ANTIBIOGRAMS AND CONJUGAL TRANSFERABILITY OF MULTIPLE DRUG RESISTANCE IN *ESCHERICHIA COLI* OF CHICKENS AND POULTRY FARM WORKERS

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ABSTRACT

Of the Escherichia coli *isolates from the intestinal tracts of chickens receiving antibiotic-supplemented feeds and from poultry farm workers, 91.6% showed resistance to at least one of the antibiotics tested. Of these, 60.4% were multiply-resistant.*

All of the chickens sampled received chlortetracycline-supplemented feeds while 50% of the chickens received sulphadimidine ethane sulphonic acid and trimethoprim in addition to chlortetracycline. Resistance to tetracycline and sulfamethoxazole trimethoprim were shown by 97.5% and 55% of the isolates respectively.

Nine out of ten E. coli *tested for transferability of their multiple drug resistance transferred at least one resistance genevia conjugation. All of the nine isolates showed partial transfer of drug resistance.*

The results show the role of supplementing feeds with antibiotics in the selection for multiple drug resistant microorganisms to persist in the population. The multiplicity of drug resistance of the E. coli *isolates and the transferability of this drug resistance through conjugation to drug sensitive* E. coli *suggest the presence of R plasmids in these microorganisms.*

INTRODUCTION

In the early 1950's, workers discovered that addition of relatively low dose of tetracycline to the feeds of chickens gave substantial growth advantage over those taking the feeds without the antibiotic. The practice was soon applied to other animals. Nearly 50% of all antibiotics produced in the United States are added directly to the feeds of

farm animals, chiefly poultry, pigs and beed cattle (Novick, 1981). Addition of antibiotics to animal feeds for growth-promoting effects is also popularly practised in the Philippines.

The rampant usage of antibiotics in animal feeds may lead to the widespread of a vast population of bacteria that are resistant simultaneously to many antibiotics such that the benefits of the drugs at times of stress are drastically reduced. This resistance may extend to drugs to which the animals and their bacteria have never been exposed to. This is because antibiotic resistance is usually carried by plasmids. These R plasmids often contain genes for resistance to multiple antibiotics. The use of any one of the drugs for which the plasmid carries resistance to will select for the entire plasmid as a whole.

These plasmids may be transferred among bacteria of the same or of different species or among species of different genera and families generally through conjugation. The hazards then become universal for animals and for man. At present times, even microorganisms such as *Hemophilus influenza, Neisseria gonorrhoeae* and *Streptococcus pneumoniae* which in the past were easily controlled with antibiotics, have become resistant to one or more of the antibiotics that have been successful in the treatment of the infections caused by them.

This study investigated the response to antibiotics of *Escherichia coli* isolated from the intestinal tracts of chicken receiving antibiotic-supplemented feeds and from poultry farm workers. The conjugal transferability of the multiple drug resistance of these isolates was also tested.

MATERIALS AND METHODS

Sources of Bacterial Isolates

Rectal swabs were taken from three different test populations. Group A is composed of 20 18-31 day-old chickens. The chickens were randomly chosen from four different chicken coops on the same poultry farm such that five chickens were sampled per coop. The chickens were given pre-starter feeds with chlortetracyline for two weeks, after which they were given feeds supplemented with sulphadimidine ethane sulphonic acid and trimethoprim from the 19th to the 21st day. This group has stopped receiving antibiotic-supplemented feeds about a week before sampling was done.

Group B is made up of 20 14 day-old chicks who have been receiving pre-starter feeds with chlortetracycline for two weeks before specimen collection was done. They did not receive sulphadimidine ethane sulfonic acid and trimethorpim-supplemented feeds. Sampling was done as described above.

Group C is made up of eight poultry farm workers who routinely handle the chickens. As a rule, they are not allowed to leave the farm during the growth of each batch of chickens.

Isolation and Identification of Bacterial Isolates

Rectal swabs taken from the test population were prevented from drying up during transport to the laboratory by immersing them in sterile normal saline solution. The swabs were streaked unto MacConkey agar plates and eosin methylene blue (EMB) plates. The plates were incubated at 37°C for 18-24 hours. Lactose-fermenting colonies typical of *Escherichia coli* were fished out and individually streaked unto nutrient agar slants. The slants were incubated at 37°C for 18-24 hours. The cultures were identified based on the reactions shown in the following biochemical tests: triple sugar iron agar reactions, indole production methyl red test, Vogues-proskauer test, citrate test and urease test.

A total of 48 *Escherichia coli* isolates were used as test microorganisms, one isolate from each chicken and farm worker.

Antibiotic-Sensitivity Testing of Bacterial Isolates

The 48 bacterial isolates were tested for their antibiotic sensitivity pattern using the modified Kirby-Brauer (1966) disc-agar diffusion method against the following antibiotics: tetracycline (Te, 30 micrograms); sulfamethoxazole trimethoprim (Sxt, sulfamethoxazole 23.5 micrograms); trimethoprim (1.5 micrograms); kanamycin (K, 30 micrograms); gentamicin (Gm, 10 micrograms); chloramphenicol (C, 30 micrograms); Ampicillin (Am, 10 micrograms); and nalidixic acid (NA, 30 micrograms).

The diameters of the zones of inhibition produced by the antibiotics on the microorganisms were measured in millimeters (mm) and the results interpreted according to the Zone Diameter Interpretation Standards.

Conjugation Experiment

Test organisms

Ten of the 48 bacterial isolates were tested for the conjugal transferability of their multiple antibiotic resistance to antibiotic sensitive *Escherichia coli.* The ten isolates which acted as donors were chosen based on the multiplicity of their antibiotic resistance and sensitivity to nalidixic acid. The latter characteristic was used to select for transconjugants.

The donor and recipient *E. coli* isolates were separately grown in brain heart infu-

sion broth (BHIB) at 37°C for 18-24 hours. They were then subcultured in fresh BHIB at 37°C for four hours to bring them to the log phase. The turbidity was adjusted against Mac-Farland standard number one to approximate 3×10^8 cells/ml. A 2.1 ratio of donor to recipient cells (2 ml and 1 ml respectively) was introduced into 2 ml of BHIB. The BHIB was incubated at 37°C for 18 hours to allow conjugation to take place.

Transconjugants (antibiotic sensitive recipient *E. coli* which were converted to antibiotic resistance after conjugation tubes on brain heart infusion agar plates containing nalidixic acid (30 micrograms) with the following drugs in individual combination: Te (30 micrograms), Sxt (25 micrograms); K (30 micrograms), Gm (10 micrograms), C (30 micrograms), Am (10 micrograms). Controls were also run together with the tests to check the efficacy of the prepared culture media with the test drugs and to recheck the characteristics of the donor and recipient *E. coli* isolates.

RESULTS

The results of the antibiotic sensitivity tests on the 48 *E. coli* isolates are summarized in the following tables:

Table 1. Antibiotic sensitivity patterns of *E. coli* isolated from 20 28-31 day old chickens fed with chlortetracycline and sulphadimidine ethane, sulphenic acid, trimethoprim-supplemented feeds.

Isolate No.	Antibiotics and concentration in micrograms	Te 30	Sxt 25	K 30	Gm 10	C 30	Am 10	NA 30
Coop. no. 1	1-1-A	R	S	I	S	S	S	I
	1-2-A	R	R	R	S	R	R	S
	1-3-A	R	R	I	S	S	I	S
	1-4-A	R	S	S	S	S	I	S
	1-5-A	R	S	I	S	S	S	S
Coop. No. 2	2-1-A	R	R	R	R	R	R	I
	2-2-A	R	I	S	S	S	S	I
	2-3-A	R	S	S	S	S	S	S
	2-4-A	R	R	S	S	I	R	I
	2-5-A	R	R	S	S	S	S	S
Coop. No. 3	3-1-A	R	I	I	S	S	S	I
	3-2-A	R	I	I	S	S	I	I
	3-3-A	R	R	R	S	S	S	S
	3-4-A	R	R	S	S	S	S	S
	3-5-A	R	R	R	S	S	S	I
Coop. No. 4	4-1-A	R	S	S	S	S	S	I
	4-2-A	R	R	R	S	S	S	I
	4-3-A	R	I	I	S	S	S	I
	4-4-A	R	I	I	S	S	S	I
	4-5-A	R	R	R	S	S	I	I

Legend: Te - Tetracycline; Sxt - Sulfamethoxazole trimethoprim; K - Kanamycin; Gm - Gentamicin; C - Chloramphenicol; Am - Ampicillin; NA - Nalidixic acid; R - Resistant; I - Intermediate; S - Sensitive

Table 11. Antibiotic sensitivity patterns of *E. coli* isolated from 20 14-day old chickens fed with chlortetracycline-supplemented feeds.

Bacterial isolated No.	Antibiotics and concentration in microorganisms	Te 30	Sxt 25	K 30	Gm 100	C 30	Am 100	NA 30
Coop. No. 1	1-1-B	I	R	I	S	S	R	S
	1-2-B	R	S	S	S	S	S	S
	1-3-B	R	R	I	S	S	S	S
	1-4-B	R	S	S	S	R	S	S
	1-5-B	R	S	S	S	S	S	I
Coop. No. 2	2-1-B	R	R	S	S	S	S	S
	2-2-B	R	R	R	S	R	S	S
	2-3-B	R	I	R	S	S	R	S
	2-4-B	R	R	R	S	R	S	S
	2-5-B	R	R	I	S	S	R	I
Coop. No. 3	3-1-B	R	S	S	S	S	S	S
	3-2-B	R	S	I	S	S	S	S
	3-3-B	R	R	S	S	S	R	S
	3-4-B	R	R	S	S	S	R	S
	3-5-B	R	R	I	S	S	R	S
Coop. No. 4	4-1-B	R	R	S	S	S	R	S
	4-2-B	R	R	I	S	S	R	I
	4-3-B	R	R	R	S	S	S	S
	4-4-B	R	I	I	S	S	R	I
	4-5-B	R	S	S	S	S	S	S

Legend: Te-Tetracycline
Sxt-Sulfamethoxazole trimethoprim
K-Kanamycin
Gm-Gentamicin
C-Chloramphenicol
Am-Ampicillin
NA-Nalidixic acid
R-Resistant
S-Sensitive
I-Intermediate

Table III. Antibiotic sensitivity patterns of E. coli isolated from 8 poultry farm workers.

Bacterial No.	Antibiotic and concentration in microgram	Te 30	Sxt 25	K 30	Gm 10	C 30	Am 1	NA 30
1C		R	R	S	S	S	S	S
2C		R	R	R	S	R	R	I
3C		I	S	I	S	S	S	S
4C		I	S	I	S	S	S	S
5C		I	S	I	S	S	S	S
6C		I	S	I	S	S	S	S
7C		R	R	R	S	S	S	S
8C		R	R	S	S	R	S	S

Legend: Te- Tetracycline
Sxt - Sulfamethoxazole trimethoprim
K - Kanamycin
Gm - Gentamicin
C - Chloremphenicol
Am - Ampicillin
NA - Nalidixic acid
R - Resistant
S - Sensitive
I - Intermediate

Based on multiplicity of drug resistance and sensitivity to nalidixic acid, ten E. coli isolates were tested for the conjugal transferability of the multiple drug resistance to drug-sensitive but nalidixic acid resistant E. coli. The results are as follows:

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Table IV. Drug resistance transferred/not transferred to antibiotic sensitive E. coli recipient in conjugation experiments with 10 multiple drug resistant E. coli isolates.

Donor isolates No.	Drug resistance transferred	Drug resistance not transferred
1 - 2 - A	Am	Te, Sxt, K, C
2 - 2 - B	K	Te, Sxt, Am, C
2 - 4 - B	K	Te, Sxt, C
3 - 3 - B	Sxt, Am	Te
3 - 5 - B	Am	Te, Sxt
4 - 1 - B	Sxt, Am	Te
4 - 2 - B	Sxt, Am	Te
4 - 3 - B	none	Te, Sxt, K
7C	Sxt, K	Te
8C	Sxt, T	C

DISCUSSION

Antibiograms of *E coli* Isolates

Out of the 48 isolates tested, 44 or 91.6% showed resistance to at least one of the antibiotics tested. The remaining 8.3% showed intermediate responses to at least two of the antibiotics and sensitivity to the rest. None of the isolates showed complete sensitivity to the drugs used in the study.

Out of the 91.6% that showed antibiotic resistance, 60.4% were multiply resistant, being resistant to at least two antibiotics tested; 22.9% were resistant to one drug but gave intermediate responses to some of the other antibiotics, and only 8.3% showed resistance to one antibiotic and sensitivity to the remaining antibiotics.

Of the *E. coli* isolated from chickens which were given chlortetracycline-supplemented pre-starter feeds for two weeks, 97.5% showed resistance to tetracycline. The remaining 2.5% (one out of 40 tested) gave intermediate response to the antibiotic. Half of the *E. coli* isolated from poultry farm workers showed resistance while the remaining 50% of *E. coli* from the same population showed intermediate response to the antibiotic.

E. coli isolated from chickens which received sulphadimidine ethane sulphonic acid and trimethoprim-supplemented feeds and *E. coli* isolated from chickens which did not receive this drug-supplemented feed both showed parallel responses to the drug. Fifty five percent of the *E. coli* from both populations were resistant, 17.5% showed intermediate response and 27.5% were sensitive to sulfamethoxazole trimethoprim. Chicken in both groups were given chlortetracycline-supplemented pre-starter feed for two weeks. This may suggest the existence of the resistance genes to both drugs in the same plasmid. The use of any one of the drugs such as chlortetracycline for which the plasmid carries resistance to will select for the entire plasmid as a whole. This may also explain the multiplicity of resistance expressed by majority of the isolates tested.

It is also possible that the genes coding for the resistance to tetracycline and trimethoprim sulfamethoxazole are found in different R plasmids. It may suggest the continued presence of the second drug in the environment as a result of contamination from previous treatment of a different batch of chickens.

The antibiograms of the *E. coli* isolates taken from chickens show the role of supplementing feeds with antibiotics in selecting for drug-resistant microorganisms to persist in the population.

E. coli isolated from the poultry farm workers also showed multiplicity of resistance to different antibiotics. This may suggest animal to man transmission of micro-

bial flora or maintenance of antibiotic-resistant *E. coli* among the workers as a consequence of the regular use of antibiotics on the farm.

Conjugal Transferability of Multiple Drug Resistance

Ten multiple drug resistant *E. coli* isolates were conjugated with a drug sensitive *E. coli*. Nine of the isolates were shown to transfer at least one resistance gene. All of the nine showed partial transfer of drug resistance. Five isolates transferred resistance to trimethoprim sulfamethoxazole either together with resistance to ampicillin, kanamycin or tetracycline. Two transferred resistance to kanamycin only and the other two isolates transferred resistance to ampicillin only. One of the isolates tested did not transfer any of its resistance genes.

Genes coding for resistance to the different antibiotics may be found on different R plasmids. Some of the plasmids may not have been accepted by the recipient because of the presence of other pre-existing plasmids in the recipient which are incompatible with the R plasmids from the donor bacteria (Corliss, Cohen and Cabellin, 1981). Likewise it is possible that repressor genes in the recipient may have prevented the expression of the accepted R plasmid. Endonuclease in the recipient may also cause unsuccessful transfer.

It is also possible that the different resistance genes are found on the same R plasmids and only part of the plasmid was transferred as a consequence of the conjugation being interrupted. Antibiotic resistance not transferred may also be chromosomally-mediated.

One of the *E. coli* isolates did not transfer any of its resistance genes. Aside from the possible explanations offered above, it may be due to the absence or dissociation of the resistance transfer factor (RTF) component of the plasmids. RTF is needed for conjugation to take place. The r determinant and RTF component of the plasmid dissociate and r determinant amplification occurs when there is antibiotic in the environment (Matsumoto & Kamio, 1978).

CONCLUSION

The antibiotic susceptibility patterns of the 48 *E. Coli* isolates from chickens and farm workers showed the roles of supplementing feeds with antibiotics and the constant presence of antibiotics in the selection for drug-resistant microorganisms to persist in population.

The multiplicity of antibiotic resistance among the *E. coli* isolates and the conjugal transfer of resistance to at least one antibiotic from nine out of ten isolates tested likewise suggest the presence of R plasmids among the test microorganisms.

The transferability of drug resistance from one bacteria to another as well as the possible transmission of resistant bacteria from animal to animal, from animal to man very clearly leads to an increase in drug-resistant microflora in the population.

The public health significance of the problem can not be over-emphasized. Drugs are rendered ineffective especially during times in which they are most needed, as during treatment of diseases. This should make us reconsider the merits as well as the consequences of routinely supplementing animal feeds with drugs to enhance growth or prevent diseases. This may backfire as the results show the selection for microorganisms resistant to the antibiotic used in the feeds.

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