MOBILIZATION OF ANTIBIOTIC RESISTANCE GENES AMONG FARM ANIMALS AND HUMAN HOSTS IN A DEVELOPING COUNTRY (SRI LANKA)

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SYNOPSIS

Out of the sixty six farm animals and five farm workers tested, thirty eight animals and all the workers were found to carry enteric strains harbouring antibiotics resistance genes. Isolates from these two sources exhibited common transferable antibiotic resistance patterns. In addition, strains isolated from farm animals were found to carry specific resistance patterns (e.g. ampicillin, tetracycline, sulphamethoxazole, and chloramphenicol) which were not found among farm workers. It was further observed that isolates from farm animals harboured plasmids which were not found among the isolates from farm workers. Thus, farm animals could be a source of R-plasmids which are probably selected under prophylactic use of antibiotic and forms means for the spread of antibiotic resistance to various human sources.

INTRODUCTION

Antibiotics are used in veterinary medicine as prophylactic and therapeutic agents. Compared to medical practice, the use of antibiotics among animals renders a greater number being subject to antibiotic load. Thus, there is a greater chance for resistance genes to appear among animals than in humans. Earlier studies have shown that "normal flora" isolated from animals were found to harbour transferable antibiotic resistance genes and in few instances R-plasmids have been isolated (1, 2, 3, 4). Thus normal intestinal flora of animals could act as a reservoir of resistance genes which could spread to human hosts under poor sanitary conditions. Bacterial resistance to furazolidone (drug which is only used in veterinary medicine) has been isolated from human infections, which suggests the mobilization of resistance genes from animals to man (5).

Sri Lanka, one of the developing country has many small farms mainly to cater the local populations. Eggs and milk are the main farm products consumed by the people. As such antibiotics are not used intensively among farm animals. However, antibiotics are incorporated into drinking water of fowls. The animal feed manufactured locally is not fortified with antibiotics. But, antibiotic incorporated animal feed is available in the market.

This study was carried out to evaluate the incidence of antibiotic resistance genes among farm animals in such a low selection environment and to monitor the spread of such resistance to human hosts via R-plasmids.

MATERIALS AND METHODS

Faecal samples were taken from the rectum of cattle (3 years), calves (6 months), goats (3 years), pigs (6 months), and rabbits (1 year). Rectal swabs were also taken from farm workers who were closely associated with these animals. Culturette swabs (AB Biodisk, Sweden) containing Stuart's transport medium were used in sampling. All these animals were from the same farm and no sick animals were included. No antibiotic had been used in animal feed except that of poultry where aureomycin and streptomycin were mixed in the drinking water as prophylactic antibiotics for the last six months. The farm workers were on antibiotics (ampicillin, tetracycline, chloramphenicol, and erythromycin) for short periods, over the past five years. In addition, water samples from this farm was tested for the presence of any coliforms.

All swabs were streaked on MacConkey agar medium containing ampicillin 100 μg/ml, chloramphenicol 20 μg/ml and tetracycline 100 μg/ml separately. These plates were incubated at 37°C for 15 hours. Bacterial colonies appearing on these plates were subcultured on slopes of nutrient agar.

Identification of bacterial strains were carried out (6).

Antibiotic Sensitivity Tests

PD medium® was used as the test medium. Later this was substituted with isosensitest agar medium® . Bacterial resistance to antibiotic were determined by the paper disc method (7).

R-plasmid transfer by Conjugation

E.coli K12 strain EC 1005 (met, nal^r) was used as the recipient strain. Conjugation was carried out (8). Antibiotic sensitivity test of these transconjugants were carried out and transferable antibiotic resistance patterns were discerned.

Screening for plasmid DNA

Bacterial strains carrying antibiotics resistance genes were screened for the presence of plasmid DNA (8). Using a blunt end of a tooth pick a fresh colony was picked from an overnight culture plate and suspended in 40 μl of 50 mM Tris hydroxy methyl amino methane pH 8.0 containing 10 mM ethylene diamine tetra acetic acid (DETA). Plasmid DNA was precipitated by centrifuging at 15,600 Xg for 15 min. in an Eppendorf centrifuge tube. The supernatant fluid was drained off and the sediment was lyophilysed at −40°C. The precipitate was resuspended in 30 μl of TE buffer (10 mM Tris hydrochloride, 50 mM EDTA).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out on horizontal (0.5%) agarose gel slab at 50 mA for 15 h. The apparatus was filled with Loening buffer (Tris acetate 40 mM, sodium acetate 28 mM, Na_2-EDTA 2 mM, pH 7.8). The gels were stained in freshly prepared ethidium bromide 2 μg/ml for 30 min., and were photographed under UV light of wavelength 254 nm using a shortwave transilluminator and polaroid-type film.

RESULTS

Out of sixty six farm animals and five farm workers sampled, thirty eight animals and all the workers were found to carry resistant strains, exhibiting resistance to more than one antibiotic. Among the animals all the pigs and goats carried resistance strains whereas 16 out of 17 cattle and 7 out of 9 calves harboured resistant strains. A very low percentage of fowls carried (24%) resistant strains and none of the rabbits exhibited antibiotic resistance (Table 1). All isolates ex-

TABLE 1
INCIDENCE OF ANTIBIOTIC RESISTANCE GENES AMONG ISOLATES FROM FARM ANIMALS AND FARM WORKERS

Source	tested	No. of strains isolated	Na	Ap	Tc	Su	Em	Cm	Ni	Tp	Gm
Cattle	17	31	0	3/11	1/14	2/29	0/0	6/29	0/8	0/0	0/0
Calves	9	14	0	2/6	3/12	2/14	0/0	4/11	0/2	0/0	0/0
Goats	5	10	0	2/3	1/4	0/6	0/0	2/5	0/0	0/0	0/0
Fowls	25	7	0	0/0	1/5	0/5	0/0	1/5	0/3	0/0	0/0
Pigs	4	11	0	2/7	2/9	1/11	0/2	3/7	0/2	0/0	0/0
Farm workers	5	15	0	0/8	2/12	1/11	0/1	7/11	0/2	0/0	0/0
Total	65	88	0	9/35	10/56	6/76	0/3	23/68	0/17	0/0	0/0

Note: $\frac{\text{No. of animals carrying transferable antibiotic resistance}}{\text{No. of animals carrying antibiotic resistance}}$

Na — Nalidixic acid; Ap — Ampicillin; Tc — Tetracycline; Su — Sulphonamide; Em — Erythromycin; Cm — Chloramphenicol; Ni — Nitrofurantoin; Tp — Trimethoprim; Gm — Gentamycin

hibiting different antibiograms were collected. A total of eighty eight gram negative aerobic bacilli were isolated from 65 animals, and all of them were *E.coli*. The majority were isloated from cattle and a few isolates were made from fowls. None of the isolates were found to be resistant to trimethoprim, gentamycin, or nalidixic acid. Incidence of resistance to ampicillin, tetracycline, sulphonamide and chloramphenicol were common and were equally distributed among different group of animals (Table 1).

Transferable resistance was observed among all animal groups, a significant number was observed among pigs and cattle (Table 1). None of the animals carried strains harbouring transferable resistance to gentamycin, trimethoprim, nitrofurantoin and erythromycin. Though the farm workers and goats carried resistant strains to ampicillin and sulphamethoxazole respectively, none of them were found to be transferable. Nine transferable resistance patterns were exhibited by these isolates (Table 2). The most common patterns were (a) ampicillin, tetracycline (b) chloramphenicol. Strains carrying transferable resistance to chloramphenicol were found among all groups of aniamls and farm workers except fowls. Out of the multiple transferable resistance patterns, resistance to sulphamethoxazole and chloramphenicol was found among isolates from cattle, pigs, and farm workers. Further, there were six transferable resistance patterns among isolates from animal source which were not found among farm workers.

Incidence of R-plasmids among farm animals and farm workers

Sixteen enteric gram negative bacteria isolated from farm animals and farm workers were screened for the presence of plasmid DNA. Except one all were found to carry multiple plasmids (Table 3). The number of plasmids carried varied from one to four. Twelve transconjugants (EC1005 *met nal*) of the above isolates were also screened for the presence of plasmid DNA. It was found that transconjugants too exhibited a similar plasmid pattern, as the corresponding wild type strains.

Plasmids of molecular sizes 44.5 kb and 6.2 kb have been found to occur in strains isolated from humans as well as goats, pigs and fowls. However, plasmids of molecular sizes 9.2 kb, 3.3 kb were only found among strains isolated from animals.

TABLE 2
FREQUENCY OF ANTIBIOTIC RESISTANCE PATTERNS AMONG ENTERIC STRAINS ISOLATED FROM FARM ANIMALS AND FARM WORKERS

Resistance patterns	Frequency among the strains isolated from the farm-animals and farm workers					
	Cattle	Calves	Fowls	Pigs	Goats	Farm workers
Ap-Tc-Su-Cm-Ni	2	1	—	2	—	1
Ap-Tc-Su-Em	—	—	—	2	—	—
Ap-Su-Cm-Ni	4	1	—	—	—	1
Ap-Tc-Su-Cm	—(1)	3	—	1	—	3
Ap-Su-Ni	1	—	—	—	—	—
Ap-Su-Cm	2(1)	—	—	—	—	1
Ap-Tc-Cm	—	—	—	2	—	1
Ap-Tc-Cm	—	—(1)	—	—	—	1
Tc-Su-Cm	5	6	2	2	—	1
Tc-Su-Ni	1	—	1	—	—	—
Tc-Cum-Ni	—	—	1	—	—	—
Su-Cm	10(1)	—	2	2(1)	3	1(1)
Tc-Su	3	2	—	—	2	2
Ap-Tu	2(1)	—	—	—(2)	1(1)	—
Ap-Su	—	1	—	—	—	—
Tc-Ni	—	—	1	—	—	—
Ap-Cm	1(1)	—	—	—	2(1)	—
Tc-Cm	—	—	—(1)	—	—	2(2)
Cm	1(3)	—(1)	—	—(2)	—(1)	—(1)
Tc	—	—	—	—	1	—
Su	—	—	—	—	1	—
Total	31	14	7	11	10	15

( ) Transferable antibiotic resistance

TABLE 3
MOLECULAR SIZE OF CONJUGATIVE PLASMIDS OF ENTERIC STRAINS ISOLATED FROM CALVES, GOATS, PIGS, FOWLS AND FARM WORKERS

Transconjugant designation	Original host	Plasmid designation	[a]Mol. size (kb)	Relevant markers	Source
Calves					
Ca8 (wild)	*E.coli*	pTVCa8	71.5	Ap Tc Su Cm	This Study
Ca8EC1005	*E.coli*	pTVCa8	71.5	Tc Su Cm	This Study
Ca9EC1005	*E.coli*	pTVCa9a	71.5)		
		pTVCa9b	48.5)		
		pTVCa9c	24.5)	Cm	This Study
		pTVCa9d	3.3)		
Goats					
G2(i) (wild)	*E.coli*	pTVG2(i)a	44.5)		
		pTVG2(i)b	43.8)		
		pTVG2(i)c	9.1)	Tc Su Cm	This Study
		pTVG2(i)d	6.2)		
G2(ii) (wild)	*E.coli*	pTVG2(ii)	69.5	Ap Tc	This Study
G2EC1005	*E.coli*	pTVG2(ii)	69.5	Ap TC	This Study
G4 (wild)	*E.coli*	pTVG4a	66.0)		
		pTVG4b	58.0)	Ap Cm	This Study
Pigs					
P1 (wild)	*E.coli*	pTVP1a	67.0)		
		pTVP1b	43.5)	Ap Tc Su Cm Ni	This Study
		pTVP1c	6.13)		
		pTVP1d	3.3)		
P2(i) (wild)	*E.coli*	pTVP2(i)	N.D	Su Cm	This Study
P2(ii) (wild)	*E.coli*	pTVP2(ii)	N.D	Ap Tc Su Cm Ni	This Study
P3(i) (wild)	*E.coli*	pTVP3(i)a	6.4)	Ap Tc Su Cm	This Study
		pTVP3(i)b	4.8)		
P3(ii) (wild)	*E.coli*	pTVP3(ii)a	69.5)		
		pTVP3(ii)b	44.5)	Su Cm	This Study
		pTVP3(ii)c	20.4)		
		pTVP3(ii)d	6.2)		
P3E1005	*E.coli*	pTVP3(ii)a	69.0)		
		pTVP3(ii)b	44.5)	Cm	This study
		pTVP3(ii)c	20.4)		
		pTVP3(ii)d	6.2)		
Fowls					
F3 (wild)	*E.coli*	pTVF3a	69.5)	Tc Su Cm	This Study
		pTVF3b	6.2)		
F3EC1005	*E.coli*	pTVF3	69.5)	Tc Cm	This Study
Farm workers					
FM1	*E.coli*	pTVFM1a	69.0)		
		pTVFM1b	44.5)		
		pTVFM1c	20.4)	Tc Su Cm	This Study
		pTVFM1d	6.2)		
FM1EC1005	*E.coli*	pTVFM1a	68.0)		
		pTVFM1b	44.5)	Su Cm	This Study
FM4	*E.coli*	pTVFM4a	66.0)		
		pTVFM4b	50.0)	Tc Cm	This Study
FM4EC1005	*E.coli*	pTVFM4a	66.0)		
		pTVFM4b	50.0)	Tc Cm	This Study

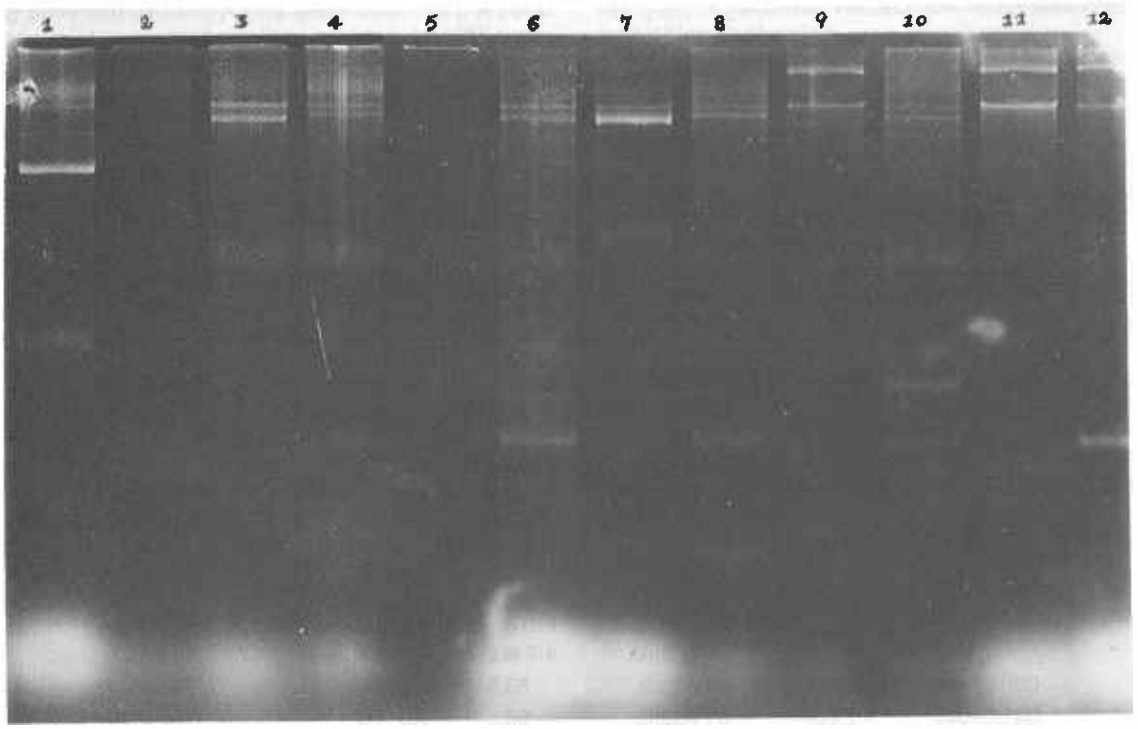

Figure 1: Agarose gels of cleared lysates of isolates from farm animals, farm workers and their transconjugants.

Lane 1.R388; 2. EC 1005; 3. P_3 (EC1005); 4. P_3; 5. FM1 (EC1005); 6 FMI; 7. C_4 (EC1005); 8. C_4; 9. G_2(i); 10. G_2(ii); 11. F_3(EC1005); 12.F_3

DISCUSSION

Farm workers were periodically subjected to antibiotic therapy for minor illness during the last three years. Similarly pigs, cattle and goats were under antibiotic therapy for very short periods, which would have increased the frequency of resistant resident organisms in respective hosts. The eating habits of these animals and the poor sanitary conditions prevailing in the farm have facilitated the spread of such resistant strains among the animals. Even in the absence of selection pressure resistant organisms could colonize an animal intestine. This has been shown where 36% of rural dogs and 15% of urban dogs which were free of antibiotic load carried multiple resistant strains of which there were strains carrying transferable resistance to ampicillin, chloramphenicol, streptomycin and tetracycline (9).

Tetracycline and sulphonamide were never used in this farm in animal therapy. Therefore incidence of resistance genes against these drugs among strains isolated from animals suggest that they could be of human origin and mobilised to these farm animals under poor sanitary conditions. Further, they could have been selected as *en bloc* along with ampicillin and chloramphenicol or streptomycin resistance genes under appropriate selection pressure among the animals. Failure of isolating strains carrying resistance to trimethoprim and gentamycin among farm animals and farm workers suggests that these resistance genes have not yet found access to this farm from the surrounding community. Resistance to nitrofurantoin has not been reported to be borne by R-plasmids. Further, *E.coli* does not possess intrinsic resistance against nitrofurantoin. None of the nitrofurantoin resistance genes were found to be transferable. This clearly shows that all nitrofurantoin genes must be of the same origin (human), and must be borne by the same non-conjugative genome (chromosomal).

Plasmids isolated from farm workers were similar to the plasmids isolated from farm animals. However, there were specific plasmids isolated from farm animals (pTVCa 8-71.5, pTVCa 9-24.5, pTVG 2(i)-9.1, pTVCa 9d-3.3) those were not present among the farm workers.

Strains isolated from farm animals carried a wide range of plasmids where there were significant numbers of small plasmids. Cryptic plasmids do play an important role in the spread of small plasmids among bacterial population. Even if a plasmid does not carry any antibiotic resistance genes their presence in the bacterial host could facilitate the spread of small non-conjugative genomes by mobilization. Further, these plasmids could also carry specific sites for intergration of other plasmids as well as transpositional sites. Thus, evaluation of large cryptic plasmid is an important parameter in monitoring the spread of antibiotic resistance in the community.

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