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Study of Antibiotic Resistance Pattern of *Escherichia coli* PS 58 Isolated from Pichhola Lake of Udaipur, Rajasthan, India

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Abstract

Escherichia coli is the major coliform faecal contaminating bacteria. In the present study an attempt has been made to isolate, identify and detect the antibiotics resistance pattern of E. coli PS 58. Strain PS 58 was isolated on nutrient agar from Pichhola lake of Udaipur and identified on the basis of its morphological and biochemical characteristics. For molecular identification Polymerase Chain Reaction was performed using 16S rRNA gene specific universal primers. 15 commonly used antibiotics i.e. gentamicin, kanamycin, polymyxin, tetracycline, erythromycin, ampicillin, penicillin, amikacin, ciprofloxacin, vancomycin, rifampicin, chloremphenicol, streptomycin, cefixime, trimethoprim were used to detect the antibiotic resistance pattern by disc diffusion method. The results revealed that the strain PS 58 was identified as E. coli (ascession no.KPO99424). It was found strongly resistant to kanamycin, ampicillin, cefixime, polymyxin, penicillin, vancomycin, rifampicin and streptomycin.

Keywords: *E .coli,* antibiotic resistance, Pichhola, lake.

Introduction

The pollution of surface water by discharge from human activities is one of the major environmental problems. Assessment of coliform bacteria can be used as a measurement for sanitary quality of any water body. *E. coli* is one of the most important members of coliform group and the presence of *E. coli* in water sample is implicit evidence for faecal contamination. It represents a threat to human and environmental health[1]. Since lakes of Udaipur are the major source of drinking water of the city monitoring bacteriological quality of lake water is of significant value in combating the problems associated with public health due to organic pollution[2].

Resistant bacteria are becoming commonplace in healthcare institutions. When bacterial strains become resistant to various antibiotics, it results in treatment failure in people who need them to treat infections, which can have serious consequences, especially in critically ill patients. The misuse of antibiotics as growth-promoters in animal production, for therapy and prophylaxis results in the spread of antibiotic resistant strains from animals to humans because of the consumption of these animal products by human being, and thus healthy individuals can become carrier hosts for multiple antibiotic-resistant bacteria[3,4]. Therefore in the present study the antibiotic resistance pattern of *E. coli* PS 58 isolated from water sample of lake Pichhola was determined.

Material and Methods

Collection of water sample: The water samples were collected from Pichhola lake of Udaipur city. Lake Pichhola is situated between longitude 73^0 40'E and latitude $24^0$34'N and covers 6.96 km^2. Water samples were collected in 125ml presterilized (at 121^0C) borosil bottles.

Isolation and Preliminary Characterization: Bacterial strain PS 58 was isolated from water sample of lake Pichhola on nutrient agar (NA) medium after an incubation of 24 hour at 37°C. It was identified by studying its morphological and biochemical characteristics. The tests include gram staining, catalase test, oxidase test, citrate utilization, urea hydrolysis, Indole production, methyl red test, Voges-Proskauer test, nitrate reduction, carbohydrate fermentation and growth on differential media like MacConkey agar and Eosine methylene blue (EMB) agar. The results will be further matched to the criteria provided in Bergey's Manual of Systematic Bacteriology[5].

Molecular characterization: DNA Isolation: The genomic DNA, from bacterial culture *E. coli* PS 58 grown overnight in nutrient broth, was extracted according to Pospiech and Neumann[6].

PCR amplification: The 16S rRNA gene based universal primers namely 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'CGGTTACCTTGTTACGACTT-3') were used for amplification of DNA in PCR. The reaction mixture (20µl) contained 10 pmol of each primer, 0.2 mM of each dNTP ($MgCl_2$) , 1X PCR buffer, 2µl of DNA solution and 1 U/µl of Taq DNA polymerase (Banglore genei). Amplification was carried out in a thermal cycler (Banglore genei) as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles

consisting of denaturation at 95°C for 30 sec, annealing at 55°c for 30 sec, and extension at 72°C for 2 min, and a final 10 minute extension step at 72°C. The final hold of PCR products were at 4°C.

Analysis of PCR Products by Agarose Gel Electrophoresis: PCR products obtained after the completion of DNA amplification were electrophoresed on a 2.0% agarose gel along with 500bp DNA ladder (Banglore genei). Electrophoresis was carried out in mini gel system (Banglore genei) by following the standard procedures given by Sambrook *et.al*[7]. The DNA bands representing PCR amplified products on gel were visualized on UV transilluminator (Banglore genei) and were photographed using Gel Doc system (Banglore genei). The amplified product was submitted to Bangalore Genei Pvt. Ltd., Bangalore, India for sequencing. The sequence of the 16S rRNA of the isolate was compared with available standard sequences of bacterial lineages in the NCBI Genebank using nBLAST.

Antibiotic susceptibility testing: Isolate *E. coli* PS 58 was subjected to susceptibility testing against 15 commonly used antibiotics. Susceptibility was determined by using disc-diffusion method of Kirby- Bauer [8] on Mueller-Hinton agar plates. A total of fifteen antibiotics namely Ampicillin (AMP10µg/disc), Amikacin (AK 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Cefixime (CFM 5 µg/disc), Chloremphenicol (C 30 µg/disc), Erythromycin (E 15 µg/disc), Gentamicin (GEN 30 µg/disc), Kanamycin (K 30 µg/disc), Penicillin (P 10 µg/disc), Polymyxin (PB 300 µg/disc), Rifampicin (RIF 30/disc), Streptomycin (S 25 µg/disc), Tetracycline (TE 30 µg/disc), Trimethoprim (TR 5 µg/disc) and Vancomycin (VA 30 µg/disc) were used. After 24 h incubation at 30°C, organisms were classified as sensitive (S), intermediately resistant (I) or resistant (R) on the basis of the size of the zone of bacterial growth inhibition according to the guidelines of the CLSI [9].

Results and Discussion

Bacterial strain PS 58 was isolated from water sample on nutrient agar (NA) medium after an incubation of 24 hour at 37°C. Isolate PS 58 was found gram negative rod shaped bacteria. The colony appears small in size, white coloured, circular, entire margin, flat surfaced on nutrient agar plate. Results of biochemical characteristics were given in table 1. It was found that strain PS 58 was catalase positive, oxidase negative, indole positive, citrate negative, MR positive, VP negative, and was able to grow on MacConkey agar and EMB agar. It produced dark pink colonies on MacConkey agar and agar and green metallic sheen colonies on EMB agar indicating lactose fermentation and acid production. The results obtained for morphological and biochemical characteristics were further matched with Bergey's Manual of Systematic Bacteriology. The isolate PS 58 was tentatively identified as *Escherichia. coli.*

Table-1
Biochemical Characteristics of isolate PS 58

Biochemical Characteristics	PS 58
Catalase activity	+
Oxidase activity	-
Citrate utilization	-
MR Reaction	+
VP Reaction	-
Indole Production	+
Nitrate reduction	+
Arginine Hydrolysis	-
Urease Activity	-
Gelatin Hydrolysis	-
H2S production	-
Starch Hydrolysis	-
Casein Hydrolysis	-
Growth on MacConkey Agar	+
Growth on EMB Agar	+
Growth on Cetrimide Agar	-
Carbohydrate Fermentation	
Lactose	+
Maltose	+
Glucose	+
Sucrose	+
Galactose	+
Raffinose	-
Rhamnose	+
Mannose	+
Fructose	+
Mannitol	+

In order to ascertain the identification of the isolate PS 58 as *E. coli* by molecular method, DNA was isolated and subjected to PCR amplification by universal primers 27F and 1492R. The amplified product gave 1500bp product on 2% agarose gel (Figure 1). On the basis of the sequence similarity of the partial 16S rRNA sequence of PS 58 compared with available standard sequences of bacterial lineages in NCBI Genebank reference strains; the isolate was identified as *Escherichia coli* PS 58. The nucleotide sequence of *Escherichia coli* PS 58 has been deposited in NCBI genebank under the accession no. KPO99424.

A very wide range of antibiotics is used for many different reasons in animal husbandry, food technology, agriculture, human and veterinary medicine practice[3]. Antibiotic resistance pattern of *E. coli* PS 58 was given in table-2. The results revealed that *E. coli* PS 58 showed resistance to more than one

antibiotic. Multiple antibiotic resistance refers to the resistance of more than one class of antibiotics. Multiple drug resistance of *E. coli* strains have also been reported from Bangladesh and other parts of the world[10-13].

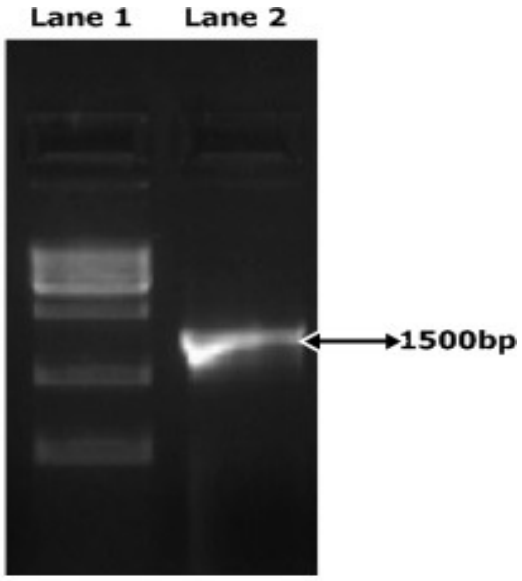

Figure-1
Gel Image of 16SrDNA amplicon, Lane 1: 1500bp DNA ladder, Lane 2: 16S rDNA amplicon band

Table-2
Antibiotic resistance pattern of *E. coli* PS 58

S.No.	Antibiotic	Zone of inhibition diameter (mm)	Antibiogram of *E. coli* PS 58
1	Ampicillin	12	R
2	Amikacin	16	I
3	Ciprofloxacin	23	S
4	Cefixime	15	R
5	Chloremphenicol	20	S
6	Erythromycin	18	I
7	Gentamicin	15	S
8	Kanamycin	12	R
9	Penicillin	0	R
10	Polymyxin	9	R
11	Rifampacin	10	R
12	Streptomycin	11	R
13	Tetracycline	14	I
14	Trimethoprim	20	S
15	Vancomycin	7	R

(R= Resistant, I= Intermediate resistance, S= Sensitive)

E. coli PS 58 was found strongly resistant to 8 antibiotics out of 15 used in the study namely kanamycin, ampicillin, cefixime, polymyxin, penicillin, vancomycin, rifampicin and streptomycin. Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment[14]. *E. coli* PS 58 showed intermediate resistance against amikacin, erythromycin and tetracycline and showed sensitivity against gentamycin, trimethoprim, ciprofloxacin, and chloremphenicol.

Resistance of *E. coli* isolates from Malaysian broiler chicken to ampicillin, tetracycline and gentamicin has been reported by Apun et. al[15]. Kinge et al.[16] studied the antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, North-West Province, South Africa and they observed resistance in *E. coli* for erythromycin, tetracycline, ampicillin, chloramphenicol and norfloxacin. Rahman et. al.[12] reported the resistance of *E. coli* isolates from broiler and layer poultry in Bangladesh against ampicillin, chloramphenicol, ciprofloxacin, streptomycin and tetracycline. Tricia et al.[17] found that *E. coli* isolates were resistant to ampicillin but not resistant to gentamicin, which is in accordance with the results of present study. Daini and Adesemowo[18] found the resistance of *E. coli* from Nigeria against gentamicin and tetracycline respectively which is contradictory with our results.

Conclusion

In this study *E. coli* PS 58 from lake Pichhola was isolated and identified. It was found strongly resistant to 8 antibiotics out of 15 used in the study namely kanamycin, ampicillin, cefixime, polymixin, penicillin, vancomycin, rifampicin and streptomycin. The findings of present study indicated that improper lake water treatment results in the contamination of water with *E. coli*, as well as other pathogenic bacteria for human and animal consumption. The high antibiotic resistance also indicates a negative impact on therapy with these classes of antibiotics. Strict quality control measures should be put in place to ensure proper treatment of lake water. This would ensure the discharge of properly treated wastewater into the lake to prevent the occurrence and spread of water- and food-borne diseases.

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