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DRUG RESISTANCE IN STRAINS OF ESCHERICHIA COLI ISOLATED FROM THE INTESTINAL TRACT OF PIGS IN NORWAY*

By

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LIVEN, EIVIND: *Drug resistance in strains of Escherichia coli isolated from the intestinal tract of pigs in Norway.* Acta vet. scand. 1979, *20*, 258—269. — Faecal samples from 95 healthy pigs and samples of jejunal content from 85 piglets suffering from colienterotoxaemia were tested for the presence of drug resistant E. coli strains. Practically all pigs in both groups harboured E. coli strains resistant to one or more of the 6 antibiotics/chemotherapeutic agents tested (oxytetracycline, streptomycin, sulphaisodimidin, neomycin, ampicillin, chloramphenicol). Almost 100 % of healthy and approx. 90 % of diseased pigs harboured strains resistant to oxytetracycline, streptomycin and sulphaisodimidin. Pigs with strains resistant to neomycin, ampicillin and chloramphenicol were less frequently found. The predominant coliform flora consisted of E. coli strains resistant to oxytetracycline, streptomycin and sulphaisodimidin in 71 % to 81 % of diseased pigs and in 47 % to 69 % of the healthy pigs. In diseased pigs ¾ of the animals had a coliform flora dominated by neomycin-resistant E. coli strains.

Of the 721 resistant E. coli strains isolated from healthy pigs, 11 % were single resistant while the corresponding figure for the 518 resistant strains isolated from diseased pigs was 6 %. Thus 89 % and 94 % of strains showed simultaneous resistance to 2 or more antibiotics. E. coli strains resistant to 3 or more drugs were found in approx. 60 % and 70 % of the isolates from healthy and diseased animals, respectively. Oxytetracycline/streptomycin/sulphaisodimidin resistance was most commonly found, approx. 22 % and 38 % of the strains from healthy and diseased pigs, respectively, showing this resistance pattern.

Transmission of drug resistance which was examined in E. coli strains originating from the diseased pigs was demonstrated in approx. 76 % of the isolates. The incidence of drug resistance transfer in single, double, triple and quadruple resistant strains was 11 %, 68 %, 97 % and 98 %, respectively.

Escherichia coli; drug resistance; transmissible drug resistance; pig.

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As in other countries with intensive pig production Escherichia coli infections in pigs represent a serious problem in Norway. Antibiotics and chemotherapeutic agents are used therapeutically and occasionally prophylactically to control these infections. Numerous reports (among others *Smith & Halls* 1966, *Mercer et al.* 1971, *Larsen & Larsen* 1972, *Linton et al.* 1974) show that drug resistant strains of E. coli of intestinal origin are widely distributed both in domestic animals and in humans. Furthermore, such strains are often resistant to several antibiotics (*Aden et al.* 1969, *Fein et al.* 1974), and there is substantial evidence that this drug resistance is transmissible in many cases (*Smith & Halls, Franklin & Glatthard* 1977).

Little information is available on the situation in Norway regarding drug resistance shown by intestinal strains of E. coli from domestic animals. The present work was therefore carried out with the intention of obtaining more information as to the extent of drug resistance, and of the type of resistance patterns existing, as well as to investigate transmissibility of drug resistance in strains of E. coli isolated from the intestinal tract of pigs in Norway.

MATERIALS AND METHODS

Samples were obtained from faecal material from 94 healthy pigs between 8 and 10 weeks of age and from jejunal content from 85 pigs suffering from colienterotoxaemia in their first week of life. The healthy pigs originated from 32 different herds and the diseased pigs from 45 herds, all in southern Norway. Diseased pigs were submitted to necropsy and microbiological examination according to standard techniques. Only samples originating from pigs with pathological changes and microbiological findings consistent with those of colienterotoxaemia were included.

Examination procedure

The samples were examined using a procedure which was in principle the same as that described by *Larsen & Larsen* (1972) and *Wierup* (1975). Each sample was diluted in saline, and 5 ml each of the dilutions 1/200 and 1/400 from faecal material (healthy pigs) and 1/3200 and 1/6400 from jejunal content (diseased pigs) were poured onto agar in Petri dishes (9 cm in

diameter) containing 24 ml of MacConkey agar. The above-mentioned dilutions gave a colony density on the MacConkey agar surface where the colonies still were distinguishable (Fig. 1). Excess material was removed and the plates dried at 37°C

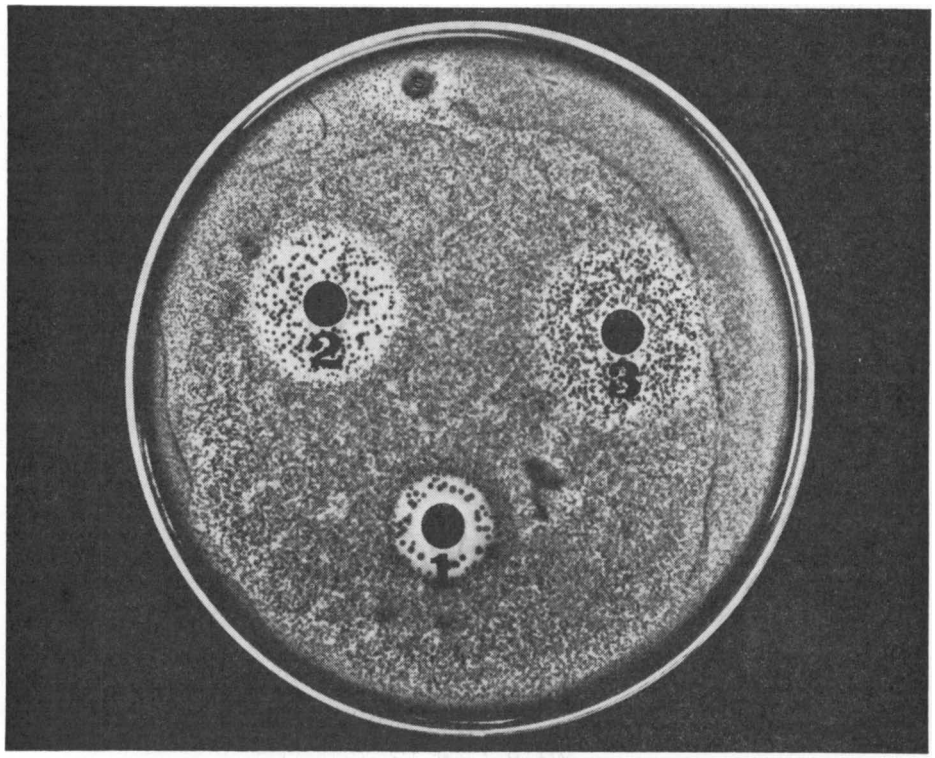

Figure 1. Various types of inhibition zones obtained by inoculating dilutions of intestinal/faecal content on MacConkey agar. Resistant coliform organisms in the zones of inhibition constitute < 25 % (discs 1 and 2) and 25—75 % (disc 3) of the organisms outside the inhibition zones.

for 30 min. Discs (AB-Biodisk) containing the following antibiotics* were then applied: oxytetracycline — 30 μg (Te), streptomycin — 30 μg (S), sulphaisodimidin — 250 μg (Su), neomycin — 30 μg (N), ampicillin — 10 μg (A) and chloramphenicol — 30 μg (C). The plates were kept at room temperature for 1—2 hrs. and then incubated at 37°C for approx. 20 hrs. Of the

* The term antibiotic will in the following text also include sulphaisodimidin.

2 plates from each sample, the one with the most optimal colony density was used to pick 2 colonies with typical coliform appearance from the various inhibition zones* where colonies were present (Fig. 1). In order to determine resistance patterns, these colonies were suspended in 5 ml saline and poured onto the surface of PDM**-Antibiotic Sensitivity Medium***. The test was subsequently carried out as recommended by AB-Biodisk. E. coli strains were identified by means of the IMViC tests (Indol, Methylred, Voges Proskauer, Citrate) and the ability to produce gas from glucose.

Evaluation of antibiotic resistance patterns shown by the E. coli strains was qualitative. Strains presenting an inhibition zone less than 1—2 mm were regarded as being resistant, while strains having a wider zone of inhibition were listed as being sensitive.

Quantitative determination of resistant E. coli in each sample was made by an estimation, on MacConkey agar, of the number of colonies within the inhibition zone compared with the number of colonies outside the inhibition zone. The proportons of resistant E. coli strains of the intestinal/faecal coliform flora were placed, on this basis, into the following percentage groups: 1) Less than 25 %, 2) between 25 and 75 % and 3) more than 75 %. Examples of the 25 and 25—75 percentage groups are shown in Fig. 1.

The strains picked from the MacConkey agar served as donor organisms in the experiments which were performed to find out whether drug resistance was of the transmissible type. Such experiments were carried out only with strains isolated from diseased pigs. From each sample only strains with different resistance patterns were used in the transfer experiment. A lactose negative, nalidixan-resistant strain of E. coli (K 12 F^-, Lac^-, Nal^+) obtained from Dr. H. Williams Smith, England was used as recipient organism. Donor and recipient strains were incubated separately in nutrient broth at 37°C for approx. 20 hrs. Subsequently 0.1 ml of the donor culture and 1 ml of the reci-

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* The inhibition zone is defined as the circular area surrounding the antibiotic-containing disc which shows 1) reduced colony density as compared to the adjacent area or 2) no visible growth of bacteria.

** Paper disc method.

*** AB-Biodisk, Pyramidvägen 7, S-17136 Solna, Sweden.

pient culture were mixed in 10 ml nutrient broth and incubated at 37°C for 20 hrs. This mixed culture was poured onto the surface of PDM-Antibiotic Sensitivity Medium containing 30 µg nalidixan per ml. Discs containing the antibiotics to which the donor strains were resistant were applied on this medium. Further inoculation and incubation procedure was identical with the procedure used for determining the resistance pattern of the E. coli strains.

In each transfer experiment, controls were carried out in order 1) to assure that the donor organisms did not grow on the agar containing nalidixan; 2) to make certain that the recipient organism had not developed resistance to the antibiotics listed and 3) to confirm the resistance patterns previously found in the donor organisms.

The Oxford strain of Staphylococcus aureus S 209 was used routinely to control the effect of the antibiotic containing discs.

RESULTS

Table 1 shows that virtually all pigs harboured antibiotic-resistant strains of E. coli in the intestinal tract. Drug resistance to the various antibiotics tested was more frequently found in strains from healthy pigs than in strains from diseased pigs. The percentage of pigs harbouring Te-, S- and Su-resistant strains was close to 100 in healthy pigs and approx. 90 in diseased pigs. Strains resistant to N, A and C were found in, respectively, 56 %, 44 % and 15 % of healthy pigs and in 26 %, 18 % and 5 % of the diseased pigs.

Table 1. Drug resistance in E. coli strains isolated from healthy and diseased pigs.

Animals	Number of pigs investigated	Number of pigs harbouring resistant E. coli strains (%)	Number of pigs (%) harbouring strains of E. coli resistant to*					
			Te	S	Su	N	A	C
Healthy pigs	94	100	99	98	98	56	44	15
Diseased pigs	85	99	88	88	92	26	18	5

* Te: Oxytetracycline; S: Streptomycin; Su: Sulphaisodimidin; N: Neomycin; A: Ampicillin; C: Chloramphenicol.

The distribution of resistant E. coli strains in the faecal and jejunal coliform flora is shown in Table 2. It can be seen that strains resistant to Te, S and Su dominated the jejunal flora in approx. 70—80 % of the diseased pigs, while in healthy pigs, strains showing the same resistant pattern dominated the faecal flora in approx. 50—70 % of the animals. Strains resistant to N were dominant in the intestinal flora in 74 % of the diseased pigs and in 26 % of the healthy pigs. The percentage of animals having a faecal/jejunal flora dominated by A- or C-resistant strains was 3 and 0 in healthy pigs and 34 and 25 in diseased pigs. Only in a few animals in both groups of pigs did the proportion of resistant E. coli strains in the coliform flora fall into the 25—75 percentage group. With the exception of the distribution of C-resistant strains this finding was most pronounced in the diseased pigs.

Table 2. Relation between the number of resistant E. coli strains and the total coliform flora in healthy and diseased animals. (Abbreviations see Table 1).

Proportion of resistant E. coli strains (percentage groups)	Number of healthy (H) and diseased (D) pigs (%) in the different percentage groups in relation to drug resistance in the strains											
	Te		S		Su		N		A		C	
	H	D	H	D	H	D	H	D	H	D	H	D
< 25	16	22	24	11	35	20	50	26	86	58	92	25
25—75	15	3	12	8	18	9	24	0	11	8	8	50
> 75	69	75	64	81	47	71	26	74	3	34	0	25

Table 3 shows the resistance patterns of the strains investigated in healthy and diseased pigs. In both groups of pigs, Te/S/Su resistant strains were found most frequently, representing 22.1 % and 38 % of the strains from healthy and diseased pigs, respectively. The T/S/Su/N resistant strains were also commonly found in both groups. The frequency of single, double and multiple resistance in E. coli strains isolated from healthy and diseased pigs, and the number of strains resistant to the various antibiotics tested are shown in Table 4. Single, double and multiple resistance was, with 1 exception found more frequently in strains from healthy pigs than in strains from diseased pigs. Triple and quadruple resistant strains comprised

Table 3. Resistance patterns of 721 strains of E. coli isolated from healthy pigs and 518 strains of E. coli isolated from diseased pigs. Figures in brackets indicate the percentage of strains representing more than 5 % of the isolates. (Abbreviations see Table 1).

Resistance pattern	Number of strains in healthy pigs		Number of strains in diseased pigs	
Te	43	(5.9)	13	
S	12		10	
Su	8		4	
N	12		0	
A	6		2	
C	0		0	
Te S	79	(10.9)	34	(6.5)
Te Su	19		15	
Te N	18		15	
Te A	0		4	
Te C	2		0	
S Su	61	(8.4)	49	(9.4)
S N	4		4	
S A	12		0	
Su N	1		1	
Su C	2		4	
Te S Su	160	(22.1)	197	(38)
Te S N	27		6	
Te S A	14		14	
Te Su A	4		0	
Te Su N	2		6	
Te Su C	1		0	
Te A C	1		0	
Te N A	2		0	
S Su A	18		16	
S Su N	8		20	
S Su C	3		0	
S A C	0		1	
Te S Su N	132	(18.3)	64	(12.3)
Te S Su A	28		24	
Te S Su C	16		12	
Te S N A	9		1	
Te S Su N A	15		2	
Te S Su N A C	2		0	
	721		518	

Table 4. Simultaneous drug resistance of E. coli strains and frequency of E. coli strains isolated from healthy and diseased pigs resistant to Te, S, Su, N, A and C. (Abbreviations see Table 1).

Animals	Number of strains tested	Number of strains (%) resistant to 1 or more (1—6) of the antibiotics tested						Number of strains (%) resistant to the antibiotics tested					
		1	2	3	4	5	6	Te	S	Su	N	A	C
Healthy pigs	721	11	27	33	26	2	0.5	80	83	66	29	15	6.5
Diseased pigs	518	6	24	50	19	0.5	—	78	87	80	23	11	3.5

respectively 1/2 and 1/5 of the strains in the diseased pigs and approx. 1/3 and 1/4 of the strains from healthy pigs. Simultaneous resistance to 2 or more antibiotics was found in 89 % and 94 % of the strains from healthy and diseased pigs, respectively, while the corresponding figures for simultaneous resistance to 3 or more antibiotics were approx. 60 % and 70 %.

The number of strains resistant to Te, S and Su was high in both groups of pigs. Also N-resistant strains were common in both healthy and diseased pigs.

The results of the experiment concerning the transfer of antibiotic resistance, which involved 93 strains isolated from diseased pigs, are presented in Table 5. Transmissible drug resistance was found in 76 % of the strains. Transmission of resistance was demonstrated in 11 % of the single resistant strains, and in 68 %, 97 % and 89 % of the double, triple and

Table 5. Transmission and type of transmissibility (partial — en bloc) of drug resistance in strains of E. coli isolated from diseased pigs.

Type of resistance pattern	Number of strains tested	Number of strains with transmissible drug resistance	Transmissibility	
			partial	en bloc
Single resistance	9	1		
Double resistance	35	24	11	13
Triple resistance	31	30	23	7
Quadruple resistance	18	16	8	8
Total	93	71	42	28

quadruple resistant strains, respectively. The extent to which drug resistance was transferred, i.e. partially or totally (en bloc), differed. Partial transmission took place in approx. 50 % of the double and qaudruple resistant strains. The corresponding figure for partial transmission in the triple resistant strains was 76 %.

An approximately equal distribution between partial and en bloc transmission was shown by strains in most of the various resistant pattern groups. However, 19 out of the 23 Te/S/Su resistant strains showed partial transmission as did all 5 of the Te/S/Su/A resistant strains. This contrasted with the situation found in the Te/S/Su/N resistant strains in which partial transmission was demonstrated in only 3 out of 10 strains.

DISCUSSION

The present investigation demonstrated that practically all of the healthy and diseased pigs, which were investigated, harboured drug-resistant E. coli strains in their intestinal tract. Most strains were resistant to oxytetracycline, streptomycin or sulphaisodimidin, while resistance to neomycin, ampicillin and chloramphenicol was more uncommon. A large proportion of the strains was resistant to 2 or more antibiotics. A high percentage of the strains were simultaneously resistant to oxytetracycline, streptomycin and sulphaisodimidin.

These results are in accordance with those presented by *Larsen & Larsen* (1974), who found that 67 %, 71 % and 96 % of strains were resistant to tetracycline, streptomycin and sulphonamides and that approx. 90 % were simultaneously resistant to 2 or more antibiotics. *Linton* (1977), however, reported a frequency of 39 %, 28 %, 33 % and 0.5 % of single, double, triple and quadruple resistant strains, respectively, while the corresponding percentages in the present investigation were 11, 27, 33 and 26 in healthy pigs and 6, 24, 50 and 19 in diseased pigs.

Although only a rough method was used to determine the proportions of resistant strains, it seems clear that a large number of pigs had a coliform flora dominated by resistant E. coli strains. This finding was more pronounced in diseased than in healthy pigs (Table 2). *Smith* (1975) found that E. coli strains resistant to oxytetracycline, streptomycin and sulphonamides constituted about 50 % of the coliform flora in approximately

half of the animals. The present investigation demonstrated, however, that the coliform flora in 3 out of 4 animals was dominated by E. coli strains resistant to oxytetracycline, streptomycin or sulphaisodimidin. The high frequency found in this investigation of diseased animals having a coliform flora dominated by neomycin-resistant E. coli strains is also in disagreement with the above-mentioned investigations.

Transmissible drug resistance was demonstrated in approx. 76 % of the resistant strains investigated. Transmission occurred with a markedly higher frequency in double, triple and quadruple resistant strains than in single resistant strains. The findings regarding the transmission of drug resistance in this investigation seem to agree with those presented by *Franklin & Glatthard* (1977), who demonstrated transmissible drug resistance in 72 % and 95 % of double and multiple resistant strains, respectively. These authors, however, demonstrated transmission of drug resistance in 63 % of the single resistant strains as compared to 11 % in the present investigation. *Larsen & Larsen* (1974) demonstrated transmissible drug resistance in approx. 55 % of the investigated strains, while *Søgaard* (1973) found transmission of drug resistance in only 28 % of the strains tested.

The difference found in this experiment between healthy and diseased pigs consisted mainly of a smaller spectrum of resistance patterns in the strains isolated from the diseased pigs, and a markedly higher number of triple resistant strains in diseased pigs. Triple resistance in the diseased pigs was, to a greater extent than in the healthy pigs, based on oxytetracycline/streptomycin/sulphaisodimidin resistant strains. The differences between the 2 groups of pigs might be due to the different ages of the animals. Several workers have demonstrated that drug resistance is more pronounced in younger than in older animals (*Guinee* 1972, *Wierup* 1975). It might also be based on the fact that the strains from healthy and diseased pigs were isolated from different parts of the gastrointestinal tract. However, the fact that the strains isolated from the diseased pigs supposedly possess special pathogenic characteristics makes it necessary to consider the possibility of a relationship between pathogenic properties and certain drug resistance patterns in these strains. In this connection *Tschäpe & Rische* (1974) emphasized the possibility of a recombination of plasmids controlling entero-

toxin production and certain antigenic structures with plasmids controlling drug resistance (R-factors). The findings presented in this paper indicate that the therapeutic effect of the mentioned antibiotics would be limited as far as colienterotoxaemia in pigs is concerned. Thus, other measures than the usage of drugs seem to be necessary to control this disease.

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SAMMENDRAG

Resistens overfor antibiotika hos stammer av Escherichia coli isolert fra tarmtraktus hos gris i Norge.

Forekomst av antibiotika/kjemoterapeutika (i det etterfølgende benevnt antibiotika) resistente stammer av E. coli ble undersøkt i fecesprøver fra 95 friske griser og i tynntarminnhold fra 85 smågriser som alle var døde av kolienterotoksemi. I praktisk talt alle dyr fra begge grupper ble det påvist E. coli-stammer som var resistente mot ett eller flere av 6 antibiotika som ble undersøkt (oxytetracyklin, streptomycin, sulfaisodimidin, neomycin, ampicillin, chloramphenicol). Hos nesten 100 % av de friske grisene og ca. 90 % av de syke grisene påvistes E. coli-stammer som var resistente mot oxytetracyklin, streptomycin og sulfaisodimidin. Dyr som hadde resistente stammer mot neomycin, ampicillin og chloramphenicol ble ikke så ofte påvist. I 71 % til 81 % av de syke dyrene og i 47 % til 69 % av de friske dyrene ble den koliforme flora dominert av E. coli-stammer som var resistente mot oxytetracyklin, streptomycin og sulfaisodimidin. Tre av 4 syke griser hadde en koliform flora som var dominert av neomycinresistente E. coli-stammer.

Av de 721 antibiotikaresistente E. coli-stammer som ble isolert fra de friske grisene var 11 % monoresistente. Det tilsvarende tall for de 518 antibiotikaresistente E. coli-stammer som ble isolert fra de syke grisene var 6 %. Således var det 89 % og 94 % av stammene fra henholdsvis friske og syke griser som var resistente mot 2 eller flere antibiotika samtidig. E. coli-stammer som samtidig var resistente mot 3 eller flere antibiotika ble påvist i ca. 60 % av isolatene fra de friske dyrene og i ca. 70 % av isolatene fra de syke dyrene. Samtidig resistens mot oxytetracyklin, streptomycin og sulfaisodimidin var det mest vanlige idet ca. 22 % og 38 % av stammene fra henholdsvis friske og syke griser hadde dette resistensmønster.

Overføring av antibiotikaresistens ble påvist i ca. 76 % av E. coli-stammer isolert fra de syke dyrene. Overføring av resistens ble påvist i 11 %, 68 %, 97 % og 98 % fra E. coli-stammer som var resistente mot henholdsvis 1, 2, 3 og 4 antibiotika på samme tid.

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