

A SURVEY OF ANTIBIOTIC RESISTANCE AMONG *E. coli* STRAINS ISOLATED FROM POULTRY IN KARACHI

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ABSTRACT

Studies were carried out to investigate the incidence of multiple antibiotic resistance among *E. coli* (total 152) isolated from poultry in Karachi to eight commonly used antibiotics: ampicillin (A), chloramphenicol (C), gentamycin (G), anamycin (K), neomycin (N), polymyxin B (P), streptomycin (S) and tetracycline (T) at the levels of 50 µg/ml, 100 µg/ml and 500 µg/ml. Tables of the results are given, showing the number of resistant strains of different patterns of antibiotic resistance at different levels. A comparison of antibiotic resistance to different number of antibiotics and the frequency of resistance to individual antibiotic at different levels is also reported. The highest frequency of resistance was against tetracycline whereas the lowest frequency of resistance was against gentamycin, Thirty R plasmids were isolated from the resistant strains and will be reported elsewhere.

INTRODUCTION

Escherichia coli is the causative agent of Hjarre's disease, coligranuloma, peritonitis, salpingitis, synovitis, amphalitis and air sac disease in poultry (Eissa, 1981, Hofstad, et al., 1978) Multiple antibiotic resistant *E. coli* have been reported from the infections of poultry throughout the World (Ezhov, 1978, Kandov, 1979, Kim et al., 1980, Lasso & Neogrady, 1980, Lubduska & Bartos, 1975, Otarov, 1980, Spais, 1979 and Tak 1977). Antibiotics are widely used for treating the infections of poultry and as feed additives (Basheer et al., 1977, Mulder et al., 1977, Quarles et al., 1977, Sheveleva & Bogorobitskaya, 1977, Smith & Tucker, 1978, William & Tucker, 1978). Indiscriminate use of antibiotics as growth promoter in poultry feed and for treating poultry infections favours the selection of resistant bacteria (Chaslus-Dancla et al., 1980, Gianelli et al., 1979, Mc Garr et al., 1977, Nazer, 1980, Pohl et al., 1980).

As there has been no systematic study on the antibiotic resistance of *E. coli* isolated from poultry in Karachi, we performed these studies to examine the antibiotic resistance of *E. coli* isolated from poultry in Karachi using eight commonly used antibiotics.

In this paper we have described the patterns of antibiotic of *E. coli* isolated from poultry in Karachi at the levels of 50 µg/ml, 100 µg/ml & 500 mg/ml. More than 20% of these strains were found to harbour transferable antibiotic resistance or R plasmids (Ansari & Khatoon, 1999 in press). Such resistance, if acquired by disease producing bacteria, results in the loss of effectiveness of antibiotics in disease treatment and as feed

additives. Furthermore, the transmission of R plasmids (which occur by conjugation) to human pathogens can also create hazards in clinical medicine.

MATERIAL AND METHODS

E. coli strains screened for their antibiotic resistance

E. coli strains (total 152) screened for their antibiotic resistance were resistance obtained from poultry research institute, Korangi, Karachi. Although these strains were obtained as pure culture, they were further purified twice on MacConkey's medium, were tested for various properties and maintained on Tryptone agar slabs.

Media

The media used in the current studies included MacConkey's agar or M.A. (Difco), triple sugar iron agar or T.S.I. (Difco), Simmons citrate agar (Merck), nutrient gelatin (Merck), glucose peptone water (Merck) and urea broth (Difco).

Other media that were made in the lab included the following: Nutrient agar (Beef extract 3 gm, peptone 10 gm, NaCl 5 gm, agar 20 gm, distilled water 1000 ml). L. Broth (Tryptone 10 gm, yeast extract 1 gm, NaCl 8 gm, distilled water 1000 ml). MacConkey's agar (M.A.), Nutrient agar (N.A.) and L. Broth were used for the growth of bacteria whereas media were used for characterization.

Antibiotics

The antibiotics used were: ampicillin trihydrate, chloramphenicol (both from Opal laboratories Ltd., Karachi), gentamycin (from Nicholas Ltd., Karachi), kanamycin sulphate (from Continental Pharma, Belgium), neomycin sulphate (from Glaxo Laboratories, Karachi), polymyxin B sulphate, streptomycin sulphate (both from Pfizer Laboratories, Karachi) and tetracycline hydrochloride (from Lederle Laboratories, Karachi). All the antibiotics were obtained in purified powdered form and stock solution (10 mg/ml) were made in distilled water. Chloramphenicol was dissolved in absolute ethyl alcohol. The solutions were sterilized by millipore filtration and kept frozen if not in use. The solution of daily use were kept refrigerated at 4°C for not more than a week.

Antibiotic resistance and sensitivity of strains

Screening for antibiotic resistance / sensitivity was performed by the methods described by Khurshid & Khatun (1984).

A broth culture of the test strains was plated on M.A. to obtain isolated colonies. Approximately 20 individual colonies were picked onto a master plate (M.A.), grown overnight and replicated on M.A. plates containing desired concentrations of each of the test antibiotics. Sterile velvet replica was used for replication. The highest concentration of an antibiotic showing growth of all replicated colonies was considered as the resistance level of the strain, for that particular antibiotic.

The minimum resistance level for any antibiotic was taken as 50 µg/ml (of the medium) below which a bacterium was considered sensitive.

RESULTS AND DISCUSSION

E. coli strains isolated from poultry in Karachi were screened for their resistance to the commonly used antibiotics: ampicillin (A), chloramphenicol (C), gentamycin (G), kanamycin (K), neomycin (N), polymyxin B (P), streptomycin (S) and tetracycline (T) at the levels of 50 µg/ml, 100 µg/ml and 500 µg/ml. Of the 152 *E. coli* strains screened, the number of resistant bacteria at the increasing levels were 145, 123 & 106 respectively. Antibiotic patterns of these strains at the three levels of antibiotics can be examined in Tables I, II and III.

A comparison of the frequency of antibiotic resistance of *E. coli* strains isolated from poultry in Karachi at three different levels of antibiotics (50 µg/ml, 100 µg/ml & 500 µg/ml) is given in Figure I.

Antibiotic resistance *E. coli* have been reported from the infection of poultry throughout the world. In Czechoslovakia, Lebduška and Bartoš (1975) isolated a large number of *E. coli* resistant to chlorotetracycline from broilers fed on chlorotetracycline. In U.S.S.R., Ezhov (1978) assessed 370 strains of *E. coli* isolated in 26 poultry farms in which colisepticaemia alone or in association with respiratory mycoplasmosis was prevalent. 80% strains were resistant to tetracycline and 48% to furazolidone. In Hungary, Lasso and Neogredy (1980) isolated *E. coli* resistant to sulfotrim, neomycin, tetracycline, chlorocid and streptomycin from a flock of 25000 broiler chickens. In Korea, Kim et al., (1980) tested 63 *E. coli* strains for drug resistance during 1978. All were resistant to bacitracin, lincomycin, oleandomycin, penicillin and erythromycin and over 90% were resistant to streptomycin and tetracycline; all were resistant to at least two drugs in common use. In U.K., Jackson (1977) tested *E. coli* strains isolated from poultry during the years 1971 to 1977 for resistance to six antibiotics using a disc diffusion technique. The percentage of strains resistant to chloramphenicol, tetracycline, neomycin, streptomycin and furazolidone were 16.8, 53.9, 42.7, 18.1 and 10.9 respectively. 52.2% and 27.8% of strains were resistant to ampicillin during 1971-1972 and 1973-1977 respectively.

From the results presented here; appears that multiple antibiotics resistance among *E. coli* strains isolated from poultry in Karachi is not uncommon as out of 152 screened for resistance, 95% were resistant to one or more antibiotics at 50 µg/ml, 81% were resistant to one or more antibiotics at 100 µg/ml and 70% were resistant to one or more antibiotics at 500 µg/ml (Tables FIII). The resistance at as high as 500 µg/ml is alarming because if bacteria resistant to such high level of antibiotics, disease treatment with antibiotics would not be possible. Several combinations of antibiotic resistance (resistance patterns), for *E. coli* isolated from poultry in Karachi were observed at three levels of antibiotics (Table I-III).

Number of antibiotic resistance patterns for *E. coli* isolated from poultry in Karachi at 50 µg/ml and 100 µg/ml were 44 and 37 respectively (Table I & II). In both the cases the most common pattern was KNST followed by ST. At 500 µg/ml, 26 different patterns of antibiotics resistance were observed (Table III). The most common pattern was T followed by KN.

The highest frequency of resistance was against kanamycin at 50 µg/ml and 100 µg/ml (Figure 1) At 500 µg/ml, the highest frequency of resistance was against kanamycin (Fig 1). The lowest frequency of resistance was against gentamycin at all the levels of antibiotics used for screening (Fig 1), and it seems to be the most effective antibiotic for treating infections of poultry caused by *E. coli*. The other antibiotics which gave good results were chloramphenicol (Fig 1). However, streptomycin, neomycin and kanamycin seem to be ineffective for treatment infections of poultry caused by *E. coli* (Fig 1). Ampicillin and polymyxin B gave fairly good results against *E. coli* (Fig 1).

Table -1
Antibiotic Resistance Patterns of *E. coli* Strains isolated
from Poultry in Karachi at 50 µg/ml

Resistance Patterns	Numbers of Isolates	Resistance Patterns	Number of Isolates
ACGNKNST	1	NPST	1
CGKNPST	2	ANP	1
ACKNST	1	AST	2
ACKNST	7	CST	2
ACNPST	1	GKN	
AGKNST	2	GST	1
AKNPST	2	KNT	2
CKNPST	2	NPS	1
ACKNT	1	NPT	1
ACPST	1	NST	2
AKNST	4	PST	5
CKNST	5	AS	1
GKNST	2	AT	1
GNPST	1	GT	1
KNPST	6	KN	1
ACST	3	KT	1
AKNS	1	PS	1
AKST	1	PT	4
APST	1	ST	23
CNST	1	A	3
KNPT	1	S	3
KNST	31	T	9

A= ampicillin
C= chloramphenicol
K = kanamycin
P = polymyxin B
T= tetracycline

G = Gentamycin
N = Neomycin
S = Streptomycin

Table II
Antibiotic Resistance Patterns of *E. coli* Strain Isolated
from Poultry in Karachi at 100 µg/ml

Resistance Patterns	Numbers of Isolates	Resistance Patterns	Number of Isolates
ACGKNPST	1	KNST	26
ACKNPST	3	AKN	1
ACKNST	5	AKP	1
AGKNPS	1	AST	2
AGKNST	1	CST	1
AKNPST	2	GCT	2
CGKNST	1	KNT	5
CGNPST	2	PST	5
ACKNT	1	AP	2
ACPST	1	GT	1
AKNST	4	KN	1
GKNST	1	PS	1
KNPST	6	PT	3
ACKN	1	ST	22
ACST	2	A	2
APST	1	P	1
CKST	1	S	1
GKNT	1	T	10
KNPT	1		

*For abbreviations see Table I

Table III
Antibiotic Resistance Patterns of *E. coli* Strains Isolated
from Poultry In Karachi at 500 µg/ml

Resistance Patterns	Numbers of Isolates	Resistance Patterns	Number of Isolates
AKNST	7	KST	2
CKNST	1	AK	2
ACKN	1	AP	1
ACST	1	AS	2
AKNS	1	KN	12
AKNT	1	KS	2
AKST	1	KT	4
KNST	6	ST	9
AKN	2	A	2
AKT	2	C	1
AST	2	K	3
KNS	7	S	10
KNT	11	T	13

*For abbreviations see Table I

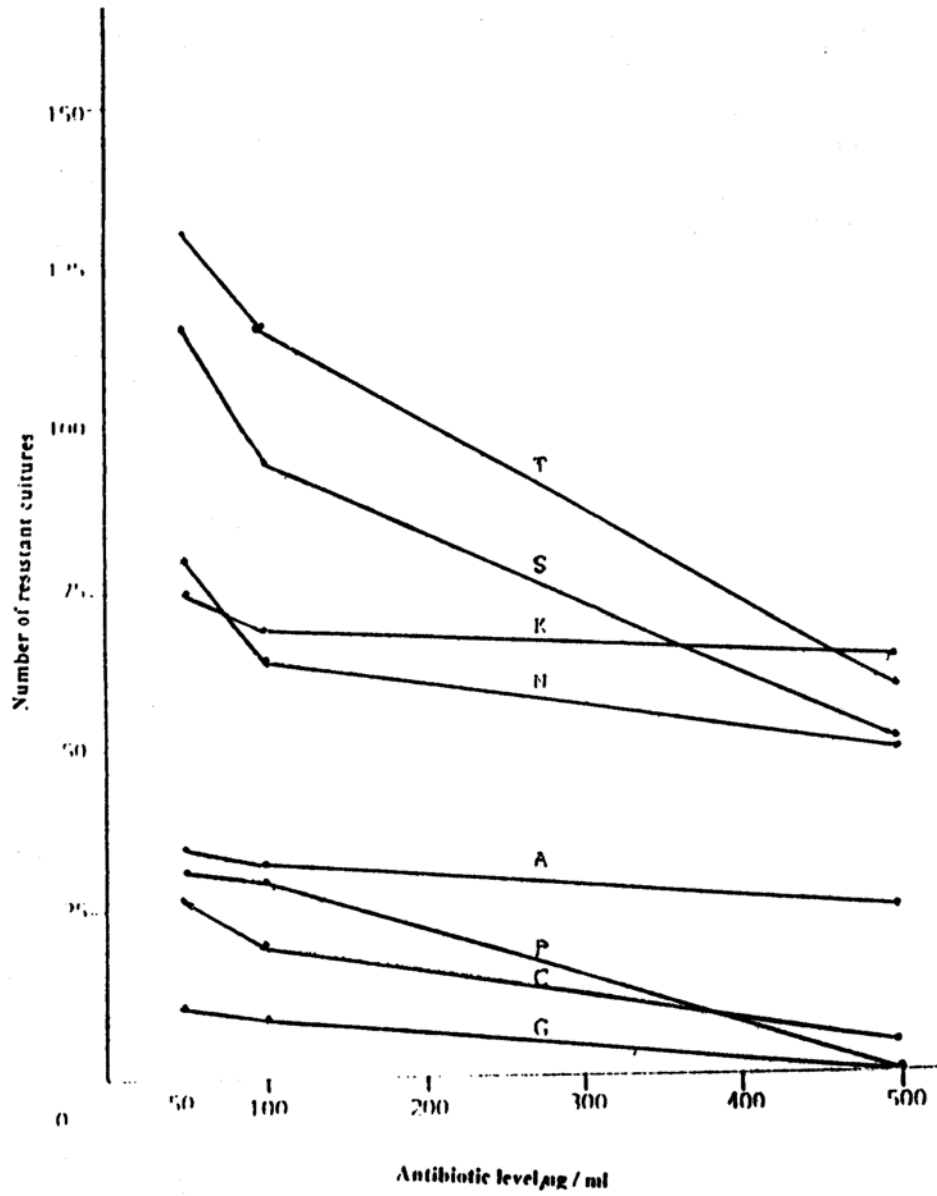


Figure I. A comparison of antibiotic resistance of *E. coli* strains isolated from poultry at three different levels of the antibiotics: Ampicillin (A), chioramphenicol (C), gentamycin (G), kanamycin (K), neomycin (N), polymyxin B (P), streptomycin (S) & tetracycline (T).

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