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A STUDY OF ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM POULTRY IN KARACHI

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ABSTRACT

Studies were carried out to investigate the incidence of multiple antibiotic resistance among *Escherichia coli* (total 152) isolated from poultry in Karachi to eight 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, 100 and 500 µg/ml. Results are showing the number of resistance strains with 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 found against tetracycline whereas the lowest frequency of resisatnce was against gentamycin. Thirty R plasmids were isolated from the resistance starins and will be reported elsewhere.

Key-words: Multiple antibiotic resistance, R plasmid, *E. coli*, poultry

INTRODUCTION

Escherichia coli (*E. coli*) is the causative agent of Hjarre's disease, coligranuloma, peritonitis, salpingitis, synuvitis, 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 (Artem'eva *et al*., 1977; Bahl and Mehrotra, 1977; Bartos and Lebduska, 1975; Ezhov, 1978; Jackson, 1977;Kandov, 1979; Kim *et al*., 1980; Lasso and Neogrady, 1980; Otarov, 1980, Smith, 1966, Spais, 1979; Tak, 1977; Walton, 1966). 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; Romvary *et al.*, 1976; Sheveleva and Bogoroditskaya, 1977; Smith and Tucker, 1978; William and 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; Kinjo, 1978; Mc Garr *et al*., 1977; Nazer, 1980; Nivas *et al*., 1976; Pohl *et al*., 1980).

As there has been no systematic study on the antibiotic resistance of *E. coli* isolated from poultry in Karachi, studies were conducted 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 resistance of *E. coli* isolated from poultry in Karachi at the levels of 50, 100, and 500 µg/ ml. More than 20% of these strains were found to harbour transferable antibiotic resistance or R plasmids (Ansari and Khatoon, unreported data). Such resistance, if acquired by disease producing bacteria, results in the loss of effectiveness of antibiotics in disease treatment and as feed additives.

MATERIALS AND METHODS

Escherichia coli strains screened for their antibiotic resistance

Escherichia coli strains (total 152) screened for their antibiotic resistance were obtained from Poultry Research Institute, Korangi, Karachi. These strains were further multiplied on MacConkey's medium, and tested for various properties. The strains were maintained on tryptone agar stabs.

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), Simmon citrate agar (Merck), Nutrient gelatin (Merck), Glucose peptone water (Merck) and Urea broth (Difco).

Other media that were made in the lab included; Nutrient agar (beef extract 3 gm, peptone 10 gm, NaCl 5 gm, agar agar 20 gm, distilled water 1000 ml) and L. Broth (tryptone 10 gm, yeast extract 1 gm, NaCI 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 other media were used for characterization.

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Antibiotics

The antibiotics used were: ampicillin trihydrate, chloramphenicol (both from Opal Laboratories Ltd., Karachi). gentamycin (from Aspro 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 solutions 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 Khursheed and. Khatoon (1984).

RESULTS AND DISCUSSION

Escherichia coli stains 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, 100, and 500 µg/ml. Of the 152 *E. coli* strains screened, the number of resistant bacteria at the increasing levels was 145, 123 and 106 respectively. Antibiotic resistance patterns of these strains at the three levels of antibiotics can be examined in Table 1, 2 and 3.

A comparison of the frequency of antibiotic resistance of *E. coli* strains isolated from poultry in Karachi at three different levels of antibiotics (50, 100, and 500 µg/ml) is given in Fig. **1**. Number of *E. coli* isolated from poultry in Karachi resistant to different number of antibiotic(s) at different levels is given in Fig. **2**.

From the results presented here, it appears that multiple antibiotic resistance among *E. coli* strains is not uncommon as out of 152 strains screened for resistance, 95% were found resistant to one or more antibiotics at 50 µg/ml, 81% were found resistant to one or more antibiotics at 100 µg/ml and 70% were resistant to one or more antibiotics at 500 µg/ml (Table 1-3). The resistance at 500 µg/ml is alarming because if bacteria become 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* were observed at three levels of antibiotics.

Table 1. Antibiotics resistance patterns of *E. coli* isolated from poultry at 50 µg/ml.

Resistance Patterns*	Number of isolates	Resistance Patterns*	Number of isolates
ACGKNST	1	NPST	1
CGKNPST	2	ANP	1
ACKNPT	1	AST	2
ACKNST	7	CST	2
ACNPST	1	GKN	1
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	11
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; G= gentamycin; K= kanamycin; N= neomycin; P= polymyxin; S= streptomycin; T= tetramycycline

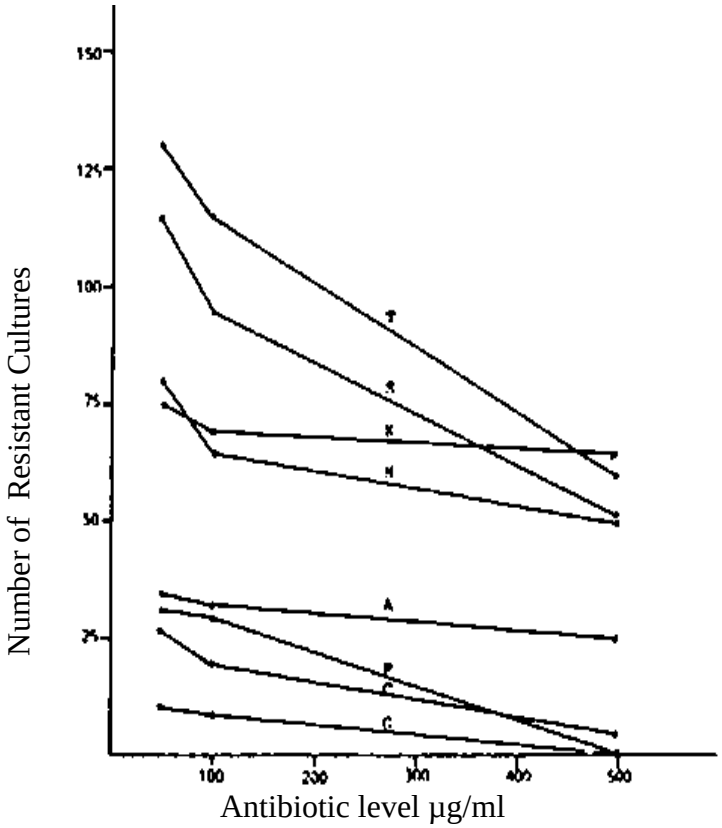

Fig.1. A comparison of antibiotic resistance of *E. coli* isolated from poultry at three different levels of the antibiotics: Ampicillin (A); Chloramphenicol (C); Gentamycin (G); Kanamycin (K); Neomycin (N); Polymyxin (P); Streptomycin (S); Tetramycycline (T).

Table 2. Antibiotics resistance patterns of *E. coli* isolated from poultry at 100 µg/ml.

Resistance Patterns*	Number 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	GST	2
CGKNST	1	KNT	5
CKNPST	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		

*A= ampicillin; C= chloramphenicol; G= gentamycin; K= kanamycin; N= neomycin; P= polymyxin; S= streptomycin; T= tetramycycline

Table 3. Antibiotics resistance patterns of *E. coli* isolated from poultry at 500 µg/ml.

Resistance Patterns*	Number 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

A= ampicillin; C= chloramphenicol; G= gentamycin; K= kanamycin; N= neomycin; P= polymyxin; S= streptomycin; T= tetramycycline

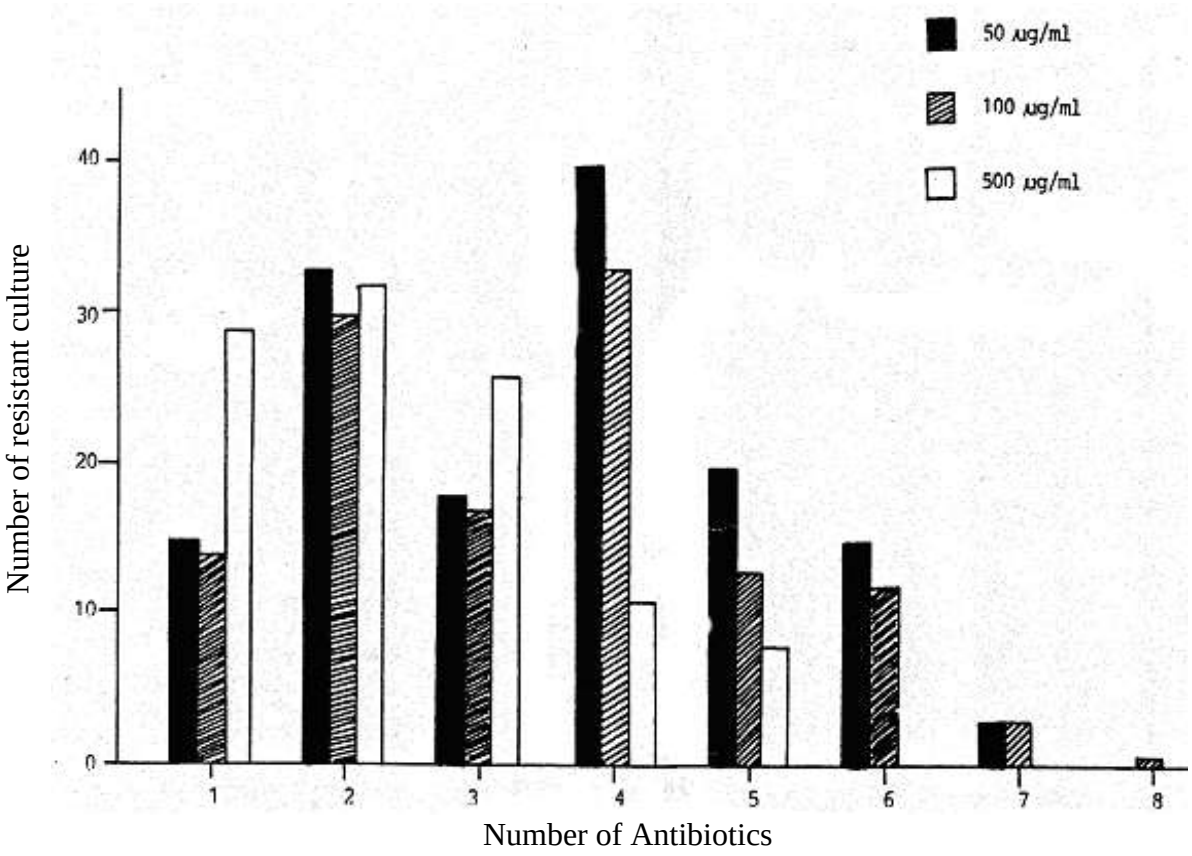

Fig. 2. A comparison of antibiotic resistance of *E. coli* isolated from poultry to different number of antibiotics at different levels.
Ampicillin (1); Chloramphenicol (2); Gentamycin (3); Kanamycin (4); Neomycin (5); Polymyxin (6); Streptomycin (7); Tetramycycline (8).

Numbers of antibiotic resistance patterns for *E. coli* at 50 and 100 µg/ml were 44 and 37 respectively (Table1, 2). In both the cases the most common pattern was KNST followed by ST. At 500 µg/ml, 26 different patterns of antibiotic resistance were observed (Table 3). The most common pattern was T followed by KN.

The highest frequency of resistance was against tetracycline at 50, and 100 µg/ml (Fig 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 seems to be ineffective for treating infections of poultry caused by *E. coli* (Fig. **1**). Ampicillin and polymyxin B gave fairly good results against *E. coli* (Fig. **1**).

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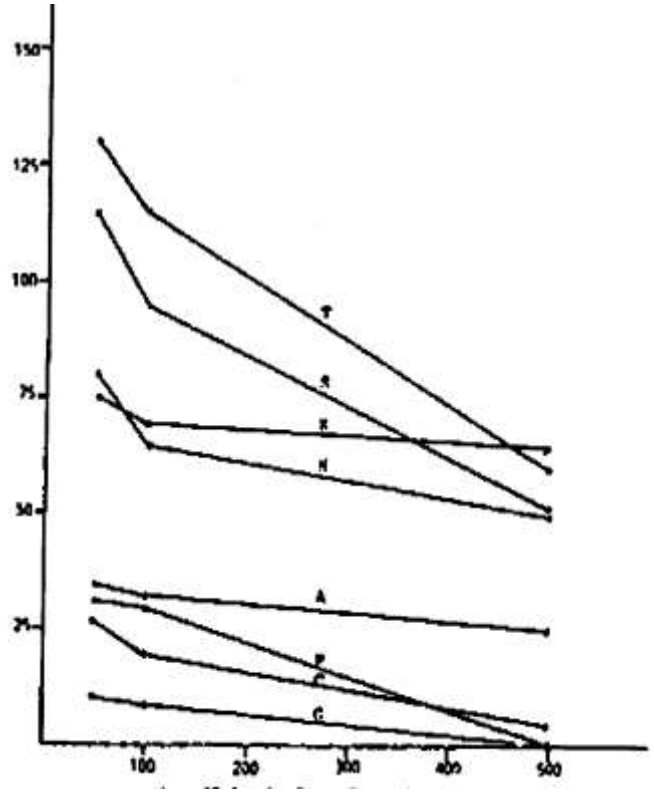
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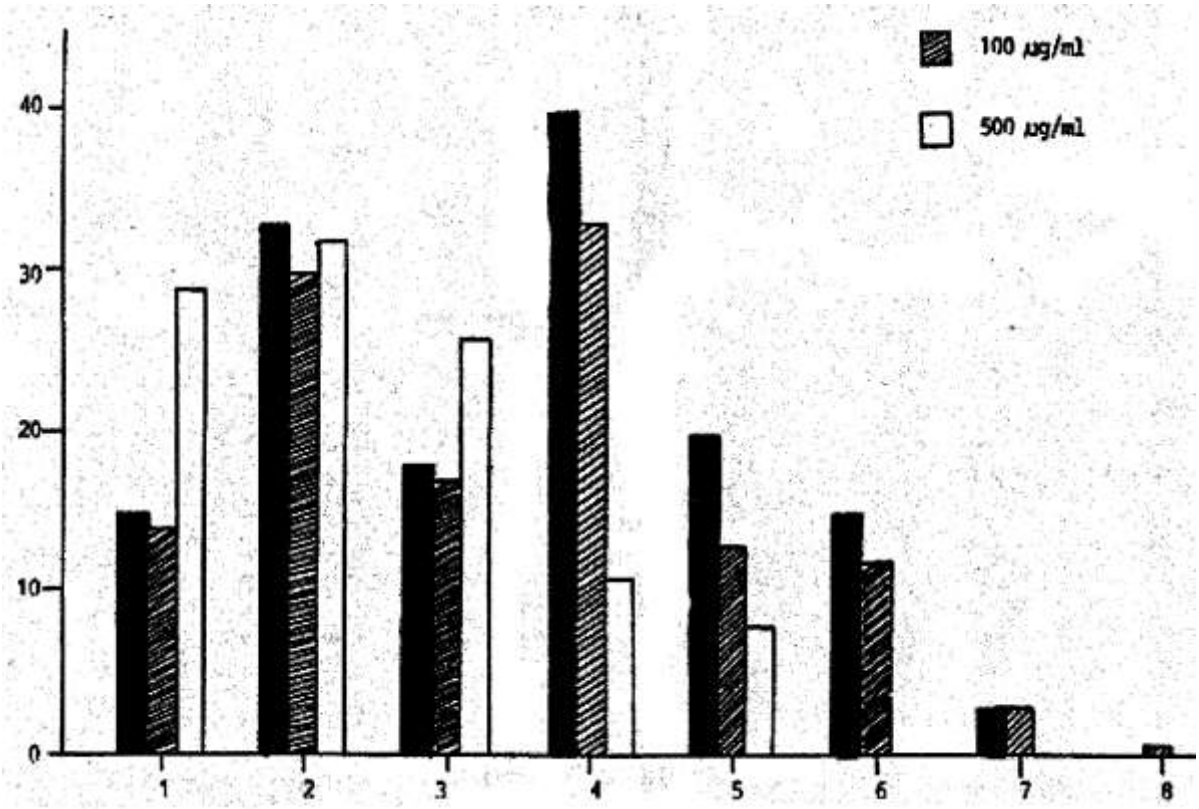
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