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A Study on Plasmid Borne Antibiotic Resistance Patterns in *Escherichia coli*

Niyaz A Dar[1], Shahid A Padder[2*], Zahoor A Bhat[3] and Abdul Q Ganai[3]

[1]Division of Plant Biotechnology, [2]Division of Plant Pathology, [3]Division of Soil Science SKAUST-K Shalimar Srinagar Kashmir (J&K) 190025

*Corresponding Author E-mail: aafan.tavkeer@gmail.com

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ABSTRACT

The objective of this study was to determine if E.coli plasmid borne genes for antibiotic resistance. This study was carried out at SKAUST-K for five different strains of E.coli : E1, E2, E3, E4 and E5. For monitoring trends in antibiotic resistance prevalence in E.coli, isolates from five healthy persons were analyzed in laboratory using Luria Broth agar plates containing respectively azithromycin, cefadroxil, cefixime, norfloxacin & amoxycllin at breakpoint concentrations with DH5α as host for plasmid transformation. The mean age of the volunteers was 30 years. Cefixime resistance was higher in the range 75-80% however maximum zone of inhibition was recorded against azithromycin. Of the entire isolates maximum zone was observed in E1 E.coli isolate. All five isolate samples of E.coli shows inducible mode of detoxification mechanism for antibiotics. Using growth inhibitory studies and also plasmids obtained from sample strains resolved at same position on agarose gel determining that they were of similar size. In the population studies, antibiotic resistance in E.coli is emerging for norfloxain & azithromycin but is higher for the primitive drugs like cefixime, cefadroxil & amoxycllin.

Key words: *Antibiotic resistance, emerging resistance, azithromycin, Norfloxacin DH5α.*

INTRODUCTION

From the last few decades, an increase in antibiotic resistance is highly prevalent in bacterial isolates worldwide, especially in the developing countries. In these countries, availability of antibiotics over the counters without prescriptions, doses of substandard quality & poor community hygiene are main factors responsible for emerging resistance[1]. Treatment of humans with antibiotics not affect only targeted pathogens but also other microbial community inhabiting skin & mucosal membrane. The emergence of resistance among commensal bacteria is a serious side effect of antibiotic usage, as it may cause extra intestinal infections[2], spread to other hosts or transfer resistance genes to other members of microbiota. Studies with *E. coli* is of great importance as it is the most common Gram negative facultative bacterium in the intestinal microbiota of man and animals[3].

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E.coli strains colonizing the large intestine vary in their virulence factors which enable bacteria to cause extra intestinal infections like urinary tract infection, peritonitis, meningitis & septicemia. In addition *E.coli* strain effectively exchanges genetic material with other pathogenic strains like *Salmonella, Vibrio, Shigella & Yersinia* as well with other pathogenic *E.coli* strains[4]. Despite these reasons the dynamics of antibiotic resistance *in E.coli* has not been studied in detail. Very few investigations have been carried to access antibiotic resistance in the Kashmir Valley & trends over time in the valley are rarely followed. For estimating trends in the resistance prevalence in *E.coli* isolates from samples of five volunteers, different antibiotics at different concentrations are used.

MATERIALS AND METHODS

Bacterial cultures used in present study have been collected from microbiology labrotory of Jehlum Valley Medical College Srinagar on blood agar plates. Single colonies were selected and streaked on fresh Luria Bertani broth agar plates. Identity was confirmed by convectional biochemical tests. Five different strains of *E.coli* E1, E2, E3, E4 and E5 were selected for current study. DH5α is used as the host for transformation of plasmids. Compounds like SDS (sodium dodecyl sulphate), Tris (hydroxyl ethyl amino ethane) were obtained from Sisco Research Laboratories (India), EDTA, potassium accetate and agarose were from Qualigen Fine Chemicals .General chemicals like glacial acetic acid, sodium hydroxide, propanol etc. were also from Qualigen Fine Chemicals. Boric acid was purchased from Thomas Baker India. Bromophenol blue & ethidium bromide were again from Qualigen Fine Chemicals. Pre mixed media like Luria Broth, Luria agar & agarose powder were obtained from Titan Biotech Limited India.

Test for microbial susceptibility to antibiotics

E. coli isolates selected for study were tested for resistance against a particular antibiotic using Kirby- Bauer test or disk diffusion method[9]. *E. coli* strains were grown in Luria-Bertani broth with aeration. Antibiotics were used at the concentrations: azithromycin, norfloxacin, cefixime, cefadroxil (1mg/ml, 1000 µg/ml & 500 µg/ml) and amoxycllin (100mg/ml, 10 mg/ml & 1 mg/ml). The diameter of zone (if formed) is measured with the help of ruler in mm. The larger the zone, the more sensitive the test organism is to the antibiotic.

Checking inducibility of antibiotic resistant operon by growth inhibition curve

Selected *E. coli* strain was exposed to different concentrations of antibiotics and growth curves were obtained. Selected strains were grown in presence or absence of 25 µg/ml antibiotic amoxicillin. An absorbance of 0.3 - 0.4 units were calculated after growing cells in secondary culture.

Plasmid isolation: Plasmid DNA was isolated by alkaline lysis described by Birnboin and Doly[5]. Supernatant was discarded and the pellet containing plasmid DNA was dried and dissolved in 300 ml of TE (10/1. pH 8.0).

Gel electrophoresis and DNA visualization: Agarose gel electrophoresis was carried out on 0.9% agarose in Tris-borate-EDTA buffer using Bromophenol blue as loading dye and a current of 40 mA was applied to run the gel for 2 hours. Agarose gel was stained with ethidium bromide at a concentration of 10mg/ml in distilled water.

Quantization of plasmid DNA: 10 µl of the isolated plasmid DNA were diluted 100 fold with distilled water, absorbance was recorded at 260 nm in UV spectrophotometer (systronics) against distilled water without DNA as control. An O.D of 1.0 at 260 nm corresponds to 50 µg of DNA/ ml.

Transformation; *E.coli* strains were transformed, positive control comprised those competent bacterial cells that received a known amount of standard preparation of supercoiled plasmid DNA. Cells were plated on LB agar plates with appropriate antibiotic.

RESULTS

It is clear from the data obtained as shown in table 1 that at the lowest concentration of azithromycin 500 µg/ml, the *E. coli* strains show moderate susceptibility as a small zone of 6mm diameter is recorded. However when concentration was raised to 1 mg/ml, a much larger zone of 13mm was obtained. Thus these zones of inhibition shows test organism are more susceptible to this particular antibiotic with increasing trend. When cefadroxil at minimum concentration of 500 µg /ml was added, no zone of inhibition was recorded, indicating that *E. coli* isolates are highly resistant to cefadroxil at this concentration but when concentration of this drug was increased to 1 mg/ml, a small zone corresponding to 7mm dia. was observed which clearly indicates that organism is moderately susceptible. Likewise when cefixime and norfloxacin were added at concentrations of 500 µg/ml, no zones were observed however when concentration was increased to 1 mg/ml, no zone was again obtained for cefixime while at the same, a small zone of 5 mm diameter was recorded against norfloxacin treatment. On subjecting *E. coli* strain to amoxicillin stress at concentration of 1 mg/ml, no zone was noted but when concentration was raised to 100 mg/ml,a small zone of 6mm dia. is seen. Thus these results clearly provide us a clue that to this particular antibiotic, these *E. coli* strains are highly resistant and tolerate very high concentrations of amoxicillin.

Table 1 Showing zones of inhibition of *E.coli* using different antibiotics

Antibiotic	Zone of inhibition in mm		
	Disk concentration		
	1 mg/ml	**10000 µg/ml**	**500 µg/ml**
Azithromycin	13	11	___
Norfloxacin	05	___	___
Cefixime	___	___	___
Cefadroxil	07	___	___
Amoxycllin	06 (100 mg/ml)	---- (10 mg/ml)	---- (1 mg/ml)

It was also clear that by administering 500 µg/ml of amoxicillin to secondary growing culture (previously grown in presence of 35 µg/ml stress) of E1 *E. coli* at A **590** corresponding to 0.3 O. D, an unusual lag period of nearly 4 hrs was observed. Thus indicating that this *E. coli* strain is somehow cooping up with the toxic levels of antibiotic until the concentration reaches to sub toxic level after which bacterial cells resume normal growth pattern.

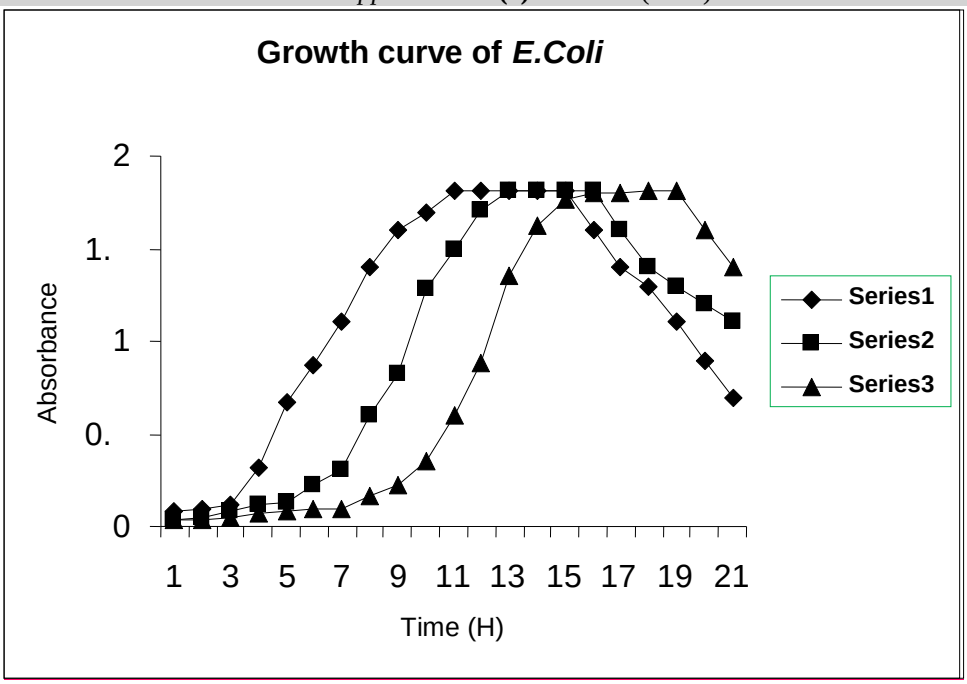

Fig. 1: Showing growth curve of *E. coli* under normal conditions (series 1), with presence of stress in primary culture (series 2) and without presence of stress in prm. culture (series 3)

When isolated plasmids were resolved on 0.9% agarose gel, it was observed that all plasmids resolved at the same position. Unfortunately no unique restriction endonuclear site was known thus plasmids could not be linearised and their size could not be determined. On transforming DH5α cells with 10 ng of plasmid DNA, we were able to get 30 transformant colonies and it was found that transformants showed same pattern of antibiotic resistance as showed by the wild type strains.

DISCUSSION

Studies in developing countries have shown that the trend in enteric pathogens is towards increasing antibiotic resistance[6]. In the current study the five drugs used are extensively used in Kashmir Valley (India) for the treatment of various infectious diseases. In the light of data, our current study shows that E1 strain showed maximum zone of inhibition (13 mm) towards azithromycin, whereas it showed higher resistance towards cefixime. Comparison of antibiotic pattern with MIC pattern in our study showed positive correlation. In Kashmir Valley most of the people are used to dental amalgam filling and for minor health problems they are using high doses of antibiotics. As Dahms *et al*[7]., reported that the level of antibiotics resistance is always higher in amalgam exposed groups. In this context our results showed that more than 95% of bacterial isolates were resistant to one or more antibiotic used.

In the current study, growth curve inhibition studies of clinical isolates of *E.coli* evoke the inducible gene expression mechanism to degrade the antibiotics present in surrounding medium. The reduction in the lag phase observed indicates that there is an inducible nature of antibiotic resistant operon operating in it. During the transformation of DH5α with the isolated plasmids, we were able to get 30 transformants, this suggests that the isolated plasmids carry resistance to almost every antibiotic as that of wild strain type, indicating that antibiotic resistance were plasmid borne. The emergence of resistance among commensal bacteria is a serious side effect of antibiotic usage in humans and veterinary medicines because the commensal may, at a later stage become cause of extra intestinal infection[8]. In Kashmir Valley people are

mainly engaged to animal husbandry and it may be one of the main causes of antibiotic resistance among the population. The future usefulness of these antibiotics will depend on effective intervention to halt the selection & spread of resistance among enteric organisms. More surveillance studies are necessary to monitor antibiotic use and antibiotic resistance over time. These data are important as a basis for the implementation of an antibiotic policy.

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