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RESISTANCE TO VARIOUS ANTIBIOTICS OF *SALMONELLA* AND *ESCHERICHIA COLI* ISOLATED FROM REGISTRABLE FARM FEEDS

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ABSTRACT

DURAND, ANETTE M., BARNARD, MARIE-LUISE, SWANEPOEL, MARTHA L. & ENGELBRECHT, MARIE M., 1987. Resistance to various antibiotics of *Salmonella* and *Escherichia coli* isolated from registrable farm feeds. *Onderstepoort Journal of Veterinary Research*, 54, 21–26 (1987)

Resistance to 20 antibiotics of 128 *Salmonella* and 97 *Escherichia coli* isolates from various registrable farm feeds was determined. A high frequency of comparatively low levels of resistance was found in both the *Salmonella* and *E. coli* isolates. This, together with an elevated frequency of multiple resistance, indicates that problems related to an effective transfer in bacterial populations of resistance to certain antibiotics are a distinct possiblity. The addition of antibiotics, such as penicillin and tetracyclines, to animal feeds can create conditions for rapid selection amongst bacteria resistant to antibiotics. The numbers of resistant bacteria in the animal environment may be increased and may lead to the development of veterinary and human health problems from the possible transfer of antimicrobial resistance from animal pathogens to human pathogens or spreading in the human population of animal pathogens resistant to antibiotics.

There is a need for caution in the use of antibiotics, particularly in animal feeds. Extended survey of, and epidemiological research on, farm feeds, manufacturing mills and animal production units are emphasized.

INTRODUCTION

Several reports on transmissible antibiotic resistance in *Samonella* and *Escherichia coli* have been published in recent years (Pocurull, Gaines & Mercer, 1971; Bisset, Abbott & Wood, 1974; Neu, Cherubin, Longo, Flouton & Winter, 1975; Cox, 1980; Cox, Luther, Newman & Ray, 1981; Altherr & Kasweck, 1982; Blackburn, Schlater & Swanson, 1984; Wernery, 1984). Multiple resistance was found in as many as 80 % of *Samonella* and *E. coli* isolates (Blackburn *et al.*, 1984). In Papua New Guinea, *Salmonella*, isolated during 1980/82 from pigs and poultry, were shown to increase their resistance rapidly to chlor- and oxytetracycline (Wernery, 1984). The problem that arises is that organisms are increasingly isolated which exhibit multiple antibiotic resistance. This may be due to the presence of antibiotics in the feeds, resulting in antibiotic-resistant organisms being selected. These feeds may be involved in further disseminating the resistant organisms. To assess the situation in the Republic of South Africa on the resistance to antibiotics of *Salmonella* and *E. coli* isolated from farm feeds registrable in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947), as amended, and its regulations, an investigation was warranted. This investigation was aimed at obtaining a general picture of the present situation and determining the need for further, more detailed work.

MATERIALS AND METHODS

Isolation of bacteria

Salmonella and *E. coli* were isolated from registrable farm feeds by various methods, and pure cultured on blood tryptose agar as described by Ogonowski, Barnard & Giesecke (1984). Single colonies of the micro-organisms were used for further serotyping and determining susceptibility with an antibiogram (Edwards & Ewing, 1955; Kauffmann, 1972).

Determination of susceptibility to antibiotics

For the determination of susceptibility to antibiotics a single colony was removed by means of a sterilized platinum loop from the agar plate, inoculated into 5 mℓ of serum broth, thoroughly mixed with the medium and incubated for 24 h at 37 ± 1 °C. A sample of 0,2 mℓ of the serum broth culture was then pipetted into a sterile plastic Petri dish and mixed with 10 mℓ of molten Diagnostic Sensitivity Test (DST) agar.[1] This was added to the inoculum in each of the Petri dishes. The plates were then left to solidify at room temperature, and discs[2] with low concentrations of antibiotics (Table 1) were placed onto the agar surface with sterile forceps. The plates with the inoculated agar and antibiotic discs were incubated for 24 h at 37 ± 1 °C. The diameter of each zone of total inhibition was then determined by means of callipers and an mm-ruler. Where no inhibition zones were formed, the isolate was considered resistant to the specific antibiotic. Inhibition zones with diameters of 8–9 mm and longer were interpreted as indicating slight resistance and no resistance (= susceptibility) to a particular antibiotic.

TABLE 1 The antibiotics used in the investigation and their concentrations

Antibiotics involved in the investigation		Concentration
Penicillin G	(Pe)	1 unit
Mithicillin	(Me)	5 µg
Ampicillin	(Am)	2 µg
Streptomycin	(Str)	10 µg
Tetracycline	(Tet)	10 µg
Chloramphenicol	(Chl)	10 µg
Neomycin	(Neo)	10 µg
Nitrofurantoin	(Nit)	50 µg
Erythromycin	(Ery)	5 µg
Tylosin	(Ty)	30 µg
Spectinomycin	(Sp)	10 µg
Clindamycin	(Cl)	2 µg
Bacitracin	(Ba)	8 units
Cephaloridine	(Ce)	5 µg
Amikacin	(Ak)	10 µg
Polymyxin B	(PoB)	100 units
Colistin Sulphate	(CS)	10 µg
Cotrimoxazole	(Co)	25 µg
Gentamycin	(Ge)	2 µg
Kanamycin	(Ka)	30 µg

It is necessary to mention here that the size of an inhibition zone does not necessarily indicate the degree of susceptibility. Antibiotics (Table 1), such as penicillin G and methicillin, were included in this study for facilitating ready comparisons with the investigations of other workers who have included them (Blackburn *et al.*, 1984; Wernery, 1984), even though these antibiotics are not recommended for treatment of gram-negative infections.

E. coli isolates were subdivided into 3 groups according to their pathogenicity, namely, non-pathogenic, potentially pathogenic and pathogenic *E. coli*.

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TABLE 2 Resistance and susceptibility patterns of *Salmonella* isolates from farm feeds and byproducts

	Antibiotics tested																			
	Pe*	Me	Am	Str	Tet	Chl	Neo	Nit	Ery	Ty	Sp	Cl	Ba	Ce	Ak	PoB	CS	Co	Ge	Ka
No. of isolates resistant	128	128	0	2	4	0	3	9	107	108	0	86	128	0	0	0	0	0	1	3
% of isolates resistant	100	100	0	1,56	3,13	0	2,34	7,03	83,6	84,38	0	76,19	100	0	0	0	0	0	0,78	2,3
No. of isolates intermediately resistant	0	0	2	0	2	0	0	4	17	2	4	1	0	0	0	0	0	0	0	0
% of isolates intermediately resistant	0	0	1,56	0	1,56	0	0	3,13	13,28	1,56	3,13	0,78	0	0	0	0	0	0	0	0
No. of isolates susceptible	0	0	126	126	122	128	125	115	4	18	124	41	0	128	128	128	128	128	127	125
% of isolates susceptible	0	0	98,44	98,44	95,31	100	97,66	89,84	3,13	14,06	96,88	32,03	0	100	100	100	100	100	99,22	97,7

* For coding see Table 1

Results

Salmonella *resistance to antibiotics*

The 128 *Salmonella* isolates investigated showed total resistance to penicillin G (Pe), methicillin (Me) and bacitracin (Ba) (Table 2). Low resistance in 1,56–100 % of the isolates to streptomycin (Str), tetracycline (Tet), neomycin (Neo), nitrofurantoin (Nit), gentamycin (Ge) and kanamycin (Ka) was determined. Intermediate resistance in not more than 13,28 % of the isolates was observed to ampicillin (Am), Tet, Nit, erythromycin (Ery), tylosin (Ty), spectinomycin (Sp) and clindamycin (Cl). The majority of isolates were fully susceptible to Am, Str, Tet, chloramphenicol (Chl), Neo, Nit, Ery, Ty, Sp, Cl, cephaloridine (Ce), amikacin (Ak), polymyxin B (PoB), colistin sulphate (CS), cotrimoxazole (Co), Ge and Ka. Susceptibility to Ery, Ty and Cl was found in 32 % or less of the isolates. To all the other antibiotics showing susceptibility, 89,84–99,22 % were observed to be susceptible to the particular antibiotic.

The 14 *Salmonella* species most frequently isolated were analysed separately (Table 3) for determining whether resistance was related to certain species.

All the species investigated (Table 3) showed 100 % resistance against Pe, Me and Ba, but not to Am, Chl, Nit, Sp, Ce, Ak, PoB, CS and Co. All the isolates resistant to Ery belonged to the species *Salmonella thompson, Salmonella escanaba, Salmonella raus, Salmonella singapore, Salmonella norwich, Salmonella madelia* and *Salmonella typhimurium*. Other species showed resistance to Ery in 66,67 % (*Salmonella tinda*)—87,5 % (*Salmonella remo* and *Salmonella chester*) of the isolates. Resistance to tylosin varied from 33,3 % (*S. thompson* and *S. norwich*) to 100 % (*Salonella seftenberg, S. remo, S. chester, S. raus, Salmonella jaja, S. singapore* and *S. typhimurium*).

An interesting phenomenon was observed in the case of Cl to which most of the isolates of *S. seftenberg, S. tinda, S. thompson, S. remo, S. chester, S. raus* and *S. norwich* were resistant, whereas other species, such as *S. escabana, S. jaja, S. singapore, S. madelia* and *S. typhimurium*, indicated susceptibility. A few isolated cases of resistance to Neo were associated with *S. thompson* (11 % of the isolates) and *S. escabana* (33,33 % of the isolates).

Species comprising only 1 or 2 isolates were all grouped under "Others" (Table 3). They showed resistance to Pe, Me and Ba. A high percentage of resistance was also observed in these species to Ery (78,26 %), Ty (86,96 %) and Cl (78,26 %). Only 4,35 % of the 23 isolates involved showed resistance to Tet (Table 3).

Nine rough *Salmonella* strains were examined for determining differences between their resistance patterns and those of the identified species. All were resistant to Pe, Me, Ty and Ba; 44 % and 77,78 % showed resistance to Cl and Ery, respectively. The rough strains showed no resistance to the other antibiotics evaluated (Table 3).

E. coli *resistance to antibiotics*

All of the 97 *E. coli* isolates investigated (Table 4) showed resistance to Pe, Me, Cl and Ba. A high percentage of resistance was found against Ery (96 % of the isolates), Ty (93 %), Ce (57 %) and Ak (56 %). The *E. coli* strains were also resistant to Am (20 % of the isolates), Str (1 %), Tet (10 %), Chl (2 %), Neo (2 %), Nit (1 %), Sp (8 %), Co (1 %), Ge (2 %) and Ka (1 %). Intermediate resistance was observed to Am, Str, Ery, Ty, Sp, Ce, Ak, Co and Ge. In these cases, 20 % or less of the isolates gave intermediate reactions. Susceptibility to antibiotics varied from 4 % (Ty) to 100 % (PoB and

TABLE 3 Percentage of *Salmonella* species resistant to various antibiotics

Antimicrobial agent	Concentration	*Salmonella* serotypes														
		S. seftenberg	*S. tinda*	*S. thompson*	*S. remo*	*S. chester*	*S. escanaba*	*S. raus*	*S. jaja*	*S. singapore*	*S. norwich*	*S. madelia*	*S. typhimurium*	Others	Rough strains	Total
		36*	9	9	8	8	6	5	4	4	3	3	1	23	9	128
Pe	1 unit	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Me	5 μg	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Am	2 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Str	10 μg	0	0	0	0	0	33,33	0	0	0	0	0	0	0	0	1,56
Tet	10 μg	0	0	11,11	0	0	50	0	0	0	0	0	0	4,35	0	3,13
Chl	10 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neo	10 μg	0	0	11,11	0	0	33,33	0	0	0	0	0	0	0	0	2,34
Nit	50 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ery	5 μg	77,78	66,67	100	87,5	87,5	100	100	75	100	100	100	100	78,26	77,78	83,59
Ty	30 μg	100	44,44	33,33	100	100	66,67	100	100	100	33,33	66,67	100	86,96	100	84,38
Sp	10 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cl	2 μg	100	11,11	66,67	100	100	0	100	0	0	66,67	0	0	78,26	44,44	67,19
Ba	8 units	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Ce	5 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ak	10 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PoB	100**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CS	10 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Co	25 μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ge	2 μg	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0,78
Ka	30 μg	0	0	11,11	0	0	33,33	0	0	0	0	0	0	0	0	2,34

* = No. of isolates involved in the investigation

** = units

TABLE 4 Resistance and susceptibility patterns of *E. coli* isolates from animal feeds and by-products

	Antibiotics tested																			
	Pe*	Me	Am	Str	Tet	Chl	Neo	Nit	Ery	Ty	Sp	Cl	Ba	Ce	Ak	PoB	CS	Co	Ge	Ka
No. of isolates resistant	97	97	19	1	10	2	2	1	93	90	8	97	97	55	54	0	0	1	2	1
% of isolates resistant	100	100	20	1	10	2	2	1	96	93	8	100	100	57	56	0	0	1	2	1
No. of isolates intermediately resistant	0	0	19	9	0	0	0	0	4	3	6	0	0	1	3	0	0	2	10	0
% of isolates intermediately resistant	0	0	20	9	0	0	0	0	4	3	6	0	0	1	3	0	0	2	10	0
No. of isolates susceptible	0	0	59	87	87	95	95	96	0	4	83	0	0	41	40	97	97	94	85	96
% of isolates susceptible	0	0	60	90	90	98	98	99	0	4	86	0	0	42	41	100	100	97	88	99

* For coding see Table 1

CS) of the isolates and were observed for Am, Str, Tet, Chl, Neo, Nit, Ty, Sp, Ce, Ak, PoB, CS, Co, Ge and Ka.

The *E. coli* serotypes were subdivided into 3 groups according to pathogenicity, namely, non-pathogens, potential pathogens and pathogens. The presumably non-pathogenic *E. coli* all showed resistance to Pe, Me, Cl and Ba (Table 5). Resistance to Ery, Ty, Ce and Ak was also high (95 %–96 % of the isolates). Lower percentages of resistance were found to antibiotics, such as Am, Tet, Neo, Nit, Sp and Ge (2 %–16 % of the isolates). Non-pathogenic *E. coli* were not resistant to Str, Chl, PoB, CS, Co and Ka.

The potentially pathogenic *E. coli* (Table 5) were resistant to Pe (100 % of the isolates), Me (100 %), Am (36 %), Tet (7 %), Ery (100 %), Ty (93 %), Sp (14 %), Cl (100 %) and Ba (100 %). They were not resistant to Str, Chl, Neo, Nit, Ce, Ak, PoB, CS, Co, Ge and Ka.

The pathogenic *E. coli* strains were resistant to Pe (100 % of the isolates), Me (100 %), Am (19 %), Str (4 %), Tet (11 %), Chl (7 %), Ery (96 %), Ty (85 %), Sp (11 %), Cl (100 %), Ba (100 %), Ce (96 %), Co (4 %), Ge (4 %) and Ka (4 %), but not to Neo, Nit, Ak, PoB and CS. In general, the 3 groups of *E. coli* strains showed only slight differences of resistance to the various antibiotics, except in the case of Ce and Ak. Thus 96 % of the non-pathogens and 96 % of the pathogens were resistant to Ce, whereas the potential pathogens all seemed susceptible to this antibiotic. Furthermore, 96 % of the non-pathogens were resistant to Ak, whereas the potential pathogens and pathogens were susceptible to this antibiotic.

Multiple resistance to antibiotics

Multiple resistance was observed in all the *Salmonella* and *E. coli* isolates investigated. The pattern observed in 46,88 % of the cases in *Salmonella* was (Pe, Me, Ery, Ty, Cl, Ba) (Table 6).

Resistance patterns of (Pe, Me, Ery, Ty, Ba) occurred in 21,88 % and of (Pe, Me, Ty, Cl, Ba) in 10,16 % of the isolates (Table 6). Most isolates of *S. seftenberg* seemed to show the former resistance pattern, formerly also applicable to *S. remo*, *S. chester* and *S. raus*. Such findings suggest the possibility that certain species of *Salmonella* may be more resistant to a certain range of antibiotics than others.

The multiple resistance pattern observed in 61,86 % of the *E. coli* (Table 7) was similar to that in the *Salmonella* (Pe, Me, Ery, Ty, Cl, Ba). Other patterns, such as (Pe, Me, Am, Ery, Ty, Cl, Ba) and (Pe, Me, Ery, Cl, Ba) were determined in 12,37 % and 5,15 % of the *E. coli* respectively.

The *E. coli* serotype, resistant to 13 of the antibiotics was pathogenic and showed the resistance pattern (Pe, Me, Am, Str, Tet, Chl, Ery, Ty, Cl, Ba, Ce, Co, Ka).

TABLE 5 Percentage of *E. coli* serotypes resistant to various antibiotics

Antimicrobial agent	Concentration	*E. coli* serotypes according to pathogenicity			
		Non-pathogenic	Potential pathogens	Pathogens	Total
		56*	14	27	97
		+058:K-; 0152:K15; 08:K49; 0 rough:K20; 032:K19; 0117:K1; 09:K26; 021:K-; 034:K-; 0114:K90; 09:K37; 095:K-; 09:K38; 036:K-; 08:K45; 08:K48; 046:K-; 085:K-; 011:K-; 0112:K68; 027:K-; 079:K-; 0 rough:K53; 08:K42; 0152:K-; 068:K10; 0113:K75; 083:K-; 028:K46; 079:K23; 08:K46; 0136:K78; 029:K-; 09:K34	0112:K68; 0117:K17; 0119:K69; 0128:K67; 0138:K81; 0139:K82; 0141:K85; 0147:K89; 0149:K91	020:K-; 021:K-; 021:K53; 028:K80; 04:K-; 06:K53; 075:K-; 08:K8; 078:K80; 08:K25; 08:K27; 09:K9; 09:K30; 09:K31; 09:K32; 09:K34; 09:K36; 09:K39	
Pe	1 unit	100	100	100	100
Me	5 μg	100	100	100	100
Am	2 μg	16	36	19	20
Str	10 μg	0	0	4	1
Tet	10 μg	11	7	11	10
Chl	10 μg	0	0	7	2
Neo	10 μg	4	0	0	2
Nit	50 μg	2	0	0	1
Ery	5μg	95	100	96	96
Ty	30 μg	95	93	85	93
Sp	10 μg	5	14	11	8
Cl	2μg	100	100	100	100
Ba	8 units	100	100	100	100
Ce	5 μg	96	0	96	57
Ak	10 μg	96	0	0	56
PoB	100**	0	0	0	0
CS	10 μg	0	0	0	0
Co	25 μg	0	0	4	1
Ge	2 μg	2	0	4	2
Ka	30 μg	0	0	4	1

* = No. of isolates involved in the investigation

** = Units

\+ = Serotypes classified in terms of pathogenicity

The corresponding *Salmonella* strain (*S. escabana*) was resistant to 9 of the antibiotics and showed the resistance pattern (Pe, Me, Str, Tet, Neo, Ery, Ty, Ba, Ka).

Discussion

Important implications for human and animal health of bacterial resistance to antibiotic remedies and the increase in resistance over some years (Pocurull *et al.*, 1971; Bisset *et al.*, 1974; Cox, 1980; Altherr & Kasweck, 1982; Blackburn *et al.*, 1984; Wernery, 1984) warranted an investigation into the possible role of farm feeds in the spreading of antimicrobial resistance and the transfer of the resistance in *Salmonella* and *E. coli*.

Various antimicrobial agents and techniques for evaluating bacterial resistance to such agents can be employed (Cox, 1980). Therefore, results from different techniques, such as the Kirby-Bauer and dilution methods are not necessarily comparable. On the other hand, results from standardized disc diffusion methods and modifications thereof, such as that used during this investigation, are more comparable. In the present study, *Salmonella* usually showed resistance to Pe, Me, Ery, Ty, Cl, Ba and to a lesser extent to Str, Tet, Neo, Nit, Ge and Ka. Cox (1980) reported *Salmonella* serotypes resistant to Tet and Ka. Timoney (1978) found that of one of the 249 strains of *S. typhimurium*, 38 % were resistant to Am, 4 % to Chl, 71 % to Ka, 67 % to Neo, 78 % to Str and 74 % to Tet. Most of the antibiotics were used at concentrations higher than those used in this investigation.

Resistance to various antibiotics has increased over time (Neu *et al.*, 1975; Wernery, 1984). Different levels of resistance have been observed in *E. coli* isolated from calves in Morocco and north-western Germany. The Moroccan isolates were highly susceptible and the German ones more resistant to antibiotics (Ghoniem, Hanschke, Amtsberg & Bisping, 1982). The present study showed percentage values of resistance more comparable with those from the Moroccan than the German isolates from calves.

Resistance to a wide range of antibiotics was observed in the *E. coli* isolated from farm feeds, even though the percentage of resistant isolates was comparatively low. That range of resistance may perhaps be attributed to cross-resistance (Plempel & Otten, 1969), inherent or acquired. Thus cross-resistance to Pe and Me, Neo, Ka and Str, Chl and Tet can be involved in the resistance to a wide range of antibiotics.

In the USA, peniclin and tetracyclines were added to feeds for young animals to promote growth and reduce chronic subclinical disease (Brander, 1973). The addition of such antibiotics to animal feeds can also create conditions for rapid selection amongst certain bacteria of antimicrobial resistance. As a result of the use of antibiotics in the animal environment and the selection for strains that are resistant to antibiotics, competition between resistant and non-resistant bacteria can take place during colonization of the intestinal tract. This was indicated by results obtained (Mamber & Katz, 1985, citing Linton, 1982) where: "It is the norm to find the larger proportion of *Escherichia coli* in pigs, poultry and calves resistant to one or more therapeutically useful antibiotics whether or not the animals are receiving antibiotics". The use of antibiotics in the animal environment seems to increase the number of resistant bacteria in the environment. This may eventually lead to the development of veterinary problems, because it may render antibiotic therapy ineffective. Veterinary problems may further escalate to a human health problem if antimicrobial resis-

TABLE 6 Multiple resistance patterns found most frequently in *Salmonella* serotypes isolated from animal feeds

Resistance pattern	Frequency No. of isolates (%)	*Salmonella* serotypes showing pattern (No. of isolates)
Pe Me Ery Ty Cl Ba	60(46,88)	*S. seftenberg* (27) Others (11) *S. remo* (7) *S. chester* (7) *S. raus* (5) *Salmonella* rough (3)
Pe Me Ery Ty Ba	28(21,88)	*S. escabana* (2) *S. jaja* (3) *S. tinda* (4) *S. madelia* (2) *S. typhimurium* (1) *S. singapore* (4) Others (5) *S. thompson* (2) *S. norwich* (1) *Salmonella* rough (4)
Pe Me Ty Cl Ba	13(10,16)	*S. seftenberg* (9) Others (1) *S. remo* (1) *S. chester* (1) *Salmonella* rough (1)
Pe Me Ery Cl Ba	10(7,81)	*S. tinda* (1) Others (2) *S. thompson* (5) *S. norwich* (2)
Pe Me Ty Ba	5(3,91)	Others (5) *Salmonella* rough (1)
Pe Me Ery Ba	3(2,34)	*S. tinda* (1) *S. madelia* (1) *S. thompson* (1)
Pe Me Ba	3(2,34)	*S. tinda* (3)
Pe Me Tet Neo Ery Ba Ka	2(1,56)	*S. escabana* (1) *S. thompson* (1)
Pe Me Str Tet Neo Ery Ty Ba Ka	1(0,78)	*S. escabana* (1)
Pe Me Str Ery Ty Ba	1(0,78)	*S. escabana* (1)
Pe Me Ery Ty Ba Ge	1(0,78)	*S. jaja* (1)
Pe Me Tet Ery Ba	1(0,78)	*S. escabana* (1)

tance be transferred from animal to human pathogens or if animal pathogens resistant to antibiotics be spread to the human population.

Because of such potential risks to public health, Swann (1969) has recommended that in general, penicillin and the tetracyclines should not be added to feeds as growth promoters but be replaced if necessary with antibiotics of economic value in livestock, with little or no application as therapeutic agents in animals and man. These are likely to impair the efficacy of prescribed therapeutic antibiotics through the development of resistant strains. Thus, zinc-bacitracin, virginiamycin and flavomycin have been proposed in the United Kingdom as additives to animal feeds. Similar provisions have become applicable in the Republic of South Africa to registered farm feeds.

However, the elevated frequency of variable levels of multiple resistance in *Salmonella* and *E. coli,* isolated during this investigation from a comparatively limited range and number of registered farm feeds, suggests that the problem of multiple resistant bacteria in such feeds requires further attention and elaboration. Elaboration is especially applicable to bacteria which, like *Salmonella*

TABLE 7 Multiple resistance patterns found most frequently in *E. coli* strains isolated from farm feeds

Resistance pattern	Frequency No. of isolates (%)	Pathogenicity of the *E. coli* strain showing the pattern (No. of isolates)
Pe Me Ery Ty Cl Ba	60(61,86)	PP (= potential pathogen) (6) P (= pathogen) (17) NP (= non-pathogen) (37)
Pe Me Am Ery Ty Cl Ba	12(12,37)	PP (3) P (4) NP (4)
Pe Me Ery Cl Ba	5(5,15)	PP (1) P (2) NP (2)
Pe Me Ery Ty Sp Cl Ba	4(4,12)	PP (1) P (1) NP (2)
Pe Me Ty Cl Ba	3(3,09)	PP (0) P (0) NP (3)
Pe Me Tet Ery Ty Cl Ba	2(2,06)	PP (0) P (0) NP (2)
Pe Me Am Tet Ery Ty Sp Cl Ba	1(1,03)	PP (1) P (0) NP (0)
Pe Me Tet Ery Ty Sp Cl Ba	1(1,03)	PP (0) P (1) NP (0)
Pe Me Cl Ba	1(1,03)	PP (0) P (1) NP (0)
Pe Me Am Tet Chl Ery Sp Cl Ba Ge	1(1,03)	PP (0) P (1) NP (0)
Pe Me Am Str Tet Chl Ery Ty Cl Ba Ce Co Ka	1(1,03)	PP (0) P (1) NP (0)
Pe Me Tet Ty Cl Ba	1(1,03)	PP (0) P (0) NP (1)
Pe Me Am Tet Ery Ty Cl Ba	1(1,03)	PP (0) P (0) NP (1)
Pe Me Am Neo Ery Sp Cl Ba	1(1,03)	PP (0) P (0) NP (1)
Pe Me Ery Ty Cl Ba Co	1(1,03)	PP (0) P (0) NP (1)
Pe Me Ery Ty Cl Ba Co Ge	1(1,03)	PP (0) P (0) NP (1)
Pe Me Am Tet Nit Ery Ty Cl Ba	1(1,03)	PP (0) P (0) NP (1)

and *E. coli,* may transfer multiple resistance to antibiotics dependent on the presence of plasmid associated with R-factors.

The elevated frequency of usually low levels of multiple resistance to the antibiotics of the *Samonella* and *E. coli* investigated suggests the availability of pools of bacteria which may be readily induced to transfer and/or increase their resistance to antibiotics depending on appropriate provocation (e.g. repeated exposure to therapeutically ineffective levels of certain antibiotics).

From the above it seems justifiable to conclude that the multiple resistance of the bacteria isolated indicates a potential risk to animal health and production. The data further emphasize the need for (i) great caution in the use of antibiotics, particularly in animal feeds; (ii) an extended survey of farm feeds, and (iii) epidemiological research on such feeds, the mills manufacturing them, and animal production units using them.

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