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Full Length Research Paper

Correlation of plasmid with drug resistance of clinical isolates of *Escherichia coli*

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According to the latest data from the Centers for Disease Control and Prevention (CDC), the six ESKAPE bacteria are responsible for two thirds of all health care-associated infections (HAIs) including *Escherichia coli*. *Klebsiella* species and their gram-negative cousin *E. coli* together accounted for 18 percent of all HAIs in 2006 to 2007, and a growing proportion of these two bad bugs carry resistance to a remarkable spectrum of antibiotics. Of the very few drugs in late-stage development, none works by a novel mechanism. The aim of our study is to correlate the plasmids with drug resistance of clinical isolates of *E. coli.* Twenty two clinical isolates of *E. coli* were collected from different diagnostic centers of Dhaka and their antimicrobial susceptibility pattern was tested. Seven multidrug resistant isolates of *E. coli* were selected and their antibiotic susceptibility pattern was tested before and after curing. Our study revealed that 100% of the isolates (22 isolates) were resistant to cephalexin, cephradine, oxacillin, penicillin and vancomycin. 95.45% of the isolates were resistant to ciprofloxacin, cloxacillin and imipenem. 90.91% isolates (21 isolates) were resistant to erythromycin. 81% of the isolates (18 isolates) were resistant to amoxicillin. 72.72% (16 isolates) were resistant to co-trimoxazole, 63.64% (14 isolates) were resistant to tetracycline. 31.81% (7 isolates) were resistant to ceftriaxone and neomycin. 22.73% (5 isolates) were resistant to gentamicin and only 13.64% of the isolates (3 isolates) were resistant to chloramphenicol. There was no significant difference in the antibiotic susceptibility pattern before and after curing indicating no correlation between plasmid and drug resistance in the 7 isolates of *E. coli*. However, isolate number 5 which was resistant to gentamicin, neomycin and imipenem became sensitive after curing. Similarly sample number 1 became sensitive to rifampicin and imipenem and sample number 7 became sensitive to imipenem after curing.

Key words: Multi-drug resistant, *Escherichia coli*, antimicrobial susceptibility, plasmid, curing.

INTRODUCTION

According to the Infectious Diseases Society of America in the January 2009 highlighted the impact of the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) as a group of particularly troublesome bacteria having the ability to "escape" the effects of current antimicrobial agents (Boucher et al., 2009). Drug resistance is an alarming problem worldwide and it is spreading rapidly due to overuse, self medication, and non-therapeutic use of antimicrobials (Slama et al., 2005). Antimicrobials themselves act as a selective pressure which allows the growth of resistant bacteria within a population and inhibits susceptible bacteria (Levy, 1994). Drug resistance property in bacteria is usually borne in R-plasmids, which can be disseminated, to diverse population and regions causing world wide problems. R-plasmids from resistance strains of an organism may transfer to a sensitive counterpart, which can show the same drug resistance in the donor strain. *E. coli* are commonly found in the lower intestine

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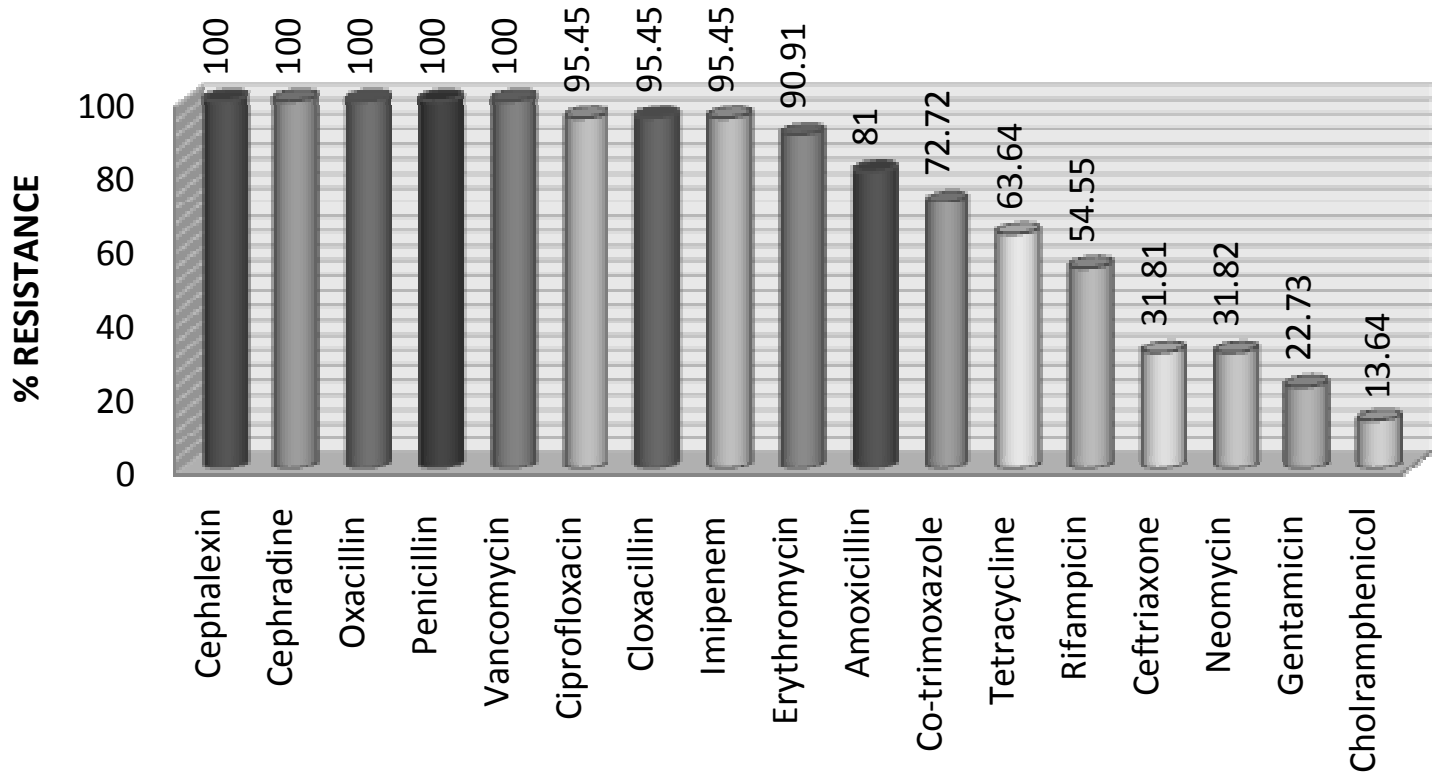

Figure 1. Percentage resistance of *E. coli* to different antimicrobials.

of warm-blooded organisms (endotherms) as normal flora of the gut, and can benefit their hosts in many ways but there are over 700 antigenic serotypes of *E. coli* some of which can food poisoning in humans, and serious illness like gastroenteritis, urinary tract infections, and neonatal meningitis (Todar, 2008). New strains of *E. coli* evolve through the natural biological process of mutation, *E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population (Brüssow e. al., 2004). In this study, plasmid profiles and drug resistance pattern of multi drug resistant clinical isolates of *E. coli* were studied before and after curing to find out whether the resistant gene which makes *E. coli* resistant to multiple drugs (antimicrobials) is present in the plasmid DNA or in the chromosomal DNA. *E. coli* is a very common organism and their resistance to multidrug is alarming for mankind.

MATERIALS AND METHODS

Collection of isolates

Twenty two isolates of *E. coli* were collected from different diagnostic centers of Dhaka. The samples were collected from pathological specimen of stool, pus and urine. The isolates were identified on the basis of their colony morphology, microscopic morphology and biochemical tests for reassurance and reconfirmation of the strain.

Antimicrobial susceptibility test

The antimicrobial susceptibility pattern of *E. coli* to 17 antibiotics namely amoxicillin, Am (30 µg/disc); ceftriaxone, Ci (30 µg/disc); cephalexin Cp (30 µg/disc); cephradine, CV (25 µg/disc); chloramphenicol C (30 µg/disc); ciprofloxacin, Cf (5 µg/disc); cloxacillin, CX (1 µg/disc); co-trimoxazole, Co (25 µg/disc); erythromycin, E (15 µg/disc); gentamicin, G (10 µg/disc); neomycin, N (30 µg/disc); oxacillin, OX (1 µg/disc); penicillin, P (10 µg/disc); rifampicin, R (5 µg/disc); tetracycline, T (30 µg/disc); vancomycin, VA (30 µg/disc) and imipenem, I (10 µg/disc) was determined by disc diffusion Kirby Bauer (A. Bauer et al., 1966) method as per recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 1997) and the result was recorded in Figure 1 for comparing.

Antimicrobial susceptibility test of *E. coli* in presence of 1% SDS solution

From the twenty two isolates 7 isolates of *E. coli* were selected and cultured in Luria broth in presence of 1% SDS solution and in absence of SDS solution. SDS solution is used as the curing agent. And antibiotic susceptibility test for these 7 samples was carried out in absence of SDS and in presence of 1% SDS by disc diffusion

Table 1. Antimicrobial susceptibility pattern of seven selected isolates of *E. coli* without SDS solution.

E. coli isolates	Zone of inhibition (mm)																
	Am^{30}	Ci^{30}	Cp^{30}	CV25	C30	Cf^{5}	CX1	Co^{25}	E^{15}	G^{10}	N^{30}	OX^{1}	P^{10}	R^{5}	T^{30}	VA^{30}	I^{10}
(1) E- 516	R	S	S	R	S	R	R	R	R	R	S	R	R	R*	S	R	R*
(2) *E. coli* -2	R	S	S	R	S	S	R	S	R	S	S	R	R	S	R	R	R
(3) E-371	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R
(4) E-210	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	S
(5) E-294	R	S	R	R	R	R	R	R	R	R*	R*	R	R	R	R	R	R*
(6) E-653	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	S
(7) E-480	R	S	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R*

Table 2. Antimicrobial susceptibility pattern of seven selected isolates of *E. coli* cultured in Luria broth in the presence of 1% SDS.

E. coli isolates	Zone of inhibition (mm)																
	Am^{30}	Ci^{30}	Cp^{30}	CV25	C30	Cf^{5}	CX1	Co^{25}	E^{15}	G^{10}	N^{30}	OX^{1}	P^{10}	R^{5}	T^{30}	VA^{30}	I^{10}
(1a) E- 516	R	S	S	R	S	R	R	R	R	R	S	R	R	S*	S	R	S*
(2b) *E. coli*-2	R	S	S	R	S	S	R	S	R	S	S	R	R	S	R	R	R
(3c) E-371	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	R
(4d) E-210	R	R	R	R	S	R	R	R	R	S	S	R	R	R	R	R	S
(5e) E-294	R	S	R	R	R	R	R	R	R	S*	S*	R	R	R	R	R	S*
(6f) E-653	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R	S
(7g) E-480	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	S*

Kirby Bauer (Bauer et al., 1966) method using the same 17 antimicrobials. The result was recorded in Tables 1 and 2. Then plasmid profile analysis of the above samples was done using gel electrophoresis plasmid isolation.

To isolate plasmid DNA from bacteria a scaled-up miniprep followed by additional purification was done. This results in relatively large amounts (several micrograms) of very pure plasmid DNA.

Separation of plasmid DNA by agarose Gel electrophoresis

Electrophoresis experiments were carried out in horizontal 1.5% agarose gel. The gel was viewed on an ultraviolet (UV) light box (short wave, ultraviolet products, Inc., San Gabriel, California, USA, 254 nm). The photographs were taken under UV illumination (using Sony cyber shot 5.1 megapixels, USA) as shown in Figure 2.

RESULTS

The results of antimicrobial susceptibility of *E. coli* to 17 antibiotics are represented in Figure 1, where percentage resistance is taken in the y-axis and different antimicrobials agents are taken in the x-axis. Then seven multi-drug resistant isolates of *E. coli* were selected, they are: E- 516, *E.coli*-2, E-371, E-210, E-294, E-653 and E-480. Then antibiotic susceptibility pattern test was carried out with these seven samples in presence of 1% SDS and in absence of SDS. The results are shown in Figure 1 and 2.

Gel electrophoresis

Plasmid profile analysis indicated that bands for plasmid DNA were absent, when multi-drug resistant isolates of *E. coli* were cultured in Luria both in presence of 1% SDS confirming that when the isolates were cultured in presence of 1% SDS, all its plasmids come out of the cells due to the formation of small pores. So no bands for plasmids were obtained in the electrophoretic pattern. But, when the same isolates were not treated with 1% SDS, the electrophoretic pattern showed the presence of plasmid DNA in Figure 2.

Discussion

Resistance to antimicrobials is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Normal intestinal flora is a reservoir for resistance genes; the prevalence of resistance in commensal *E. coli* is a useful indicator of antibiotic resistance in bacteria in the community. Oral administration of antibiotics can also influence the normal intestinal micro flora and can lead to an overgrowth of resistant the correlation between antibiotic resistance and plasmid profile may indicate that the genetic information is plasmid borne (Myaing et al., 2005).

In our six months period of study, it was observed that

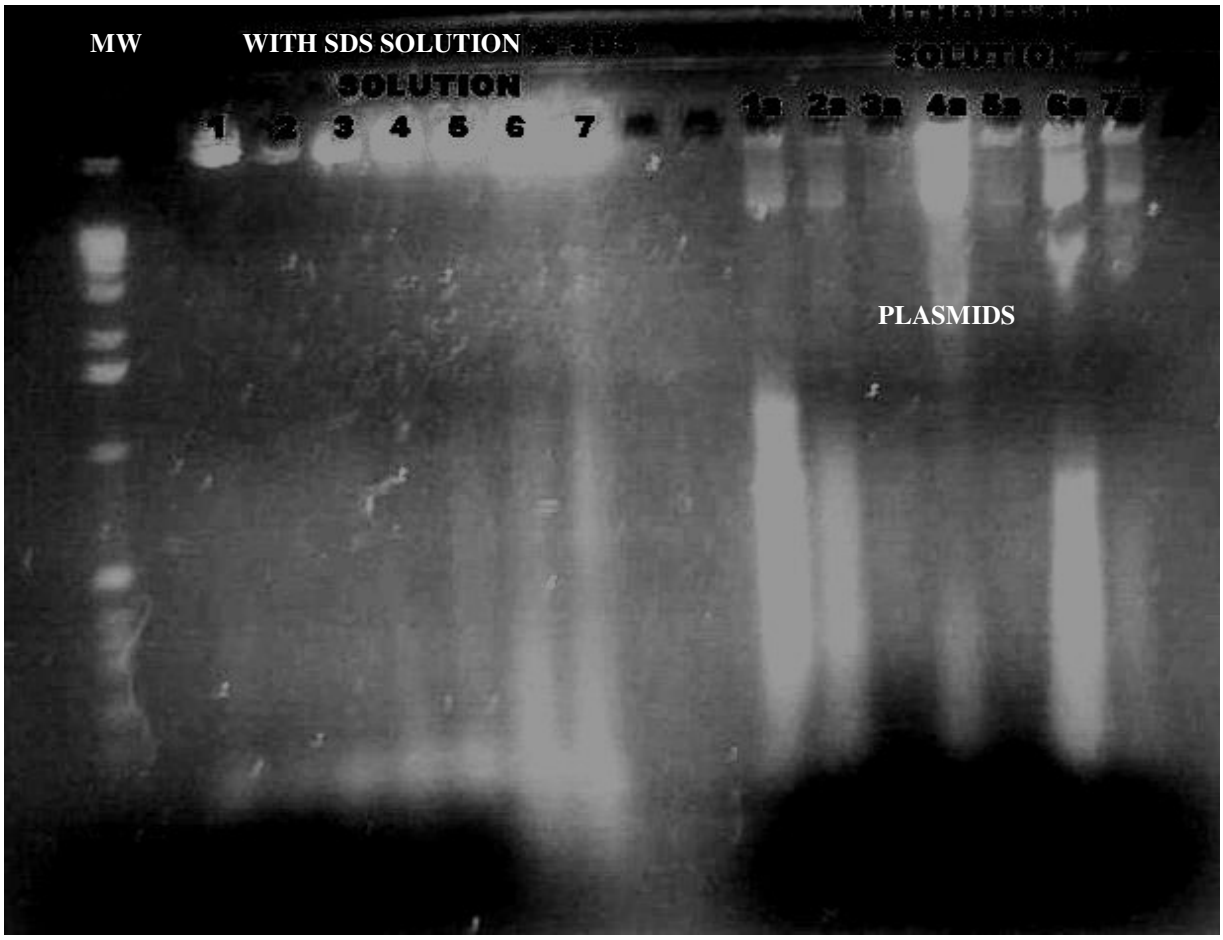

Figure 2. Agarose (1.5%) gel electrophoresis of plasmid DNA from *E. coli* isolates grown in Luria broth in absence and in presence of 1% SDS.

the isolates of *E. coli* collected from different pathological specimen showed different degree of sensitivity to different antimicrobials. Seventeen different antimicrobial agents were used to test the susceptibility. Most of the *E. coli* isolates of this study are resistant to the first line antibiotics that are commonly prescribed by the physicians. All the isolates (100%) were found to be resistant to cephalexin, cephradine, oxacilline, penicillin and vancomycin. 95.45% of the isolates were resistant to ciprofloxacin, cloxacillin and imipenem. 90.91% isolates were resistant to erythromycin. 81% of the isolates were resistant to amoxicillin. 72.72% resistant to co-trimoxazole, 63.64% to tetracycline. 31.81% of the isolates were resistant to ceftriaxone and neomycin. 22.73% is resistant to gentamicin and only 13.64% of the isolates were resistant to chloramphenicol.

However, in the present study isolate number 5 was resistant to Gentamicin, Neomycin and Imipenem when cultured in absence of 1% SDS solution but became sensitive when cultured in presence of 1% SDS solution. Since isolate number 5 has lost its plasmid (Figure 2) after being cultured in presence of 1% SDS (that is, the SDS treated sample lacks plasmid inside the cells) it appears that gentamicin, neomycin and imipenem resistant genes may be present in the plasmid. Similar phenomenon was observed in case of isolate numbers 1 and 7. Isolate number 1 becomes sensitive to rifampicin and imipenem and isolate number 7 becomes sensitive to imipenem after being cultured in presence of 1% SDS, though both the isolates were resistant to respective antibiotics when cultured in absence of SDS. So it appears that in sample number 1, 5 and 7 the resistant gene for the respective antibiotics may be present in the plasmids.

Conclusion

In the study, the result obtained indicates that there appears to be no correlation between plasmid and antibiotic resistant of *E. coli* though there might be exceptions. So, further studies are needed to be carried out in future to confirm this issue. If the gene responsible for multidrug resistance can be located, genetic

engineering and further research can be done to prevent *E .coli* from becoming resistance.

REFERENCES

Bauer A, Kirby WMJ, Sherris C, Truck M (1966). Antibiotic susceptibility testing by a standard single disc method. Am. J. Clin. Pathol., 44: 493.

Boucher HW, Talbot GH, Bradley JS (2009). Bad bugs, no drugs: no ESKAPE! An update from Infectious Diseases Society of America. Clin. Infect. Dis., 48: 1–12.

Brüssow HH, Canchaya C, Hardt WD (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol. Mol. Biol. Rev., 68(3): 560–602.

Levy SB (1994). Balancing the drug-resistance equation. Trends Microbiol., 2(10): 341-2.

Myaing TT, Saleha AA, Arifah AK, Raha AR (2005). Antibiotic resistance and plasmid carriage among *Escherichia coli* isolates from chicken meat in Malaysia applications of gene-based technologies for improving animal production and health in developing countries, pp. 521-527.

National Committee for Clinical Laboratory Standard (1997). Methods for dilution antimicrobial tests for bacteria that grow aerobically, 3rd end. approved standard. NCCLS, Pennsylvania. Document, M7-A3.

Slama TG, Amin A, Brunton SA (2005). A clinician's guide to the appropriate and accurate use of antibiotics: The Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria. Am. J. Med., 118 (Suppl 7A): 1S–6S.

Todar K (2008). Pathogenic *E. coli*. Online Textbook of Bacteriology. Department of Bacteriology, University of Wisconsin–Madison, USA.