Communications

Susceptiblity to antibiotics of *Escherichia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad

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ADESIYUN (A.A.), KAMINJOLO (J.S.). Sensibilité aux antibiotiques de souches d'*Escherichia coli* isolées de bétail atteint ou non de diarrhée à l'île de la Trinité. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 260-262

La sensibilité de souches d'*Escherichia coli* provenant de veaux, porcelets, agneaux et chevreaux a été testée par rapport à sept antibiotiques. Au total, 264 souches sur 289 (91,3 p. 100) isolées chez des animaux diarrhéiques et 173 souches sur 198 (87,4 p. 100) provenant d'animaux non diarrhériques ont montré une résistance vis-à-vis d'un ou de plusieurs antibiotiques. La différence n'est pas significative ($P \geq 0,05$; X^2). Indépendemment de l'état de santé, les isolats d'agneaux sont moins résistants (75,0 p. 100) et ceux de porcelets plus résistants (96,7 p. 100) avec une différence significative ($P \leq 0,001$; X^2). Les souches d'*E. coli* sont plus résistantes à la streptomycine (81,3 p. 100) et à la tétracycline (78,9 p. 100) et moins résistantes au chloramphénicol (4,3 p. 100) et à la gentamycine (4,7 p. 100). En outre, quelle que soit l'origine des isolats, le profil de résistance prédominant est vis-à-vis de la streptomycine-tétracycline (27,9 p; 100). Il est conclu que la résistance très marquée aux antibiotiques reflète leur utilisation excessive dans le milieu local. *Mots clés* : Bovin - Porcin - Ovin - Caprin - Diarrhée - *Escherichia coli* - Résistance aux produits chimiques - Antibiotique - Antibiogramme - Ile de la Trinité - Antilles.

Introduction

Escherichia coli beside being a normal inhabitant of the intestinal tract, is associated with a variety of pathologic conditions including gastroenteritis in man and animals (14).

Indiscriminate use of antimicrobial agents has resulted in the development of resistance among *E. coli* strains (13, 16), with therapeutic and public health implications. Similarly, humans have been infected through exposure to multi-resistant *E. coli* from animals and meat (3, 12, 18).

Unlike in some developed countries where the use of antibiotics as growth promoters has been banned (13), in Trinidad and Tobago, antimicrobial agents are still used at subtherapeutic levels as growth promoters. The present study therefore determined the prevalence of antibiotic resistant strains of *E. coli* in diarrhoeic livestock in Trinidad.

Materials and Methods

Sources of E. coli *strains*

In a comprehensive study aimed at determining the bacterial, parasitic and viral pathogens associated with diarrhoeic and non-diarrhoeic livestock (calves, piglets, lambs and kids) in Trinidad, faecal samples or rectal swabs were taken from the experimental animals. Details of the sampling protocol employed in the study have been earlier described (1).

Isolation and identification of E. coli

Rectal swabs or faecal matters were plated for isolation on eosin methylene blue (EMB) agar and incubated for 24 to 48 h at 37 °C. Colonies with a characteristic metallic sheen appearance on EMB were picked and stained according to Gram's method. Isolates that were Gram-negative rods were further subjected to biochemical tests using standard methods (4).

Determination of E. coli *antibiograms*

The disc diffusion method of BAUER *et al* (2) was used to determine the antibiograms of the *E. coli* strains. The antibiotic discs (Difco) and concentrations used were as follows : ampicillin (10 mcg), chloramphenicol (30 mcg), gentamycin (10 mcg), kanamycin (30 mcg), neomycin (30 mcg), streptomycin (10 mcg) and tetracycline (30 mcg). The resistance and sensitive status of strains were determined according to inhibitory zone sizes and disc manufacturer recommendations.

The choice of antibiotics was based on their current use and availability in Trinidad.

Results

The prevalence of resistance to antibiotics of *E. coli* strains isolated from diarrhoeic and non-diarrhoeic livestock is shown in table I. A total of 487 strains of *E. coli* were tested and 437 (89.7 %) strains exhibited resistance to one or more of the tested antibiotics. Resistance was very high in isolates from piglets, 177 (96.7 %) of 183 isolates ($P \leq 0.001$; X^2) and calf strains, 196 (88.3 %) of 222 isolates ($P \leq 0.05$; X^2), being significantly different from the rate of occurrence of resistance in strains isolated from lambs, 51 (75.0 %) of 68 isolates. Thirteen *E. coli* strains (92.9 %) out of 14 isolated from kids were resistant. For all species, the differences in the prevalence of resistance in *E. coli* strains isolated from diarrhoeic and non-diarrhoeic controls were not statistically significant ($P \geq 0.05$; X^2). For diarrhoeic animals, the prevalence of resistance of *E. coli* strains was significantly higher in piglets, 97.3 % ($P \leq 0.001$; X^2) and calves, 91.4 % ($P \leq 0.01$; X^2) than that detected for strains isolated from diarrhoeic lambs. Similarly, animal management system had no effect on prevalence of resistant *E. coli*.

Resistance to streptomycin and tetracycline was prevalent among isolates of *E. coli* from all sources, *i.e.* 396 (81.3 %) and 384 (78.9 %), respectively of 487 isolates (table II). Regardless of the source, the resistance was less marked for chloramphenicol (4.3 %) and gentamycin (4.7 %). Resistance to kanamycin, neomycin and ampicillin was found in 184 (37.8 %), 178 (36.6 %) and 123 (25.3 %) isolates, respectively.

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Reçu le 15.5.1992, accepté le 18.9.1992.

TABLE I Resistance of **E. coli** *strains from animal sources to antibiotics tested.*

		Occurrence of resistance* among *E. coli* strains isolated from animals				
		Diarrhoeic		Non-diarrhoeic		
Specie	No. tested	No. tested	No. (%) resistant	No. tested	No. (%) resistant	Total No. (%) resistant
Calves	222	128	117 (91.4)	94	79 (84.0)	196 (88.3)
Piglets	183	113	110 (97.3)	70	67 (95.7)	177 (96.7)
Lambs	68	41	30 (73.2)	27	21 (77.8)	51 (75.0)
Kids	14	7	7 (100.0)	7	6 (85.7)	13 (92.9)
Total	487	289	264 (91.3)	198	173 (87.4)	437 (89.7)

** Résistant to one or more of the seven antibiotics tested.*

TABLE II Distribution of antimicrobial resistance by **E. coli** *strains isolated from four animal species.*

Antibiotic	Concentration (mcg)	Calves	Piglets	Lambs	Kids	All sources
Streptomycin	10	186 (83.8)	158 (86.3)	41 (60.3)	11 (78.6)	396 (81.3)
Tetracycline	30	171 (77.7)	168 (91.8)	35 (51.5)	10 (71.4)	384 (78.9)
Kanamycin	30	81 (36.5)	74 (40.4)	22 (32.4)	7 (50.0)	184 (37.8)
Neomycin	30	74 (33.3)	80 (43.7)	18 (26.5)	6 (42.9)	178 (36.6)
Ampicillin	10	58 (26.1)	54 (29.5)	10 (14.7)	1 (7.1)	123 (25.3)
Gentamycin	10	7 (3.2)	11 (6.0)	5 (7.4)	0 (0.0)	23 (4.7)
Chloramphenicol	30	8 (3.6)	11 (6.0)	2 (2.9)	0 (0.0)	21 (4.3)
Total number of strains tested		222	183	68	14	487

( ) %.

TABLE III Predominant antimicrobial resistance patterns of 487 strains of **E. coli** *isolated from various animals.*

Resistance pattern*	Number of strains (%)
S-Te**	136 (27.9)
A-K-N-S-Te	76 (15.6)
K-N-S-Te	52 (10.7)
Te	25 (5.1)
A-S-Te	19 (3.9)
N-S-Te	10 (2.1)
S	10 (2.1)
A-N-S-Te	4 (0.8)
A-C-G-K-N-S-Te	4 (0.8)
G-K-N-S-Te	3 (0.6)
A-C-K-N-S-Te	3 (0.6)
None of the above***	27 (5.5)
Sensitive to all antibiotics	50 (10.3)

** Overall, 38 antimicrobial resistance patterns were observed.*
*** S, streptomycin ; Te, tetracycline ; A, ampicillin ; K, kanamycin ; N, neomycin : C, chloramphenicol ; and G, gentamycin.*
**** Each of the other 27 patterns not shown had only one isolate with exception of K-S-Te and A-G-K-S-Te with two isolates each.*

The predominant antibiotic resistance patterns of the 487 *E. coli* strains tested are shown in table III. The prevalent patterns were streptomycin-tetracycline (27.9 %), ampicillin-kanamycin-neomycin-streptomycin-tetracycline (15.6 %) and kanamycin-neomycin-streptomycin-tetracycline (10.7 %), regardless of animal source of *E.coli.* strains. Resistance to tetracycline was the most prevalent among the various patterns. A total of 38 resistance patterns were observed.

Discussion

It was of therapeutic significance that almost 90 % of all tested *E. coli* strains were resistant to one or more antibiotics. Also of importance was the finding that a high proportion of the isolates were multi-resistant. This is because in Trinidad, all the antibiotics tested, with a possible exception of chloramphenical and gentamycin, are currently used in different preparations in the livestock industry. The therapeutic implications of the findings should therefore be strongly emphasized.

It was hardly a surprise that strains of *E. coli* isolated from piglets and calves were the most resistant while strains from lambs exhibited the lowest resistance to antibiotics. This was because it has been reported that antibiotic resistant strains of *E. coli* are regularly excreted by calves (7) and pigs (11) while they are rarely excreted by sheep (9) and adult cattle (8). The fact that in this study as many as 75 % of the isolates from sheep (lambs) were resistant therefore highlights the rather high prevalence of resistance to antibiotics among *E. coli* strains of animal origin in Trinidad.

It was of interest to observe that regardless of animal species, the prevalence of resistance to antibiotics was similar for strains isolated from diarrhoeic and non-diarrhoeic animals. This finding may be partly explained by the fact that the pathogenicity of the *E. coli* strains was not determined for establishing their aetiological significance.

In all animal species, resistance to streptomycin and tetracycline was clearly predominant. The prevalence of resistance to streptomycin and tetracycline found here was much higher but still comparable than that reported elsewhere (5, 6). The relative sensitivities of isolates to chloramphenicol, gentamycin, ampicillin and kanamycin are in agreement with earlier reports (6, 17). The rather low prevalence of resistance to chloramphenicol and gentamycin by the strains appeared to result from the very uncommon use of these antibiotics in veterinary practice in the country. Development of resistance of *E. coli* to tetracycline has earlier been reported as a problem worldwide due to its use as a growth promoter in animals and a ban of its use in animals in Britain in 1971 has led to a reduction of the prevalence of resistance among isolates (13). Studies have also established that at subtherapeutic level tetracycline acts as a selector of an already established resistance (7, 10). That isolates from piglets had the highest prevalence of resistance to tetracycline compared to isolates from calves, lambs and kids where resistance to streptomycin was highest, was therefore not unexpected. The on-going widespread practice in Trinidad of oral administration of chlortetracycline preparations to pigs as growth promoters may be responsible for this. This should be a source of concern to the autorities because it has economic, clinical and public health implications (15).

The zoonotic significance of the findings in this study is that people in contact with the young livestock sampled here are at a risk of exposure to resistant strains of *E. coli* which may also be pathogenic for man. OYENIYI (12) earlier established the occurrence of such a spread among people working with poultry.

Conclusion

It is evident that the rather high prevalence of resistance to antibiotics among strains of *E. coli* isolated from livestock in Trinidad should be of public health concern. It is therefore imperative that efforts should be made to control the use of antibiotics in the livestock industry particularly their use as growth promoters or at subtherapeutic levels. It is only when such an action is taken that chemotherapy in veterinary practice in Trinidad will be more cost-effective and the risk of humans associated with livestock being exposed to resistant *E. coli* reduced.

Acknowledgements

The University of the West Indies, Saint-Augustine Campus Research Fund Committee is greatly acknowledged for funding the project. The assistance of Drs R. LOREGNARD, S. JOHNSON of the Quarantine station and other field veterinarians is appreciated. We thank Mrs Aweeda NEWAJ-FYZUL for her technical assistance and Mrs Lu-Ann JOSEPH for typing the manuscript.

ADESIYUN (A.A.), KAMINJOLO (J.S.). Susceptibility to antibiotics of *Escherichia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (3-4) : 260-262

The sensitivity of strains of *Escherichia coli* isolated from calves, piglets, lambs and kids in Trinidad to seven antibiotics was determined. Two hundred and sixty-four (91.3 %) of 289 strains isolated from diarrhoeic animals and 173 (87.4 %) of 198 strains from non-diarrhoeic animals exhibited resistance to one or more antibiotics. The difference was not statistically significant ($P \geq 0.05$; X^2). Regardless of health status, isolates from lambs were least resistant (75.0 %) and those from piglets most resistant (96.7 %) and the difference was significant ($P \leq 0.001$; X^2). Strains of *E. coli* were most resistant to streptomycin (81.3 %) and tetracycline (78.9 %) and least resistant to chloramphenicol (4.3 %) and gentamycin (4.7 %). The predominant antibiotic resistance pattern for isolates from all sources was streptomycin-tetracycline (27.9 %). It was concluded that the widespread prevalence of resistance to antibiotics reflects their misuse in the local environment. *Key words* : Cattle - Pig - Sheep - Goat - Diarrhoea - *Escherichia coli* - Chemical resistance - Antibiotics - Antibiogram - Trinidad - West Indies.

References

1. ADESIYUN (A.A.), KAMINJOLO (J.S.), LOREGNARD (R.), KITSONPIGGOTT (W.). Campylobacter infections in calves, piglets, lambs and kids in Trinidad. *Br. Vet. J.*, 1992, **148** : 547-556.

2. BAUER (A.W.), KIRBY (W.M.M.), SHERRIS (J.C.), TURCK (M.). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Path.*, 1966, **45** : 493-496.

3. BENSINK (J.C.), BOTHMANN (F.P.). Antibiotic-resistant *Escherichia coli* isolated from chilled meat at retail outlets. *New Zealand Vet. J.*, 1991, **39** : 126-128.

4. COWAN (S.T.), STELL (K.F.). Manual for identification of medical bacteria. 2nd ed. Cambridge, Cambridge University Press, 1974. 238 p.

5. FARRIS (A.S.), WIERUP (M.), JACOBSSON (S.O.). Antibiotic resistance and transferable antibiotic resistance of *Escherichia coli* isolated from calves on a modern farm with therapeutic problems and unsatisfactory management conditions. *Nordisk Veterinaermed.*, 1979, **31** : 20-24.

6. GOYETTE (G.), HUGGINS (R.). Drug sensitivity of *Escherichia coli* isolates from piglets and calves with neonatal diarrhoea. *Med. Vet. Quebec*, 1987, **17** : 36.

7. HOWE (K.), LINTON (A.H.). The distribution of O-antigen types of *Escherichia coli* in normal calves, compared with man, and their R-plasmid carriage. *J. Appl. Bacteriol.*, 1976, **40** : 317-330.

8. HOWE (K.), LINTON (A.H.), OSBORNE (A.D.). A longitudinal study of *Escherichia coli* in cows and calves with special reference to distribution of O-antigen types and antibiotic resistance. *J. Appl. Bacteriol.*, 1976, **40** : 331-340.

9. LINTON (A.H.). Antibiotic and antibiosis in agriculture. *In* : Proceedings of the University of Nottingham School of Agriculture, 1977.

10. LINTON (A.H.), HOWE (K.), OSBORNE (A.D.). The effects of feeding tetracycline, nitrovin and quindoxin on the drug-resistance of coliaerogenes bacteria from calves and pigs. *J. Appl. Bacteriol.*, 1975, **38** : 255-275.

11. LINTON (A.H.), HANDLEY (B.), OSBORNE (A.D.), SHAW (B.G.), ROBERTS (T.A.), HUDSON (W.R.). Contamination of pig carcasses at two abattoirs with special reference to O-serotypes and antibiotic resistance. *J. Appl. Bacteriol.*, 1977, **42** : 89-110.

12. OYENIYI (A.A.). Direct transmission of *Escherichia coli* from poultry to humans. *Epid. Infect.*, 1989, **103** : 512-522.

13. SMITH (H.W.). Persistence of tetracycline resistance in pig *E. coli*. *Nature*, 1975, **258** : 628-631.

14. SOJKA (W.J.). Enteric diseases in newborn piglets, calves and lambs due to *Escherichia coli*. *Vet. Bull.*, 1971, **41** : 509-522.

15. WALTON (J.R.). The public health implications of drug resistance bacteria in farm animals. *Annls N.Y. Acad. Sci.*, 1971, **182** : 358-361.

16. WASHINGTON (J.A.). The effects and significance of subminimal inhibitory concentrations of antibiotics. *Rev. Inf. Dis.*, 1979, **1** : 781-786.

17. WIERUP (M.). Antibiotic resistance and transferable antibiotic resistance of *Eschericia coli* isolated from Swedish calves 5 and 30 days old. *Nordisk Veterinaermed.*, 1975, **27** : 77-84.

18. WILLIAM-SMITH (H.). Transfer of antibiotic resistance from animal and human strains of *Escherichia coli* to resident *E. coli* in the alimentary tract of man. *Lancet*, 1969, **1** : 1174-1176.