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Drugs Susceptibility Reactions Patterns and Multiple Drug Resistance of *Escherichia coli* Isolated from Diarrhoeic Calves in Sudan

[1]O. Salwa Ellaithi, [1]E.D.M. ElAmin and [2]A.E. Abdalla
[1]Central Veterinary Research Laboratories, P.O. Box 8067-Alamart, Khartoum, Sudan
[2]Department of Clinical Studies, Faculty of Veterinary Medicine,
University of Khartoum, P.O. Box 32, Khartoum North, Sudan

Abstract: Drug sensitivity test was performed on forty-four *E. coli* isolates using nine antimicrobial drugs. The latter were ampicillin, chloramphenicol, erythromycin, gentamycin, nalidixic acid, neomycin, streptomycin, sulfamethoxazole/trimethoprim and tetracycline. A considerable variation in their pattern of sensitivity was shown. Almost all isolates showed sensitivity to chloramphnicol. On the other hand, the strains were all insensitive to erythromycin. Forty-one patterns of drug susceptibility reactions were obtained. Each pattern was represented by a single strain with the exception of three of them which included two strains each.On the whole, 26 patterns of drugs resistance that ranged between resistance to a single drug and seven drugs were encountered A scheme of drug susceptibility patterns is put forward for use in routine clinical diagnosis as well as epidemiological investigations.

Key words: Drugs, reactions, resistance, ampicillin, susceptiobility, epidmiological

INTRODUCTION

In Sudan, the susceptibility of *E. coli* strains isolated from diarrhoeic calves and goat kids to different antibiotics was studied by Kamal (2000). He found that they were sensitive to Gentamycin and were all almost resistant to Streptomycin. Similar results were also obtained by Cid *et al.* (1996) who found the Streptomycin was the least effective. Antibiotics have been used for prevention and treatment of colibacillosis, particularly oxytetrcycline, chloroteteracycline and streptomycin.

It has been the practice in some farms to feed antibiotics as a prophylactic measure and the greatest value was obtained when these substances were given orally for several days beginning within 24 h of birth. This has resulted in the development of resistant strains of *E. coli* (Buxton and Frazer, 1997).

Multiple drug resistance has definitely assumed a world-wide spread (Shears, 2001). Certainly, the Sudan is no exception, although the size and magnitude of the problem are not clearly defined.

MATERIALS AND METHODS

Bacteria: Forty-four isolates of *E.coli* isolated from diarrhoeic calves were used.

Antimicrobial drug susceptibility test: This test was essentially carried out as described by (Bauer *et al.*, 1996; Benson, 1980).

Table 1: Antimicrobial drugs used, their concentration and the standard zones of growth inhibition of resistant, intermediate and susceptible *E. coli* isolates

Name of Antibiotics or chemotherapeut -ic agents	Conc. (mg)	Standard zones of growth inhibition (in mm)*		
		Resistant	Intermediate	Susceptible
Ampicillin	10	13 mm	14-16	17
Chloramphinicol	30	12	13-17	18
Erythromycin	15	13	14-22	23
Gentamycin	10	12	13-14	15
Neomycin	30	14	15-18	19
Nalidixic acid	30	13	14-18	19
Streptomycin	10	11	12-14	15
Trimethoprim/ sulphathyaxol	1.25/23.75	10	11-15	16
Tetracycline	10	13	14-16	17

*Adopted from NCCLS (1992)

For preparation of the bacterial suspension, the growth in Nutrient Broth (Oxoid,CM67) was diluted with sterile normal saline to a degree of turbidity visually equivalent to that of the standard prepared by adding 0.5 ml of 1% BcI_2 to 99.5 mL of 1% H_2SO_4 (0.36N) before being plated out onto Mueller-Hinton Agar (Scharlaw Microbiology) and incubated overnight at 37°C.

The antimicrobial drugs used and their corresponding concentrations are shown in Table 1.The diameter of the inhibition zone, including that of the antimicrobial disk, was measured. The inhibition zone produced by an antimicrobial drug was compared with its corresponding standard zone of inhibition (Table 1) to determine whether the specified *E. coli* isolate was Sensitive (S), Intermediate (I) or Resistant (R) to the drug in question.

Corresponding Author: O. Salwa Ellaithi,Central Veterinary Research Laboratories, P.O. Box 8067-Alamart, Khartoum, Sudan

Table 2: Antimicrobial drugs susceptibility reactions of 44 *E.coli* isolates form diarrhoeic calves

Isolate No.	C	Amp	G	Sxt	S	E	N	NA	T
1	S	R	S	S	S	R	R	S	S
2	S	R	S	S	R	I	S	S	R
4	S	R	S	S	I	I	S	S	R
5	S	R	S	S	I	R	S	S	S
7	S	R	S	S	I	I	I	R	S
8	S	S	S	S	R	R	R	R	S
9	S	S	I	S	S	R	I	I	R
11	S	R	S	S	R	I	S	S	S
14	S	S	S	S	I	I	I	S	R
19	S	R	R	R	R	R	I	R	S
20	S	S	S	S	S	R	I	S	S
22	S	S	S	S	I	R	R	I	R
24	S	S	S	S	S	I	S	I	S
25	S	S	S	S	R	R	I	S	R
26	S	R	R	S	R	I	R	S	S
30	R	R	R	R	R	R	R	R	R
31	S	I	I	S	I	R	I	I	S
33	S	S	S	S	I	I	I	S	I
34	S	I	S	S	R	R	I	S	S
35	S	S	S	S	S	I	I	S	S
39	S	S	S	S	R	R	R	I	R
40	I	S	S	S	R	R	R	S	S
43	S	S	S	S	S	R	I	S	R
47	S	I	R	S	R	R	I	S	R
48	S	S	S	S	R	I	R	S	I
49	S	R	S	S	S	I	R	S	S
50	S	I	I	S	S	R	R	I	R
51	I	S	S	S	I	R	R	I	R
52	S	I	S	S	I	R	I	S	S
55	S	R	S	S	I	R	S	S	S
58	S	S	S	S	I	R	I	S	S
59	R	R	R	R	R	R	R	S	S
60	S	R	S	R	R	R	I	S	R
61	S	I	S	S	I	R	R	I	S
63	S	I	S	S	I	R	I	S	S
66	S	S	R	S	I	R	R	I	R
67	S	I	S	S	I	R	I	I	R
70	S	R	S	S	S	I	I	S	R
72	S	S	S	S	S	R	R	I	R
75	S	R	S	S	R	I	S	S	R
78	S	S	S	S	I	I	R	S	R
79	S	I	S	S	I	R	R	I	R
80	S	S	S	S	R	I	R	I	R
81	S	R	R	R	R	R	R	I	R

C = Chloramphenicol, AM = Ampicillin, GM = Gentamycin, SXT = Sulfamethoxazole/trimethoprim,N = Neomycin, S = Streptomycin, E = Erythromycin, TE = Tetracycline, NA = Nalidixic acid, R = Resistant, I = Intermediate, S = Sevsitive.

Table 3: Antimicrobial drugs susceptibility reactions patterns of 44 *E. coli* isolates from diarrheic calves using nine antimicrobial drugs.

SPN	ISN	C	SXT	GM	NA	TE	AMP	S	N	E
1	35	S	S	S	S	S	S	S	I	I
2	20	S	S	S	S	S	S	S	I	R
3	58	S	S	S	S	S	S	I	I	R
4	52,63	S	S	S	S	S	I	I	I	R
5	34	S	S	S	S	S	I	R	I	R
6	49	S	S	S	S	S	R	S	R	I
7	1	S	S	S	S	S	R	S	R	R
8	5, 55	S	S	S	S	S	R	I	S	R
9	11	S	S	S	S	S	R	R	S	I
10	33	S	S	S	S	I	S	I	I	I
11	48	S	S	S	S	I	S	R	R	I
12	43	S	S	S	S	S	S	I	I	R
13	14	S	S	S	S	R	S	I	I	I
14	78	S	S	S	S	R	S	I	R	I
15	25	S	S	S	S	R	S	R	I	R
16	70	S	S	S	S	R	R	S	I	I
17	4	S	S	S	S	R	R	I	S	I
18	2, 75	S	S	S	S	R	R	R	S	I
19	24	S	S	S	I	S	S	S	S	I
20	61	S	S	S	I	S	I	I	R	R
21	72	S	S	S	I	R	S	S	R	R
22	22	S	S	S	I	R	S	I	R	R
23	80	S	S	S	I	R	S	R	R	I
24	39	S	S	S	I	R	I	R	R	R
25	67	S	S	S	I	R	I	I	I	R
26	79	S	S	S	I	R	I	I	R	R
27	8	S	S	S	R	3	S	R	R	R
28	7	S	S	S	R	S	R	I	I	I
29	31	S	S	I	I	S	I	I	I	R
30	9	S	S	I	I	R	S	S	I	R
31	50	S	S	I	I	R	I	S	R	R
32	26	S	S	R	S	S	R	R	R	I
33	47	S	S	R	S	R	I	R	I	R
34	66	S	S	R	I	R	S	I	R	R
35	60	S	R	S	S	R	R	R	I	R
36	81	S	R	R	I	R	R	R	R	R
37	19	S	R	R	R	S	R	R	I	R
38	40	I	S	S	S	S	S	R	R	R
39	51	I	S	S	I	R	S	I	R	R
40	59	R	R	R	S	S	R	R	R	R
41	30	R	R	R	R	R	R	R	R	R

S= Sensitive, I= Intermediate, R=Resistant bacteria , SPN= Susceptibility Pattern Number, ISN = Isolate(s) No.(s)., C =Chlorumpenicol., AMP = Ampicillin., CN =Gentamycin, S = Streptomycin, E = Erythromycin, N = Neomycin, NA= Nalidexic acid, TE=Tetracycline.

RESULTS

The diameter of growth inhibition zone including that of the drug disc was measured for each drug. The tested *E. coli* isolates were found to be highly sensitive to chloramphenicol (40 isolates, 90. 9%) followed by sulphamethexazol/trimethoprim, (39 isolates, 88.6%) then gentamycin (34 isolates, 77.2%).Different levels of sensitivities to nalidixic acid (59%), tetracycline (45%), ampicillin (44%), neomycin (41%), streptomycin (23%) and erythromycin (0%) were also displayed by *E. coli* isolates tested (Table 2). States of intermediate susceptibilities to sulphamethoxazol/ trimethoprim (0%), chloramphenicol (5%), tetracycline (5%), gentamycin (7%), ampicillin (20%), nalidixic acid (32%), erythromycin (34%), streptomycin (39%) and neomycin (41%) were also observed (Table 2). Not a single *E. coli* isolate was sensitive to all antimicrobial drugs used. Neverthless, isolates nos. 24,33 and 35 were not resistant to any of these drugs but they showed intermediate susceptibilities to erythromycin and nalidixic acid, erythromycin, neomycin, streptomycin and tetracycline and erythromycin and neomycin, respectively (Table 2).

The drugs susceptibility reactions recorded from the 44 *E. coli* isolates from diarrhoeic calves were arranged in 41 susceptibility patterns (Table 3). On the other hand, twenty-six different patterns of resistance were obtained (Table 4) of which multiple drug resistance had accounted

Table 4: Resistance patterns of nine antimicrobial drugs to 44 isolates of *E. coli* from diarrhoeic calves

SN	ISN	RAD	NDI	F %
1	20,31,43,52,58 and 63	E	1	6(13.64%)
2	14	Te	1	1(2.27%)
3	5 and 55	Amp and E	2	2(4.54%)
4	7	Amp and NA	2	1(2.27%)
5	49	Amp and N	2	1(2.27%)
6	11	Amp and S	2	1(2.27%)
7	4 and 70	Amp and Te	2	2(4.54%)
8	61	E and N	2	1(2.27%)
9	67	E and Te	2	1(2.27%)
10	48	N and S	2	1(2.27%)
11	78	N and Te	2	1(2.27%)
12	1	Amp, N and E	3	1(2.27%)
13	2 and 75	Amp, S and Te	3	2(4.54%)
14	22,51,79,72 and 50	E, N and Te	3	5(11.36%)
15	40	E, N and S	3	1(2.27%)
16	25 and 80	E, S and Te	3	2(4.54%)
17	26	Amp, GM, N and S	4	1(2.27%)
18	66	E, GM, N and Te	4	1(2.27%)
19	8	E, N, NA and S	4	1(2.27%)
20	39	E, N, S and Te	4	1(2.27%)
21	37	GM, N, S and Te	4	1(2.27%)
22	60	Amp, E, S, SXT and Te	5	1(2.27%)
23	19	Amp, E, GM, NA, S and Te	6	1(2.27%)
24	81	Amp, E, GM, N, S, SXT and Te	7	1(2.27%)
25	59	Amp, C, E, GM, N, S and SXT	7	1(2.27%)
26	30	Amp,C,E,GM,N,NA,S,SXTandTe	9	1(2.27%)

SN= Serial Number, ISN= Isolate Number, RAD= Resistant Antimicrobial Drugs, NDI= Number of Drugs Involved, F= Frequency (%)AMP= Ampicillin, C= Chloramphenicol, E= Erythromycin, GM= Gentamycin, N= Neomycin,NA= Naldixic Acid, S= Streptomycin, SXT=Sulphamethoxazol/Trimethoprim and Te = Tetracycline

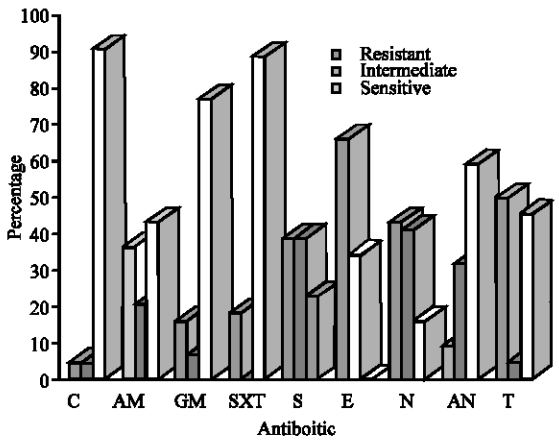

Fig. 1: Antimicrobial reaction of 44 *E.coli* isolates from diarrhoeic calves produced by nine drugs. AM = Ampicillin, C = Chloramphenicol, E = Erythromycin, GM = Gentamycin, NA = Nalidixic- acid, N = Neomycin, S = Streptomycin, SXT = Sulfamethoxazole/Trimethoprim, T = Tetracyclin

for 77.27% whereas resistance for a single drug only accounted for 15.91%. The remaining 6.81% were represented by the aforementioned three isolates, viz isolates nos. 24,33 and 35. Antimicrobial drug sensitivity of the 44 isolates to the nine drugs is also demonstrated in Fig. 1.

DISCUSSION

In the present study, different isolates of *E. coli* reacted differently to the drugs used (Table 2 and 3). The most efficacious drug was chloramphenicol, to which 90% of the test isolates were susceptible, followed by sulphamethoxazole/trimethoprim (89%), gentamycin (77%) and naladixic acid (59%). Lower efficacies were recorded by tetracycline (45%), ampicillin (44%), streptomycin (23%) and neomycin (16%) whereas erythtromycin was not sensitive to any isolate examined though it showed an intermediate state to 15 isolates (Table 2). In Sudan Kamal (2000) studied the susceptibility of *E. coli* strains isolated from diarrhoeic calves and goat kids to different antibiotics. He found that they were sensitive to gentamycin and were all almost resistant to streptomycin. This finding is in agreement with our findings where streptomycin had a very weak effect. Similar results were also obtained by Cid *et al.* (1996) who found that streptomycin was the least effective (93% of strains were resistant), followed by sulphadiaxine (89%) and then tetracycline, kanamycin, neomycin and ampicillin. Further comparisons with analogous studies which are carried out in other countries will not do much benefit as considerable area to area variation in sensitivity patterns has become a common knowledge (Radostits *et al.*, 1994).

Multiple drug resistance has definitely assumed a world-wide spread (Shears, 2001). Certainly, the Sudan is

no exception, although the size and magnitude of the problem are not clearly defined. Results reported in this study demonstrate that, despite the small number of isolates examined, multiple drug resistance is a serious problem and has attained a hazardous level (77.27%). Practices such as indiscriminate use of antimicrobial drugs, exhaustive use of certain antibiotics or other drugs, their supply without prescription and their use in subcurative doses can constitute the root of the problem, elements of its aggravation and pillars of its sustainability. Moreover, it is well kwon that certain plasmids encode for antimicrobial drugs resistance and *E. coli* has plasmid-mediated resistance to some of them (Gross, 1983). Consequently, failure of antibiotics' treatment in controlling some cases of diarrhoea caused by *E. coli* strains in newborn animals (Salih *et al*., 1997) may be partially explained by presence of drug resistance. If it is tenable to assume that the intermediate state is one-way transitional state that eventually leads to the state of resistance, the future use of antimicrobial drugs for the treatment of *E. coli* infection in this country would be an onerous, if not impossible, task.

Forty-one different patterns of drug sensitivity reaction were obtained following recording drug sensitivity reactions (sensitive, intermediate and resistant) of 44 isolates of *E. coli* to nine antimicrobial drugs. The incredible set of drugs had almost differentiated each isolate from the other and constitutes a highly promising prospect of an excellent discriminating scheme; only three patterns (nos. 4, 8 and 18; Table 3) contained two isolates each. The remaining 38 patterns were each represented by a single isolate of *E. coli*. Future application of the same set of antimicrobial drugs may inaugurate the introduction of a valuable epidemiological tool for the differentiation between *E. coli* strains, locating the focus of infection in an outbreak and tracing its extent of spread. The validity of the high potential of discrimination of this scheme of drug sensitivity patterns will be sanctioned by reproduction of comparable, if not similar, results.

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REFERENCES

Buxton, A. and G. Frazer, 1977. Animal Microbilogy Immunology, Bacteriology, Mycology, Diseases of Fish and Laboratory Methods, (1st Edn.), Blackwell Scientific Publication, London, UK., 1: 93-102.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Truck, 1966. Antimicrobial resistance in the tropics: Epidemiological surveillance of antimicrobial resistance in the tropics. Am. J. Clin. Path., 45: 493-496.

Benson, H.J., 1980. Microbiological application, a laboratory manual in general microbiology. (3rd Edn.), WMC, Brown Company Publishers, College Division. Dubuque, Iowa, pp: 194-197.

Cid, D., M. Blanco, J.E. Blanco, Ruiz, Santa, J.A. Puiterira, R. De la Funete and J. Blanco, 1996. Serogroups, toxins and antibiotic resistance of *E. coli* strains isolated from diarrhoeic goat kits in Spain.Vet. Microbiol., 53: 349-354.

Gross, R.J., 1983. *E. coli* diarrhoea. J. Infect., 7: 177- 192.

Kamal, E.H.A., 2000. Studies on colibacillus diarrhoea in calves, sheep and goats. M. Sc. Thesis. U.K.

NCCLS, 1992. Performance standards for antimicrobials disc susceptibility tests. M2-A2. National Committee for Clinical Laboratory Standards, Villanova. Pa.

Radostits, O.M., D.C. Blood and C.C. Gay, 1994. Veterinary Medicine, (8th Edn.), (ELBS) Bailliere Tindall, London.

Shears, P., 2001. Antimicrobial resistance in the tropics: Epidemiological surveillance of antimicrobial resistance in the tropics. Transac. Roy. Soci. Trop. Med. Hyg., 95: 127-130.

Salih, O.S.M., M.T. Shigidi, H.O.S. Mohamed, P. Mc Dough and Y.F. Chang, 1997. Camel Newsletter, ACSAD, P.O.Box 2440, Damascus Syria, 13: 34-43.