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STUDIES ON DIARRHOEA IN LAMBS IN ASSIUT GOVERNORATE: II- *IN VITRO* MINIMUM INHIBITORY CONCENTRATION (MIC) OF SOME ANTIMICROBIAL AGENTS AGAINST *E.COLI* STRAINS ISOLATED FROM DIARRHOEIC LAMBS AND THEIR PLASMID PROFILE

(With 3 Tables and 3 Figures)

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دراسات عن الاسهال فى الحملان بمحافظة أسيوط
II- اختبار حساسية بعض المضادات الحيوية ضد معزولات الميكروب القولونى من اسهالات الحملان باستخدام أقل جرعة مثبطة وكذلك توصيف البلازميدات الموجودة فى هذه المعزولات

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أجريت الدراسة على ٥٠ عترة من الميكروب القولوني المعزولة من حـــالات الاســهال فــي الحملان وذلك لدراسة حساسية تسعة مضادات حيوية ضدها معمليا باستخدام طريقة أقل جرعـة مثبطة في الاجار وقد أسفرت النتائج عن حساسية كل المعـــزولات لكـــل مـــن التوبرامبســين والانروفلوكساسين بنسبة ١٠٠% وكانت النسبة مع الجنتاميسين والكلورامفينكول ٩٢% لكـــل منهما بينما الاموكسيسلين وحامض النالديكسك والاوكسى تتراسيكلين اعطـــت نســبة حساســية ٦٤% , ٣٤% , ٢٤% على التوالى وقد لوحظ أن كـــل العـــترات المختـــبرة قـــاومت تـــأثير الاريثروميسين والفلوموكين بنسبة حساسية صفر % وقد تم تحديد اقل جرعة مثبطة والتي تـــم قياس درجات الحساسية بناء عليها كما تم تحديد أقل جرعة مثبطة لكل من ٥٠% , ٩٠% مـــن العزلات ضد كل مضاد حيوى على حدة وكذلك تمت مناقشة هذه النتـــائج . وبدراســـة تعـــدد المقاومة البكتيرية للميكروب الواحد أسفرت النتائج عن وجود نسب ٢% , ٣٢% , ٤٢% , ١٨ % , ٦% من كل العزلات المختبرة يقاوم مقاومـــة متعـــددة لعـــدد ٢ , ٣ , ٤ , ٥ , ٦ مـــن المضادات الحيوية على التوالى. وبالاستقصاء عن وجود البلازميدات في عترات الميكـــروب القولونى المعزولة من اسهالات الحملان وجد أن ٧٠% من العزلات المختبرة تحتوى على عدد

من البلازميدات . وبالتحليل الالكتروفوريسى للبلازميدات المستخلصة وجد أنها تتراوح بين ١- ٥ بلازميدات في العترة الواحدة باحجام جزيئية مختلفة تتراوح بين ٢.٥ - ٢٧ كيلوبيز.

SUMMARY

Fifty *E.coli* strains isolated from diarrhoeic lambs were tested to determine *in vitro* their antimicrobial susceptibility to nine antimicrobial agents using minimum inhibitory concentration diluted in agar media. All strains were sensitive to both tobramycin and enrofluxacin with susceptibility 100%. Both gentamycin and chloramphenicol showed susceptibility 92%, while amoxycillin, nalidixic acid and oxytetracycline showed susceptibility percentages 64%, 34% & 24% respectively. All tested strains resist both erythromycin and flumoquine resulting susceptibility 0%. Multidrug resistance study revealed that 2%, 32%, 42%, 18% and 6% of the tested isolates resisted double, triple, quadruple, quintuple and sixtuple antimicrobial agents respectively. Screening for plasmid presence using electrophoresis analysis revealed that 70% of the tested isolates contained plasmids. The extracted plasmid contents were 1-5 per a single *E.coli* strain with molecular size ranged from 2.5 up to 27 Kilo base pairs (Kb).

Key words: *Lambs, diarrhoea, antimicrobial agents (MIC), E.coli, Plasmid*

INTRODUCTION

Escherchia coli strains are considered as an important cause of diarrhoea referred to colibacillosis in all species of farm animals (Wray et al., 1993). In such condition, the antimicrobial therapy is an important tool in reducing the enormous losses (Blanco et al., 1996).

The use of antimicrobial drugs as a prophylactic agent to prevent diseases or as food additives in domestic animals has resulted in an increase of drug resistance (Nazer 1978). Urassa et al. (1997) suggested that there is a quasi-direct relationship between antibiotic use and bacterial resistance as the main factor responsible for the development and spread of antimicrobial resistance is the injudicious use at different time periods. Recent study (Pean, 2000) concluded that it is difficult to establish a relationship between the use of antimicrobial agent and the frequency of resistance. Anyhow, the farm animals constitute a reservoir of antimicrobial resistant strains.

Concerning to its great importance, *E.coli* antimicrobial resistance in diarrhoeic lambs was widely studied (Adesiyun & Kaminjolo, 1992; Blanco et al., 1996; Cid et al., 1996 and Orden et al., 2000 b). *E.coli* is not usually regarded as a cause of enteritis in older animals; hence, only *E.coli* strains isolated from lamb in colibacillosis infections are suitable for antibiotic resistance testing (Wise et al., 1985).

Bacteria are carrying genes encoding for antibiotic resistance which called plasmids replicate autonomously (Wagner and Hahn 1999). Many new antibacterial drugs have been developed and used. Plasmids conferring resistance to them have often appeared within a short time.

The practical importance of plasmid-determined genes is recognized with the discovery of transferable drug resistance (Datta and Nagent 1984). This transmission of drug resistance is occured when plasmids or bacterial strains containing plasmids are transmitted between different animals even of different species (Chaslus-Dancla et al., 1987), rather than the potential cross infection of human beings from animals and vice versa via such plasmids (Singh et al., 1992).

The present study aimed to evaluate the antimicrobial susceptbility of *E.coli* strains isolated from diarrhoeic lambs to several antimicrobial agents. Also to perform plasmid profile analysis of these multidrug resistant strains.

MATERIAL and METHODS

Fifty *E.coli* strains belonging to serotypes [O 86 B7, O11 B4 and O44 K74 (L)] and some others untypable strains isolated from diarrhoeic lambs- through an investigation for the same authors under press- were used in the present study.

In vitro antimicrobial susceptibility tests were performed by the agar dilution method according to NCCLS (1993) against nine antimicrobial agents; amoxycillin (Am), oxytetracycline (Oxt), chloramphenicol (Chl), gentamycin (Gt), tobramycin (Tb), erythromycin (Er), enrofluxacin (En), flumoquine (Fl) and nalidixic acid (Nd). Antimicrobial agents were 100% potancy and provided kindly by Amoun-Egypt. Preparation of sterile initial stock antibiotic solutions were prepared as recommended by the manifacturers. Nutrient agar was used for the agar dilution procedures. Ten sensitivity agar-antibiotic dilutions were achieved; 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.4, 0.2 & 0.1 µg/ ml media for each tested antibiotic.

Fom the initial purified strains, the standardized inocula were prepared by inoculation of each strain into 2 ml brain heart infusion broth incubated at 37°C until the turbidity reached a density corresponding to McFarland nephlometer standard 0.5.Then bacterial cultures were diluted 1:10 in sterile normal saline (0.9 NaCl). 10 µL from each obtained diluted bacterial culture would yeild a standardized spot inoculum of approximately 10^4 cfu (Orden et al., 2000 a). The inoculated plates were incubated at 37°C for 24 h.

MIC was the lowest concentration of an antimicrobial agent which completely suppressed a visible bacterial growth. MIC breakpoint for each antimicrobial agent was as recommended by NCCLS guidelines. MIC_{50} & MIC_{90} were determined at which 50% and 90% respectively of the tested strains were completely inhibited. Any bacterial growth at the tested antibiotic concentration equals its breakpoint was considered as a resistant reaction (Fig 2).

Screening for the presence of plasmid DNA in *E.coli* strains was done by the alkaline lysis technique. The extracted DNA for each strain was subjected to electrophoresis through 0.7% agarose gel stained by ethidium bromide 0.5 µg/ml gel and running 100 m A-120 V. (David *et al.* 1991).

RESULTS

The results are manifested in Tables 1, 2 & 3 and Figures 1, 2 & 3.

DISCUSSION

Diarrhoea in neonatal lambs causes major economic losses. In such colibacillosis syndrome, antibiotic therapy is frequently ineffective partly due to the presence of drug-resistant strains and the failure to identify drug sensitivity (Orden et al., 2000 b). Studies on the antimicrobial resistance of intestinal *E.coli* from different young animals showed increased incidences of resistance over the years as a result of widespread use of the antimicrobial drugs (Lepelletier et al., 1999).

For achieving a valuable antimicrobial susceptibility testing - as mentioned in the promise - the present study was concerning with *E.coli* strains isolated from diarrhoeic lambs only. All tested *E.coli* strains were sensitive and susceptible to both tobramycin and enrofluxacin - 100% susceptibilty- (Table 1 & Fig 1). MIC ranges were (0.78-3.13) and (0.1-0.2) µg /ml respectively. The very narrow MIC range showed the similarity of

all tested strains in their sensitivity to enrofluxacin. Also it showed complete growth inhibition at the most high dilution indicating the highest sensitivity of *E.coli* strains all over the present study. Similar results were obtained by Watts et al. (1993); Blancho et al. (1997) and Sayed et al. (1998) as the tested *E.coli* strains showed the highest susceptibility percentage to enrofluxacin which is a flouroquinolone antibacterial drug. While Pean (2000) detected *E.coli*-flouroquinolone resistant strains.

In the present study, the tested *E.coli* strains showed gentamycin susceptibility (92%) with both MIC_{50} and MIC_{90} as 3.13 µg/ml. MIC range was (0.78-12.5) µg/ml - (Table 1 & Fig. 1). Adesiyun and Kaminjolo (1992), Urassa et al. (1997) and Orden et al. (2000 b) concluded that *E.coli* strains were sensitive to gentamycin with 95, 80 and 95% respectively. The most recent study determined MIC_{50} and MIC_{90} as 0.5 & 32 µg/ml and a very wide MIC range was obtained (0.5-512/ml) indicated the great variations among tested strains from highly sensitive to greatly resistant in the same study. Gentamycin resistance was detected by Wray et al. (1986); Chaslus-Dancla et al. (1987), David et al. (1991) and Adesiyun & Kaminjolo (1992).

E.coli strains tested in the present work showed 92% chloramphenicol susceptibility with both MIC_{50} and MIC_{90} 12.5 µg/ml. MIC range was (1.56-25 µg/ml) Table (1) & Fig. (1). Similar results were obtained by Jakson (1981) and Adesiyun & Kuminjolo (1992) as 92 and 95% of *E.coli* strains were sensitive to chloramphenicol respectively. But 50% of the tested *E.coli* strains were resistant for chloramphenicol in both studies of Cid et al. (1996) and Orden et al. (2000 b). The latter determined MIC_{50} and MIC_{90} as 16 & 512 µg/ml with a very wide MIC range (1-512 µg/ml).

Ampicillin susceptibility testing in the present work showed that 64% of *E.coli* strains were sensitive with MIC_{50} and MIC_{90} 6.25 & 12.5 µg/ml respectively. MIC range was (0.78-25 µg/ml) Table (1) & Fig (1). Cid et al. (1996) obtained that 70% of *E.coli* strains were susceptible. While *E.coli*-ampicillin resistant strains were detected by Wray et al. (1993), Urassa et al. (1997) and Orden et al. (2000 b) who concluded that 50, 80 and 50% -respectively- of the tested strains were resistant. Orden et al. (2000 b) determined MIC_{50} and MIC_{90} values as 16 and 512 with MIC range (1-512) µg/ml showing very wide range of high resistance.

Through the present investigation, the tested *E.coli* strains showed low nalidixic acid susceptibility (34%) with MIC_{50} and MIC_{90} values 6.25

& 25 µg/ml. MIC ranged from 3.13 up to 25 µg/ml (Table 1 & Fig. 1). Nalidixic acid which is one of the first generation of quinolone antibacterial drug was recommended by Krishnamohan et al. (1995) for the treatment of enterobacterial infections. They concluded that all *E.coli* strains were sensitive. Chaslus-Dancla et al. (1987), Basile et al. (1996) and Sayed et al. (1998) recovered *E.coli*-nalidixic acid resistant strains coinciding with the present findings.

Oxytetracycline susceptibility testing showed a high resistance result in the present work (24% susceptibility) with both MIC_{50} and MIC_{90} ≥ 50 µg/ml (Table 1 & Fig 1). Oxytetracycline was widely discussed by Nazer et al. (1978), Chaslus-Dancla et al. (1987) and Blancho et al. (1996). They reported that with *E.coli* antimicrobial testing, it showed the highest resistance prevalences. David et al. (1991); Adesiyun & Kamiyolo (1992) and Cid et al. (1996) determined *E.coli*-oxytetracycline resistance prevalence as 91, 79 and 70% respectively. This may be due to the widespread and frequent usage of tetracycline in veterinary fields. So, a growing number of various bacterial species acquire resistance to the bacteriostatic activity of tetracycline (Schnappinger and Hillen 1996).

The present results revealed that all tested *E.coli* strains were resistant completely to both erythromycin and flumoquine up to the least dilution (50 µg/ml) with susceptibility 0% (Table 1 & Fig. 1). Flumoquine was the third quinolone tested in our study, where its results coincided with that obtained by Krishnamohan et al. (1995) who tested *E. coli* against three quinolones concluding that flumquine had the least susceptibility prevalence. Sayed et al. (1998) tested *E.coli*-also-against three quinolones concluding that both enrofluxacin and flumoquine showed 100% sensitivity more than nalidixic acid. So the increasing incidence of quinolone-resistance is a matter for concern.

Coste et al. (1984) noticed that adminstration of an antibiotic to conventional lambs hosting has the same antibiotic resistant -*E.coli* resulted in significant inactivation of the antibiotic in the intestine against *E.coli* sensitive strains. This is due to the presence of transferable plasmids carrying encoding genes in *E.coli*-resistant strains (Jakson 1981). These genes when are transmitted to *E.coli*-resistant against another antibiotic, it will develop a multidrug-resistant *E.coli* strain. The seriousness of the problem arises from the fact that once, multidrug resistant organisms develop, they can persist in the host or in the environment in absence of antibiotic selection (Hinton et al., 1986).

In the present investigation, all tested *E.coli* strains were multidrug-resistant (2% were double, 32% were triple, 42% were quadruple, 18% were quintuple and 6% were sixtuple antibiotic resistant) table (2). Multidrug resistance was widely studied. Nazer (1978) detected 65% of the tested *E.coli* resisted six antibiotics, David et al. (1991) concluded that all tested *E.coli* strains resisted at least four antibiotics. Ombui et al. (1995) detected 29% of the tested *E.coli* strains as multiresistant ones, Cid et al. (1996) showed that 55% were quadruple and 33% were sixtuple antibiotic resistant strains. Urassa et al. (1997) reported that more than 80% of tested strains were resistant for at least three different antibiotics. More recent study (Orden et al., 2000 b) recorded that 77% of the tested *E.coli* strains were resistant for at least double, 67% could resist at least four, and 32% resist at least eight antibiotics. These great significant differences in multidrug resistance percentages necessistates searching for the transferable plasmids encoding genes of antibiotic resistance.

Resistance to one or more antibiotics may be present on the same plasmid (Jackson, 1981). In the present study, 70% of the tested strains indicated the presence of plasmids which their content ranged (1-5) per strain with molecular size ranged (2.5-27) Kilobar pairs (Kb)- table (3) and Fig. (3). David et al. (1991) and Ombui et al. (1995) detected (1-8) and (1-5) plasmids per an *E.coli* strain respectively.

Great variations in plasmid molecular sizes as David et al. (1991), Adesiyun and Kaminjolo (1992), Cloeckaert et al. (2000) and Keys et al. (2000) detected *E.coli* plasmids with molecular size ranging (1.5-34.7), 100, (110-125) and (186-204) Kb respectively.

The seriousness of a plasmid presence appears when it is a transferable one. Resistance with the highest MIC value is normally indicative of its transferability (Wise et al., 1985). Nazer (1978), Baldini *et alýýý*. (1983) and Wise et al. (1985) showed that 26%, 97% and 91% of the tested *E.coli* antibiotic resistant were carrying transferable plasmids.

From the present investigation, it is concluded that it is of great importance to study the antibiotic resistance pattern of *E.coli* isolated from diarrhoeic lambs before treatment as the widespread prevalence of an antibiotic resistance reflects the misuse of it in the local environment (Adesiyun and Kaminjolo, 1992). Also to study the seriousness of the extracted plasmids, further studies might be in need to determine their transferability.

REFERENCES

Adesiyun, A.A. and Kaminjolo, S. (1992): Susceptibility to antibiotics of *Eschericia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad. Rev. Elev. Med. Vet. Pays Trop. 45 (3-4): 260-262.

Baldini, M. M.; Kaper, J. B.; Levine, M. M.; candy, D. C. and Moon, H. W. (1983): Plasmid-mediated adhesion in enteropathogenic *Eschericia coli*. J. Pediatr. Gastroentrol. Nutr. 2 (3): 534-538.

Basile, S. P. K.; Truong, Q. C.; Lafont, J. P.; Gutmann, X, Y.; Osman, M. and Moreque, N. J. (1996): Resistance to flouroquinolones in *Eschericia coli* isolated from poultry. Antimicrobial agents and chemotherapy, 40 (6): 1504-1507.

Blanco, J.; Cid D, Blanco, J. E.; Blanco, M.; Ruiz-Santa Quiteira, J. A. and De la Fuente R. (1996): Serogroups, toxins and antibiotic resistance of *Eschericia coli* strains isolated from diarrhoeic lambs in Spain. Vet. Microbiol. 49 (3-4): 209-217.

Blanco, J.E.; Blanco, M.; Mora, A. and Blanco, J. (1997): Prevalence of bacterial resistance to quinolones and other antimicrobial among avian *Eschericia coli* strains isolated from septicemic and healthy chickens in Spain. J. Clin. Microbiol. Aug.; 35 (8): 2184-2185.

Chaslus-Dancla, E.; Gerbaud, G.; Martel, J. L.; Lagorce, M, Lafont, J. P. and Courvalin, P. (1987): Probable transmission between animals of a plasmid encoding aminoglycoside 3-N- acetyltransferase IV and dihydrofolate reductase I. Vet. Microbio. Oct. 15 (1-2): 97-104.

Cid, D.; Piriz, S.; Ruiz-Santa-Quiteria, J. A.; Valle, J.; Valdillo, S. and De La Fuente, R. (1996): *In vitro* susceptibility of *Eschericia coli* strains isolated from diarrhoeic lambs and goat kids to 14 antimicrobial agents. J. Vet. Pharmacol. Ther. Oct. 19 (5): 397-401.

Cloeckaert, A.; baucheron, S.; Flaujac, G.; Schwarz, S.; Kehrenberg, C, Martel, J. and Chaslus-Dancla, E. (2000): Plasmid-mediated florfenicol resistance encoded by the flo R gene in *Eschericia coli* isolated from cattle. Antimicrobial agents and Chemptherapy, 44 (10): 2858-2860.

Coste, M.; Gouet, P. and Escoula, L. (1984): Ampicillin inactivation in the caecum of axemic, gnotoxenic and conventional lambs: interaction with resistant or sensitive *Eschericia coli*. J. Gen. Microbiol. 130 (6): 1325-1330.

Datta, N.and Naget, M. E. (1984): Plasmids. in Bacterial variation. ch. 6 of Toply and Wilson's: Principals of bacteriology, virology and immunity. Vol.1 7 th ed. Edward Arnold, Butler and Tanner Ltd. London.

David, B. P.; Purushothaman, V. and Venkales, R. A. (1991): Comparison of plasmid profile analysis, antibiogram testing, resistotyping and biotyping in identification of *Eschericia coli* isolates from poultry. Vet Rec. Aug. 3; 129 (5): 94-97.

Hinton, M.; Kaukas, A.; and Linton, A. H.; (1986): The ecology of drug resistance in enteric bacteria. J. Appl. Bacteriol. (Symposium suppl.) 77-92.

Jackson, G. (1981): A survey of antibiotic resistance of *Eschericia coli* isolated from farm animals in Great Britain from 1971 to 1977. Vet. Rec. 11; 108 (15): 325-328.

keyes, K. C.; Hudson, J. J.; Mauer, S.; Thayer, D. G.; and Lee, M. D. (2000): Dectetion of florfenicol resistance genes in *Eschericia coli* isolated from sick chickens. Antimicrob. Agents Chemther. 44: 421-424.

Krishnamohan, Y.; Shoba, K, Dorairajan, N. and Punniamurthy, N. (1995): *In vitro* sensitivity studies of *Eschericia coli* to Quinolone antibiotics. Indian Vet. J. July, 752-753.

Lepelletier, D.; Caroff, N.; Reynaud, A. and Richet, H. (1999): *Eschericia coli:* epidemiology and analysis of risk factors for infections caused by resistant strains. Clin. Infect. Dis. 29 (3): 548-552

National Committee for Clinical Laboratory Standards (NCCLS), (1993): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard No. M7-A3, 2nd Edn. NCCLS, Wayne, PA, USA.

Nazer, A. H. (1978): Transmissible drug resistance in *Eschericia coli* isolated from healthy dogs, cattle, sheep and horses. Vet. Rec. 23-30; 103 (26-27): 587-589.

Ombui, J. N.; Macharia, J. K. and Nduhiu, G. (1995): frequency of antimicrobial resistance and plasmid profiles of *Eschericia coli* strains isolated from milk. East Afr. Med. J. 72 (4): b228-230.

Orden, J. A.; Ruiz-Santa-Quiteria, J. A.; Garcia, S.; Cid-D and De la Fuente, R. (2000 a): *In vitro* susceptibility of *Eschericia coli* strains isolated from diarrhoeic dairy calves to 15 antimicrobial agents. J. Vet. Med. B 47, 329-335.

Orden, J. A.; Ruiz-Santa-Quiteria, J. A.; Garcia, S.; Cid-D and De la Fuente, R. (2000 b): Quinolone resistance in *Eschericia coli* strains from diarrhoeic lambs in Spain. Vet. Rec. Nov. 11; 147 (20): 576-578.

Pean, Y. (2000): Surveillance of bacterial antibiotic resistance. Presse Med. 2; 29 (37) 2069-2071.

Sayed, A. M.; Ibrahim, R.S.; Abdel-Aziz, A. M. and Sayed, A. M. (1998): Microbial agents increminated in reduced hatchability of duck embryos. II. Antimicrobial *in vitro* sensitivity testing of Salmonella emek and *E. coli* isolates using minimum inhibitory concentration (MIC) Assiut Vet. Med. J. 40 (79) : 73-82.

Schnappinger, D. and hillen, W. (1996): Tetracyclines: antibiotic action, uptake and resistance mechanisms. Arch. Microbiol. Jun, 165 (6): 359-369.

Singh, M.; Sanyal, S. C. and Yadav, J. N. (1992): Enterotoxogenic drug resistant plasmids in animals isolates of *Eschericia coli* and their zoonotic importance. J. Trop. Med. Hyg. Oct; 95 (5): 319-321.

Urassa, W.; Lyamuya, E. and Mhalu, F. (1997): Recent tends on bacterial resistance to antibiotics. East Afr. Med. J. 74 (3): 129-133.

Wanger, J. and Halhn, H. (1999): Increase of bacterial resistance in human medicine by resistance genes of bacteria from meat supplying animals. Berl. Munch. Tierartl Wochenschr , 112 (11): 380-384.

Watts, J. L.; Salmon, S. A.; Yancy, R. J.; Neressian, B, and Kounev, Z. V. (1993): Minimum inhibitory concentration of bacteria isolated from septicaemia and air sacculitis in duck. J. Vet. Diagnostic Investigation, 5 (4): 625-628.

Wise, P. J.; Towner, K. J.; Webster, C. A.; Slack, R. C. and Jones, T. O. (1985): Trimethoprim resistance plasmids in *Eschericia coli* isolated from diarrhoea in cattle, pigs and sheep. J. App. Bacteriol. 58 (6): 555-561.

Wray, C.; Hedges, R. W.; Shannon, K. P. and Bradley, D. E. (1986): Apramycin and gentamycin resistance in *Eschericia coli* and salmonella isolated from farm animals. J. Hyg. (Lond.) 97 (3): 445-456.

Wray, C. I.; McLaren, M.; and Carroll, P. J. (1993): *Eschericia coli* isolated from animals in England and Wales between 1986 and 1991. Vet. Rec. 133, 439-442.

Table (1): In vitro antimicrobial susceptibility of *E.coli* strains isolated from diarrhoeic lambs

Anti microbial agents	MIC µg/ml													MIC range	Break point	drug susceptibility
	50	25	12.5	6.25	3.13	1.56	0.78	0.4	0.2	0.1	MIC_{50}	MIC_{90}			
Am	-	5	13	24	-	3	5	-	-	-	6.25	12.5	(0.78-25)	12.5	64%
Oxt	36	8	3	-	-	3	-	-	-	-	≥50	≥50	(1.56- ≥50)	25	24%
Chl	-	4	37	3	2	4	-	-	-	-	12.5	12.5	(1.56-25)	25	92%
Gt	-	-	4	1	38	5	2	-	-	-	3.13	3.13	(0.78-12.5)	12.5	92%
Tb	-	-	-	-	41	5	4	-	-	-	3.13	3.13	(0.78 - 3.13)	6.25	100%
Er	50	-	-	-	-	-	-	-	-	-	≥50	≥50	≥50	6.25	0%
En	-	-	-	-	-	-	-	-	12	38	0.1	0.2	(0.1 - 0.2)	12.5	100%
Fl	50	-	-	-	-	-	-	-	-	-	≥50	≥50	≥50	6.25	0%
Nd	-	10	11	12	17	-	-	-	-	-	6.25	25	(3.13 - 25)	6.25	34%

Am : Amoxycillin Oxt : Oxytetracycline Chl : Chloramphenicol
Gt : Gentamycin Tb : Tobramycin Er : Erythromycin
En : Enrofluxacin Fl : Flumequine Nd : Nalidixic acid

Table (2): Multidrug resistance pattern of *E. coli* strains isolated from diarrhoeic lambs against 9 antimicrobial drugs.

Drug resistance pattern	No. of strains	percentage
Double :	1	2%
Er & Fl	1	2%
Triple :	16	32%
Er , Fl & Oxt	11	22%
Er , Fl & Am	5	10%
Quadruple:	21	42%
Er , Fl , Nd & Oxt	15	30%
Er , Fl , Nd & Am	3	6%
Er , Fl , Nd & Gt	3	6%
Quintuple:	9	18%
Er, Fl , Nd , Oxt & Am	7	14%
Er , Fl , Nd , Oxt & Chl	2	4%
Sixtuple:	3	6%
Er , Fl , Nd , Oxt , Am & Chl	2	4%
Er , Fl , Nd , Oxt , Am & Gt	1	2%

All E. coli strains tested were resistant at least against double antibiotics

Table (3): Plasmid profile analysis of 10 *E. coli* strains corresponding to their own multidrug resistance

No. of plasmids contained	Molecular size of plasmid DNA (Kb)	Multidrug resistance pattern
0	-	Er , Fl & Oxt
1	16.2	Er , Fl & Oxt
5	3.1 , 4.2 , 11.4 , 17.5 & 27	Er , Fl , Nd , Oxt & Chl
2	17.5 & 21.5	Er , Fl , Nd & Oxt
3	3.1 , 4.2 & 17.5	Er , Fl , Nd & Oxt
1	17.5	Er , Fl , & Oxt
2	2.5 & 17.5	Er , Fl & Am
2	2.5 & 17.5	Er , Fl & Am
0	-	Er , Fl & Oxt
0	-	Er , Fl & Oxt

Molecular size average readings obtained after gel electrophoresis running 100 m A-120 V.

Kb : Kilobase pairs

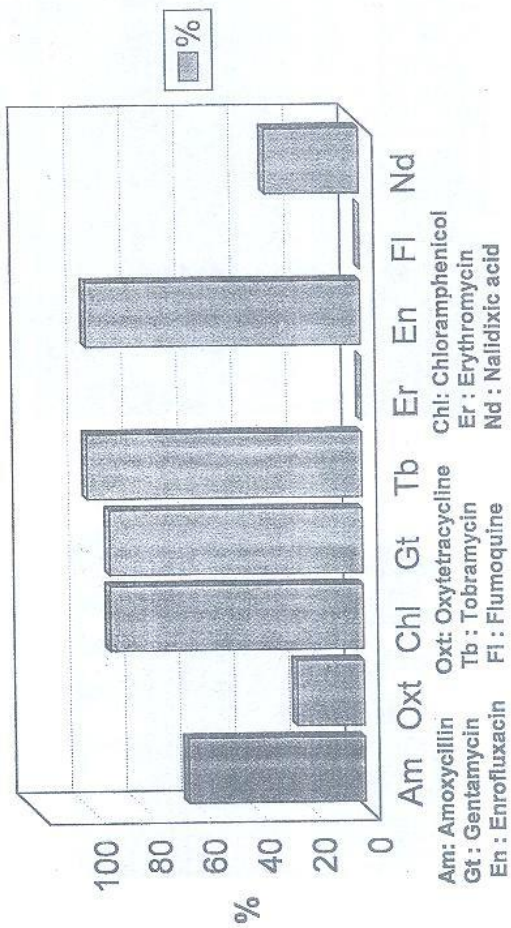

Fig. (1): Antimicrobial susceptibility percentages of E.coli strains to different nine antimicrobial agents.

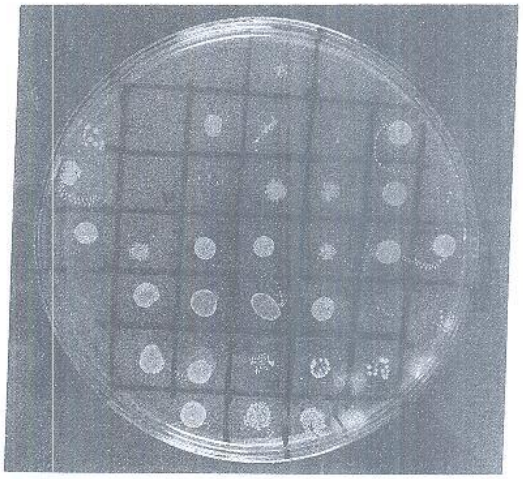

Fig. (2): A sensitivity agr plate containg 25 µg/ml Oxt (its break point)
A & B : resistant strains
C : sensitive strain

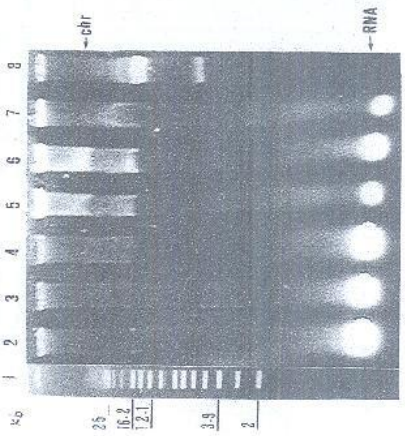

Fig. (3): Plasmid profile analysis of *E.coli* strains isolated from diarrhoeic lambs