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Antimicrobial Resistance of *E. coli* Isolates from Pig Farm Workers, Nondiarrhoeic and Diarrhoeic Piglets

P.Y. CHOO, A. SALAM ABDULLAH and A.R. CHE NYONYA[1]
Department of Animal Science,
Faculty of Veterinary Medicine and Animal Science
Universiti Pertanian Malaysia,
43400 Serdang, Selangor, Malaysia
[1]*Department of Biochemistry and Microbiology*
Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia,
43400, Serdang, Selangor, Malaysia

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ABSTRAK

Kajian kerintangan antibiotik ke atas 274 pencilan E. coli *daripada pekerja ladang, anak babi yang tidak cirit birit dan anak babi yang mengalami cirit-birit terhadap 11 agen antimikrob telah dijalankan untuk menentukan bentuk resistan antibiotik* E. coli. *Lima ladang babi terlibat dalam kajian ini. Kerintangan antimikrob adalah lebih tinggi serta ketara (P<0.05) dan hampir 100% bagi sulphasoxazole, streptomycin dan tetracycline pada pencilan babi bila dibandingkan dengan pencilan manusia. Purata peratusan kerintangan antibiotik di antara ladang tidak menunjukan perbezaan yang ketara (P>0.05) tidak kira sumber pencilannya. Analisis statistik menunjukan bahawa kerintangan antibiotik adalah rendah serta ketara (P<0.05) bagi pencilan dari pekerja ladang bila dibandingkan dengan pencilan-pencilan dari anak babi yang tidak cirit birit dan yang mengalami cirit-birit. Peratusan pencilan yang kerintangan terhadap sekurang-kurangnya 8 antibiotik ialah 27.7, 5.6 dan 1.0 masing-masing bagi babi yang cirit-birit, babi yang tidak cirit birit dan pekerja ladang. Kajian ini juga menunjukan bahawa purata kerintangan antimikrob adalah lebih tinggi bagi ladang yang besar (P<0.01) dan bagi ladang yang tidak mempunyai doktor veterinar yang tetap (P<0.05).*

ABSTRACT

Antimicrobial resistance of 274 E. coli *isolates from farm workers, nondiarrhoeic and diarrhoeic piglets to 11 antimicrobial agents was investigated to determine the antimicrobial resistance pattern of* E. coli. *Five pig farms were involved in this study. Antimicrobial resistance was significantly higher (P<0.01) and almost 100% for sulphasoxazole, streptomycin and tetracyline in porcine isolates compared with human isolates. The mean percentages of antibiotic resistance between farms were not significantly different (P>0.05) irrespective of source of isolate. Statistical analysis showed that the antibiotic resistances were significantly lower (P<0.05) in farm worker isolates than in nondiarrhoeic and diarrhoeic piglet isolates. The percentages of isolates resistant to at least eight antibiotics were 27.7, 5.6 and 1.0 for isolates from diarrhoeic, nondiarrhoeic piglets and farm workers respectively. The present study also indicated that the mean antimicrobial resistance was significantly higher in larger farms (P<0.01) and in farms without a resident veterinarian (P<0.05).*

INTRODUCTION

Frequent and often indiscriminate use of veterinary drugs, particularly the antimicrobial agents, either in animal feeds as a growth promotant or for the treatment of diseases has resulted in the increase and spread of antimicrobial-resistant bacteria in animals both overseas (Findland 1975; Dubel *et al.* 1982) and in Malaysia (Khor *et al.*

1982; Tan and Chin 1982; Bahaman and Liman 1985). This endangers both animal and human health because it is generally accepted that resistant bacterial strains of animal origin can reach humans in contaminated food products.

Salam *et al.* (1986) have shown that the prevalence of antimicrobial-resistant organisms is correlated with the therapeutic use of antimicrobial agents in the farm. They found in an institutional pig farm that *Staphylococcus aureus* was resistant to penicillin and streptomycin (the two antibiotics commonly used in this pig farm) as well as tetracycline and ampicillin. Other studies have shown multiple antibiotic resistance in *E. coli* isolated from pigs (Chin 1983; Bahaman and Liman 1985; Linton 1986).

Although a number of studies abroad have shown the spread of antimicrobial-resistant organisms, particularly *E. coli,* from animal to man (Levy *et al.* 1976; Linton *et al.* 1977; O'Brian *et al.* 1982; Linton 1986), no such study has been carried out in Malaysia. Therefore, the objective of the present study is to determine the relationship between the antimicrobial resistance between *E. coli* isolated from pigs and pigfarm workers.

MATERIALS AND METHODS

Five commercial pig farms were selected for the study, based on farm size, the availability of a resident veterinarian, and location of the farm. Farm 1 was an institutional farm, with no other farms within a 10-km radius. Farms 2 and 5 were the closest–at least 5 km apart, whereas Farms 3 and 4 were located in an intensive pig-farming area of 86 farms with about 90,000 pigs. Two farms (Farms 1 and 5) have resident veterinarians.

The utilization of antibiotics at subtherapeutic and therapeutic levels in feed and for the treatment of diseases respectively in these farms over the last five years is shown in Table 1 and Table 2.

All farms used streptomycin, tetracyline, sulphonamides with or without trimethoprim combinations, tylosin, penicillin and chloramphenicol in the treatment of livestock diseases within the last five years. Gentamycin, neomycin and kanamycin were not used in Farm 1.

All categories of livestock feeds were medicated. The diets prepared were creep, weaner, grower and breeder rations. Creep feeding was introduced to piglets two weeks old. Tetracy-

TABLE 1
Antimicrobial drug usage (therapeutic) in pig farms in the last 5 years

Drug	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Ampicllin	Yes*	Yes	Yes	Yes	Yes
Penicillin	Yes	Yes	Yes	Yes	Yes
Chloramphenicol	Yes**	Yes	Yes	Yes	Yes
Gentamycin	No	Yes	Yes	Yes	Yes
Kanamycin	No	Yes	Yes	Yes	Yes
Neomycin	No	Yes	Yes	Yes	Yes
Streptomycin	Yes	Yes	Yes	Yes	Yes
Tetracylines	Yes	Yes	Yes	Yes	Yes
Sulphomides + Trimethoprim	Yes	Yes	Yes	Yes	Yes
Tylosin	Yes	Yes	Yes	Yes	Yes

*Since 1987

**terminated usage 2 years before the study

TABLE 2
Antimicrobial drug usage (in feed) in pig farms in the last 5 years

Drug	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Neomycin	Yes	No	No	No	No
Tetracyclines	Yes	Yes	Yes	Yes	Yes
Sulphonamides + trimethoprim	No	Yes	Yes	Yes	Yes
Tylosin/ Erythromycin	No	No	Yes	Yes	Yes
Penicillin	No	Yes	Yes	No	Yes
Lincomycin	No	No	No	Yes	No
Virginiamycin	Yes	No	No	Yes	No

cline was used subtherapeutically in all farms. Other antimicrobial agents commonly used at subtherapeutic level were sulphonamides, penicillins, tylosin, lincomycin and viginiamycin. The antibiotic levels varied between 50-200 ppm in pig feed rations. Except for Farm 1, subtherapeutic feeding was not withdrawn in finisher feeds.

The lactating sows and suckling pigs were housed in individual farrowing pens with crates on slatted plastic-coated flooring in all farms. Diarrhoeic piglets were normally medicated with a neomycin-kaolin-pectate drench and with antimicrobial therapy peculiar to farm usage.

Sample Collection and Treatment

Approximately 2 g of faeces from five individual non-diarrhoeic suckling piglets (3-4 weeks old) from different non-diarrhoeic litters were collected in presterilized MacCartney bottles. About 6-9 ml of stool samples from individual diarrhoeic piglets in different litters from the non-diarrhoeic specimens were collected by abdominal squeezing and thumping of diarrhoeal contents into sterile test-tubes.

Human faecal samples were collected from 4-5 farm volunteers from each farm into presupplied sterilized autoclave plastic bags.

All samples were kept in ice (4°C) in polystyrene ice chests or kept in a refrigerator, transported to the laboratory and kept at -20°C until processed.

One gram of a composite faecal sample from a respective study group for each farm was serially diluted in phosphate buffer and plated onto MacConkey agar. Twenty to twenty-five isolated colonies growing on agar plates of the highest dilutions, with 30 to 200 colonies per plate, were randomly selected. These isolates were picked according to the proportion of lactose and non-lactose fermenters. The cultures were reisolated in MacConkey agar at 37°C overnight. Single isolated colonies on MacConkey agar were used in the identification of species using the API-20E Enteric system. Gram staining was performed to confirm gram-negative status. *E. coli* isolates were purified in MacConkey agar and were subcultured in stock culture agar slants and refrigerated. Data was analysed using analysis of variance and comparison of means by Kruska-Walls test to determine the significance of difference between the means.

Antibiotic Susceptibility Testing

Pure cultures were subjected to antimicrobial susceptibility testing by the Kirby Bauer technique on Mueller-Hinton agar on duplicate plates. *E. coli* ATCC strain 25922 was used as the control organism. Antimicrobial susceptibility discs (Difco) were used to determine resistance. The antimicrobial agents used were Ampicillin-Ap (10μg), Cephalothin-Cf (30 μg), Chloramphenicol-C (30 μg), Neomycin-N (30 μg), Streptomycin-S (10 μg), Tetracycline-Te (30 μg), Trimethoprim-Tr (5 μg) and Sulphasoxazole-Su (300 μg).

Zone inhibition diameters were measured with vernier callipers (+ 0.01 mm) and intepreted

TABLE 3
Incidence of antimicrobial resistance of *E. coli* in pigs and farm workers

Source of Isolates	Number of Isolates/Number Resistant	Per cent Resistance
Non-Diarrhoeal Pigs		
Farm 1	23/23	100
Farm 2	23/23	100
Farm 3	18/18	100
Farm 4	18/18	100
Farm 5	19/19	100
Overall	101/101	100
Diarrhoeal Pigs		
Farm 1	15/15	100
Farm 2	17/17	100
Farm 3	19/19	100
Farm 4	18/18	100
Farm 5	19/19	100
Overall	88/88	100
Farm Workers		
Farm 1	15/4	26.7
Farm 2	19/3	15.8
Farm 3	20/13	65.0
Farm 4	19/6	31.6
Farm 5	12/8	66.7
Overall	85/34	41.2

as resistant, intermediate or sensitive according to National Committee for Clinical Laboratory Standards (NCCLS).

RESULTS

A total of 101, 88 and 85 faecal isolates of *E. coli* from nondiarrhoeic and diarrhoeic piglets and farm workers respectively were tested (Table 3).

All faecal isolates from nondiarrhoeic and diarrhoeic piglets were resistant to at least one antimicrobial agent in contrast to 41.2% of isolates from pig farm workers. The incidence of multiple resistance was observed in all isolates from diarrhoeic piglets and in 96.3 + 2.55% of isolates from nondiarrhoeic piglets. However, the mean frequency of multiple resistance in farm workers (29.9 + 6.62%) was significantly lower ($P<0.01$) than in nondiarrhoeic and diarrhoeic piglets.

In isolates from nondiarrhoeic piglets, the antimicrobial resistance was high for tetracycline (98.3%), streptomycin (91.6%) and sulphasoxazole (75.7%) and low for gentamycin and nalidixic acid (0.9%) (Table 4). The resistance frequencies were 35.5, 32.3, 31.4 and 24.7% for chloramphenicol, kanamycin, neomycin and ampicillin respectively.

In isolates from diarrhoeic piglets, the resistance to tetracycline, streptomycin and sulphadimidine was 95.5, 92.3 and 90.9% respectively. The resistance to nalidixic acid was only 1%. However, the resistance to ampicillin, gentamycin and kanamycin and trimethoprim was significantly higher ($P<0.05$) in nondiarrhoeic pig isolates (Table 4) even though these antibacterial drugs were not used in feeds in these farms. The resistant bacteria emerged as a result of extensive therapeutic use of these

TABLE 4
Antimicrobical resistance of isolates of *E. coli* in non-diarrhoeal and diarrhoeal pigs and farm workers

	Antimicrobial Agent (% Resistance)								
Samples	AP	C	Gm	K	N	S	Te	Tr	Su
Non-Diarrhoeal Pigs									
Farm 1	4	17	0	0	0	74	91	17	61
Farm 2	26	4	4	12	13	100	100	13	65
Farm 3	27	89	0	100	100	100	100	17	100
Farm 4	56	67	0	44	39	100	100	28	100
Farm 5	11	0	0	5	6	84	100	0	63
Mean	24.8[a]	35.4[b]	0.8[d]	32.4[a]	31.4[b]	91.6[d]	98.2[d]	15.0[c]	75.8[d]
Diarrhoeal Pigs									
Farm 1	130	20	13	27	47	100	100	47	60
Farm 2	82	77	12	41	47	94	94	100	100
Farm 3	37	37	11	47	42	34	100	32	100
Farm 4	33	67	0	83	83	83	89	56	94
Farm 5	100	68	32	63	63	100	95	95	100
Mean	53.0[b]	53.8[b]	13.6[b]	52.2[b]	56.4[b]	92.2[d]	95.6[d]	66.0[d]	90.8[d]
Farm Workers									
Farm 1	7	20	0	7	7	13	13	13	27
Farm 2	5	5	0	5	5	16	11	5	16
Farm 3	25	5	0	5	5	50	50	5	65
Farm 4	0	11	0	11	11	26	32	0	11
Farm 5	25	0	0	0	0	16	33	0	25
Mean	12.4[a]	8.2[a]	0[a]	5.6[a]	5.6[a]	24.2[c]	27.8[c]	4.6[c]	26.8[c]

a, b Values in different columns bearing different superscripts are significantly different (p <0.05).

c, d Values in different columns bearing different superscripts are significantly different (p < 0.01).

antibacterials and colonized the intestine of non-diarrhoeic piglets. Antimicrobial resistance of diarrhoeal isolates to chloramphenicol varied from 20 to 76.5% between farms but was not significantly different (P>0.05) from the nondiarrhoeic isolates. There was also no significant difference between the resistance of either isolate to streptomycin, tetracycline and sulphasoxazole.

The faecal *E. coli* isolates from farm workers showed resistances not exceeding 30% for all antimicrobial agents tested. The resistance observed was high for tetracycline (27.7%), streptomycin (24.4%) and sulphadimidine (26.6%), Therefore, the antimicrobial resistance in isolates from farm workers was similar to isolates from diarrhoeal and non-diarrhoeal porcine origin although at a significantly lower frequency (P<0.01). Resistance to ampicillin and chloramphenicol was lower, at 12.4% and 8.2% respectively. Resistance against cephalothin, gentamycin, neomycin, kanamycin and trimethoprim was lowest.

The sample sizes of faecal isolates by source and farm were too small for conclusive comparison. However, statistical analysis showed that antibiotic resitances were significantly lower in farm workers' samples than in i) nondiarrhoeic piglets for chloramphenicol, neomycin (P<0.05); tetracycline, streptomycin and sulphadiazine (P<0.01) and ii) diarrhoeic isolates for ampicillin, chloramphenicol, gentamycin, kanamycin, neomyin (P<0.05); tetracycline, streptomycin, sulphasoxazole and treimethoprim (P<0.01).

However, there was no significant difference (P>0.05) between the variance of mean of

TABLE 5

Resistance to number of antibiotics

	Per cent Resistant to at Least			
Samples	2	4	6	8
Non-Diarrhoeai Pigs				
Farm 1	87	17	0	0
Farm 2	100	26	43	0
Farm 3	100	100	89	11
Farm 4	100	94	39	17
Farm 5	95	11	0	0
Mean	96.4[d]	49.6[d]	34.2[c]	5.6[a]
Diarrhoeal Pigs				
Farm 1	100	53	27	13
Farm 2	100	100	82	29
Farm 3	100	63	47	11
Farm 4	100	89	72	22
Farm 5	100	100	90	63
Mean	100.0[d]	81.1[d]	63.6[d]	27.6[b]
Farm Workers				
Farm 1	27	13	13	0
Farm 2	16	11	5	0
Farm 3	55	20	5	5
Farm 4	26	11	11	0
Farm 5	25	0	0	0
Mean	29.8[c]	11[c]	6.8[c]	1.0[a]

a,b Values in the same column bearing different superscripts are significantly different ($P < 0.05$).

c, d Values in the same column bearing diferent superscripts are significantly different ($P < 0.01$).

antimicrobial resistance between farms for all three groups of isolates.

A total of 27, 33 and 16 antibiotypes were observed from nondiarrhoeic pigs, diarrhoeic pigs and farm workers respectively. The more common antibiotypes were i) in nondiarrhoeic pig isolates, TeSSu (26.7%), TeS (13%) and TeSSuNKC (10.9%); ii) in diarrhoeic isolates, TeSSuNKCAPTr (17.1%), TeSSuCApTr (13.6%) and TeSSu (9.1%); and iii) in farm workers, TeSSu (71.%) and TeSSuAp (4.7%) were most common.

The median resistance was 3.8, 6.5 and 0.8 antibiotics for nondiarrhoeic piglets, diarrhoeic piglets and farm workers respectively. Table 5 shows 27.7% of diarrhoeic porcine isolates were resistant to at least eight antibiotics in comparison with 5.6 and 1.0% observed in nondiarrhoeic pig and farm worker isolates ($P<0.05$) respectively. With respect to resistance to at least five antibiotics, 74.3% of diarrhoeic were resistant compared with 39.9 and 6.8% in nondiarrhoeic pigs and farm workers isolates, respectively.

The mean multiple antimicrobial resistance (MAR) index, which is defined as the mean number of resistance observed over the number of antibiotics tested expressed as a percentage (Krupperman 1983), showed that diarrhoeic pigs had the highest MAR index (Table 5).

There were significant differences ($P<0.05$) in MAR index between the three groups of isolates. No significant difference in the mean MAR index between farms was observed.

DISCUSSION

In all five farms under study, the frequency of antimicrobial resistance observed in diarrhoeal swine isolates was significantly higher than those of nondiarrhoeic isolates for ampicillin, gentamycin, kanamycin and trimethoprim ($P<0.05$), although these four antibacterial drugs were not included in feeds. This suggests that the indiscriminate therapeutic use of antimicrobial drugs is more likely to cause resistance than the use of these drugs in feeds as a growth promotant. Our results, which showed a higher prevalence of antimicrobial resistance in large herds with no veterinary supervision than in small herds with veterinary supervision, support our hypothesis. This finding also concurs with a study on pigs in an institutional farm (Salam *et al.* 1986) on the pattern of resistance of *E. coli* and *Staphylococcus aureus*, which showed increased resistance to drugs in the treatment of livestock in the farm.

On an individual farm basis, Farm 1 had lower antibiotic resistance frequencies for chloramphenicol, ampicillin and kanamycin. In this farm the use of chloramphnicol had been terminated two years earlier. Ampicillin was recently introduced in the treatment of piglet diarrhoea while kanamycin was not used. However, in Farm 5 where ampicillin was the therapeutic drug of choice, all diarrhoeic strains isolated were resistant to it. Individual farm preference for the use of chloramphenicol (Farm 2) and kanamycin (Farm 4) for therapeutic use was associated with higher antibiotic resistance frequencies for these drugs in porcine isolates.

The observation of antibiotic resistance patterns of human isolates cannot be compared with piglets' isolates directly as they may not be epidemiologically linked. An extensive epidemiological survey is therefore required involving a larger number of farms. However, the antibiogram characteristics of faecal isolates of *E. coli* from farm workers is significantly lower than nondiarrhoeic piglet isolates for chloramphenicol and neomycin ($P<0.05$), streptomycin, tetracycline and sulphadimidine ($P<0.01$). It is, therefore, consistent with the finding that most antibiotic resistance in human pathogens relates to the use of antimicrobial drugs in human, rather than in veterinary medicine (Prescott and Baggot 1988). Our results also indicated that the bacteria may develop resistance and colonize human intestines irrespective of whether the drugs are used therapeutically or prophylactically in farms. Thus, it is unfair to ban or withdraw the antibiotic usage in animal feeds as a growth-promotant while allowing the indiscriminate use of these drugs therapeutically.

The farm workers involved in our study did not report increased incidences of diarrhoeal episodes while working with livestock. This agrees with the findings of Linton *et al.*(1977), that there is little evidence that strains of *E. coli* enteropathogenic for man arise from animals.

Although no significant differences ($P>0.05$) were observed between the mean resistances to antibiotics tested between farms for all three groups of isolates, it was observed that there was a trend towards higher incidence of antimicrobial resistance with increasing herd size (Farm 1 versus Farms 2-5). Lower antimicrobial resistance prevalence was observed in nondiarrhoeic swine isolates and in farms with veterinary supervision (Farm 1 and 5 versus Farms 2, 3 and 4). However, statistical analysis was not performed as the sample sizes were too small for comparison, and this observation must be substantiated by more extensive epidemiological studies. Another observation which tended to agree with the findings of Bahaman and Liman (1985) was that multiple antimicrobial resistance to the number of antibiotics was higher in large than in small farms. This may explain the differences observed between Farms 1 and 5 (Farm 1 had 50 sows versus Farm 5 which had 500 sows).

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