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STUDIES ON TRANSFORMATION OF RESISTANT PLASMID BETWEEN *SALMONELLA* AND *E. COLI*

HANAN, M. IBRAHIM** MERVAT, S. HANAFI** ; AMANY, I. EL-BIALY**; and HATEM , M. E*.

* Microbiology of Department, Faculty. Vet. Med., Cairo University

** Serology Unit, Animal Health Research Institute, Dokki, Egypt.

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SUMMARY

Plasmid profile analysis of 10 multiple drug resistant *Salmonella* serovars with (resistant to ampicillin, chloramphenicol, oxytetracycline, nalidixic acid and streptomycin) was performed using agarose gel electrophoresis. There were 8 isolates harbouring plasmid with molecular weight ranged from 16 - 31.5 kbp while the plasmid of 2 isolates could not be detected . Successful transformation of resistant plasmid was done between *S. Kentucky* and *S. Typhimurium* as donor bacteria and *E. coli* XL1 as recipient bacteria by electroporation. Transconjugants were obtained by conjugation assay between multiple drug resistant (MDR) *S. Typhimurium* as donor bacteria and each of *S. Enteritidis* , *S. Typhi* , *E. coli* : XL1 and *E. coli* : O157H7 as recipient bacteria

INTRODUCTION

Salmonella enterica is a major endemic and epidemic pathogen in animals and human worldwide and has a great effect on animal production . The frequency of resistance among food-borne pathogens has increased dramatically . Presumably due to extensive use of antimicrobial agents in human and veterinary medicine. Furthermore, resistance to combination of several classes of antimicrobials has led to the emergence of multiple drug resistant (MDR) strains that may pass from food animals to human (Gebreyes and Altier, 2002).

Successful antimicrobial therapy has been threatened by the emergence of multiple resistant strains of Salmonella due to resistant (R) plasmids that may be transferred to other bacterial organisms by conjugation or transduction (Rouahi et al. , 2000 and Wain et al. , 2003).

Plasmid analysis technique demonstrated significant homology between Salmonella and E. coli plasmids from animal and human (John, 1993). The new antibacterial resistant gene detection may provide a unique tool for analyzing food-born transmission of highly antibacterial resistant organism .Transfer of resistance between enteric organisms and possibly the transfer of the resistance determinants from commensals to more virulent organisms could initiate or complicate the problem (Winokur et al. , 2001). Multi drug resistant genome is transferred through one coherent piece of DNA (often called a plasmid) (WHO, 2005) .

Therefore, the aim of the present study was screening of plasmid profile and gel electrophoresis for multidrug resistant *Salmonella* strains as well as trials for transforming sensitive *E. coli*: XL1 by using resistant extracted plasmid from MDR Salmonella into resistant one through electroporation and conjugation assay.

MATERIALS AND METHODS

Ten Salmonella isolates which were recovered from diseased poultry (*S. Arizona*, *S. Enteritidis*, *S. Kentucky* and *S. Typhimurium*), cattle (*S. Montevideo* and *S. Sandiego*) and feedstuff (*S. Hadar* and *S. Virchow*) as well as diseased human (*S. Paratyphi* A and *S. Typhi*) were investigated. Determination of antibiotic sensitivity using agar disk diffusion technique (ADDT) for these 10 isolates against 16 antibacterial agents was carried out according to National Committee for Clinical Laboratory Standards (NCCLS) (2002) .

Preparation and purification of plasmid DNA (miniprep)were performed according to Ausubel et al. (1987), while the identification of the extracted plasmid DNA using agarose gel electrophoresis was performed according to Sambrook et al. (1989).

Bacterial transformation between DNA plasmid of MDR *S. Typhimurium* and *S. Kentucky* as donor bacteria and an overnight saturated culture of sensitive *E. coli*: XL1 as recipient bacteria (standard strain) by electroporation .This technique was done in Biotechnology Center for Services and Researches (BCSR) Cairo- University.

DNA plasmid (in Tris EDTA) was mixed with standard *E. coli*: XL1 and incubated on ice for 30-60 sec. then the gene pulser apparatus for 25 uf was setted and the puse controlled to 200 O. the gene pulser setted at 2-5 KV. For 2 mm or 1.5KV. for 1mm cuvettes. The cell suspension was transferred to a test tube and incubate at 37c° for hour while shaking. Cells was plated on LB agar with antibotics (50µg chloramphenicol and 50µg ampicillin), This technique according to Pfaller et al. (1994) and Winokur et al. (2001). Finally bacterial transformation by conjugation was performed by inoculating 10 ml. nutrient broth with 1 ml.

mixture of MDR *S. Typhimurium* as donor bacteria and 1 ml.of each, sensitive *E. coli*: O157H7, *E. coli*: XL1 as well as sensitive *S. Enteritidis* and *S. Typhi* as recipient bacteria. After overnight incubation at 37 c° . Transconjugates were selected by plating cells onto Muller- Hinton agar supplemented with 50 µg chloramphenicol and 50 µg ampicillin. This conjugation performed according to Wain et al. (2003).

RESULTS

The present study was planned to study the plasmid profile in ten MDR isolates of Salmonella. Plasmid extractions are given in (Table, 1) and Photo. (1 and 2). The present results revealed that eight isolates of *Salmonella* serovars harboured natural single plasmid and two strains showed no plasmid in their plasmid extraction; *S. Hadar* (feedstuff) and *S. Sandiego* (cattle) . Regarding to 8 Salmonella serovars which harboured plasmid, Four of them had plasmids ranging from 16 - 18 kbp. Those include *S. Arizona*, *S. Enteritidis*, *S. Kentucky* (poultry) and *S. Virchow* (feedstuff) with 16.4, 16.9, 17.4 and 17.4 kbp, respectively. As well as four *Salmonella* serovars had plasmids ranging from 20 - 32 kbp which were found in *S. Typhimurium* (poultry), *S. Montevideo* (cattle), *S. Paratyphi* A and *S. Typhi* (human) with 20.7, 25.5, 29.7 and 31.2 kbp, respectively (Table 1).

The standard electroporation protocols results revealed transformed *E. coli*: XL1 that grow on LB agar containing both 50µg ampicillin and chloramphenicol. A transferable plasmid inside those transformed *E. coli* was observed in (Table, 2) and Photo (2).

Moreover, plasmid of transformed *E. coli*: XL1 ranged from 24.5 - 28.7 kbp for *E. coli* resistant plasmid of *S. Kentucky* and *S. Typhimurium* respectively.

Regarding to conjugation assay (Table, 3). There were successful transfer between *S. Typhimurium* and all recipients bacteria. This transconjugants became resistant to ampicillin and chloramphenicol when subjected to ADDT.

Table (1) : Plasmid profile analysis of 10 resistant Salmonella isolates .

No.	Serovar	Source	Plasmid profile analysis			Resistance pattern
			Presence	Lanes	MW kbp	
1	*S. Arizona*	Poultry	+Ve	Lane 1	16.4	C, NA, S
2	*S. Enteritidis*		+Ve	Lane 2	16.9	Amp, NA, OT, S
3	*S. Kentucky*		+Ve	Lane 3	17.4	Amp, C, NA, S
4	*S. Typhimurium*		+Ve	Lane 5	20.7	Amp, C, NA, S
5	*S. Montevideo*	Cattle	+Ve	Lane 10	25.5	Amp, C, NA, S
6	*S. Sandiego*		-Ve	Lane 8	-	CXM, C. S
7	*S. Paratyphi A*	Human	+Ve	Lane 6	29.7	CXM, S, CFR
8	*S. Typhi*		+Ve	Lane 7	31.2	Amp, CXM, OT, S
9	*S. Hadar*	Feedstuff	-Ve	Lane 9	-	C, NA, S
10	*S. Virchow*		+Ve	Lane 4	17.4	Amp, C, OT, S

+Ve : Presence of plasmid. -Ve : Absence of plasmid.

C: Chloramphenicol, NA: Nalidixic acid, S: Streptomycin ,

Amp: Ampicillin, OT: Oxytetracycline , CXM: Cefuroxium sodium, CFR: Cefadroxil.

Table (2): Electroporation transformation of R plasmid between *S. Typhimurium* , *S. Kentucky* (donors) and *E. coli* XL1 (recipients).

Serovar	Source	Plasmid profile analysis			Resistance pattern
		Presence	Lanes	MW kbp	
Donors					
S. Kentucky	Poultry	+Ve	Lane 1	15.5	Amp, C, NA, S
S. Typhimurium		+Ve	Lane 2	18.2	Amp, C, NA, S
Recipients					
E. coli XL1 With R 1	Reference	+Ve	Lane 3	24.5	Amp, C, NA, S
E. coli XL1 With R 2	strain	+Ve	Lane 4	28.7	Amp, C, NA, S

E. coli XL1 With R 1 : Recipient *E. coli* R plasmid of *S. Kentucky*.

E. coli XL1 With R 2 : Recipient *E. coli* R plasmid of *S. Typhimurium*.

C: Chloramphenicol, NA: Nalidixic acid,

S: Streptomycin , Amp: Ampicillin,

Table (3): The rate of transfer of antibacterial resistance by conjugation within 24 hours between donor and recipient bacteria.

Serovar	Sensitivity before conjugate				Sensitivity after conjugate			
	C_{30}		Amp_{10}		C_{30}		Amp_{10}	
	IZ_{mm}	IP	IZ_{mm}	IP	IZ_{mm}	IP	IZ_{mm}	IP
Donor bacteria *S. Typhimurium*	12	R	10	R	8	R	7	R
Recipient bacteria								
S. Enteritidis	28	S	16	S	12	R	7	R
S. Typhi	25	S	20	S	10	R	9	R
E. coli (XL1)	25	S	18	S	8	R	10	R
E. coli (O157H7)	20	S	15	M	5	R	8	R

R: Resistance.
S: Sensitive.
M: Moderate sensitivity.
IZ mm: Inhibitory zone

C_{30}: Chloramphenicol 30µg.
Amp_{10}: Ampicillin 10 µg.

I P: Interpretation

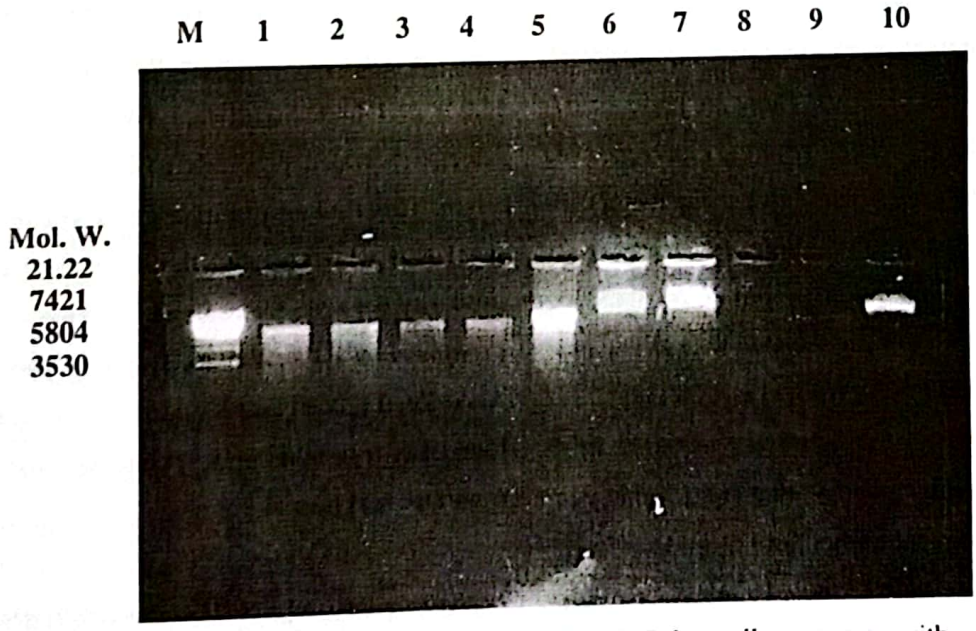

Photo (1): Lanes of plasmids of MDR *Salmonella* serovars with molecular marker from left to right.

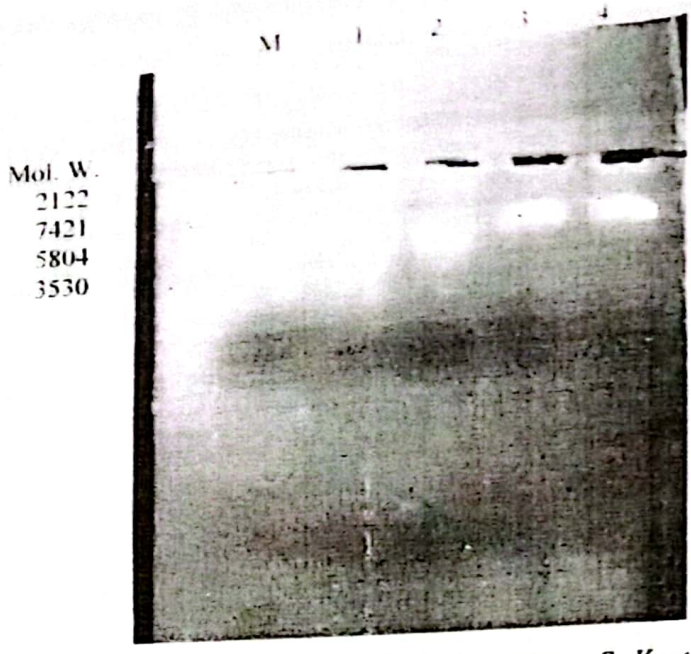

Photo (2): Lanes of plasmids of *S. Typhimurium* , *S. Kentucky* (1, 2) and transformed *E. coli* XL1 (3,4).

DISCUSSION

Resistance to multiple antimicrobial agents in bacterial pathogens is an emerging global problem that is having a serious impact on the treatment of infections diseases. There are undoubtedly many factors associated with the emergence of resistance. An understanding of these factors in crucial if we are to limit the spread of resistance . A significant proporation of drug resistance in bacteria is known to be associated with the acquisition of plasmid DNA (Wain et al. , 2003).

The present results revealed that eight isolates of Salmonella serovars harboured plasmids. S. Arizona, *S. Enteritidis*, *S. Kentucky* and *S. Virchow* had plasmid about 16.4, 16.9, 17.4 and 17.4 kbp, respectively. While *S. Typhimurium*, *S. Montevideo* , *S. Paratyphi* A and *S. Typhi* had plasmid with molecular weight 20.7, 25.5, 29.7 and 31.2 kbp, respectively (Table, 1). Our results agree to large extent with Yun et al. (2003) who found that plasmid of *Salmonella* ranged between 13.2 - 21.1 kbp. Significant variations were not detected between plasmids of human and cattle origin. This finding resembles to that of Corre et al. (1999) who revealed that there were clonal similarity between human and bovine strain of *S. Typhimurium* . Our results appear lowered than Abou Zeed (2000) and Ibrahim et al. (2001) who found two plasmid, in *S. Typhimurium* ranging from 69 - 90 kbp.

In the present study, *S. Hadar* (feed) and *S. Sandiego* (cattle) showed no plasmid in their extraction, this due to potential plasmid instability

exists in both sources or the plasmid may be of low copy number resulting in negative results during gel electrophoresis. Similar observation had been reported by Hamada et al. (2003) and Yan et al. (2003) who found plasmid fraction *S. Typhimurium* and absent in *S. Montevideo*.

The genetic exchange is a major significant cause of antibacterial drug resistance through transfer of genetic material producing epidemic bacterial infection. The genetic element exchange through bacteriophage, plasmid mediated conjugation or by simple transformation between bacterial population.

On concern to bacterial transformation by electroporation in these investigation revealed presence of transferable plasmid in these transformed *E. coli* as observed in (Table, 2) and (Photo, 1).

Moreover, plasmids of transformed *E. coli*: XL1 ranged from 24.5 and 28.7 kbp for *E. coli* resistant plasmid of *S. Kentucky* and *S. Typhimurium* respectively. Our result agree to large extent with Bolton et al. (1999).

Regarding to the conjugation assay, results revealed that conjugation occurred between donor bacteria *S. Typhimurium* and recipient bacteria (*E. Enteritidis, S. Typhi, E. coli*:XL1 and *E. coli*: O157:H7). There are successful transfer between *S. Typhimurium* and all recipient bacteria. These transconjugants become resistant to amplicillin and chloramphenicol.

Similar observation have been reported by Llanes et al. (1999) and Gebreyes and Altrer (2002) while Hamada et al. (2003) failed to transfer resistance gene from *S. Infantis* to *S. Litchfield*.

In conclusion practical measures should be applied in live stocks to prevent the spread of antibiotic resistance bacteria which could be transmitted to human via food chain causing relative serious hazard. At the same time antibiotic policy may be more strictly applied and strict public health control measures have to be implemented at a national and international level, this might decrease both of the incidence of infection and antibiotic resistance rate for intercycle of Salmonella transmission.

So, the multiple drug resistance *Salmonella* serovars harboured plasmids which can be transformed to other bacteria of the same species or same family by electroporation or conjugation . Therefore practical measures should be applied in live stocks to prevent the spread of antibiotic resistance genes to human.

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