Original article

Plasmid mediated multiple antibiotic resistance in Escherichia coli isolated from community acquired infection of urinary tract in Aligarh Hospital

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Abstract

This study was to investigate the current trends of multiple drug resistance in bacteria against antibiotics for the proper empirical treatmen. Clinical isolates were collected from community-acquired infection of urinary tract patients in Aligarh India from March 1999 to August 1999. Antibiotic susceptibility test was performed, using the disc diffusion method followed by plasmid isolation by the method of Kado and Liu. Transfer experiments were performed by the method of Lederberg and Cohen. Clinical study revealed that this infection was more common in young women. Various strains of E. coli isolated during the course of study were found to show multiple antibiotic resistance which was further characterized as plasmid-borne drug resistance. This study shows that E. coli may be one of the important causative agents of urinary tract infection (UTI) in young women.

Keywords: plasmid; drug resistance; UTI

INTRODLICTION

Urinary tract infections (UTIs) are amongst the most common infections described in outpatients setting [1,2]. UTIs are also the most common infection in acute and long term care hospital patients [3,4]. Escherichia coli is the most frequently found bacteria in both; community acquired and hospitalized UTI patients [5,6,7].

Increasing resistance in bacterial pathogens is of worldwide concern. The prevalence of antimicrobial resistance in both out and hospital patients with UTI is increasing and can vary according to geographical and regional location [8,9]. In UTI infection, antimicrobial therapy is initiated even before the results of urine culture are available. Hence, there exists a great need for antimicrobial resistance surveillance at local, national and international level. There is no doubt that antimicrobial therapy is necessary when urinary tract infection (UTI) develops in pregnancy. The aim of therapy is to maintain sterile urine throughout pregnancy without causing toxicity to the mother or the fetus. However, the best mode of achieving this aim is still unclear. There is no consensus on the choice of antimicrobials, duration of therapy or on the prophylactic use of antimicrobials in pregnancy [10,11].

The aim of this study is to determine antibiotic susceptibility of E. coli prevalent in community acquired UTI infection. We have also explored the susceptibility data as well as possibility of having UTI among young women. Moreover, we are also interested to find whether the resistant markers are present on the plasmid.

MATERIAL AND METHODS

A total of 168 urine samples were collected from patients with community-acquired UTI during March 1999 to September 1999 from Aligarh hospital, India. Strains were plated on to the Mac Conkey agar

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plates. Pink colonies were observed. A well-isolated single colony was picked and incubated in nutrient broth, at 37℃ for 12-16 hrs. then the inoculum was spread on eosine methylene blue agar plates and incubated at 37℃ for 24 hrs., green colonies having a metallic sheen were observed. Well-isolated colonies were picked and then re-streaked on to the hard agar plates. Hi-Media kits´manufacturer instructions were followed to identify E. coli. Hi25TM Enterobacteriaceae identification kit and Hi E. coliTM Identification Kit were used. Characterized strains were stored at -80℃ for further studies.

Antimicrobial susceptibility testing was performed by the disk diffusion method, according to National Committee for Clinical Laboratory Standards document (Now called as, Clinical Laboratory Standard Institute) M7-A5 [12]. E. coli ATCC 25922 strain was used as reference strain. Isolates showing an intermediate level of susceptibility were classified as resistant. The following antimicrobial agents at the indicated concentrations were tested: Amp (ampicillin,25), Ch (chloramphinicol,30), Er (erythromycin,15), Ka (kanamycin,30), Nx (norfloxacin,10), Rf (rifampicin,30), St (strteptomycin, 25), Sm (sulphamathizole,300), Tr (tetracycline, 30). Throughout this study, results were interpreted according to the National Committee for Clinical Laboratory Standards criteria for broth microdilution and disk diffusion methods [12].

A preparation of plasmid was made by the method of Kado and Liu [13] and was detected on 0.7% agarose gel. The molecular mass of palsmids was obtained by comparing the relative mobility of their EcoR1 digested plasmid DNA with known marker (1 Kb DNA ladder) on 0.7% agarose gel. Electrophoresis was carried out at 100 V. The gel (5 mm thick) was stained with 0.5 g of ethedium bromide per ml for one hour at 28℃ [13].

The transfer of plasmid to recipient E. coli C600 cells was achieved by using the method of Lederberg and Cohen [14]. C600 cells grown overnight and were made competent by suspension in 0.1 M $MgCl_2$ for 30 minutes on ice and then in 0.1M $CaCl_2$ under similar conditions. To 1.0 ml competent cells 2 g of plasmid DNA was added and incubated on ice for 30 minutes followed by heat shock at 42 ℃ for 3 minutes. These cells were then allowed to grow in LB for 2 hours at 37 ℃. The cells were spread onto the different plates, each of having a single antibiotic of the same concentration as the respective disc used previously.

To analyze the UTI among the patients of different age groups, Pearson Chi-square Test was performed to check the significant difference among different groups. A difference was considered significant if the probability that chance would explain the results was reduced to less that 5% ($P < 0.05$). This test was performed, using SPSS version 11.5.

RESULTS

A total of 168 samples were collected from patients with community acquired UTI during March 1999 to September 1999 from Aligarh hospital, India. One hundred and two (102) of them were identified as E. coli. Our study revealed that out of 61% of E. coli infection 17% were male patients, whereas remaining were females (83%). Moreover, the prevalence of UTIs among women was also studied with reference to their age group. It was observed that 59.8 % (61/102) women were from the age group of 20-30 years of young females. This figure was reduced to 22.5% (2/102) among the women of 31-45 years of age (Table 1). Other studies on UTIs in outpatients have shown E. coli to be the most common etiological agent (>80%), with preponderance in young females (30 times > young males).

A total of 102 samples were identified as E. coli among 168 UTI samples. Their antibiotic sensitivity and resistance were tested by the method stated above. Our data showed that 90% isolates were resistant against ampicilin. Present studies also showed that 60-79% isolates were resistant against chloramphinicol, erythromycin, rifampicin, sulphamathizole, tetracycline. Norfloxacin showed intermediate resistance (23%). The most effective antibiotics in our study against E. coli were found to be kanamycin and streptomycin (2% and 0% resistance respectively). Figure 1 shows the agarose gel electrophoretic profile of plasmids isolated from some selected strains. A single plasmid of 18 kb was found in all multiple resistant strains. To provide evidence that resistance markers were actually plasmid borne, we carried out the transformation experiments employing R-plasmids isolated from the E. coli strains. The expression of resistance markers in all transformants suggested that antibiotic resistance was plasmid mediated. The transformation frequencies of C600 cells with R-plasmid (pEC186, pEC56, pEC188, pEC193, pEC196, pEC191) are shown in table 2. Our data also revealed that almost all the isolates included in this study were found resistant to two or more antibiotics (Table 2).

SPSS was used to perform Pearson Chi-square

Test. P-values for the data obtained among different age groups of patients were found to be significant ($p < 0.05$) as shown in table 1.

Table 1. Physical profile of UTI patients

Sex of Patients	Age group (Years)	[a]No. of UTI samples identified as *E. coli*	p-value
Males	10-50	18	0.001
Females	20-30	61	0.001
Females	31-45	23	0.001

P value was compared among different age groups.

[a] Total number of samples tested were 168

Table 2 Transformation frequencies of the E. coli C600 with R-plasmids (pEC163, pEC56, pEC34, pEC79, pEC86, pEC90) isolated from six-selected E. coli strains of UTI patients.

R-plasmids	Resistant markers present in isolates	No. of cells/ml	Total no. of antibiotics resistant transformants per plate (0.1 ml)	Transformation frequency	[a,b]Resistant markers presentin transformants
pEC163	Ch, Er, Sm, Tr, Amp	$5 * 10^7$	$3.4 * 10^2$	$6.8 * 10^{-5}$	Ch, Er, SmTr
pEC56	Amp, Ka, Nx, Ch Rf, Sm	$5 * 10^7$	$2.8 * 10^2$	$5.6 * 10^{-5}$	Amp, Ka, Nx Rf, Sm
pEC34	Amp, St, Ka, TrCh	$5 * 10^7$	$1.5 * 10^2$	$3 * 10^{-5}$	Amp, St, Ka, Tr
pEC79	Amp, Nx, Rf, Ch	$5 * 10^7$	$1.6 * 10^2$	$3.2 * 10^{-5}$	Amp, Nx, Rf
pEC86	Rf, Nx, Tr, Amp	$5 * 10^7$	$2 * 10^2$	$4 * 10^{-5}$	Rf, Nx, Tr
PEC90	Amp, Sm	$5 * 10^7$	$2 * 10^3$	$4 * 10^{-4}$	Amp, Sm

[a] Amp (ampicillin,25), Ch (chloramphinicol,30), Er (erythromycin,15), Ka (kanamycin,30), Nx (norfloxacin,10), Rf (rifampicin,30), St (strteptomycin,25), Sm (sulphamathizole,300), Tr (tetracycline,30)

[b] Each number indicated against antibiotic is concentration in g/disc

DISCUSSION

Our study shows the distribution and antibiotic susceptibility of E coli strains isolated from patients with community acquired UTIs in J. N. M. C. H, Aligarh during March 1999 to September 1999. A majority of the E coli strains causing UTI were isolated from young female patients (59.8 %). The data was found to be significant, statistically. It has been extensively reported that women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors [15,16].

Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country [15]. The most effective antibiotics in our study against E. coli were found to be kanamycin and streptomycin, are also supported by previous studies [17]. The progressive appearance of a number of bacterial species with multiple antibiotic resistance is the characteristic of plasmid[18]. A single plasmid of 18 kb was found in all multiple resistant strains which has also been shown by other workers [16,19].

A single plasmid of 18 kb was found in all multiple resistant strains as shown in figure 1. Similar pattern of EcoR1 digested plasmids from the different isolates having varying antibiogram is probably due to the fact that EcoR1 sites are not altered because of the presence of different markers [20]. Moreover, some of the markers are not transferred, might be present on genomic DNA as indicated in the table 2. The frequencies obtained with recipient strains in E. coli C600 were comparable to those obtained by Bopp et al (1983) [21] in case of E. coli recipient, transformed with Pseudomonas putida derived Rp4 plasmids. All the test E. coli transformants were found to have two or more resistance marker out of 5-7 markers.

Our study concludes that E. coli is one of the important causative agents of urinary tract infection in young women. Moreover, multiple antibiotic resistance in E. coli were identified as plasmid mediated in the present study. It is quite alarming to note that almost all the isolates included in the present study were found resistant to two or more antibiotics. It has become a major problem for the public health, which might also threaten the lives of patients admitted in the hospital. Therefore, it is an important and most urgent issue to be addressed by the policy makers to formulate strict antibiotic prescription policy in our country.

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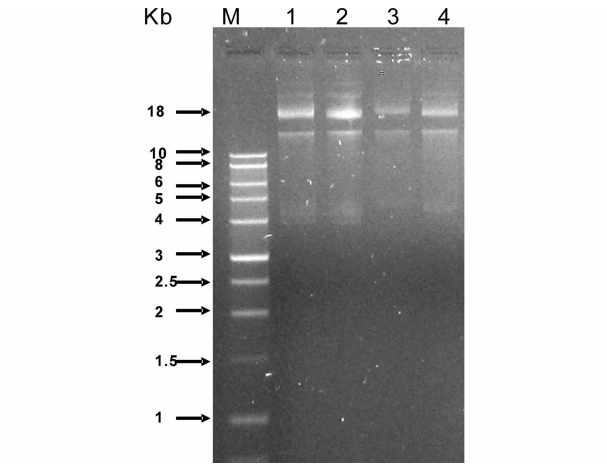

Legends to figures

Figure 1. Electrophoretic profile of R-plasmids isolated from some selected strains. Each plasmid DNA (2 g) was partially digested by EcoR1 (20 Units). The reaction was performed at 37 o C for one hour. Lane, M is marker (1 Kb DNA ladder). Lane 1 to 4 is plasmid from E. coli isolates (pEC34, pEC79, pEC86, pEC90 respectively).

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