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MICROBIOLOGY
Research Article

Transfer of Resistance Plasmids between *Escherichia coli* Isolates from Domestic Livestock

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Received: February 19, 2016; **Published:** March 15, 2016

Abstract

Drug-resistant commensal *Escherichia coli* isolates constitute a significant reservoir of antibiotic resistance determinants that can be transferred to those bacteria pathogenic for animals and/or humans. Bacterial conjugation is believed to play a major role in the dissemination of antibiotic resistance genes and virulence plasmids. In this study, we used conjugation assay to investigate the mobility of resistance plasmids between drug resistant *Escherichia coli* isolated from domestic animals and plasmid-free laboratory strain (gene-hog DH10B) as a recipient. Plasmid DNA was extracted using the alkaline SDS method and separated by agarose gel electrophoresis. Antibiotic susceptibility testing and plasmid profiling were used to confirm the transfer of resistance plasmid in the transconjugants. The result showed that the isolates harbored plasmids of different sizes in the range of 1 to 120 KB. The rate of transferability of the plasmids among the isolates from different sample sources varied; highest conjugation efficiency was observed in isolates fromgoat ranging from 1.0×10^{-6}- 9.4×10^{-7}. The isolates from other sourcesviz: cattle, poultry and pig had conjugation efficienciesin the range of 1.2×10^{-6}- 8.7×10^{-7},, 1.0×10^{-6}- 9.4×10^{-7} and 1.4×10^{-7}- 3.0×10^{-6} respectively. The results of this study suggest that conjugation could be an important mechanism for horizontal gene transfer between commensal and pathogenic bacteria. A better understanding of the mechanism and magnitude of resistance gene transfer may provide a strategy to reduce the potential for dissemination of these genes.

Keywords: *Plasmid Transfer; Antibiotic Resistance; Escherichia coli; Nigeria*

Introduction

Antimicrobial agents are extensively used in animal therapy, for prophylaxis and metaphylaxis, and in some geographical regions for growth promotion. Many of these drugs belong to the same families of antimicrobial compounds that are used for treating humans [1]. Surveillance studies conducted in different countries generally report an increase in the level of resistance in *Escherichia coli* isolates to major classes of antibiotics used for the treatment of livestock and companion animals [2,3]. It has been suggested that an important factor in the emergence and dissemination of resistance is the selective pressure exerted following antibiotic exposure. The potential for transmission of *E. coli* clones between different animal hosts and humans has been documented previously [4,5]. In addition, transmission of genetic determinants of resistance *in vitro* and *in vivo* has been described in several studies [6-8]. The dissemination of resistance markers can be attributed to a number of independent genetic events collectively known as horizontal gene transfer (HGT). Most of the genes conferring antibiotic resistance are not specific to one bacterial host. HGT has been attributed to mobile and mobilizable genetic elements such as plasmids, transposons, and integrons. [9].

The main mechanisms of horizontal gene transfer are conjugation (mobile genetic elements are being transferred from a donor to a recipient cell), transformation (uptake of naked DNA), and transduction (bacteriophages as transporters of genetic information). Conjugation is considered as the principal mode for antibiotic resistance transfer since many antibiotic resistance genes are situated on mobile

elements, such as plasmids and conjugative transposons. Conjugation of broad-host-range plasmids enables DNA to be transferred over genus and species borders, whereas transformation and transduction are usually more limited to the same species [10]. When considering a medical point of view, the transfer of antibiotic resistance determinants from commensal bacteria to pathogens is of utmost importance, and it is clear that commensal bacteria should not be seen as devoid of antibiotic resistance determinants because of their non-pathogenicity.

While antibiotic-resistant zoonotic food-borne pathogens constitute an obvious threat to public health, the problem of resistance in other bacteria colonizing animals cannot be ignored. Data on the genetic mechanisms of antibiotic resistance in both food producing and companion animals in Nigeria are limited. This study was undertaken to investigate the rate of transfer of resistant plasmids between drug resistant *Escherichia coli* isolated from four domestic livestock comprisingcattle, goats, poultry and swine in three states of South East Nigeria.

Materials and Method

The materials and methods were as in our previous published studies [11,12] respectively.

Briefly, fresh fecal droppings were randomly collected from animal facilities in Owerri, Imo State; Okija, Anambra State; Aba, Abia state and were cultured for isolation of *E. coli*. The isolates were screened for antibiotic susceptibility using the disc diffusion method on Mueller-Hinton agar (Oxoid, England). PlasmidDNA was extracted using the alkaline SDS method. The extracted plasmids were separated by agarose gel electrophoresis with 0.8% agarose in 1x TAE buffer. Conjugation assay was carried out using plasmid-free rifampicin-resistant recipient (gene-hog DH10B) in all matings to have a selectable marker for selection against the donor. Resistance to tetracycline and ampicillin was used as a selection marker for transconjugants from recipients. To control for recipient mutations, unmated recipient strains were also spread directly onto LB plates containing tetracycline or ampicillin. After conjugation, the putative transconjugants were enumerated and the conjugation efficiency of the donor cells was calculated by using the formula:

$$\text{Conjugation efficiency} = \frac{\text{Number of transconjugants}}{\text{Number of donor cells added}}$$

The CFU of the donor cells were determined by 6 x 6 dilutions in a 96 well plates with multichannel pipette. The presence of plasmids in the transconjugants was confirmed by plasmid profiling.

Results

In the conjugation experiments, the drug resistant E. coli isolates from animal sources successfully transferred antibiotic resistance plasmids to the recipient (*E. coli* DH10B). Antibiotic susceptibility testing and plasmid profiling were used to confirm the transfer of resistance plasmid to the transconjugants. Plasmid profiling result showed that, the drug resistant *E. coli* isolates harbored plasmids of different sizes in the range of 1 to 120 KB. Thehighest conjugation efficiency was observed in isolates from goat ranging from 1.0×10^{-6} - 9.4×10^{-7}. The isolates from other sources viz: cattle, poultry and pig had conjugation efficiencies in the range of 1.2×10^{-6}- 8.7×10^{-7},, 1.1×10^{-6}- 7.9×10^{-7} and 1.4×10^{-7}- 3.0×10^{-6} respectively. Tables 1-4 below showed the detailed conjugation efficiency of individual sample, the size and number of plasmidsharbored by each isolate.

Considering the results from different sample sources, out of the 29 *E. coli* isolates from cattle, plasmid transfer in eight isolates was not successful. A total of 30 plasmids with different sizes was detected in this sample source while the conjugation efficiency was in the range of 1.2×10^{-6}- 8.7×10^{-7},. The isolates from goat harbored a total of 29 plasmids with size ranging from 1-120; no plasmid was detected in four isolates and thus conjugation efficiency was not determined. The highest rate of transfer of plasmid, 9.4×10^{-7}observed in this study was found in isolate from goat specimen. In poultry specimens, a total of 15 isolates were examined and 17 plasmids detected with four isolates harboring multiple plasmids. Conjugation efficiency was not determined in four isolates because no plasmid was detected in the isolates. The plasmid transfer rate in the isolates was generally high when compared to other sample sources; only one sample had low rate of $1.1 \text{ X } 10^{-6}$. The 17 isolates from pig specimens harbored 24 plasmids with size ranging from 1.4-120. Plasmid

transfer in 9 isolates from this group was not successful and the lowest conjugation efficiency range found in this study, 1.4 x 10^{-7}- 3.0 x 10^{-6} was observed in isolates from pig.

S/N	STRAIN ID	Origin	PLASMID PROFILE		DONOR	Transcnju	Conjugation efficiency
			Number of Plasmids	SIZE (KB)	CFU/ML	CFU/ML	
1	CA 1	COW	0				ND
2	CA 2	COW	1	4	1.2 X 10^{8}	75	6.4 X 10^{-7}
3	CA 5	COW	4	2, 4, 5,120	1.6 X 10^{8}	38	2.4 X 10^{-7}
4	CA 6	COW	1	3	2.5 X 10^{8}	86	3.4 X 10^{-7}
5	CA 7	COW	1	120	1.8 X 10^{8}	75	4.3 X 10^{-7}
6	CA 9	COW	1	120	2.5 X 10^{8}	16	6.3 X 10^{-8}
7	CA 11	COW	0		2.6 X 10^{8}	197	7.7 X 10^{-7}
8	CA 8	COW	0		2.0 X 10^{8}	119	5.9 X 10^{-7}
9	CA 12	COW	0				ND
10	CA 13	COW	2	2.5, 120	2.0 X 10^{8}	702	3.4 X 10^{-6}
11	CA 14	COW	0				ND
12	CA 15	COW	0				ND
13	CA 16	COW	2	1.3, 120	2.0 X 10^{8}	250	1.3 X 10^{-6}
14	CA 17	COW	1	2.8	1.9 X 10^{8}	210	1.2 X 10^{-6}
15	CA 18	COW	1	10	1.6 X 10^{8}	139	8.7 X 10^{-7}
16	CA 19	COW	2	1, 2.5	1.7X 10^{8}	75	4.5 X 10^{-7}
17	CA 20	COW	1	1.5	1.8 X 10^{8}	390	2.1 X 10^{-6}
18	CA 22	COW	1	2.8	1.7 X 10^{8}	200	1.2 X 10^{-6}
19	CA 23	COW	1	1.5	1.6 X 10^{8}	248	1.5 X 10^{-6}
20	CA 24	COW	1	120	1.6 X 10^{8}	500	3.3 X 10^{-6}
21	CA 25	COW	2	95, 120	1.7 X 10^{8}	13	7.5 X 10^{-8}
22	CA 26	COW	0				ND
23	CA 27	COW	0				ND
24	CA 28	COW	2	2.5, 95			ND
25	CA 29	COW	3	1, 4,120	1.8 X 10^{8}	260	1.4 X 10^{-6}
26	CA 30	COW	0				
27	CA 31	COW	2	95,120	2.4 X 10^{8}	508	2.1 X 10^{-6}
28	CA 32	COW	1	95	1.9 X 10^{8}	230	1.2 X 10^{-6}
29	CA 33	COW	0				ND

Table 1: *Conjugation Efficiency of E. coli Isolates from Cattle.*

Key: *Transcnju: Transconjugants; ND: Not determined; Number: Plasmid Number; Size (KB): Plasmid size in kilo base pair; CFU/ML: Colony forming unit per mil*

S/N	Strain ID	Origin	Plasmid Profile		Donor	Transcnju	Conjugation efficiency
			Number of Plasmids	Size (KB)	CFU/ML	CFU/ML	
1	GO 2	GOAT	4	2, 5, 20, 95	1.4×10^{8}	123	8.8×10^{-7}
2	GO 3	GOAT	4	1, 4, 10, 55	2.1×10^{8}	111	5.3×10^{-7}
3	GO 4	GOAT	2	2.5, 3.5	2.3×10^{8}	141	6.3×10^{-7}
4	GO 6	GOAT	2	1, 120	2.3×10^{8}	85	3.7×10^{-7}
5	GO 7	GOAT	2	2.5, 4.5	1.8×10^{8}	420	2.3×10^{-6}
6	GO 9	GOAT	0				ND
7	GO 10	GOAT	3	2.8, 95,120	1.6×10^{8}	154	9.4×10^{-7}
8	GO 8	GOAT	1	120			ND
9	G0 12	GOAT	1	120	2.3×10^{8}	429	1.8×10^{-6}
10	GO 13	GOAT	0				ND
11	GO 16	GOAT	2	2.8, 120	9.6×10^{7}	159	6.4×10^{-6}
12	GO 17	GOAT	1	1.5			ND
13	GO 20	GOAT	2	1.5, 2.5	2.3×10^{8}	162	7.6×10^{-7}
14	GO 21	GOAT	1	2.5	1.4×10^{8}	102	7.0×10^{-7}
15	G0 18	GOAT	2	3, 95			ND
16	GO 23	GOAT	1	2.5	1.8×10^{8}	196	1.1×10^{-6}
17	GO 24	GOAT	1	4	1.8×10^{8}	180	1.0×10^{-6}
18	GO 26	GOAT	0				ND
19	GO 29	GOAT	0				ND

Table 2: *Conjugation efficiency of E.coli isolates from goat.*

S/N	Strain ID	Origin	Plasmid Profile		Donor	Transcnju	Conjugation efficiency
			Number Of Plasmids	SIZE (KB)	CFU/ML	CFU/ML	
1	PL 2	POULTRY	1	95	2.3×10^{8}	150	6.4×10^{-7}
2	PL 3	POULTRY	1	55	2.2×10^{8}	100	4.6×10^{-7}
3	PL 7	POULTRY	0		1.9×10^{8}	4	2.1×10^{-8}
4	PL 8	POULTRY	2	1.5, 5	1.8×10^{8}	140	7.9×10^{-7}
5	PL 9	POULTRY	4	2.8,7,55,120	1.8×10^{8}	8	4.4×10^{-8}
6	PL 10	POULTRY	2	1.3, 120	2.3×10^{8}	150	7.4×10^{-7}
7	PL 12	POULTRY	1	3	2.5×10^{8}	117	4.7×10^{-7}
8	PL 13	POULTRY	2	2, 120	2.5×10^{8}	114	4.5×10^{-7}
9	PL 14	POULTRY	1	120	2.4×10^{8}	185	7.8×10^{-7}
10	PL 16	POULTRY	0		1.6×10^{8}	181	1.1×10^{-6}
11	PL 17	POULTRY	1	120			ND
12	PL 18	POULTRY	0				ND
13	PL 19	POULTRY	0				ND

14	PL 20	POULTRY	1	120	1.6×10^{8}	878	5.5×10^{-8}
15	PL 21	POULTRY	1	2.5			ND

Table 3: *Conjugation efficiency of E.coli isolates from poultry.*

Key: *Transcnju: Transconjugants; ND: Not determined; Number: Plasmid Number; Size (KB): Plasmid size in kilo base pair; CFU/ML: Colony forming unit per mil*

S/N	strain ID	Origin	Plasmid Profile		Donor	Transcnju	Conjugation efficiency
			Number of plasmids	SIZE (KB)	CFU/ML	CFU/ML	
1	PG 1	PIG	2	1.4, 4			ND
2	PG 2	PIG	1	2			ND
3	PG 3	PIG	1	2.5	1.6×10^{8}	33	2.1×10^{-7}
4	PG 4	PIG	1	2.5			ND
5	PG 6	PIG	0				ND
6	PG 7	PIG	1	120			ND
7	PG 8	PIG	0				ND
8	PG 9	PIG	0				ND
9	PG 11	PIG	2	1.4, 4			ND
10	PG 12	PIG	2	1.4, 4			ND
11	PG 14	PIG	2	1.5, 5	1.5×10^{8}	22	1.4×10^{-7}
12	PG 15	PIG	2	3.5, 120	1.6×10^{8}	226	1.4×10^{-6}
13	PG 16	PIG	3	5, 7, 120	1.7×10^{8}	500	3.0×10^{-6}
14	PG 17	PIG	1	95	2.2×10^{8}	300	1.4×10^{-6}
15	PG 18	PIG	4	5,7,95,120	1.1×10^{8}	290	2.7×10^{-6}
16	PG 19	PIG	1	5	1.7×10^{8}	475	2.9×10^{-6}
17	PG 20	PIG	1	120	1.7×10^{8}	253	1.5×10^{-6}

Table 4: *Conjugation efficiency of E. coli isolates from pig.*

Key: *Transcnju: Transconjugants; ND: Not determined; Number: Plasmid Number; Size (KB): Plasmid size in kilo base pair; CFU/ML: Colony forming unit per mil*

Discussion

Transfer of antibiotic resistance genes among bacteria is a threat to both human and veterinary medicine. In this study, the transfer of resistance plasmid was demonstrated in drug resistant commensal *E. coli* isolates from food animals and laboratory strain.The study showed that the broad-host-range plasmid carrying multiple resistance genes could be transferred to other bacteria under laboratory conditions and that this event made the recipient strains antibiotic resistant. The results also show that the antibiotic resistance genes present in the general horizontal gene pool can be transferred from commensal *E. coli* isolates to other pathogenicorganisms. It has been shown that bacteria can acquire antibiotic resistance from genetic mutations or via the natural transfer of resistance genes from other bacteria (horizontal gene transfer; [12-14].

Bacterial conjugation is a horizontal gene transfer mechanism that is favored in environments where bacteria are concentrated. Conjugation promotes the dissemination of genetic material via plasmids [13-16]. Under laboratory conditions, conjugation is usually performed in the absence of antibiotics to avoid the growth inhibition of susceptible mating cells. However, it has been reported that

sub-MICs of antibiotics alter the global transcription patterns of bacteria, promoting DNA transfer and recombination [17]. These counterintuitive effects can emerge as levels of antibiotic in the body fall to so-called 'sub inhibitory' levels [18]. The increased number of transconjugants observed in this study was mostly due to the high efficiency of conjugation in the presence of antibiotic selection makers. The results suggest that the strains harboring different antibiotic resistance genes tended to form more channels to acquire both hereditary and nonhereditary antibiotic resistance from neighboring cells when they were co-cultured in the sensitive antibiotics. The survival strategy may be widespread in nature. The bacteria may exchange resistance genes to convert themselves into super-resistant bacteria [18,19].

The transfer of resistance genes in bacteria is often accompanied by transmission of flanking genes, including those conferring resistance to other antimicrobials and affecting expression, including multiple promoters and novel replicase genes. In our study, transconjugants generated from all the animal sources had the same drug resistance profile as the donor strain, demonstrating resistance gene transfer and the potential for rapid dissemination of multiple antibiotic resistance genes to the same and other species. E. coli has evolved as a significant pathogen by the acquisition of genes from 53 different species, including pathogenicity islands and virulence factors responsible for attachment, colonization, and production of toxins, and gene transfer to other bacteria has been observed [20]. The advent of antibiotic resistance organisms has rendered virtually most antibiotics obsolete and has severely limited treatment options, and a huge concern is that these pathogens may also acquire other genes, increasing virulence [21].

Conclusion

In conclusion, we have demonstrated that drug resistance plasmids can be transferred from commensal strains to pathogenic strains under laboratory conditions. The detection of transconjugants was done by plating, not only does this transfer occurs at rather high transfer efficiency, but the acquisition of the plasmid also makes the recipient resistant to multiple antibiotics. In worst-case scenarios, infections with these plasmid-mediated antibiotic resistant pathogens can lead to exacerbation of the health condition, treatment failure and thus compromise human and animal health. Identification of resistance genes allied to the investigation of their transfer potential among bacteria of animal origin provides valuable information regarding this important resistance gene pool and the dynamics of resistance gene exchange. Further studies are required to establish the true nature of the animal resistance reservoir.

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