

ANTIBIOGRAM TYPING OF *ESCHERICHIA COLI* OF BOVINE DIARRHOEIC ORIGIN

W. Manohar Paul¹, M. Rajeswaran², R.Senthil kumar³ &
N.Daniel Joy Chandran⁴

Department of Veterinary Microbiology, Madras Veterinary College, Chennai-7

ABSTRACT

One hundred and four isolates of E.coli isolated from cases of bovine diarrhea were subjected to antibiogram testing using 17 antibiotics and antibacterials. By disc diffusion technique the isolates were differentiated into 91 different antibiotic resistance pattern indicating high order of reliability and sensitivity (88%). Nearly all the isolates were susceptible to norfloxacin, enrofloxacin, ofloxacin. The drug of choice for bovine diarrhea due to E.coli warrants manufacture of patent preparations containing norfloxacin, enrofloxacin and ciprofloxacin.

Keywords: *Escherichia coli*, antibiogram, bovine diarrhoeic origin, antibiotic diffusion discs.

INTRODUCTION

With increased spread of multiresistance *E.coli* in man and animals microbiologist have sought various systems which distinguish new epidemic strains from endemic ones. Chemotherapy continues to be indispensable in the control of this disease. For chemotherapy to be successful selection of proper antibiotics is essential. In the present study the antibiotic sensitivity of one hundred and four isolates of *E.coli* recovered from cases of bovine diarrhea was determined by employing commonly available antibiotics to find out the effective antibiotic treatment for the disease due to *E.coli*. The study also involved typing of *E.coli* isolates by their antibiotic resistant phenotypes

One hundred and four isolates of *E.coli* were identified from bovine diarrhoea based on morphological and biochemical characteristics in

accordance with Holmes and Gross (1990). They were further subjected to disc assay tests (Bauer *et al.* 1966) using seventeen antibiotics and antibacterials.

Antibiotic Sensitivity Testing

Antibiotic sensitivity test was done (Bauer *et al.* 1966) with slight modifications (Anonymous 1977). The growth on the top surface of 3 to 5 colonies of test organism were used as inoculum in 5 ml of trypticase soy broth. After 5-6 hr of growth at 37 degree Celsius the growth was diluted with normal saline to a density visually equal to Mc Farland 0.1. Muller Hinton agar (HiMedia) plates were dried in the incubator at 37 degree Celsius for 30 minutes before inoculation. The diluted bacterial growth was streaked in 3 different planes on Muller Hinton agar using a sterile swab.

1. Retired Professor, Department of Veterinary Microbiology, Madras Veterinary College, Chennai-07

2 & 3 Undergraduate Scholars, Madras Veterinary College, Chennai-7

4. Professor, Director of Research, TANUVAS, Chennai-51

Table 1
Multiplex resistance pattern of one hundred and four isolates of *Escherichia coli*
(Bovine diarrhoeic origin)

Serial number of antibiogram group	Antibiotic resistance pattern	Total number of isolates to antibiogram pattern	No. of isolate conforming showing resistance
1	A,B,CP,D,E,G,AK,N,NT,PM,P,T,TM	3[E-35,3-54,3-62]	3 (2.8%)
2	A,CP,CF,D,E,G,AK,NT,PM,P,T,TM	1[E-66]	3 (3%)
3	A,B,CP,D,E,AK,N,NT,PM,P,T,TM	2[E-96,E-103]	
4	A,B,CP,D,E,G,NT,PM,P,T,TM	1[E-31]	8 (8%)
5	A,B,CP,E,AK,N,NT,PM,P,T,TM	1[E-32]	
6	A,B,E,G,AK,N,NT,NF,OF,P,T	1[3-57]	
7	A,B,CP,E,G,AK,NT,PM,P,T,TM	2[E-68,E-87]	
8	A,CP,D,E,G,AK,NT,PM,P,T,TM	1[E-84]	
9	A,CP,D,E,G,AK,N,PM,P,T,TM	1[E-89]	
10	A,B,CP,D,G,N,NT,PM,P,T,TM	1[E-102]	21 (20%)
11	A,B,CP,D,E,G,AK,NT,P,T	1[E-5]	
12	A,B,CP,D,E,G,AK,P,T,TM	1[E-13]	
13	A,B,CP,D,E,G,AK,PM,P,TM	1[E-15]	
14	A,B,CP,D,E,N,PM,P,T,TM	1[E-28]	
15	A,B,CP,E,N,NT,PM,P,T,TM	3[E-29,E-30,3-95]	
16	A,CP,E,G,AK,N,NT,PM,P,TM	1[E-39]	
17	A,B,D,G,AK,N,NT,P,T,TM	1[E-40]	
18	A,B,CP,D,E,NT,PM,P,T,TM	1[E-41]	
19	A,B,CP,D,E,N,NT,PM,P,T	1[E-44]	
20	A,B,CP,D,G,N,PM,P,T,TM	2[E-49, E-82]	
21	A,B,CP,G,AK,N,PM,P,T,TM	1[E-65]	
22	A,B,CP,D,N,NT,PM,P,T,TM	1[E-80]	
23	A,CP,CF,D,E,AK,PM,P,T,TM	1[E-90]	
24	A,CP,E,G,AK,N,PM,P,T,TM	1[E-93]	
25	A,B,CP,D,E,AK,N,NT,P,TM	1[E-94]	
26	A,B,CP,D,E,AK,N,PM,P,TM	1[E-100]	
27	A,B,CP,G,AK,N,NT,PM,P,T	1[E-101]	20 (19%)
28	A,CP,D,E,N,NT,PM,P,T,TM	1[E-53]	
29	A,B,CP,D,E,N,NT,PM,P	1[E-18]	
30	A,B,CP,E,G,AK,N,PM,P	1[E-8]	
31	A,B,CP,D,NT,PM,P,T,TM	1[E-10]	
32	A,B,CP,D,G,NT,PM,T,TM	1[E-16]	
33	A,B,D,E,NT,PM,P,T,TM	2[E-20,E-92]	
34	A,CP,D,E,G,AK,N,PM,P	[E-24]	
35	A,B,CP,D,E,PM,P,T,TM	3[E-37,E-48,E-50]	
36	A,B,CP,E,N,NT,P,T,TM	1[E-42]	20 (19%)
37	A,B,D,E,N,PM,P,T,TM	1[E-46]	
38	A,B,D,E,G,AK,N,NT,P	1[E-52]	
39	A,CF,EF,AK,N,PM,P,T,TM	1[E-58]	
40	A,B,D,E,AK,NT,PM,P,TM	1[E-77]	
41	A,B,CP,E,N,NT,PM,P,T	1[E-79]	
42	A,B,CP,D,N,NT,PM,P,T	1[E-83]	
43	A,B,E,G,AK,NT,PM,P,TM	1[E-97]	
44	A,B,CP,E,AK,NT,PM,P,T	1[E-25]	
45	A,B,D,AK,N,PM,P,T,TM	1[E-26]	

conti. .

Table 1
Multiple resistance pattern of one hundred and four isolates of *Escherichia coli*
(Bovine diarrhoeic origin)

Serial number of antibiogram group	Antibiotic resistance pattern	Total number of isolates to antibiogram pattern	No. of isolate conforming showing resistance
46	A,B,CP,E,NT,PM,P,T	1[E-7]	17(16%)
47	A,B,CP,E,G,PM,P,TM	1[E-21]	
48	A,B,E,G,NT,P,T,TM	1[E-27]	
49	A,B,CP,E,N,PM,P,TM	2[E-33, E-64]	
50	A,B,CP,G,N,P,T,TM	1[E-34]	
51	A,D,N,NT,PM,P,T,TM	1[E-43]	
52	A,B,CP,E,G,AK,PM,P	1[E-45]	
53	A,CP,D,E,PM,P,T,TM	1[E-47]	
54	A,B,CP,E,N,NT,PM,P	1[E-59]	
55	A,B,CP,D,G,P,T,TM	1[E-60]	
56	A,CP,D,E,G,NT,PM,T	1[E-69]	
57	A,B,CP,N,NT,PM,P,TM	1[E-73]	
58	A,B,D,NT,PM,P,T,TM	1[E-75]	
59	A,CP,E,N,NT,PM,P,TM	1[E-85]	
60	A,B,D,E,NT,PM,P,TM	1[E-88]	
61	A,B,CP,E,AK,N,PM,P	1[E-104]	
62	A,B,E,PM,P,T,TM	1[E-2]	13(12%)
63	A,B,CP,E,NT,PM,TM	1[E-9]	
64	A,B,E,G,AK,PM,TM	1[E-14]	
65	A,CP,D,E,PM,P,T	1[E-22]	
66	A,B,CP,E,G,P,TM	1[E-54]	
67	A,B,CP,G,AK,N,PM	1[E-67]	
68	A,B,CP,E,NT,P,TM	1[E-70]	
69	A,B,CP,E,NT,PM,T	1[E-71]	
70	A,B,CP,E,PM,P,TM	1[E-72]	
71	A,B,D,E,G,AK,T	1[E-74]	
72	A,B,CP,E,N,PM,P	1[E-86]	
73	A,B,D,E,AK,P,TM	1[E-91]	
74	A,D,ENT,P,T,TM	1[E-98]	
75	A,B,E,PM,P,TM	1[E-3]	8(8%)
76	A,D,E,PM,T,TM	1[E-11]	
77	A,E,NT,PM,T,TM	1[E-17]	
78	A,B,CP,NT,P,TM	1[E-19]	
79	A,B,D,E,P,TM	1[E-38]	
80	A,D,E,N,PM,TM	1[E-55]	
81	A,CP,D,PM,P,T	1[E-61]	
82	A,G,NT,P,T,TM	1[E-99]	
83	A,PM,P,T,TM	2[E-1, E-36]	10(10%)
84	A,CP,D,N,P	1[E-4]	
85	A,B,CP,D,NT	1[E-6]	
86	A,B,C,NT,PM	1[E-12]	
87	A,CP,PM,P,TM	2[E-23, E-63]	
88	A,E,N,P,T	1[E-51]	
89	A,CP,E,NT,TM	1[E-76]	
90	A,CP,E,P,TM	1[E-81]	
91	A,B,CP,TM	1[E-78]	1(1%)

E – no in brackets indicate the serial number of the isolate of *Escherichia coli*.

A – Ampicillin, AK-Amikacin, B-Bacitracin, CP-Chloramphenicol, CF-Ciprofloxacin,

D-Doxycycline, EF-Enrofloxacin, E-Erythromycin, G-Gentamicin, N-Neomycin,

NT-Nitrofurantoin, OF-Ofloxacin, PM-Polymyxin B, P-Pencillin G, T-Tetracycline,

TM-Trimethoprin, NF-Norfloxacin.

The plates were dried at room temperature for 5 minutes, followed by placing discs of antibiotics by means of a sterile forceps. The antibiotic discs supplied by HiMedia were used. Six discs were placed equidistant hexagonally in each petridish of 15 cm diameter and such plates were used for each test organism. After overnight incubation at 37 degree Celsius the diameter of each zone of inhibition was measured, recorded and interpreted accordingly to the zone size interpretative charts (Brown and Blowers 1978).

RESULTS AND DISCUSSION

The antibiogram patterns of the hundred and four isolates of *E.coli* of bovine diarrhoeic origin were determined and among them 91 multiple resistance patterns were observed. The multiple antibiotic resistance exhibited by one hundred and four isolates of *E.coli* to 17 antibiotics and antibacterials are presented in Table I. Among 104 isolates of *E.coli* 55 exhibited (53%) resistance to 9 or more and 49 (47%) resistance to 4 to 8 number of antibiotics and antibacterials. All the hundred and four isolates showed 100% resistance to ampicillin while 88% of them resistance to penicillin, more than 70% of isolates exhibited resistance to bacitracin, erythromycin, polymixin B and trimethoprin. Nearly 99% of isolates were susceptible to the newly introduced antibiotics such as ciprofloxacin, norfloxacin and enrofloxacin. Antimicrobial susceptibility was used as an epidemiological marker to study dissemination of multiresistant *E.coli*.

Our results suggest that the drug of choice for bovine diarrhoea due to *E.coli* should ciprofloxacin, ofloxacin, norfloxacin and enrofloxacin. The usage of ampicillin and chloramphenicol have to

be dispensed with because of rapid emergence of resistant strains (Schroeder *et al.*, 2002). It is stressed that regular antibiotic sensitivity should be conducted before starting the treatment for disease to avoid dissemination of drug-resistant *E.coli* from diseased animals to healthy ones

REFERENCES

- Anonymus. 1977. Report of the Expert committee on Biological Standardization. 28 Report. Technical Report Series 610. World Health Organization, Geneva. pp. 110-114.
- Holmes B and Gross R.J. (1990) Coliform Bacteria. *Topley and Wilson's Systematic Bacteriology*. 8th edn., 1990.
- Bauer A W, Kirby M M, Sherris J C and Truck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45: 493-96.
- Brown D and Blowers R. 1978. Disc methods of sensitivity testing and semiquantitative methods. *Laboratory Methods in Antimicrobial Agents Chemotherapy*. pp. 8-30 (Eds) Reeves D S, Philips I J D, Williams and Wise N. Churchill Livingstone, London.
- Schroeder, C.M., C.Zhao, C.DebRoy, J. Torcolini, S. Zhao, D.G. While, D.D. Wagner, P.F. McDermott, R.D. Walker, and J.Meng. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. *Appl. Environ. Microbiol.*