern of MIC of ***E.coli*** as seen in Cephalexin. Ciprofloxacin, Tetracycline and Ampicillin were showing more or less similar MIC pattern. Azithromycin was showing the maximum MIC (52%) at 1 mg\ml and minimum MIC (8%) at 0.25 mg\m

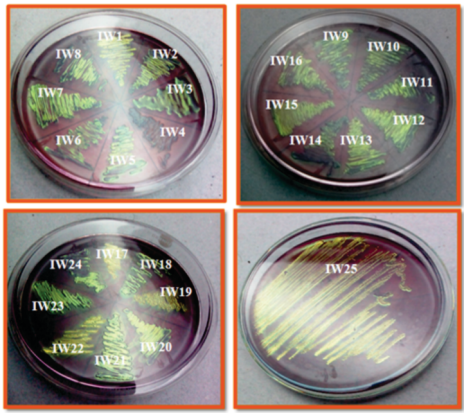

Fig1: Pure isolates of different *E.coli*

Table 1: Biochemical Characterization Isolates Obtained From Industrial Effluent

Isolate	Indole Test	Methyl Red Test	Vogues-Proskeur Test	Simmons Test	
				Ammonium Citrate test	Ammonium Acetate Test
Isolate1	+ve	+ve	+ve	+ve	-ve
Isolate2	+ve	+ve	+ve	+ve	-ve
Isolate3	+ve	+ve	+ve	+ve	-ve
Isolate4	+ve	+ve	+ve	+ve	-ve
Isolate5	+ve	+ve	+ve	+ve	-ve
Isolate6	+ve	+ve	+ve	+ve	-ve
Isolate7	+ve	+ve	+ve	+ve	-ve
Isolate8	+ve	+ve	+ve	+ve	-ve
Isolate9	+ve	+ve	+ve	+ve	-ve
Isolate10	+ve	+ve	+ve	+ve	-ve
Isolate11	+ve	+ve	+ve	+ve	-ve
Isolate12	+ve	+ve	+ve	+ve	-ve
Isolate13	+ve	+ve	+ve	+ve	-ve
Isolate14	+ve	+ve	+ve	+ve	-ve
Isolate15	+ve	+ve	+ve	+ve	-ve
Isolate16	+ve	+ve	+ve	+ve	-ve
Isolate17	+ve	+ve	+ve	+ve	-ve
Isolate18	+ve	+ve	+ve	+ve	-ve
Isolate19	+ve	+ve	+ve	+ve	-ve
Isolate20	+ve	+ve	+ve	+ve	-ve
Isolate21	+ve	+ve	+ve	+ve	-ve
Isolate22	+ve	+ve	+ve	+ve	-ve
Isolate23	+ve	+ve	+ve	+ve	-ve
Isolate24	+ve	+ve	+ve	+ve	-ve
Isolate25	+ve	+ve	+ve	+ve	-ve

Table 2: Minimum inhibitory concentration of antibiotics under the influence of different *E.coli isolates*

Antibiotics	Total percent of isolates being inhibited/ resistance against different Antibiotics					
Tetracycline	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.625**	**0.3125**
	No of isolates being inhibited	25(100%)	21(84%)	10(40%)	1	_	_
Ampicillin	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**
	No of isolates being inhibited	7(28%)	_	_	_	_	_
Doxycycline	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**
	No of isolates being inhibited	24(96%)	7(28%)	6(24%)	4(16%)	3(12%)	_
Cephalexin	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**
	No of isolates being inhibited	8(32%)	4(16%)	2(8%)	2(8%)	3(12%)	_
Cefadroxil	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**
	No of isolates being inhibited	11(44%)	5(20%)	5(20%)	5(20%)	3(12%)	_
Ciprofloxacin	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.625**	**0.3125**

	No of isolates being inhibited	24(96%)	24(96%)	23(92%)	14(56%)	11(44%)	_
Azithromycin	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.625**	**0.3125**
	No of isolates being inhibited	13(52%)	1(4%)	1(4%)	_	_	_
Amoxicillin	**MIC(mg/ml)**	**1**	**0.5**	**0.25**	**0.125**	**0.625**	**0.3125**
	No of isolates being inhibited	2(8%)	2(8%)	_	_	_	_
Erythromycin	**MIC(mg/ml)**	**25**	**20**	**15**	**10**	**5**	**1**
	No of isolates being inhibited	18(72%)	_	_	_	_	_

Waste water originated from domestic and industrial discharge in the river where *E.coli* is indicator organism of water contamination. External contact and ingestion of bacteria from fecal contamination can cause disturbance in Health. Municipal waste is also responsive for *E.coli* contamination in the river. Antibiotic resistance bacteria are increasing day by day and are serious threat to human health. *E.coli* is one of the most common bacteria in human intestinal tract. It is known as fecal contaminant in water and food. Although ***E.coli*** is not a pathogenic but some time it causes traveler's diarrhea, food born disease and urinary tract infection. The bacterium *E.coli* is one of the best free living microorganisms. It has various species pathogenic and nonpathogenic. It is widely used as indicator for fecal contamination in water bodies. External contacts and ingestion of bacteria from fecal contamination can cause disturbance in health. Waste water originated from domestic and industrial discharge in the rivers. India is the land of agriculture in which rivers contributing major roles in this sector.

The study demonstrates how industrial waste water contributes to the development of Antibiotic resistance in ***E. coli*** and multiple antibiotic resistance activities. In the industrial waste may act as a possible source of transfer of these highly resistance pathogens and their genes to human that could be treat for the treatment of disease by commercially available antibiotics (**Osterblad *et al.*, 2004**). Antimicrobial drugs have been proved remarkably effective for the control of bacterial infections. However, it was soon evidenced that bacterial pathogens were unlikely to surrender unconditionally, and some pathogens rapidly became resistance to many of the first effective drugs. Over the past few decades, antimicrobials have become increasingly available for a broad range of pathogens. Due to the widespread use of these drugs, new forms of antimicrobial resistance have emerged (**Osterblad *et al.*, 2004**). Antibiotic resistance profile for ***E. coli*** isolates has well been documented by various workers. Opportunistic pathogens presenting broad-spectrum antibiotic resistance have emerged extensively in hospital waste water/ environments, causing serious infections in immune compromised hosts **(Franklin et al., 1999).** It is likely that we detected a higher number of resistance bacteria **(*E.coli*)** by using a selective medium, in the wastewater. The results of this study indicate that bacteria carrying **MDR** factors present in industrial wastewater in India. This will lead to a situation where bacteria carrying **MDR** factor enter the community through contaminated water sources and spread the diseases.

CONCLUSION

Our result revealed that antibiotics resistance *E.coli* in industrial waste water might be due to untreated hospital waste discharge in the industrial waste water or misuse of antibiotics in environment. On the basis of above said facts, efforts must be taken to prevent dumping of antibiotics without treatment (WHO-Guidelines) into the environment; complete destruction of antibiotics before disposal. We expect that the results of this study will be helpful for further scientific enquiry in Rampur (U.P) India and other countries regarding the presence of emerging contaminants like antibiotics in environment and its impact on public health.

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