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PREVALENCE OF ANTIMICROBIAL-RESISTANT *ESCHERICHIA COLI* INFECTIONS IN DIARRHOEIC PIGLETS

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SUMMARY

The prevalence of antimicrobial resistance *Escherichia coli* infection in diarrhoeic piglets was studied. Sixty-five samples were collected from 10 farms in Penang, Perak and Selangor. *Escherichia coli* isolated from the samples were subjected to antimicrobial sensitivity test. The results showed that *Escherichia coli* was highly resistant to oxytetracylcine (100%), nalidixic acid (96.8%), trimethoprim-sulfadimthoxine (95.1%), chloramphenicol (91.9%), enrofloxacin (90.3%), ampicilllin (85.5%), kanamycin (74.2%) and neomycin (71.3%). However, *Escherichia coli* was sensitive to apramycin and colistin sulphate. Most of the *Escherichia coli* isolates showed multiple resistance to the antimicrobials tested in this study.

Keywords: Antibiotic resistance, *Escherichia coli*, faeces, piglets

INTRODUCTION

Diarrhoea, normally caused by *E. coli,* is a common problem encountered by suckling piglets. The highest incidence of life-threatening diarrhoea occurs during the first 3-5 days of life with less serious diarrhoea occurring later (Alexander, 1994). The infections can occur as early as a few hours after the piglets are born. The faeces of the diarrhoea usually will be alkaline due to the secretory diarrhoea caused by the enterotoxins produced by *E. coli*. If the piglets are not treated early, death may result due to severe dehydration and loss of electrolytes. Several farms in Malaysia have reported heavy losses in their piglets during the first week of life and many of these were thought to be due to colibacillosis (Joseph, 1977; Bahaman and Liman, 1985). Thus, antimicrobials as prophylactic medication is commonly used at the preweaning stage. However, excessive use of antimicrobials may increase the risk of resistance in animals and human pathogens (Aarestrup, 2000). This will result in economic losses due to an increased number of untreated cases in the diarrhoeic piglets. Additionally, antibiotic residue in the meat products is another public health concern. The prevalence of antimicrobial-resistant *E. coli* in pigs has been reported over the last 20 years (Bahaman and Liman, 1985). However, no such report particularly in diarrhoeic piglets has been documented since then. This information is important to help the field veterinarian or farmers to select better antibiotics. Thus, the objective of the present research was to study the prevalence of antibiotic-resistant *E. coli* in diarrhoeic piglets.

MATERIALS AND METHODS

Samples collection and transportation

Sixty-four samples were collected from 10 pig farms located in different areas in the country. Eighteen samples were collected from three different farms from Val Dor, Penang. Twenty-six samples were collected from one farm at Segari, and three different farms in Bidor, Perak. Twenty samples were collected from three different farms in Tanjung Sepat, Selangor. Approximately seven faecal samples of diarrhoeic piglets from different litters were collected in each farm. The faecal samples from the rectum of the diarrhoeic piglets were collected using sterile swabs and kept in Amies Transport Media in an ice chest containing ice blocks. Information on the types of antimicrobials used for therapeutic and prophylactic purposes in each farms were noted.

Isolation of E. coli

All the samples were kept in the fridge at a temperature of 4° C before being cultured on MacConkey agar. The samples were cultured on MacConkey agar using sterile wire loop and then incubated at 37° C for 24 hours. Three lactose fermented colonies that formed on MacConkey agar were randomly selected and subcultured on blood agar and incubated at 37° C for 24 hours. After incubation, the colonies formed on blood agar were observed for any sign of haemolysis. For biochemical tests, the colonies formed on blood agar were inoculated in TSI, SIM, citrase, and urease medium and incubated at

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37° C for 24 hours. Oxidase test was also performed as one of the biochemical tests for determination of *E. coli*. The *E. coli* colony from each sample was subcultured onto nutrient agar slant as stock culture.

Antimicrobial sensitivity test

The stock cultures of *E. coli* were subcultured on blood agar and incubated at 37° C for 24 hours. The bacteria colonies were transferred to the sterile test tubes containing about 3 ml of normal saline and then cultured on Mueller Hinton agar. The concentrations of the solution were adjusted by comparing with McFarland Standard 0.5 and streaked over Mueller Hinton agar using a sterile swab. The inoculum were left to dry for 10 minutes before placing the antimicrobial discs on the agar.

Antimicrobial sensitivity test was carried out using 12 antimicrobials disc: ampicillin 10μg (AM), cefadroxil 30μg (CFR), enrofloxacin 5μg (ENR), gentamicin 10μg (GM), kanamycin 30μg (K), apramycin 15μg (APR), chloramphenicol 30μg (C), trimethoprim-sulphadimethoxine 23.75μg (TSD), oxytetracycline 30μg (TE), colistin sulphate 10μg (CL), neomycin 10μg (N), and nalidixic acid 30μg (NA). Six different antimicrobial discs were placed on a plate agar that was inoculated with the culture. The plates with antimicrobial discs were inverted and then incubated aerobically for 18 hours at 37° C. The *E. coli* strain, ATCC 25922, was used as control.

The inhibition zone around each antimicrobial disc was used to determine the strain as sensitive, intermediate or resistant (Table 1). The results within the intermediate zone were classified as resistant.

RESULTS

The results of the antimicrobial sensitivity tests are presented in Table 2. CL and APR were the most sensitive antimicrobial against *E. coli* compared with other antimicrobials. However, the APR was less sensitive compared with the CL. The resistance percentage of CL for the *E. coli* isolated from Tanjung Sepat was 5%, whereas for other areas, it was 0%. The *E. coli* isolated from Tanjung Sepat was more resistant to APR than the samples from other areas. *E. coli* resistance to GM and CFR was similar. However, GM had a lower percentage of resistance compared with N, K, AM, ENR, C, TSD, NA and OTC. The resistance percentages for the N, K, AM and C were similar. Similar results were also found between AM, ENR, C, TSD and NA. The samples from Val Dor had a higher resistance percentage against TSD compared to other areas. *E. coli* resistance to OTC had the highest percentage among the antimicrobials. A high resistance of *E coli* to NA and OTC was found in three areas.

Figure 1 shows number of faecal samples against the number of tested antimicrobials that are resistant to the *E. coli* isolated from all farms. More than 95% of the *E. coli* isolated from the samples were resistant to more than 6 types of antimicrobials tested in this study.

DISCUSSION

The results of the antimicrobial sensitivity show that the degree of antimicrobial resistance in *E. coli* isolated from diarrhoeic piglets was very high and the use of antimicrobials in the livestock industry especially in the swine industry should be given more attention. More than 50% of the *E. coli* isolated showed resistance to 10 types of antimicrobials in the present study. The development of *E. coli* resistance indicates excessive use of antimicrobials in the livestock industry.

Farmers tend to use antimicrobials as aprophylactic and as a growth promoter in their farms. Cefadroxil, trimethoprim-sulfadimethoxine, oxytetracycline, and colistin sulphate are the common antimicrobials used in the

Table 1: Diameter of inhibition zone according to the type of antimicrobials that were tested in this study

No.	Antimicrobial		Disc potency	Zone diameter (millimetre)			
				Control	Resistant	Intermediate	Sensitive
1.	Ampicillin	(AM)	10 μg	16-22	≤13	14-16	≥17
2.	Cefadroxil	(CFR)	30 μg	15-21	≤14	15-17	≥18
3.	Trimethoprim -sulfadimethoxine	(TSD)	23.75 μg	23-29	≤10	11-15	≥16
4.	Oxytetracycline	(OTC)	30 μg	18-25	≤14	15-18	≥19
5.	Colistin sulphate	(CL)	10 μg	11-25	≤8	9-10	≥11
6.	Neomycin	(N)	10 μg	17-23	≤12	13-16	≥17
7.	Gentamicin	(GM)	10 μg	19-26	≤12	13-14	≥15
8.	Kanamycin	(K)	30 μg	17-25	≤13	14-17	≥18
9.	Apramycin	(APR)	15 μg	15-20	≤11	12-14	≥15
10.	Nalidixic acid	(NA)	30 μg	22-28	≤13	14-18	≥19
11.	Enrofloxacin	(ENR)	5 μg	32-40	≤16	17-22	≥23
12.	Chloramphenicol	(C)	30 μg	21-27	≤12	13-17	≥18

Table 2: Percentage of resistance in *E. coli* isolated from different areas

Locations	CL	APR	GM	CFR	N	K	AM	ENR	C	TSD	NA	OTC
Val Dor	0.0	18.7	56.3	68.8	68.7	68.7	93.7	93.7	93.7	100	100	100
Segari and Bidor	0.0	19.2	65.4	65.4	65.4	73.0	92.3	92.3	96.2	92.3	92.3	100
Tanjung Sepat	5.0	30.0	45.0	65.0	80.0	80.0	70.0	85.0	85.0	95.0	100	100
Mean	**1.7**	**22.6**	**55.6**	**66.4**	**71.4**	**73.9**	**85.3**	**90.3**	**91.6**	**95.8**	**97.4**	**100**

CL: colistin sulphate 10μg, APR: apramycin 15μg, GM: gentamycin 10μg, CFR: cefadroxil 30μg, N: neomycin 10μg, K: kanamycin 30μg, AM: ampicillin 10μg, ENR: enrofloxacin 5μg, C: chloramphenicol 30μg, , TSD: trimethoprim-sulphadimethoxine 23.75μg, and NA: nalidixic acid 30μg, TE: oxytetracycline 30μg.

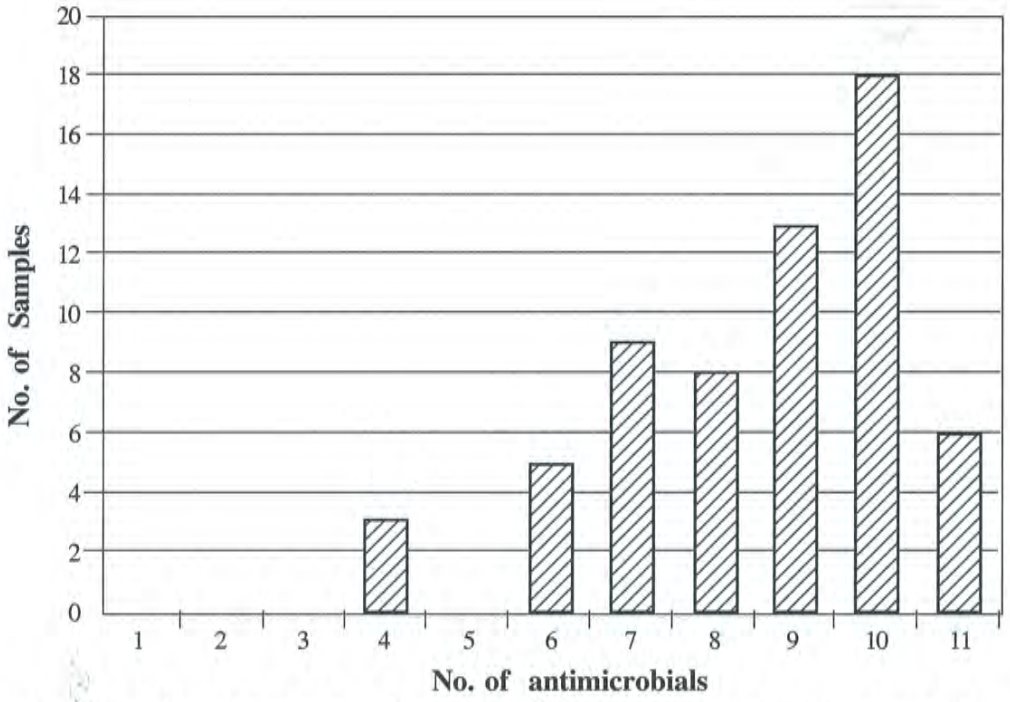

Figure 1: Number of faecal samples against number of tested antimicrobials that are resistant to the *E. coli* isolated from all farms

premix of the diets of pigs. Premix use in the diets of the animals normally depends on the market price of pork. If market price is higher, more premix will be used or *vice-versa*. Constant and long term use of a single antimicrobial may predispose the *E. coli* to develop resistance to the antimicrobial (Baum and Marre, 2005). Additionally, use of antimicrobials at a lower rate than the recommended dose will further encourage *E. coli* to develop resistance. Some farmers will also use water soluble antimicrobials for the animals when the environment jeopardises the health of the animals especially during rainy seasons.

Consistent use of the same antimicrobial groups in a farm tends to promote bacterial resistance to the antimicrobials used. In order to avoid development of resistance, different types of antimicrobials need to be used from time to time. In this study, apramycin and colistin sulphate had the lowest resistance by the *E. coli*; this could be due to the antimicrobials not being commonly used in the pig farms. Thus, with low exposure of the bacteria to this antimicrobial, resistance development chances will be slim.

The percentage of resistance in *E. coli* isolated for neomycin, oxytetracycline, chloramphenicol, and nalidixic acid were 71.3%, 100%, 91.9% and 96.8% respectively. If the present results are compared with the findings obtained by Bahaman and Liman (1985) and Choo

(1991), resistance to these antimicrobials has been found to have increased tremendously. Among these antimicrobials, nalidixic acid has seen the most significant change as the percentage of resistance was 90% higher than the values obtained in previous studies (Bahaman and Liman, 1985). This indicates that nalidixic acid has been used extensively and constantly for the past 20 years in the swine industry.

More than 95% of *E. coli* developed multiple antibiotic resistance in the present study. This result is consistent with the findings of Radu *et al.* (1997), who reported that more than 90% of *E. coli* strains isolated from frozen beef and duck intestine samples were resistant to two or more antibiotics tested. Bahaman and Liman (1985) reported that multiple antimicrobial resistant *E. coli* was prevalent in the commercial pig farms whilst in the smallholders, single antibiotic resistant *E. coli* was more dominant. The use of antimicrobials in the farm is closely related to affordability and financial status of the farmers.

CONCLUSION

The degree of antimicrobial resistance in *E. coli* isolated from 10 selected pig farms was very high. The most probable cause for this condition could be due to excessive use of antimicrobials in the pig farms. However, we should not rule out the possibility that the *E. coli* may have acquired resistance from other bacteria in the environment and feed given to the animals in the farms. The results from this study indicate that *E. coli* resistance has become more serious compared to the studies done over the past 20 years.

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