

Study of Antimicrobial Susceptibility and Plasmid analysis of *Escherichia coli* in Rajshahi, Bangladesh

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Multiple drug resistant *Escherichia coli* isolated from Rajshahi metropolis, which were resistant against ampicillin, tetracycline, chloramphenicol, erythromycin, kanamycin, streptomycin and nalidixic acid at 90, 70, 45, 45, 35, 30 and 10% respectively. Moreover, plasmid DNAs having molecular weight of 5.0 to 8.1 MD and molecular size of 28.4 to 39.0 kb has been isolated from these drug resistant mutants.

Key words: *Escherichia coli*, antimicrobial susceptibility, plasmid DNA and resistance

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Introduction

Bacterial resistance to antimicrobial agents is a major world wide problem because of introduction of new antimicrobial agents is usually followed sooner or later by emergence of bacterial resistance to these drugs (Patwary, 1994).

The drug resistance in bacterial population is may be due to a genetic and non-genetic mechanism (Choudhury, 1988). Regarding genetic mechanism most drug resistant microbes emerged as a result of genetic changes and subsequent processes by antimicrobial drugs. The drug resistance may be chromosomal DNA or plasmid DNA mediated. Plasmids are autonomously replicated DNA which are extra-chromosomally located in the micro-organisms. The plasmid mediated drug resistance is caused due to the presence of drug resistant gene(s) harboring on the plasmid DNA. These gene(s) confer the drug resistance phenomenon in the host organism (Meyer's *et al.*, 1976). Plasmids carrying drug resistance phenotype are known as R-factor which is responsible for the spread of multiple drug resistance among bacteria. R-factor consists of two components i.e. resistance transfer factor (RTF) and resistance determinant 'r'. The complete plasmid (RTF + r) is called R-factor. (Patwary, 1994) *E. coli* is one of the serious pathogen that can cause tremendous therapeutic problem by developing resistance against antibiotics. As a result of drug resistance to several antibiotics in *E. coli* it has become a serious problem not only in the developing countries where it is endemic but also an important problem of treating drug resistant *E. coli* infection in the developed countries (Tauxe *et al.*, 1990).

E. coli is referred to as the colon bacillus because it is the predominant facultative species in the large bowel. The major species of *E. coli* are found in the lower portion of intestine of human and warm blooded animals where it comprises the normal intestinal flora (Pelczar *et al.*, 1993). *E. coli* is the causative agent of many life threatening diseases like UTI, pyelonephritis, bacteremia and diarrhoeal diseases. In this research work tested the antimicrobial susceptibility of twenty *E. coli* strains in an attempt to establish their antibiotic resistance pattern and also isolated plasmid DNAs from these drug resistant mutants *E. coli* and characterize the plasmid DNAs.

Materials and Methods

Collection of bacterial samples: Twenty-eight bacterial samples were collected from two pathological laboratories at Rajshahi during June - December 1989. These samples were isolated from different sources of the patients, such as, stool, urine etc. and were cultured into food media. These samples were then sub-cultured in LB and Mac Conkey agar plates.

Identification of the bacteria: *E. coli* was identified on the basis of gross morphology along with cultural characteristics and the manner in which the bacteria did response to various biochemical tests according to Collins *et al.* (1987).

Antibiotic susceptibility tests of the bacteria: Antibiotic susceptibility tests of the collected strains of *E. coli* were done by antibiotic disc diffusion method (Bauer *et al.*, 1966) using filter paper discs. Discs were impregnated in various antibiotic solutions. These discs were then seeded in plates in which a 24hr. culture of the strain grown at 37°C were spread on a Mac Conkey agar plate by using sterilized glass spreader. The plates were then kept at 4°C for 4hr so that the antibiotics can diffused in the agar and finally the plates were incubated at 37°C for 16 hr. After incubation the plates were observed in order to calculate the diameter of clear zone produced around each disc. Such clear zone produced around each disc is the index of sensitivity to the corresponding drug. Eight commonly used antibiotics, viz. ampicillin, amoxycillin, chloramphenicol, kanamycin, streptomycin, tetracycline,

nalidixic acid, nitrofurantoin and erythromycin were used. In order to establish antibiotic susceptibility profile of the collected *E. coli* strains, the clear zone produce around each disc were measured in millimeter.

Extraction of Plasmid DNA: Plasmid DNAs were extracted from each of the drug resistant *E. coli* strains in a method according to Sambrook *et al.* (1989).

Agarose gel electrophoresis of the extracted DNA: DNA extracted from each of the sample strain was subject to gel electrophoresis with 0.8% agarose gel according to Meyer's *et al.* (1976).

Results

Identification of the collected bacterial samples: Identification of bacterial sample for different morphological and biochemical tests were performed according to the procedures described by Collins *et al.* (1987). Twenty strains out of twenty eight strains were identified as *E. coli* on the basis of their biochemical behavior and morphological characteristics (Table 1).

Establishment of antibiotic susceptibility profile: In an attempt to establish antibiotic susceptibility profile each of the twenty *E. coli* strains were tested for their susceptibility against eight commonly used antibiotics by disc diffusion method. About 90% of the collected drug resistant mutant *E. coli* strains were resistant to ampicillin, tetracycline, chloramphenicol, erythromycin, kanamycin, streptomycin nalidixic acid at 90, 70, 60, 45, 35, 30 and 30% respectively. All the strains were absolutely sensitive to nitrofurantoin (Fig. 1).

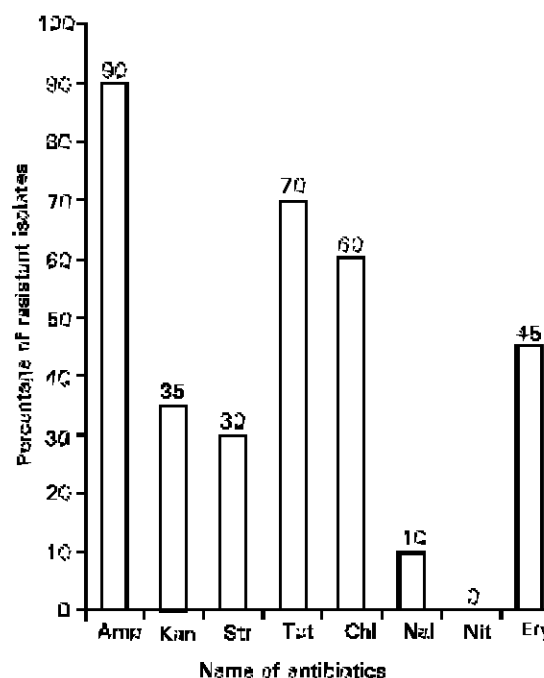


Fig. 1 Percentage(%) of resistance to different antibiotics of *E. coli*. Here Amp, Ampicillin; Kan, Kanamycin; Str, Streptomycin; Chl, Chloramphenicol; Tet, Tetracycline; Nal, Nalidixic acid; Nit, Nitrofurantoin and Ery, Erythromycin.

Table 1: Results of various biochemical tests specific for *E. Coli*

S. No	Fermentation test						MIU medium			KIA medium				Comments
	Lac	Man	Glu	Suc	Ox	Cit	Mot	Ind	Urea	Slop	Butt	H ₂ S	Gas	
S ₁	-	-	+	-	+	-	+	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₂	+	+	+	-	-	-	+	+	-	Y	Y	-	+	<i>E. Coli</i>
S ₃	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₄	-	+	-	-	-	-	-	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₅	-	-	+	-	-	-	+	+	-	R	R	-	-	Not <i>E. Coli</i>
S ₆	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₇	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₈	-	+	-	-	-	-	-	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₉	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₁₀	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₁₁	-	+	-	-	-	-	-	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₁₂	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₁₃	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₁₄	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₁₅	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₁₆	-	+	-	-	-	-	-	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₁₇	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₁₈	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₁₉	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₂₀	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₂₁	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₂₂	-	+	-	-	-	-	-	-	-	R	R	+	-	Not <i>E. Coli</i>
S ₂₃	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₂₄	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₂₅	+	-	+	+	+	-	-	+	-	R	Y	-	-	<i>E. Coli</i>
S ₂₆	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>
S ₂₇	-	-	-	+	-	-	+	-	-	R	R	-	-	Not <i>E. Coli</i>
S ₂₈	+	+	+	-	-	-	+	+	+	Y	Y	+	-	<i>E. Coli</i>

Symbols: + = Positive test; - = Negative test; R = Red pink (Alkaline reaction); Y = Yellow (Acid reaction)

Key: Lac, Lactose fermentation; Man, Mannose fermentation; Glu, Glucose fermentation; Suc, Sucrose fermentation; Ox, Oxidase test; Cit, Citrate utilization; Mot, Motility test; Ind, Indol test; Urea, Urease test.

Table 2: Estimated molecular size and weight of different plasmid DNAs extracted from different *E. Coli* Strains

No. Of strains	Molecular size (kb)	Molecular weight (MD)
2	38.0	7.5
3	34.0	7.1
6	32.0	6.8
7	33.0	6.9
9	37.5	7.3
10	35.2	5.8
12	36.0	6.4
13	37.4	7.4
14	36.8	7.1
15	28.4	4.3
17	30.7	5.0
18	31.0	5.5
19	38.7	7.8
20	39.0	8.1
21	38.2	7.3
23	38.8	7.9
24	37.0	6.8
25	37.2	6.9
26	36.9	6.3
28	37.0	6.8

Characterization of Plasmid DNAs by agarose gel electrophoresis: Plasmid DNAs were extracted from each of the twenty experimental drug resistant mutant *E. coli* and subjected to agarose gel electrophoresis using 0.8% agarose according to Meyer's *et al.* (1976). Based on the electrophoretic mobility of the DNAs on the gel during electrophoresis, molecular weight and molecular size of the plasmid DNAs were determined. Here we have used λ DNA (*Hind* III digested) as marker DNA. From the result it was revealed that molecular weight of the plasmid DNAs extracted from twenty different drug resistant *E. coli* varied from 5.0 to 8.1 MD and the molecular size varied from 28.4 to 39.0 kb (Table 2).

Discussion

Plasmid DNAs, possibly carrying drug resistant gene(s) were characterized and twenty eight bacterial samples were

collected during June-December 1989 from two pathological laboratories of Rajshahi metropolis, Bangladesh. Twenty out of twenty eight bacterial strains were screened as *E. coli* on the basis of biochemical reaction pattern and their morphological characteristics. All these twenty *E. coli* strains were subjected to antimicrobial susceptibility test in an attempt to establish their antimicrobial susceptibility profile. Eight commonly used antimicrobial drugs were used in the susceptibility test. It was revealed that different samples were resistant to different antimicrobial drugs and their efficiency varied from antibiotics to antibiotics. From ampicillin to nitrofurantoin the range varied from 0.00 to 89.47%. These findings were in accord with the Azad *et al.* (1999). They have documented reports of isolation of multidrug resistant *E. coli* which were resistant to at least eight commonly used antibiotics including ampicillin, tetracycline and chloramphenicol. Antimicrobial susceptibility testing of intestinal micro-organisms like *E. coli* is important consideration because the administration of antimicrobial substances can alter the intestinal microbial balance and resulted in the suppression of certain beneficial bacterial groups. The altered microbial balance may results in intestinal disorders (Kobayashi *et al.*, 1973).

Plasmid DNAs were isolated from each of the twenty drug resistant mutant *E. coli* strains by modern protocol according to Sambrook *et al.* (1989). The DNAs were subjected to agarose gel electrophoresis with 0.8% agarose according to Meyer's *et al.* (1976).

From the pattern of bands observed in the gel after staining with ethyl bromide solution (EtBr), molecular weight and molecular size of the plasmid DNAs were calculated. The molecular weight of the plasmid DNAs isolated from twenty *E. coli* strains were varied from 5.0 to 8.1 MD and the molecular size were varied from 28.4 to 39.0 Kb. In this case we have used λ DNA (*Hind* III digested) as marker DNA.

These findings were in consistent with the findings of others. (Martinez *et al.*, 1987; Flint *et al.*, 1987 and Wachsmuth *et*

al., 1983). They reported that about strains of *E. coli* harbored plasmid of varying molecular weight and molecular size. In this investigation we have been revealed that each of the twenty drug resistant *E. coli* harbored single plasmid.

However, more research is necessary in order to determine the exact mechanism of drug resistance in these *E. coli* and to identify the resistance transfer factor (RTF) and 'r' determinant of these plasmid DNAs. In conclusion it can be said that increasing incidences of drug resistance in *E. coli* to different antibiotics including the broad spectrum antibiotic tetracycline heralds the coming therapeutic problem in the treatment of infections cause by this micro-organism.

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