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SHORT COMMUNICATION

MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX AND PLASMIDS IN *ESCHERICHIA COLI*

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SUMMARY

A total of 198 strains of *Escherichia coli*, 134 from frozen beef samples and 64 from duck intestine samples, were examined for resistance to six antibiotics and for presence of plasmid DNA. More than 90% of the strains were resistant to two or more antibiotics tested, with the most common resistance being penicillin (100%), cephalothin (76.8%) and streptomycin (64.1%). The least resistance was observed to ampicillin (9.6%) and kanamycin (12.6%). Multiple antibiotic resistance indexing of the strains showed that more than 90% originated from high risk contamination environments where antibiotics were often used. Plasmids ranging in size from 1.2 to 65 MDa were detected in 44 of 64 (68.7%) isolates from duck intestine samples, and 79 of 134 (59%) isolates from beef samples. The plasmid analysis indicated the presence of more than a single clone with the same antibiotic resistance pattern. The data obtained in this study indicated that animals form a reservoir for multiple-resistant and plasmid-containing *Escherichia coli* in the study area.

Keywords: Multiple antibiotic resistance, plasmid, *Escherichia coli*.

The widespread use of antibiotics as therapeutic agents and as feed additives in food-producing animals may lead to the development of antibiotic resistant micro-organisms (O'Brien *et al.*, 1982). Human infections with resistant micro-organisms present difficulties in therapy by limiting the choice of antibiotics. Therefore, any source of antibiotic-resistant micro-organisms must be viewed with concern, and the occurrence of antibiotic-resistant *E. coli* in animals represents a potential hazard to human health especially to people who consume products made from improperly prepared meat or post-contamination from pet animals. Once these organisms enter and colonize the human gut they may transfer their resistance to bacteria already present in the gut or to sensitive pathogens with which their host may become infected (Linton *et al.*, 1977). The present study, which was part of a survey of antibitoic resistant micro-organisms (ARM) (Son *et al.*, 1995; Son *et al.*, 1996; Son *et al.*, 1997a; Son *et al.*, 1997b; Rusul *et al.*, in press), was undertaken to assess the extent to which food animals are contaminated with antibiotic-resistant strains of *E. coli*.

The frozen beef and intestine samples from duck (*Anas domestica*) were collected from retail outlets around Selangor and the Federal Territory and isolates of *E. coli* determined by standard laboratory procedures (Edwards and Ewing, 1972) were isolated. Disk conducted as described by Bauer *et al.* (1966). The *Escherichia coli* strains were tested against the following antibiotic discs (BBL Sensi-Disc, Becton Dickinson): ampicillin, 10 mg; cephalothin, 30 mg; kanamycin, 30 mg; penicillin, 5 mg; streptomycin, 25 mg; and trimethoprim-sulfametoxazole, 25 mg. Bacteria were suspended in saline to give a density of two McFarland standard, diluted 1:20, and streaked over Mueller Hinton agar plates using a sterile cotton swab. The antibiotic discs were placed on the agar surfaces sufficiently separated from each other so as to prevent overlapping of the inhibition zones. After 30 min, the plates were inverted and incubated at 37°C overnight. Characterization of strain as sensitive, intermediate or resistant was based on the size of inhibition zones around each disc and the manufacturer's instruction. The multiple antibiotic resistance (MAR) index is defined as a/b where 'a' represents the number of antibiotics to which the particular isolates was resistant and 'b' the number of antibiotics to which the isolate was exposed (Krumperman, 1983). Organisms were screened for plasmid DNA by the procedure of Birnboim and Doly (1979) and were characterized as described previously (Son *et al.*, 1997a).

A total of 198 *Escherichia coli* strains (64 from duck intestine samples and 134 from frozen beef samples) were isolated. The results of the antibiotic

Table 1. Frequency of antibiotic resistant *Escherichia coli* strains from beef and duck.

Antibiotics	Percentage of resistance		
	Strains from beef (n = 134)	Strains from duck (n = 64)	Mean
Ampicillin	6	17.2	9.6
Cephalothin	68.7	93.8	76.8
Kanamycin	0.8	37.5	12.6
Penicillin	100	100	100
Streptomycin	53.7	86	64.1
Trimethoprim-sulfamethoxazole	39.6	93.5	57.6

Resistance to cephalothin and penicillin was greater than 70%. The least resistance was observed for ampicillin (9.6%) and kanamycin (12.6%). Resistance to trimethoprim-sulfamethoxazole (57.6%) and streptomycin (64.1%) was intermediate. Differences were found in antibiotic susceptibility depending on the sample source. The strains from beef showed marginally less resistance to ampicillin compared to the strains from duck samples. There was no variation in resistance to penicillin among the isolates from both sources. Resistance to cephalothin, kanamycin, streptomycin and trimethoprim-sulfamethoxazole was comparatively higher among the strains from duck intestine samples.

The multiple antibiotic resistance (MAR) index of the *E. coli* strains are shown in Table 2. MAR index values higher than 0.2 are considered to have originates from high risk sources of contamination like humans, commercial poultry farms, swine and dairy cattle where antibiotics are often used. MAR index values of less than or equal to 0.2 indicate a strain originated from animals where antibiotics are seldom or never used (Krumperman, 1983). The MAR index for the *E. coli* isolates from beef samples ranged from 0.1 to 0.8, while the MAR index for the isolates from duck intestine samples ranged between 0.5 to 1.0. Only one strain from duck intestine sample exhibited resistance to all six antibiotics tested. Over 90% of the *E. coli* isolates were resistant to two or more antibiotics. The logical explanation for the MAR index is that more than 90% of the antibiotic resistant strains from both sources originated from high risk sources of contamination like swine, poultry, cattle and human environments where antibiotics are often used. There is a large body of literature reviewed by Novick (1981) demonstrating that the subtherapeutic use of antibiotics in animal husbandry has promoted the emergence and maintenance of MAR pathogenic bacteria in the faecal environments of these animals. In addition, the wide use and abuse of antibiotic in human therapy has produced MAR pathogenic micro-organisms in the faeces of human as well. The release of pathogenic bacteria in the faeces results in dispersal into the environments where plasmid exchange between bacteria is readily facilitated (especially in aquatic systems) and results in a higher frequency of MAR forms. It has been suggested that isolates with an identical MAR index and the same resistance profile may have a common origin (Kaspar *et al.*, 1990).

Table 2. Multiple antibiotic resistance (MAR) index of *Escherichia coli* strains from beef and duck and the percentage of occurrence.

MAR index	Percentage of occurrence	
	Strains from beef (n=134)	Strains from duck (n=64)
0.1	17	-
0.2	-	-
0.3	40	
0.4	-	
0.5	46	6
0.6	28	29
0.7	-	-
0.8	3	28
0.9	-	-
1.0	-	1

Approximately 44 of 64 (68.7%) isolates from duck intestine samples and 79 from 134 (59%) isolates from frozen beef samples harboured plasmid DNAs (data not shown). The plasmids ranged from 1.2 to 65 megadalton (MDa). The total number of plasmids in any given bacterial population can affect the results of the strain analysis (Tacket *et al.*, 1984). Without the plasmid profiles, we could have thought that the *E. coli* with similar antibiotic resistance patterns were derived from the same ancestral strain. In this study, plasmid analysis helped us to recognize the presence of more than a single clone with the same resistance pattern, and reflects the additional and diverse microbial contamination that can, and usually does, occur during distribution and preparation of raw food for retail sale in the study area. The value of plasmid analysis in ecological studies is self-evident.

The isolation of multiple-resistant *E. coli* containing plasmids demonstrates that the problem of multiple-resistant bacteria exists in Malaysian food sources. The continued agricultural use of medicated feeds and their application in the rapidly developing animal husbandry industries will accentuate current problems of antibiotic-resistant microflora in foods, and foster greater disemmination of virulent and resistant bacterial pathogens in the natural environment and in the human food chain.

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REFERENCES

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standard single disk method. *Am. J. Clin. Pathol.* **36:** 493-496.

Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7:** 1513-1523.

Edwards, P.R. and Ewing, W.H. (1972). Identification of *Enterobacteriacea*, 3rd ed. Burgess Publishing. pp 7-47.

Kaspar, C.W., Burgess, J.L., Knight, I.T. and Colwell, R.R. (1990). Antibiotic resistance indexing of *Escherichia coli* to identify the sources of faecal contamination in water. *Can. J. Microbiol.* **36:** 891-894.

Krumperman, P.H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high risk sources of faecal contamination of foods. *Appl. Environ. Microbiol.* **46:** 165-170.

Linton, A.H., Howe, K., Bennett, P.M., Richmond, M.H. and Whiteside, E.J. (1977). The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. *J. Appl. Bacteriol.* **43:** 465-469.

Novick, R.P. (1981). The development and spread of antibiotic resistant bacteria as a consequence of feeding antibiotics to livestock. *Ann. New York Acad. Sci.* **368:** 23-59.

O'Brien, T.F., Hopkins, J.D., Gilleece, E.S., Medeiros, A.A., Kent, R.L., Blackburn, B.O., Holmes, M.B., Reardon, J.P., Vergeront, J.M., Schell, W.L., Christenson, E., Blissett, M.L. and Morse, E.V. (1982). Molecular epidemiology of antibiotic resistance in *Salmonella* from animals and human beings in the United States. *New Engl. J. Med.* **307:** 1-6.

Rusul, G., Son, R., Abdul Hamid, S.N. and Arumugaswamy, R.K. (1997). Antibiotic resistance of *Listeria* species isolated from food sources in Malaysia. *Lett. Appl. Microbiol (In press).*

Son, R., Ansary, A., Salmah, I. and Maznah, A. (1995). Survey of plasmids and resistance factors among veterinary isolates of *Salmonella enteritidis* in Malaysia. *World J. Microbiol Biotech.* **11:** 315-318.

Son, R., Karim, M.I.A., Rusul, G. and Yusoff, K. (1996). Plasmids and antimicrobial resistance among *Campylobacter jejuni* isolated from retail fresh poultry. *AsPac J. Mol. Biol. Biotech.* **4(2):** 106-111.

Son, R., Rusul, G. and Karim, M.I.A. (1997a). Conjugal transfer of plasmids and antibiotic resistance among *Escherichia coli* isolated from animals in a rural area in Sarawak (Malaysia). *J. Appl. Microbiol.* **82:** 240-244.

Son, R., Rusul, G., Sahilah, A.M., Zainuri, A., Raha, A.R. and Salmah, I. (1997b). Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Telapia (*Telapia mossambica*). *Lett. Appl. Microbiol* **24:** *(In press).*

Tacket, C.O., Shahid, N., Huq, M.I., Alim, A.R.M.A. and Cohen, M.L. (1984). Usefulness of plasmid profiles for differentiation of *Shigella* isolates in Bangladesh. *J. Clin. Microbiol.* **20:** 240-244.

RINGKASAN

INDEK KETAHANAN ANTIBIOTK BERBILANG (MAR) DAN PLASMID DALAM ESCHERICHIA COLI

Sejumlah 198 strain Escherichia coli, *134 daripada sampel daging sejuk beku dan 64 daripada sampel usus itik telah diperiksa ketahanannya terhadap enam antibiotik dan untuk kewujudan DNA plasmid. Lebih dari 90% strain ini tahan terhadap dua atau lebih antibiotik diuji, dengan antibiotik paling umum ialah penisilin (100%), sefalotin (76.8%) dan streptomisin (64.1%). Ketahanan paling sedikit dicerapkan ialah terhadap ampisilin ampisilin (9.6%) dan kanamisin (12.6%). Pengindeksan ketahanan antibiotik berbilang strain ini menunjukkan lebih 90% berpunca daripada pencemaran persekitaran risiko tinggi di mana antibiotik sering diguna. Plasmid yang saiznya berjulat diantara 1.2 hingga 65 MDa dikesan dalam 44 dari 64 (68.7%) isolat sampel usus itik dan 79 daripada 134 (59%) isolat sampel daging. Analisis plasmid menunjukkan wujudnya lebih dari satu klon yang mempunyai pola ketahanan antibiotik sama. Data yang diperolehi dalam kajian menunjukkan haiwan yang merupakan reservoir untuk* Escherichia coli *mengandungi plasmid berbilang tahan dalam kawasan kajian.*