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Original Research Paper

Incidence of Antibiotic Resistance Transfer Among *Escherichia coli* from Hospital Environment

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ABSTRACT

Infections caused by antibiotic resistant organisms are difficult to treat. The aim of this study was to analyse dissemination of antibiotic resistance and resistant transfer in *Escherichia coli* from pregnant women. Forty one multi drug resistance strains of *E. coli* were isolated from the urine of pregnant women in Erode district. The isolates showed high resistant to tetracycline and ampicillin antibiotics. Evaluation of the isolates for their drug resistance revealed distinct antibiotic resistant pattern and showed the increasing dissemination risk of drug resistance especially tetracycline and ampicillin. The presence of a multi plasmid in both donor and transconjugants, but not in the recipients, provided physical evidence for the transfer of antibiotic resistance and also indicated that the drug resistance mediated by plasmids. Then the strains of transcojugants were assessed for plasmid curing by acridine orange. The data signify the presence of an undesirably high level of transferable antibiotic resistance in the healthy pregnant women.

INTRODUCTION

The ubiquity of bacteria possessing transferable antibiotic resistance presents obvious health hazards and dictates the need for an experimental system that will provide a greater understanding of the *in situ* behaviour of R plasmids (Althrr & Kasweck 1982). Antibiotic consumption is believed to be the main factor associated with the increase in antibiotic resistance in human faecal flora in both outpatients and hospitalized patients (Levy & Marshall 1988, Shanahan et al. 1994). Although antibiotic resistance is becoming a major threat to human health world wide, information concerning the dissemination and geographical distribution of antibiotic resistant bacterial pathogens remains scattered (Files 1999, Levy 1998).

Escherichia coli is one of the main opportunistic bacterial pathogens causing extra intestinal infections, such as urinary tract infections (UTI: e.g. cystitis and pyelonephritis) sepsis and meningitis. Plasmid transfer by conjugation between isogenic strains was quite efficient (Stuy 1979). Not surprisingly, perhaps, some of these plasmids transferred from donor to recipient cell by a process requiring cell to cell contact. Bacterial plasmid allows the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (David et al. 2004). During bacterial conjugation, the single stranded DNA molecule is transferred through cell envelopes of donor and the recipient cell.

Hence, conjugation is an important discovery in the study of bacterial antibiotic resistance. Resistance factors have now been found in a host of different environment and in a variety of different organisms but mainly in the Enterobacteriaceae. This study was undertaken to accomplish two goals: the first is to provide detailed descriptive information about antibiotic resistance profiles of

Escherichia coli isolated from urine of pregnant women, and the second is to find out the horizontal dissemination of antibiotic resistance plasmid among *E. coli*.

MATERIALS AND METHODS

Source and identification: The 102 urine samples collected from pregnant women in Government and private hospitals in Erode, Tamilnadu. The mid stream urine was collected on voiding in sterile plastic containers (Hi-media, Mumbai). Then the bacteria were identified using biochemical and selective media.

Antibiotic susceptibility test: The presence of drug resistance was performed by using Kirby Bauer disc diffusion method (Bauer et al. 1966) on Muller Hinton agar by using commercially available antibiotic disc of ampicillin, tetracycline, kanamycin, streptomycin and rifampicin (Hi-media, Mumbai). The plates were incubated at 37ºC for 24 hrs. Zones of inhibition were measured and resistance pattern was evaluated.

Transfer of drug resistance: Gene transfer was studied in nine antibiotic resistant strains of *Escherichia coli*. The overnight culture of donor and recipient were mixed in volume ratio of 1:1. Suspensions were incubated for 15 hrs without shaking, to prevent the disturbance during mating process. At the time of incubation period, 5, 10 and 15 hr samples were withdrawn and conjugation interrupted by violent agitation for 1 minute. Tripticase soya agar was prepared with the donor and recipient antibiotic markers. Vortexed 0.1mL of conjugated samples were spread on the TS agar. The plates were observed at the end of incubation period (Armand et al. 1989).

Plasmid isolation: The presence of plasmids in the marine and industrial bacterial isolates was determined using a modification of alkaline lysate method (Brinboim & Doly 1979, Surzycki 2000). Plasmid DNA was diluted in phosphate-buffered saline (PBS) (1:100), and the concentration and purity of the extracted plasmids were determined spectrophotometrically. The optical density (OD) of the DNA was measured at 260 and 280 nm. The OD260 allowed calculation of the DNA concentration in the sample, where an OD260 of 1 corresponds to approximately 50 µg/mL of double stranded DNA. The ratio of the OD260 nm and OD280 nm provides an estimate for the purity of DNA. Plasmid DNA was separated by electrophoresis on a 0.8% agarose gel (w/v) at 50 volts. Lamda *Eco* R1 was used in each gel as molecular weight marker. The gel was stained with ethidium-bromide, visualized under UV transillumination.

Plasmid curing: Multiple drug resistant 'R' plasmid curing was detected by acridine orange curing method for the transconjugant. Then 1 mL of overnight culture was added in 1.9 mL of nutrient broth (pH 7.2) containing 0.1 mL acridine orange (Shahid & Malik 2004).

RESULTS AND DISCUSSION

A total of 102 samples were collected, among this 41 strains were identified as *E. coli* and pattern of resistance to five drugs was determined. It was found that 22 or 53.6 % of the strains were completely resistant to all five antibiotics, whereas 19 strains showed sensitivity to one or more drugs. All the 41 strains showed 100% resistance to ampicillin and tetracycline. 95% of strains were resistant to kanamycin, 78% of strains to streptomycin and 80% of strains to rifampicin. Twenty two (53.6%) strains were MDR against ampicillin, tetracycline, kanamycin, streptomycin and rifampicin (amp-tet-kan-strp). Ten (24.4%) strains were MDR to amp-tet-kan-rif, seven (17%) strains to amp-tet-rif and two (5%) strains to amp-tet. Totally four different antibiotic phenotypic patterns were obtained. The results showed more resistance against the antibiotics tested in frequently isolated *Escherichia coli*.

All the strains of *E. coli* from UTI showed 100% resistance to the antibiotics ampicillin and tetracycline. The tetracycline and chloramphenicol resistant plasmid carried by Australian clinical isolates of *E. coli* (David et al. 2004). More than 34.9% of *E. coli* were resistant to more than one antibiotic among the 347 resistant strains (Doern et al. 1999). In this study there were 53.6% *E. coli* strains resistant to all the antibiotics.

The conjugation pairs of donor were selected depending upon their antibiotic resistant profile and recipient was *E. coli* (CSH57, Str^r, F^-). The following strains of *E. coli* 22, 18, 04, 34 and 27 with antibiotic marker of Amp-Tet-Rif-Kan, *E. coli* 26 and 21 with Amp-Tet- Rif and *E. coli* 12 and 07 with Amp-Tet were used as donor for conjugation. Totally 9 conjugation pairs were undergone for conjugation experiment. Among 9, seven were successfully transferring their antibiotic resistant factor to the recipient CSH57. These seven transconjugant showed tetracycline resistance, indicating that the transfer frequency of tetracycline resistant plasmid was higher than that of the other antibiotic resistant plasmid. The transconjugant in the 5 hrs incubation showed no transconjugant colonies. The transconjugant colonies were high in 15 hrs incubation (Table 1). The antibiotic resistance transfer observed could be attributed to both the transformation as well as conjugation (Lorenz & Sikorski 2000). The conjugative plasmids transfer themselves between most bacteria, thus, being one of the main causal agents of the spread of antibiotic resistance among the pathogenic bacteria (Matxalen et al. 2002). The multiple antibiotic resistant coliform resistance to kanamycin, tetracycline and ampicillin were transferable at different rates (Althrr & Kasweck 1982). Although 75% of MDR coliforms were capable of transferring resistance at some level only 25% were capable of transferring resistance at rates greater than 10^3 transconjugant per initial donor.

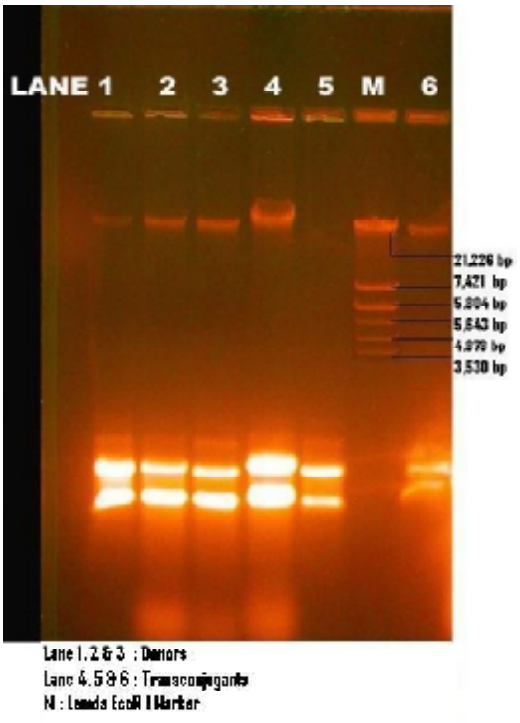

Fig. 1: Plasmids profile of donor and transconjugant *E. coli.*

The plasmid content of the donor and transconjugant were electrophoretically compared using alkaline lysis techniques. All the donor and transconjugant were subjected to alkaline lysis plasmid isolation and transconjugants containing multiple plasmids (David et al. 2004). Present studies showed 2 to 3 plasmid bands in the transconjugants (Fig. 1). Gene conferring resistance to tetracycline is among the most abundant of the identified drug resistance elements (Levy & Marshall 1998). The *tet* genes often occur on mobile genetic elements, such as plasmid, transposons and integrons. Plasmids harbour tetracycline resistance may also carry other antibiotic resistance and virulence factor gene (Olasz et al. 2005).

Plasmid curing was determined by acridine orange treated cells compared with the nontreated cells (control). 49-60% of transconjugant colonies were cured by acridine orange (Table 2). After curing, they showed sensitivity to some of the antibiotics to which they were resistant be-

Table 1: Antibiotic resistant marker transfer from donor to recipient.

S.No	Donor	Antibiotic Marker	Recipient	Transconjugant[a]			Transferred Marker
				5 hrs	10 hrs	15 hrs	
1.	*E.coli* 22	A^r-T^r-R^r-K^r-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	15×10^4	25×10^4	A^r-T^r-R^r-K^r
2.	*E.coli* 26	A^r-T^r-R^r-K^s-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	17×10^5	31×10^5	A^r-T^r-R^r
3.	*E.coli* 18	A^r-T^r-R^r-K^r-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	11×10^6	28×10^6	A^r-T^r-R^r-K^r
4.	*E.coli* 21	A^r-T^r-R^r-K^s-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	26×10^5	44×10^5	A^r-T^r-R^r
5.	*E.coli* 04	A^r-T^r-R^r-K^r-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	7×10^6	10×10^6	A^r-T^r-R^r-K^r
6.	*E.coli* 12	A^r-T^r-R^s-K^s-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	Nil	Nil	NTR
7.	*E.coli* 34	A^r-T^r-R^r-K^r-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	18×10^5	21×10^5	A^r-T^r-R^r-K^r
8.	*E.coli* 07	A^r-T^r-R^s-K^s-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	nil	Nil	NTR
9.	*E.coli* 27	A^r-T^r-R^r-K^r-S^s	*E.coli*-CSH 57,S^r,F^-	Nil	23×10^4	35×10^4	A^r-T^r-R^r-K^r

NTR- No transconjugant recovered, a-Transconjugant in different incubation periods
A-ampicillin, T-tetracyclin, R-rifampicin, K- kanamycin, S-streptomycin, Nil-no transconjugants colonies observed

Table 2: Effect of plasmid curing in transconjugants.

S.No.	Transconjugants	Colony Forming Unit (CFU)		Plasmid curing in %
		AOT	AONT	
1.	EcTn 1	37×10^5	75×10^5	49.3
2.	EcTn 2	54×10^4	75×10^5	49.3
3.	EcTn 3	56×10^4	108×10^4	51.9
4.	EcTn 4	51×10^5	96×10^5	53.1
5.	EcTn 5	37×10^6	66×10^6	56.1
6.	EcTn 6	49×10^5	96×10^5	50.0
7.	EcTn 7	46×10^5	86×10^5	53.5

EcTn: *E. coli* transconjugants, AOT: Acridine Orange treated cells, AONT: Acridine Orange nontreated cells (Control)

fore the curing treatment, indicating that the R-plasmid mediated the antibiotic resistance. Plasmids are eliminated from the host bacteria after exposure to the sublethal concentrations of the intercalating dyes such as acridine orange, ethidium bromide, etc. Curing with acridine orange inhibits the replication of the plasmid without interfering with the bacterial cell replication leading to the production of the plasmid free cells. Loss of the plasmid is concomitant with the loss of resistance to the antibiotic encoded by the plasmid (Shahid & Malik 2004). It is reported that a single plasmid may code for the resistance up to ten different antibiotics simultaneously (Committee for Veterinary Medical Products, U.K. 1999).

CONCLUSION

Incidence of drug resistance among healthy population has been found that the patients undergoing antibiotic therapy often excrete *E. coli* capable of transferring drug resistance, consequently it was of interest to determine whether healthy hospital pregnant women were carrier of *E. coli* strains exhibiting infections drug resistance. The results of this survey indicated that infectious drug resistance is probably the most common form of resistance among pathogenic *E. coli* strains from urine of pregnant women. Dissemination of the resistant bacteria is not only a problem of the resistant pathogens themselves, but also availability of the resistant genes to the nonpathogens via horizontal gene transfer in practically every environment. Tetracycline is not used to treat *E. coli* infection in humans, but

resistance to tetracycline is still common in *E. coli,* which suggest that resistance has been transferred to commensal *E. coli* during treatment of other pathogens in human.

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