

# ANTIMICROBIAL DRUG RESISTANCE IN ENTEROTOXIGENIC *ESCHERICHIA COLI* K88, K99, F41 AND 987P ISOLATED FROM PIGLETS IN INDONESIA

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## ABSTRACT

Piglet neonatal diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) containing K88, K99, F41 or 987P fimbrial antigen is known to occur in commercial piggeries in Indonesia. Antimicrobial drugs are commonly used for treatment with little apparent success. A representative sample of ETEC strains containing either K88, K99, F41 or 987P fimbrial antigen collected in 1985 and 1988 were tested for their susceptibility to the antibiotics commonly used in the piggeries studied. The antibiotics included ampicillin, streptomycin, neomycin, oxytetracycline, kanamycin, trimethoprim/sulphonamides, chloramphenicol together with some antibiotics not yet in use in the piggeries which included erythromycin, nitrofurantoin, gentamicin and polymyxin B sulphate. Of the 73 *E. coli* K88 serotypes isolated in 1985, 16.4% were resistant to ampicillin, 69.9% to streptomycin and/or sulphonamides, 28.8% to neomycin, 94.5% to oxytetracycline, 31.5% to kanamycin and 56.2% to chloramphenicol. None of the isolates were resistant to trimethoprim/sulphonamides, nitrofurantoin or polymyxin B sulphate. Of the serotypes tested 92.5% were resistant to at least 2 antibiotics and some were resistant to up to 7 antibiotics. Of the 500 ETEC containing either K88, K99, F41 or 987P fimbrial antigen isolated in 1988, 27.8% were resistant to ampicillin, 62% to streptomycin, 54.4% to neomycin, 95.8% to oxytetracycline, 23.2% to erythromycin, 45.2% to kanamycin, 21.8% to trimethoprim/sulphonamides, 16.2% to chloramphenicol, 66.6% to sulphonamides and 1 isolate was resistant to gentamicin and nitrofurantoin. None were resistant to polymyxin B sulphate. Of the serotypes tested 95.8% were resistant to at least 2 antibiotics and some were resistant to up to 9 antibiotics. The presence of multiple resistance to the commonly used antibiotics is the likely cause of the failure of antibiotics to control neonatal scours associated with ETEC and the associated high mortality of neonatal pigs seen in the piggeries studied.

## ABSTRAK

Diare neonatal pada anak babi yang disebabkan oleh *Escherichia coli* enterotoksigenik yang mempunyai antigen perlekatan K88, K99, F41 atau 987P banyak terjadi pada peternakan babi di Indonesia. Obat-obatan antibiotika sering dipakai untuk pengobatan diare neonatal pada anak babi, akan tetapi kurang atau tidak efektif. Kasus diare dan kematian anak babi neonatal tetap tinggi. Sejumlah isolat *E. coli* K88, K99, F41 atau 987P yang diasingkan dari kasus diare neonatal dalam tahun 1985 dan 1988 diuji sensitifitasnya terhadap obat antibiotika yang sering dipakai untuk pengobatan dan pencegahan diare pada peternakan babi, antara lain: ampisilin, streptomisin, neomisin, oksitetrasiklin, kanamisin, trimetoprin/sulfonamida, khlorampenikol, bersama dengan beberapa macam antibiotika yang belum banyak dipakai pada peternakan babi, antara lain: eritromisin, nitrofurantoin, gentamisin dan polimiksin B sulfat. Dari 73 isolat *E. coli* K88 yang diisolasi dalam tahun 1985 memberikan reaksi resisten terhadap ampisilin 16,4%, streptomisin dan sulfonamida 69,9%, neomisin 28,8%, oksitetrasiklin 94,5%, kanamisin 31,5% dan khlorampenikol 56,2%. Resisten terhadap 2 sampai 6 macam obat antibiotika sebanyak 90,4% (66/73). Semua isolat tersebut masih sensitif terhadap trimetoprin/sulfonamida, nitrofurantoin dan polimiksin B sulfat. Sebanyak 500 isolat *E. coli* K88, K99, F41 atau 987P yang diisolasi dalam tahun 1988 menunjukkan resisten terhadap ampisilin 27,8%, streptomisin 23,3%, kanamisin 45,2%, trimetoprin/sulfonamida 21,8%, khlorampenikol 16,2%, sulfonamida 66,6% dan resisten terhadap gentamisin dan nitrofurantoin masing-masing 1 isolat. Resistensi terhadap 2 sampai 9 macam obat antibiotika sebanyak 95,8% (479/500). Semua isolat tersebut masih sensitif terhadap polimiksin B sulfat. Adanya tingkat resistensi yang tinggi terhadap obat antibiotika yang sering dipakai secara kontinyu mengakibatkan pengobatan dan kontrol diare neonatal akibat *E. coli* K88, K99, F41 dan 987P dengan obat tersebut pada daerah yang diteliti tidak efektif lagi.

## INTRODUCTION

Enterotoxigenic *Escherichia coli* strains are capable of causing diarrhoeal disease in new born piglets and calves. This disease had been reported in many parts of the world (Tzipori, 1985).

Despite the predominance of Islam in Indonesia, the commercial production of pigs is developing rapidly, leading to the emergence of problems asso-

ciated with intensification. One of the more serious problems on most farms is neonatal colibacillosis (Supar, 1986; 1987). Enterotoxigenic *E. coli* (ETEC) have been recognized as a cause of neonatal diarrhoea in pigs in Indonesia. Supar (1986) isolated *E. coli* containing K88 antigen which produced heat labile enterotoxin (Supar, 1987) from piglets up to 14 days of age which had diarrhoea. *E. coli* containing K99 fimbrial antigen were isolated by Supar *et al.* (1988)

from piglets up to 21 days of age with diarrhoea and *E. coli* containing F41 and 987P antigen were isolated by Supar *et al.* (1989a; 1989b) from pigs up to 35 days of age which had diarrhoea. The significance of the disease is indicated by the high neonatal mortality rate of 20%–30% of piglets within the first 2 weeks of age (unpublished data). Antimicrobial drugs are commonly used on commercial farms in Indonesia for the treatment of neonatal scours, with little apparent success (unpublished data).

De Lopez *et al.* (1982a) have reported that ETEC of porcine and bovine origin exhibited multiple antimicrobial drug resistance. The *E. coli* strains of porcine origin were resistant to at least 6 antimicrobial drugs and those from bovine origin to at least 7 antimicrobial drugs.

Antibiotic sensitivity tests of ETEC have not been reported from Indonesia. The purpose of the present study was to test a representative number of ETEC of the K88, K99, F41 and 987P serotypes against a range of antimicrobial drugs, including those drugs commonly used to treat neonatal scours in pigs in Indonesia. The antimicrobial drug sensitivity data will help determine which drugs are suitable for use for the control of neonatal scours in piggeries in Indonesia.

## MATERIALS AND METHODS

### *E. coli* Isolates

*E. coli* were selected from 2 previous surveys undertaken in 1985 (Supar; 1986) and 1988 (Supar *et al.*, 1988; 1989a). The isolates from the 1985 survey included 73 *E. coli* containing K88 fimbrial antigen, which were known to produce heat labile toxin (Supar, 1987), and 20 K88-negative isolates from both normal piglets and from some with diarrhoea.

The isolates from the 1988 survey included 500 strains containing either K88, K99, K99F41, F41 or 987P fimbrial antigens which were isolated from piglets with diarrhoea from piggeries in the Jakarta Capital Territory and the Bogor area. All isolates were maintained on Dorset's egg medium.

The *E. coli* reference strain ATCC 25922 was included as a standard to assure the reliability and reproducibility of the antibiotic sensitivity test.

### Antibiotic Sensitivity Test

#### *Inoculum preparation*

The inoculum was prepared using a method modified from Simmons and Craven (1980) and the

Pasteur antibiogram manual (1981). Briefly, each test organism, including the *E. coli* reference strain, was subcultured onto McConkey agar and incubated at 37°C overnight. Four or 5 similar colonies were selected and inoculated into 1% tryptone water, pH 7.4 and incubated at 37°C for 16–18 hours. The culture was then diluted 1:1,000 in 10 ml of 0.9% NaCl. Glass Petri dishes containing Mueller Hinton agar (4 mm depth of agar per plate) and which had been dried at 40°C for about 30 minutes were inoculated by flooding with 3–4 ml of the diluted culture and then left at room temperature for 4–5 minutes. After this time the excess inoculum was removed and the plates were left to dry at 37°C for about 15 minutes.

### Antibiotic discs

The following antibiotic impregnated discs were used: ampicillin (10 µg), streptomycin (10 µg), neomycin (30 i.u.), oxytetracycline (30 i.u.), kanamycin (30 µg), trimethoprim/sulphonamides (25 µg), sulphonamides (300 µg), chloramphenicol (30 µg), gentamicin (10 i.u.), polymyxin B sulphate (300 i.u.), erythromycin (15 µg) and nitrofurantoin (300 µg). The antibiotic discs were manufactured by either Oxoid (United Kingdom) or Pasteur Institute (France) and were stored at 4°C prior to use.

The antibiotic discs were applied individually to the agar plates following inoculation with organisms. Five antibiotic discs were applied to each 15 cm plate to avoid overlapping of the zones of inhibition. After applying the discs the plates were then left at room temperature for 30 minutes and then incubated at 37°C overnight.

### Reading and evaluation

The diameter of the zone of inhibition around the antibiotic disc was measured using a millimeter rule held on the back of the plate which was illuminated by reflected light. Sensitive, intermediate and resistant strains of *E. coli* were identified by comparing the diameter of the zones of inhibition for each antibiotic disc to those published in the Pasteur antibiogram manual (1981).

## RESULTS AND DISCUSSION

The *E. coli* strain ATCC 25922 was sensitive to all the antimicrobial agents that were used in this assay and was included in order to control the quality of each

batch of medium and each batch of antimicrobial sensitivity tests. The mean diameters of the zone of inhibition and standard deviation of up to 18 replicate tests for each of the antibiotics for ATCC strain 25922 are shown in Table 1. The standard deviation of the

**Table 1.** Antibiotic sensitivity of *E. coli* reference strain 25922

Antibiotic	No of tests	Mean diameter of inhibition (mm)	Standard deviation
Ampicillin (10 µg)	18	20.7	0.9
Streptomycin (10 µg)	18	19.9	0.3
Neomycin (30 iu)	18	19.9	1.9
Oxytetracycline (30 µg)	18	25.9	0.5
Kanamycin (30 µg)	18	23.5	1.4
Trimethoprim/sulphonamides (25 µg)	18	29.1	1.4
Sulphonamides (300 µg)	18	24.4	1.2
Chloramphenicol (30 µg)	18	26.1	0.8
Nitrofurantoin (300 µg)	18	25.4	0.7
Polymyxin B sulphate (300 iu)	18	14.3	1.3
Erythromycin (15 µg)	10	12.1	0.9
Gentamicin (10 iu)	10	23.3	1.4

replicate tests were within the acceptable level described for precision control by Simmons and Craven (1980) and the Pasteur antibiogram manual (1981).

*E. coli* K88 serotypes isolated in 1985 from 3 different piggeries (AT, BT, SH) were tested against 8 antimicrobial drugs which were available for use on the farms at that time. The antibiotics were ampicillin, streptomycin, neomycin, oxytetracycline, kanamycin, trimethoprim/sulphonamide, sulphonamides, chloramphenicol. Two other antibiotics, nitrofurantoin and polymyxin B sulphate, were also included as it was expected that these antibiotics would be used on the farms in the near future. The antibiotic resistance of the 73 serotypes tested are shown in Table 2. A high number of isolates were resistant to streptomycin (76.3%), oxytetracycline (95.7%) and sulphonamides (74.2%) with moderate numbers resistant to chloramphenicol (50.5%) and kanamycin (39.8%). None of the isolates were resistant to trimethoprim/sulphonamides, nitrofurantoin or polymyxin B sulphate. The pattern of resistance of the 20 *E. coli* K88-negative isolates tested was similar to that of the *E. coli* K88 isolates, although 2 of the K88-negative isolates were resistant to trimethoprim/sulphonamides.

**Table 2.** Antimicrobial drug resistance of *E. coli* isolated from piglets in 1985

Piglets/ piggery	<i>E. coli</i>		No of <i>E. coli</i> isolates resistant to :									
	Serotypes	No	AM	S	N	OT	K	SXT	C	SSS	FT	PB
Diarrhoea												
AT	K88	55	4	45	3	51	5	—	41	33	—	—
BT	K88	10	—	6	10	10	10	—	—	10	—	—
SH	K88	8	8	—	8	8	8	—	—	8	—	—
Subtotal		73	12	51	21	69	23	—	41	51	—	—
% of resistance			16.4	69.9	28.8	94.5	31.5	—	56.2	69.9	—	—
Diarrhoea												
AT, BT, SH	K88-ve	10	2	10	4	10	10	2	4	8	—	—
Normal	K88-ve	10	2	10	—	10	4	—	2	10	—	—
Subtotal		20	4	20	4	20	14	2	6	18	—	—
% of resistance			20	100	20	100	70	10	30	90	—	—
Total		93	16	71	25	89	37	2	47	69	—	—
% of resistance			17.2	76.3	26.9	95.7	39.8	2.2	50.5	74.2	—	—

**Note:** AM = Ampicillin  
S = Streptomycin  
N = Neomycin  
OT = Oxytetracycline  
K = Kanamycin  
SXT = Trimethoprim/sulphonamides  
C = Chloramphenicol  
SSS = Sulphonamides  
FT = Nitrofurantoin  
PB = Polymyxin B sulphate

Five hundred of 2,000 *E. coli* isolates containing either K88, K99, F41 or the 987P antigen which had been collected from 5 different piggeries in 1988 were tested against the same 10 antibiotics as those isolates from 1985 as well as the antibiotics erythromycin and gentamicin. The last 2 antibiotics were added as these were then being used in the piggeries for control of neonatal scours. The antibiotic resistance of the 500 isolates is shown in Table 3. Resistance was present to all of the antibiotics except polymyxin B, although only 1 isolate was resistant to nitrofurantoin and only

1 to gentamicin (Table 3). The level of resistance to oxytetracycline was very high (95.8%) which is similar to the situation found in other countries (Timbs *et al.*, 1980a; 1980b; Mudigdo and Peranginangin, 1982; De Lopez *et al.*, 1982a; Dey *et al.*, 1983). In addition to that, of the *E. coli* isolates tested, more than 50% were resistant to streptomycin, neomycin and sulphonamides. These antimicrobial agents have been used continuously over the years for the treatment of colibacillosis in piglets on the farms sampled (unpublished data), furthermore they now appear to have

**Table 3.** Antimicrobial drug resistance of *E. coli* K88, K99, F41 and 987P isolated from piglets in 1988

Piggery	<i>E. coli</i> serotypes	No of isolates tested	No of <i>E. coli</i> isolates resistant to											
			AM	S	N	OT	E	K	SXT	C	SSS	GM	FT	PB
G	K88	22	1	22	19	22	—	19	19	1	16	—	—	—
IB	K88	31	7	12	16	20	2	16	6	10	18	—	1	—
BT	K88	39	4	34	9	38	9	—	4	4	36	—	—	—
L	K88	9	—	9	9	9	2	9	9	—	9	—	—	—
Subtotal		100	12	77	53	89	13	44	38	15	79	—	1	—
% of resistance			12	77	53	89	13	44	38	15	79	—	1	—
G	K99	107	59	89	89	107	15	74	53	48	60	—	—	—
R	K99	37	12	27	27	35	5	25	6	6	25	—	—	—
IB	K99	15	15	15	15	15	1	15	5	4	15	—	—	—
BT	K99	10	5	10	7	10	2	5	—	—	8	—	—	—
L	K99	10	1	10	6	10	2	2	—	—	9	—	—	—
Subtotal		179	92	145	144	177	24	121	64	58	117	—	—	—
% of resistance			51.4	81	80.4	98.9	13.4	67.6	35.6	32.4	64	—	—	—
G	F41	29	—	2	4	29	5	—	—	—	—	—	—	—
R	F41	64	1	7	4	63	42	—	—	—	50	—	—	—
IB	F41	22	2	7	8	22	7	6	—	—	22	—	—	—
BT	F41	3	3	3	3	3	—	3	—	—	2	—	—	—
L	F41	1	1	1	1	1	—	1	—	—	1	—	—	—
Subtotal		119	7	20	20	118	54	10	—	—	75	—	—	—
% of resistance			5.9	16.8	16.8	99.2	45.4	8.4	—	—	63	—	—	—
G	987P	22	3	18	19	19	2	18	2	3	16	1	—	—
R	987P	26	7	17	9	26	11	7	1	1	5	—	—	—
IB	987P	33	9	23	18	30	3	16	—	2	27	—	—	—
BT	987P	8	3	5	5	8	2	5	1	1	8	—	—	—
L	987P	6	3	4	4	6	5	5	3	1	6	—	—	—
Subtotal		95	25	67	55	89	23	51	7	8	57	1	—	—
% of resistance			26.3	70.5	57.9	89.7	24.2	53.7	7.4	8.4	60	1.1	—	—
G	K99F41	7	3	1	—	6	2	—	—	—	5	—	—	—
% of resistance			42.9	14.3	—	85.7	28.6	—	—	—	71.4	—	—	—
Total		500	139	310	272	479	116	226	109	81	333	1	1	—
% of resistance			27.8	62	54.4	95.8	23.2	45.2	21.8	16.2	66.6	0.2	0.2	—

**Note:** AM = Ampicillin      E = Erythromycin      SSS = Sulphonamides  
S = Streptomycin      K = Kanamycin      GM = Gentamicin  
N = Neomycin      SXT = Trimethoprim/sulphonamides      FT = Nitrofurantoin  
OT = Oxytetracycline      C = Chloramphenicol      PB = Polymyxin B sulphate

little impact on piglet mortality rates which ranged between 20% and 35% within the first 3 weeks of age (unpublished data).

In the K88 strains, it is interesting to note the emergence of resistance since 1985 to trimethoprim/sulphonamides with a level of resistance approaching nearly 100% on 1 of the farms sampled. The drug had been used since 1986 on that farm for the control of piglet diarrhoea. On the other hand, resistance to chloramphenicol fell from 56% to 15% overall, in that time. This is probably due to a directive prohibiting the use of chloramphenicol in food producing animals which was implemented by the Directorate General of Livestock Services of the Department of Agriculture in recent year. The pattern of resistance between farms for each serotype is also of interest. On all farms and for all serotypes, the *E. coli* isolated were almost universally resistant to oxytetracycline. Furthermore, for the K88, K99 and 987P isolates, the pattern of resistance was similar with the exception of farm G which had a higher incidence of *E. coli* K99 isolates resistant to chloramphenicol. The 987P isolates were less resistant to trimethoprim/sulphonamides and chloramphenicol. Lower levels of resistance were generally present in the F41 isolates other than to oxytetracycline and to erythromycin where a level of resistance of over 60% was found on farm R.

Multiple resistance was commonly observed on all farms for all of the serotypes. Of the K88 isolates from piglets with diarrhoea made in 1985, 90.3% were resistant to 2 or more antibiotics (Table 4). Nearly 60% of strains were resistant to 4 antibiotics, usually streptomycin, neomycin, oxytetracycline and sulphonamides. The antibiotic resistance pattern of the K88 and non-K88 *E. coli* isolates was similar, which suggested, as had De Lopez *et al.* (1982b), that the genes conferring antibiotic resistance did not share the same plasmid which determined the K88 fimbrial antigen.

Of the 500 isolates tested since 1988, 95% were resistant to 2 or more antibiotics (Table 5). Of the 100 K88 serotypes the level of multiple antibiotic resistance remained unchanged over 3 years at around 90%. The K88 isolates were commonly resistant to 3 to 6 different antibiotics, however the K88 isolates from 1988 had developed a resistance to trimethoprim/sulphonamides (Table 5).

For the 179 K99 serotypes studied, 98.3% showed multiple resistance, with 23% of the isolates being resistant to a total of 8 antibiotics (Table 5). On each of the farms the predominant *E. coli* type was resistant to 6 antibiotics. Of the 119 F41 serotypes studied, 96% had multiple resistance with most isolates resistant to either 2 (43%) or 3 (40%) antibiotics.

**Table 4.** Multiple resistance of *E. coli* isolated from piglets in 1985 to 10 different antimicrobial drugs

Piglets/ piggery	<i>E. coli</i> serotypes	No of isolates tested	No of <i>E. coli</i> isolates resistant to (n) antibiotics							
			n = 1	2	3	4	5	6	7	8
Diarrhoea										
AT	K88	55	7	6	5	31	3	3	—	—
BT	K88	10	—	—	—	6	4	—	—	—
SH	K88	8	—	—	—	6	2	—	—	—
Subtotal		73	7	6	5	43	3	—	—	—
% of resistance			9.6	8.2	6.8	58.9	12.3	4.1	—	—
Diarrhoea										
AT, BT, SH	K88-ve	10	—	—	4	4	2	—	—	—
Normal	K88-ve	10	—	2	—	—	6	—	2	—
Subtotal		20	—	2	4	4	8	—	2	—
% of resistance			—	10	20	20	40	—	10	—
Total		93	7	8	9	47	17	3	2	—
% of resistance			7.5	8.6	9.7	50.5	18.3	3.2	2.2	—

**Note:** Antimicrobial drugs tested:

Ampicillin	Trimethoprim/sulphonamides
Streptomycin	Chloramphenicol
Neomycin	Sulphonamides
Oxytetracycline	Nitrofurantoin
Kanamycin	Polymyxin B sulphate

**Table 5.** Multiple resistance of *E. coli* K88, K99, F41 and 987P isolated from piglets in 1988 against 12 different antimicrobial drugs

Piggery	<i>E. coli</i> serotypes	No of isolates tested	No. <i>E. coli</i> isolates resistant to (n) antibiotics								
			n = 1	2	3	4	5	6	7	8	9
G	K88	22	—	1	2	—	—	18	1	—	—
IB	K88	31	9	—	4	2	4	6	5	1	—
BT	K88	38	—	—	11	18	8	1	—	—	—
L	K88	9	—	—	—	—	—	7	1	1	—
Subtotal		100	9	1	17	20	12	32	7	2	—
% of resistance			9	1	17	20	12	32	7	2	—
G	K99	107	1	12	15	6	12	12	3	31	15
R	K99	37	2	8	1	—	9	10	2	5	—
IB	K99	15	—	—	—	—	—	5	5	5	—
BT	K99	10	—	—	3	—	2	5	—	—	—
L	K99	10	—	—	4	3	1	2	—	—	—
Subtotal		179	3	20	23	9	24	34	10	41	15
% of resistance			1.7	11.2	12.8	5	13.4	19	5.6	22.9	8.4
G	F41	29	3	18	6	2	—	—	—	—	—
R	F41	64	2	22	38	2	—	—	—	—	—
IB	F41	22	—	11	4	—	2	5	—	—	—
BT	F41	3	—	—	—	—	—	3	—	—	—
L	F41	1	—	—	—	1	—	—	—	—	—
Subtotal		119	5	51	48	5	2	9	—	—	—
% of resistance			4.2	42.9	40.3	4.2	1.7	6.7	—	—	—
G	987P	22	2	1	2	2	6	6	3	—	—
R	987P	26	—	9	6	5	2	3	—	1	—
IB	987P	33	2	5	8	2	7	5	3	1	—
BT	987P	8	—	1	1	1	3	—	1	—	1
L	987P	6	—	1	—	—	2	1	1	—	1
Subtotal		95	4	17	17	10	20	15	8	2	2
% of resistance			4.2	17.9	17.9	10.5	21.1	15.8	8.4	2.1	2.1
G	K99F41	7	—	—	5	2	—	—	—	—	—
% of resistance					71.4	28.6					
Total		500	21	89	110	46	58	89	25	45	17
% of resistance			4.2	17.8	22	9.2	11.6	17.8	5	9	3.4

Note: Antimicrobial drugs tested:

Ampicillin	Erythromycin	Sulphonamides
Streptomycin	Kanamycin	Gentamicin
Neomycin	Trimethoprim/sulphonamides	Nitrofurantoin
Oxytetracycline	Chloramphenicol	Polymyxin B sulphate

Of the 95 987P isolates studied, 96% also showed multiple resistance, and the percentage resistance at each level was similar. Only 7 K99F41 isolates were tested with all showing multiple antibiotic resistance, 71% of isolates were resistant to 3 antibiotics and 29% to 4 antibiotics.

Two important points have emerged from this study. The first is that the failure of antibiotics to control neonatal mortalities in piglets in the field is readily explained by the high level of multiple resis-

tance of each of the enterotoxigenic strains studied. The antimicrobial drugs, streptomycin, neomycin and sulphonamides have been used continuously on piggeries in Indonesia for the control of colibacillosis during the time of this study, however the level of neonatal mortality is still 20%—30% during the first 2 weeks of life (unpublished data), suggesting that the use of these drugs is having little impact on the losses due to neonatal scours. The finding that over 50% of *E. coli* isolates collected since 1988 were resistant to

these drugs is therefore not surprising. Similar problems were reported in Singapore by Timbs *et al.* (1980a and 1980b). They reported that there was no significant difference in weight gain, diarrhoea and mortality between piglets given antibiotics and those untreated. Parenteral antibiotic treatment was also ineffective in preventing diarrhoea of piglets associated with ETEC (Timbs *et al.*, 1980a). Antibiotic sensitivity tests of *E. coli* isolated from diarrhoeic piglets showed a wide range of resistance to the commonly used antibiotics, including tetracycline and its derivatives, neomycin, streptomycin and sulphamezanthine (Timbs *et al.*, 1980a and 1980b).

The second point is the emergence of resistance in K88 strains to trimethoprim/sulphonamide which has been routinely used since 1986 for the treatment of diarrhoeic piglets. Resistance to this antibiotic is also present in K99 strains and at a low level in 987P strains. Three drugs, gentamicin, nitrofurantoin and polymyxin B have not been used on any of the piggeries studied and of the 500 isolates tested, only 1 was resistant to nitrofurantoin and 1 to gentamicin. There is clear evidence of the selection of multiple antibiotic resistance through the use of antibiotics in the field.

In view of the lost efficacy of antibiotics to control neonatal colibacillosis studies are proceeding with the development of a multivalent *E. coli* vaccine for swine in Indonesia.

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