Plasmid profile analysis of multidrug resistant *E. coli* isolated from UTI patients of Nagpur City, India

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Abstract

Infections caused by *Escherichia coli* (*E. coli*) have become a significant worldwide public health problem with India being no exception. Furthermore, the situation is worsening due to advent of increased antibiotic resistance due to the evolution of multi-resistant antibiotic plasmid genes. Extended Spectrum B-lactmases (ESBLs) are plasmid mediated and these enzyme producing organisms exhibit co resistance to many other classes of antibiotics. The wide spread presence of drug resistant *E. coli* and other pathogens in our environment necessitates regular monitoring of antibiotics susceptibility trends in the clinical isolates obtained from different regions to provide the basis for developing National and International prescription programs that can be used for delineating guidelines to maintain the desired effectiveness of antibiotics. In the present study, out of 135 isolates, 76 isolates were of *E.coli*. These isolates were tested for antibiotic resistance and plasmid profiles. The results revealed that more than 50% of the isolates exhibited multi-drug resistance. Out of 76 *E.coli* isolates, 40(52.6%) were found to possess plasmids. Some isolates possess single sized plasmid while other had multiple plasmids with different size ranged from 2.3 kb to 26 kb, very high antibiotic resistance was detected from isolates possessing high molecular weight plasmids (23kb). The studies show good prospects for further research in the same area to explore and assign definite cause for antibiotic resistance and multi drug resistance.

Keywords: Plasmid, UTI, Antibiotic resistance, *E. coli,* ESBL

Introduction

E. coli is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics [1,2] isolated from humans. It has been observed that antibiotic susceptibility of bacterial isolates is not constant, but dynamic and varies with time and environment [3]. Studies by Russo and Johnson [4] have reported the magnitude of extraintestinal infections by *E. coli* in the range of 6 to 8 million with 0.1 million cases per year of sepsis in the United States. *E. coli* are the most common cause of urinary tract infections (UTIs) in women and, because of its high incidence, have been studied during numerous epidemiological studies [5]. Many factors that are responsible for acquiring UTI, and amongst main risk factors, history of UTI is the predominant factor [6,7].

The therapeutic steps during the treatment of UTIs, involves a short course of antimicrobial drug, such as antibiotics viz. ampicillin, chloramphenicol, colistin methane sulphonate, kanamycin, nalidixic acid, nitrofurantoin, streptomycin, norfloxacin, Trimethoprim-Sulfamethoxazole (SMP-SMX) etc. The antibiotic resistance studies using urinary tract isolates of *E. coli* from the outpatient clinics have been reported to have shown increased resistance to certain antibiotics, for e.g. ampicillin [8] and ciprofloxacin [9]. The cause of many epidemics was confirmed as *E. coli*, which was identified because of the

distinctive drug resistance profile of some clonal groups, which contributed to its recognition in many countries, such as UK and other areas of Europe and the United States [10,11].

Understanding the molecular epidemiology of resistance plasmids has been a major issue since investigators/scientists became aware of its (plasmids') role in the spread of antimicrobial drug resistance. However, understanding this epidemiology has been complex because of the diversity and dynamic nature of these elements. The plasmid replication system, which dictates the plasmid's behavior (host range, copy number) is the major plasmid landmark from a biologic standpoint; it is used for plasmid classification and identification [12]. However, their number (plasmid copies) also plays a critical role in imparting various characteristics to the pathogen, such as resistance towards different antibiotics.

The approach that we stick was to screen *E. coli* isolates on the basis of ESBL production and analyzed their plasmids.

Materials and Methods

Sample Collection and Bacteriology

Urine samples were collected randomly from various (Government and private) pathological laboratories of Nagpur City situated in Maharashtra State of India. A total of 135 samples were screened using standard isolation and identification procedure for detection of *E.coli*, [13]. The media used during the study included, MacConkey Agar, Eosin Methylene Blue Agar, Endo Agar, Nutrient Agar and Nutrient Broth (Hi-Media, Mumbia).The cultures were incubated at 37^0C for 24 hrs respectively. These *E.coli* isolates were subcultured on Nutrient Agar slants and maintained at 4^0C for further studies.

Double –Disk synergy Test

All clinical isolates of *E.coli* collected by UTI patients resistant to at least ceftazidime, ceftriaxome and cefotaxime were subjected to double disk synergy test. [23]. A clear inhibition zone of cephalosporin towards augmentin disc is interpreted as positive for ESBL production.

Antibiotic susceptibility testing

Susceptibility of isolates to different antibiotics were tested following Kirby Bauer disc diffusion method [14] using Muller Hinton Agar against selected antibiotics, namely Ampicillin (A) 25mcg, Chloramphenicol (C) 50mcg, Colistin Methane Sulphonate (Cl) 100mcg, Kanamycin (K) 30mcg, Streptomycin (S) 30mcg and Tetracyclin (T) 100mcg (Hi-Media, Mumbai). Amoxicillin (30 μg), amoxicillin-clavulanic acid (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), gentamicin(10μg),), ofloxacin (30 μg), levofloxacin (30 μg), ciprofloxacin (30 μg) (Oxoid, UK) The sensitivity test was standardized using *E. coli* (ATCC 25922). Inhibition zone size was interpreted using standard recommendation of National Committee for Clinical Laboratory Standards [15] now known as Clinical Laboratory Standard Institute (CLSI) [20]

Plasmid analysis

Plasmid DNA was extracted from cultured cells following alkaline lysis method of plasmid preparation [16]. The samples were processed using gel electrophoresis to identify the number of plasmid copies present in different isolates. For this purpose, an agarose gel of 0.8% was used. Staining of DNA fragments was carried out using ethidium bromide and they were visualized by UV-Trans illumination. Standard DNA molecular weight markers were

used to estimate the Plasmid size. The standard DNA molecular weight Markers used in the present study were, 1kb ladder, 1kb plus DNA ladder and lambda DNA/*MIu* I digest.

Results

Out of 135 urine Samples analyzed *E.coli* was present in 76 samples. Further, the resistance ability of these 76 *E. coli* isolates was tested against different antibiotics, 40 isolates showed presence of plasmids, which corresponded to 52.65% of the total isolates.

Antibiotic resistance pattern

Amongst the isolates, the number and size of plasmid varied significantly. Detailed results of the antibiotic resistance screening tests and the summary of antibiogram profiles obtained are presented in (**Table 1**). The results showed that more than 50% of isolates were multidrug resistant i.e., were resistant to four or more antibiotics. **Figure 1** shows the multidrug resistance exhibited by one of the *E. coli* isolates.

Table 1. Antibiogram and plasmid profile of *E.coli*

Sr. No.	Antibiotics resistance pattern	No. of isolates showing similar antibiotic resistance profile	No. With plasmids
1	A, C, Nf, S, T, Ofx	6	2
2	A, C, K, Na, T	4	2
3	A, Na, Nf, S, T, Ofx, Fox,	2	1
4	A, C, S, T	2	1
5	A, Ti, T, Tr, Sx, Amx, Fox,	4	3
6	A, Na, S, T	2	1
7	A, Ti, T, Tr	3	3
8	A, C, S, T, Aug, Cip	2	2
9	A, C, Nf, T	5	1
10	A, Nf, Ti, T, Na, Tr, Aug,G Fox, Amx	10	2

Key
A: Ampicillin, **C**: Chloramphenicol, **Cl**: Colistin Methane Sulphonate, **K**: Kanamycin, **Nf**: Nitrofurantoin, **S**: Streptomycin, **T**: Tetracycline, **Tr**: Ticarcillin, **Sx**: Sulphamethoxazole and **G**: Gentamicin, **Aug:** Augmentin, **Cip:** ciprofloxacin, **Amx:** Amoxicillin, **Ofx:** Ofloxacin, **fox:** cefoxitin.

Gel Electrophoresis (GE)

On the basis of gel electrophoresis, the plasmid copies were found to vary between 1 and 3 (**Figure 2**). The maximum numbers of plasmid copies of 3 were recorded form a total of 7 *E. coli* isolates. Although all these isolates showed multidrug resistance, the resistance pattern was not same in all the cases i.e. each one showed few antibiotics same and few different (**Table 1**). Plasmid size ranged from 2.3 kb to 6.5 kb (1kb ladder; Figure 1), while with lambda DNA/*MIu* I digest ladder, the plasmid size was found to be 26 kb (**Figure 2**).

Figure 1. Antibiogram of *E. coli* isolate (E39) showing resistant to multiple antibiotics
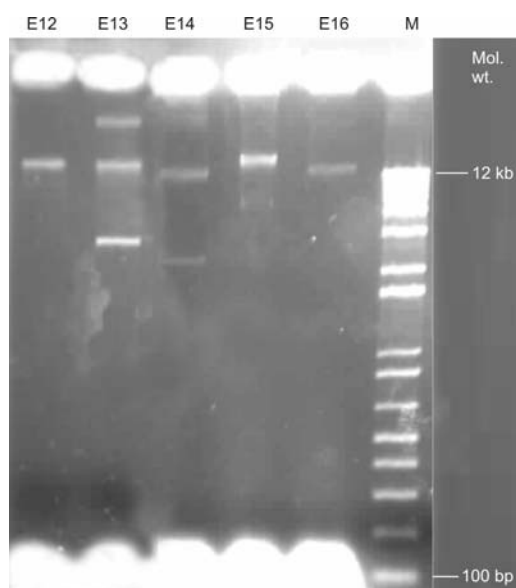
Figure 2. Plasmid profile of test isolates of *E.coli* lane1:E12 to lane5: E16 and lane 6:1kb plus DNA Ladder
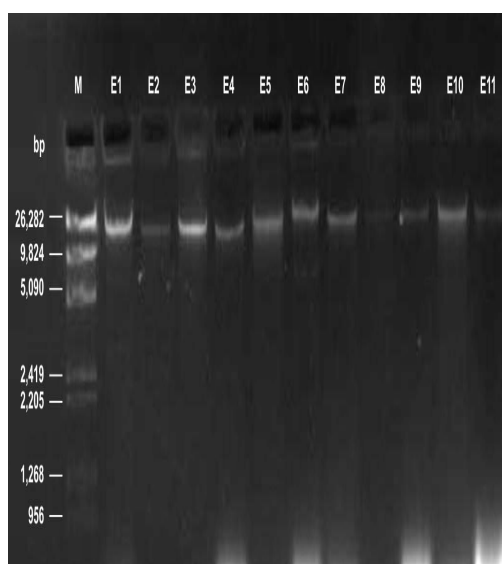
Figure 3. Plasmid profile of *E. coli* isolates; Lane 1: Marker Lambda DNA/M1u1 digest, Lane 2: to Lane 12 – E2 to E11 *E. coli* test isolates

Discussion and Conclusion

It has been reported that pathogenic isolates of *E.coli* have relativity high potential for developing resistance [9]. Besides, amongst the enteric pathogens, resistance of E. coli was observed to be increasing, especially to first line, broad spectrum antibiotic, such as ampicillin and others. In the present investigation, high resistance of *E.coli* to numerous antimicrobial agents (antibiotics) was observed. These results are congruent to the results reported by [17], who found 100% resistance of their *E.coli* isolates to ampicillin. Out of the total, 23 (57.7%) E. coli isolates showed resistance to Nitrofurantoin, such high number of resistance by E. coli, these results are contradictory to those reported by [18], who reported that the *E.coli* isolated from UTI patients to be highly sensitive to nitrofurantoin (Nf). Thus, the results from present study provide an entirely opposite response of *E. coli* to the antibiotic Nitrofurantoin. The situation indicates a threat and a possibility that the *E. coli* could have become resistance to many more antibiotics to which it showed susceptibility earlier.

In view of these results, the studies on *E. coli,* focusing on the changes on molecular level, could provide valuable insights for its management. In this study, we found a large number of plasmids having molecular weight of 26 kb. All the isolates with plasmid of 26kb showed multidrug resistance. Clinical isolates of *E. coli* are known to harbour plasmids of different molecular size ranging from 2-3 kb to 6.5 kb and maximum 26 kb. Danbara [19] have also reported the plasmid size between 3.9kb and 50kb in *E.coli* strains isolated from patients suffering from traveler's diarrhea. The clinical isolates of *E. coli*, along with many others are constantly exposed to the hospital environment where they gain resistance to numerous antibiotics by various mechanisms. This drug resistance increases as a function of time and their (microorganism's) exposure to many factors (antibiotics, chemicals, etc). Besides, the bacteria acquire resistance through different routes, such as natural or intrinsic resistance (inaccessibility of the target, multidrug efflux systems and drug inactivation), mutational resistance (drug target site modification, reduced permeability or uptake, metabolic by pass and derepression of multidrug efflux), extrachromosomal or acquired resistance (drug target site modification, reduced permeability or uptake, metabolic by pass and derepression of multidrug efflux). All these mechanisms of antibiotic resistance warrant a detailed investigation of multiple factors, with prioritization of the studies of molecular characterization. Multiple drug resistance among UTI isolates in USA was reported to be 7.1% in 2000 [20]. Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E.coli* and for the possible co-selection of antimicrobial resistance mediated by multi drug resistance plasmids [21]. Thus, the studies confirm the important role of plasmid numbers and plasmid size that controls the resistance characteristics in *E. coli*.

Acknowledgment

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