

PLASMID PROFILING AND CURING OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* RECOVERED FROM RETAIL CHICKEN MEAT

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ABSTRACT

Confirmed *E. coli* (n = 25) were analyzed for the presence of plasmids and cured using temperature and sodium dodecyl sulphate (SDS) treatments. Plasmid profiling of the isolates showed that 96 % isolates were harboring plasmids of varying size and number. The average plasmid number among the isolates was 2.28, ranging from 0 – 5 plasmids per isolate and plasmid size varied from 100bp – 12kb which was distributed randomly in these isolates. Statistically no significant correlation ($p>0.05$) was found between antibiotic resistance and plasmid profiles. Plasmid curing study showed 3.9% and 16.7% curing efficiency with that of temperature and SDS treatment, respectively. Statistical analysis revealed that resistant isolates, treated with either physical or chemical treatments produced significant results ($p<0.05$) of plasmid curing. Antimicrobial resistance genes might be located on plasmid and statistically no relationship was observed between antibiotic resistance and plasmid profiles.

Key words: Plasmid profiles, plasmid curing, *E. coli*, antibiotic resistance

INTRODUCTION

Escherichia coli (*E. coli*) is a gram negative, non-spore forming, rod shape, facultative anaerobe, having peritrichous flagella and belongs to family *Enterobacteriaceae* (Kubitschek, 1990). Food of animal origin is major vector for this pathogen to reach its predilection sites in new susceptible host (Newell *et al.*, 2010). Indiscriminate use of antibiotic during chicken rearing results in development of drug resistance in commensal bacteria of GIT. Similarly retail chicken meat may get contaminated during processing (Akond *et al.*, 2009). Antibiotic resistance genes are either located on chromosomes or on mobile genetic elements such as plasmids, transposon and integrons. Transmission of plasmid mediated antimicrobial resistance occurs through variety of mechanisms. Plasmids are self replicating DNA molecules present in bacteria and are responsible for horizontal transmission of antibiotic resistance genes among bacteria of same genera and sometime other genera (Wonglumsom *et al.*, 2011). Plasmids in *E. coli* carry different genes which imparts specific characteristic to bacteria e.g. antibiotic resistance, antibiotic production, virulence genes and sex pilli which helps *E. coli* to survive in adverse condition and enable the other bacteria to compete with environmental conditions (Hardy, 1986). Plasmid profiles have been reported to use as epidemiological tool and to determine the transmission pattern of antibiotic resistance genes among microorganisms (Wonglumsom *et al.*, 2011).

Plasmid curing is the elimination of plasmid DNA from bacterial isolates in order to determine the

relationship of multidrug resistance with plasmid DNA (Raj, 2012). Various chemical and physical agents when exposed in sub-inhibitory concentrations cause the elimination of plasmid. The current study is unique and is designed to perform plasmid profiling and curing of previously isolated multi-drug resistant *Escherichia coli* from retail chicken meat samples.

MATERIALS AND METHODS

Biochemical Confirmation: Previously isolated multidrug resistant (ciprofloxacin (5µg), gentamycin (10µg), ampicillin (10µg), streptomycin (10µg), sulphamethoxazole (25µg), amoxicillin (10µg) and levofloxacin (5µg)) *E. coli* isolates recovered from retail chicken meat were used. Glycerol stocks of *E. coli* (n = 25) were revived by culturing in tryptose soy broth and placed at 37°C for 24 hours. The isolates were confirmed biochemically (Ahmed, 2010).

Plasmid Profiling: The *E. coli* culture were inoculated in TSB and placed at 37°C for 24 hours. *E. coli* culture (0.5mL) were transferred in a microfuge tube and same volume of phenol: chloroform: isoamyl-alcohol (25:24:1) were added in it. The phenol was saturated with Tris EDTA buffer (10mM Tris, 1mM EDTA with final pH 7.5) before mixing with chloroform and Isoamylalcohol. The microfuge tube containing mixtures were vortexed at the maximum speed for one minute. Then the tubes were centrifuged at 12,000 rpm for 5 minutes. After centrifugation, the upper aqueous phase (0.45mL), leaving the interphase intact, were collected in another microfuge tube containing 0.5 mL isopropanol. The

microfuge tubes were mixed well and centrifuged immediately at 12,000 rpm for five minutes.

The supernatant was discarded, 70% ethanol (0.5mL) added to the side of the tube carefully and centrifuged at 14,000 rpm for 10 minutes. The supernatant was discarded and pellet air dried. Then 25 μ L of deionized water was added to dried pellet and stored at -20°C till further use (Zhang and Cahalan, 2007).

The resultant plasmids were separated using gel electrophoresis. Agarose gel (0.8%) in 1X TBE buffer were prepared and placed in Gel electrophoresis tank containing 1X TBE buffer. The current was supplied about 90V for 1 hour and the resulting bands were visualized under UV transilluminator and compared with 100bp and 1kb ladder (Wonglumsom *et al.*, 2011; Farshad *et al.*, 2010)

Plasmid curing: *E. coli* isolate was cultured in TSB for 18 hours at 37°C in duplicate test tubes. Loop-full culture (0.5 McFarland) was inoculated in a microfuge tube containing freshly prepared 1mL TSB. The microfuge tube was placed on thermostat with set temperature of 45°C for 18 hours as mentioned previously (Raj, 2012). *E. coli* culture was inoculated in test tubes (Triplicate) containing 10 % SDS in TSB. The tubes were placed at 37°C for 24, 48 and 72 hours respectively (Salisbury *et al.*, 1972).

The curing of plasmid in selected isolates was confirmed by antibiotic sensitivity testing using tetracycline and streptomycin as per previously defined protocol (Fortina and Silva, 1996; Chigor *et al.*, 2010).

RESULTS AND DISCUSSION

Plasmid Profiling: Plasmid mediated analysis of different multi drug resistant *E. coli* isolates from retail chicken meat were observed by agarose gel electrophoresis which showed plasmid bands in different combinations (Fig. 1). Samples (n=25) were processed and 24 (96%) multi drug resistant isolates were found harboring plasmid of different sizes (low, medium or high molecular weights). The average plasmid number among the isolates was 2.28, but ranging from 0 – 5 plasmids per isolate. The highest number of plasmid was 5 which were observed in sample number 7. Only one isolate (4%) was harboring 5 plasmids, five isolates (20%) had 4 plasmids, one isolate (4%) showed 3 plasmids, twelve isolates (48%) had 2 plasmids, five isolates (20%) harbored only 1 plasmid and no plasmid was observed in only one isolate (4%).

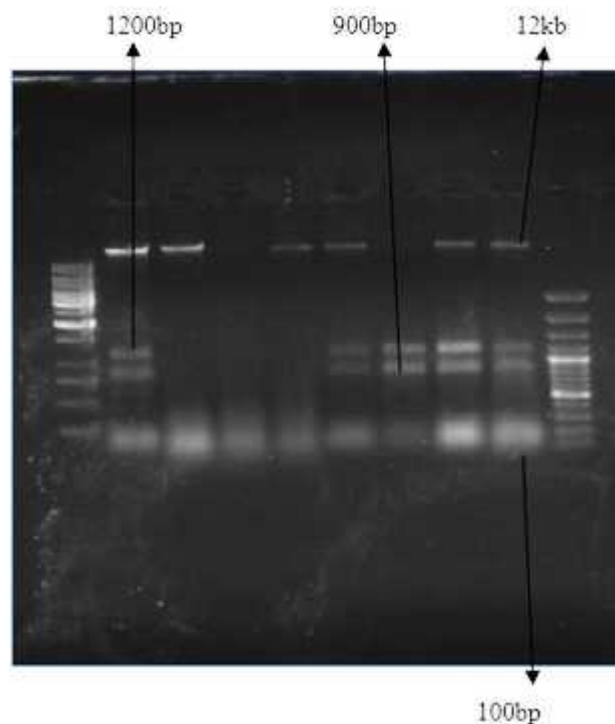


Fig. 1. Banding pattern of plasmid DNA

The plasmid size was found in different combinations which ranged from 100bps-12000bps in all isolates (Fig. 2). In previous study, the plasmid number ranged from 1- 5 with size range 2.9 - 66kb (Al Bahry *et al.*, 2006). Another study showed that the range of plasmid number was from 1 - 7. The study conducted in Bangladesh showed that the plasmid number, from *E. coli* isolates, was ranging from 1 - 5 and size ranging from 0.5 – 40kb (Alam *et al.*, 2010). The plasmid analyses study conducted on uropathogenic *E. coli* isolated from children showed the average copy number of 5.5 (ranging from 1 – 10) with plasmid size from 1 – 33kb (Farshad *et al.*, 2012). While previous studies also showed that some isolates were harboring only 1 plasmid of size ranging from 5 – 9kb (Growther *et al.*, 2012). Similarly in another study low plasmid copy number was observed ranging from 0 – 3, as most of isolates showed 0 or 1 plasmid (Hussain *et al.*, 2007). The slight variation in results may be due to difference in origin of isolation of *E. coli*, geographical distribution of the bacteria and exposure to different antimicrobials. The difference in plasmid size might be due to the fact that the spread of resistance genes is evolutionary process which requires lot of energy. In order to carry small sized plasmid, less energy is required than large sized plasmids.

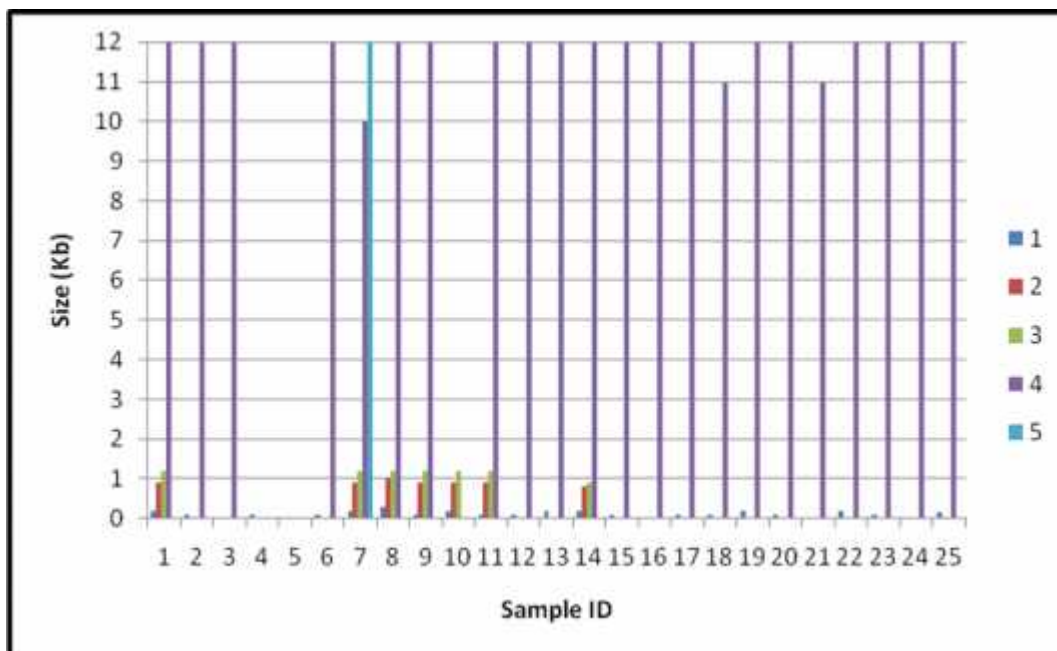


Fig. 2. Graphical representation of plasmid profiles (number and size) of *E. coli*

Relationship between plasmid profiles and antibiotic resistance: Using previous data collected on antibiotic resistance study, relationship of present plasmid mediated analyses and antibiotic resistance showed that out of 25 *E. coli* isolates only two isolates (8%) were resistant to 6 antibiotics and both of them were harboring 2 plasmids. Six isolates (24%) were showing resistance against 5 antibiotics with 1 – 5 plasmid copy number. While isolates, showing resistance to 4 or less antibiotics, were harboring plasmid in different copy numbers. All these isolates had different plasmid size. Another study showed that some strains resistant to only one antibiotic had more than one plasmid while others containing 1 or 2 plasmids were resistant to a large number of antibiotics (Al Bahry *et al.*, 2006). In a study on *Vibrio* strains isolated from cultured shrimps reported that some strains were resistant to four antibiotics, others were resistant to two antibiotics and all harbored one plasmid of 21.2 kb pair (Aja *et al.*, 2002). Statistically, there was no significant correlation ($p > 0.05$) exists between antibiotic resistance and plasmid profiles. Results of present study are similar with study on plasmid profiling of *E. coli* isolates on different samples including chicken (Wonglumsom *et al.*, 2011) which indicates that all antibiotic resistance genes are not located on plasmids. Some of the resistance genes could be present on bacterial chromosomes.

Plasmid curing: Plasmid curing analyses of selected isolates revealed the average curing efficiency was 3.9% and 16.7% with respect to physical and chemical method when subjected to MHA plate culturing containing tetracycline and streptomycin. The curing of extracted plasmid revert back previously resistant isolates into

sensitive form. The curing efficiency in the study was 4% when *Lactobacillus* strains (having plasmid for peptidase activity and lactose metabolism) were exposed to elevated growth temperatures reverted to wild forms (Fortina and Silva, 1996). In another study, high curing efficiency (60% to 100%) was observed when ampicillin resistant *Pseudomonas aeruginosa* was treated with high temperature (Ahmed, 2004). In another study, effect of SDS treatment on drug resistance and phage sensitivity was not observed after 48 hours of incubation but after 72 hours and 10 - 30% cells showed susceptibility to previously resistant antibiotics, although resistance to chloramphenicol and sulphonamides remained stable (Salisbury *et al.* 1972). In another study, high curing efficiency (17% to 83%) with chemical treatment was observed when drug resistant *Pseudomonas aeruginosa* was treated with SDS (10%) (Ahmed, 2004). Statistical analysis reveals that resistant isolates when treated with either physical or chemical treatments produced significant results ($p < 0.05$) of plasmid curing. While comparing physical and chemical treatments, chemical treatment produced significant results ($p < 0.05$) comparing to physical treatment as shown in figure 03. The plasmid curing using temperature and SDS causes elimination of resistance plasmid and revert the resistant strains into susceptible form. The small variation in curing efficiency might be depends upon permeability through outer membrane and location of genes responsible for resistance as SDS has to produce its effect after penetrating through cell membrane. The possible reason might be in disruption plasmid DNA replication or inactivation of necessary enzymes.

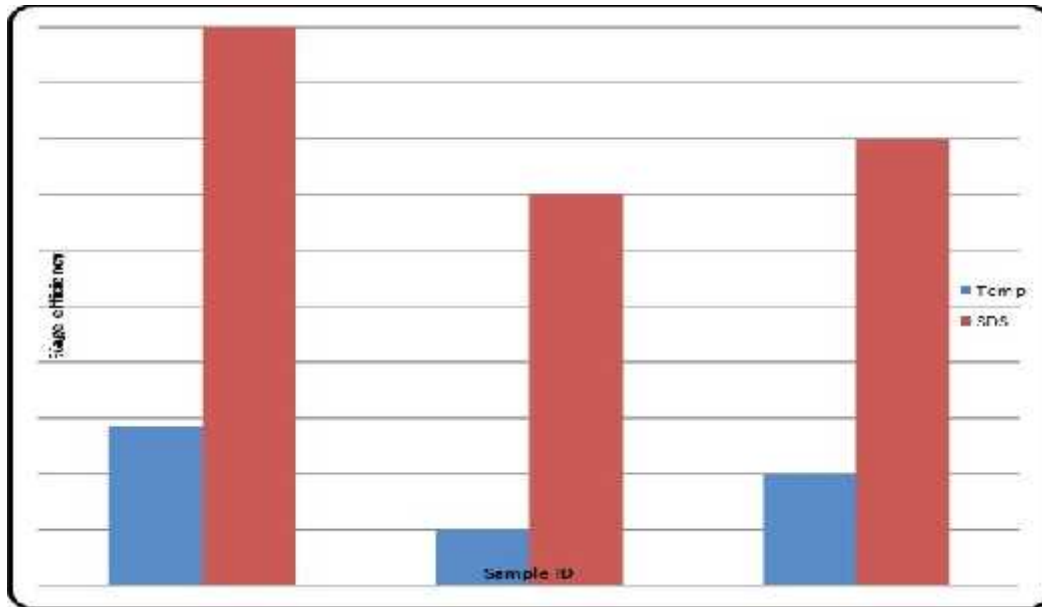


Fig. 3: Graphical representation of treatment of plasmid curing by physical and chemical methods

Conclusion: Present study revealed that plasmids were randomly distributed in *E. coli* isolates. The genes for multidrug resistance might be located on plasmid DNA or chromosomal DNA. Overall no consistent relationship was observed between antimicrobial resistance and plasmid profiles. Plasmid curing converts resistant isolates into susceptible form (if resistance is plasmid mediated). The data of above mentioned study is consistent with limited number of isolates; further studies are required on other MDR bacteria causing serious infections.

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