TRANSFERABLE ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES OF *ENTEROBACTERIACEAE* IN MALAYSIA

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SUMMARY

Enterobacteriaceae *isolated from clinical sources were examined for antibiotic resistance and the ability to transfer resistance to* ***Escherichia coli.*** *Twenty-nine out of 80 strains tested transferred part or all of their resistance genes. The strains carrying R plasmids included the genera* ***Escherichia, Klebsiella, Salmonella, Enterobacter, Proteus, Providencia*** *and* ***Citrobacter.*** *These results indicate that R plasmids possibly play a major role in the emergence of antibiotic resistance among clinical isolates of* ***Enterobacteriaceae.***

INTRODUCTION

Antibiotic resistance mediated by R plasmids is now recognised as a worldwide problem with serious medical and public health implications. R plasmids not only render their bacterial hosts resistant to a considerable number of antibiotics but also enable them to transfer this property to other bacteria.

The occurrence of R plasmids in clinical isolates of *Enterobacteriaceae* has been reported in many countries. [1,2,3,4,5,6] In Malaysia, the occurrence of R plasmids in *Salmonella typhi* [7] and *Salmonella* spp. [8] have been previously reported.

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In an attempt to assess the extent of the clinical importance of R plasmids, a study was undertaken on the occurrence of transferable antibiotic resistance in clinical isolates of *Enterobacteriaceae.*

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains were all from clinical sources. The strains were isolated from urine, stool, blood and pus specimens received by the Bacteriology Division, Institute for Medical Research, between January to December, 1981.

Antibiotic Sensitivity Tests

All isolates were routinely screened for antibiotic sensitivity by the Comparative Disc diffusion method. [9] Strains resistant to at least 3 antibiotics

TABLE I
DISTRIBUTION OF R PLASMIDS AMONG CLINICAL ISOLATES

Species	No. of Strains Tested	No. of Strains with R. Plasmids
Salmonella spp.	31	15
Escherichia coli	22	5
Klebsiella spp.	10	5
Shigella spp.	8	0
Enterobacter spp.	3	1
Proteus spp.	3	1
Providencia spp.	1	1
Citrobacter spp.	1	1
Serratia spp.	1	0
Total	80	29

were selected for conjugation experiments. These strains were retested for their antibiograms by replication using a multipoint inoculator (Denley-Tech Ltd., England) onto antibiotic incorporated agar plates and examining for growth after 24 hours. Strains not inhibited by 30 mcg/ml of ampicillin, chloramphenicol, kanamycin, tetracyclin, streptomycin; 10 mcg/ml of gentamicin, neomycin; 50 mcg/ml of nalidixic acid and 300 mcg/ml of sulphadiazine were considered resistant. The plates were prepared by incorporating the relevant concentrations of the antibiotics into Diagnostic Sensitivity Test agar (Oxoid).

Conjugation Experiments

Conjugation experiments were carried out by a method described previously. [8] The recipient organism used was *Escherichia coli* W1802, resistant to nalidixic acid. Appropriate dilutions of the conjugation mixtures were plated on selection plates. The selection plates consisted of MacConkey Agar (Oxoid) containing 50 mcg/ml of nalidixic acid and one of the following antibiotics : ampicillin (30 mcg/ml), chloramphenicol (30 mcg/ml), gentamicin (10 mcg/ml), kanamycin (30 mcg/ml), tetracycline (30 mcg/ml). Representative transconjugant colonies growing on these selection plates were tested by multipoint inoculation to ascertain the patterns of antibiotic resistances transferred.

TABLE II
FREQUENCIES OF INDIVIDUAL RESISTANCES

	A	C	G	K	N	S	Su	T
No. of strains	74	54	24	39	35	71	78	55
Frequency (%)	93	68	30	49	44	89	98	69

Key: A = Ampicillin, C = Chloramphenicol, G = Gentamicin, K = Kanamycin, N = Neomycin, S = Streptomycin, Su = Sulphadiazine, T = Tetracycline.

RESULTS

Eighty multiple-resistant strains were selected for conjugation experiments from a total of 550 strains of *Enterobacteriaceae* isolated.

Twenty-nine out of 80 (36 percent) of these antibiotic-resistant strains were shown to possess R plasmids. (Table I). The strains carrying R plasmids included the genera *Escherichia, Klebsiella, Salmonella, Enterobacter, Proteus, Providencia* and *Citrobacter.*

Table II shows the frequencies of the individual resistances of the 80 strains. Resistance to sulphadiazine was most common (98%) followed by ampicillin (93%), streptomycin (89%), chloramphenicol (68%), tetracycline (69%), kanamycin (49%) and neomycin (44%). Resistance to gentamicin was least common (30%).

Conjugation experiments showed that 29 of the strains were able to transfer all or part of their

TABLE III
R. PLASMIDS IN CLINICAL ISOLATES OTHER THAN *SALMONELLAE*

Strain	Source	Resistance Pattern	Patterns Transferred
E.coli	Pus	ACGKNSSuT	ACGKSU/AGKNSu/AGKSu/CN
E.coli	Pus	AKNSSuT	AKNSSu/A
E.coli	Urine	ACNSSuT	ACSSuT
E.coli	Urine	AKNSSuT	AKNSSuT*/KN
E.coli	Urine	ACSSuT	AT/T
Klebsiella spp.	Urine	ACGKNSSu	ACGKNSu
Klebsiella spp.	Blood	ACGKNSSuT	ACGKNSSuT*/ ACNSSu
Klebsiella spp.	Urine	AGKNSSuT	AGKNSSuT*/AKNSSuT/ASSuT
Klebsiella spp.	Urine	ACKNSSuT	ACKNSSuT*/ACKNSSu
Klebsiella spp.	Urine	ACGKSSuT	ACGKSSu
Enterobacter spp.	Pus	ACGKNSSuT	ACGKNSSuT*
Providencia spp.	Urine	ACGKNSSuT	ACGKNSSuT*/ACGKNSu/ACKNSSuT
Proteus spp.	Pus	ACGKNSSuT	ACGKNSSuT*
Citrobacter spp.	Pus	ACKNSSuT	AKSSu/CSSuT/N

* Complete pattern transferred

resistances to the recipient *E. coli*. The antibiograms, clinical source and the resistance patterns transferred are shown in Tables III and IV.

DISCUSSION

The study demonstrated that 29 out of 80 strains (36%) of *Enterobacteriaceae* isolated from clinical sources carried R plasmids. They included 15 strains of *Salmonella* spp., 5 of *E. coli*, 5 of *Klebsiella* spp. and 1 each of *Enterobacter* spp., *Proteus* spp., *Providencia* spp. and *Citrobacter* spp. These R plasmid carrying strains were isolated from stool (14), urine (8), pus (5) and blood (2) specimens.

In a study of the occurrence of transferable antibiotic resistance in clinical isolates of enteric bacteria, [10] 24% of randomly selected bacteria belonging to the genera of *Klebsiella, E. coli, Shigella* and *Salmonella* were shown to possess R plasmids. Egawa *et al* [11] studied 51 strains of *Enterobacteriaceae* isolated from patients at the Boston City Hospital and showed that 20 strains possessed R plasmids.

These results demonstrate that R plasmids play a major role in antibiotic resistance in the *Enterobacteriaceae*. The importance of R plasmids is due to the fact that they confer resistances to several antibiotics simultaneously. In our study 26 out of 29 (90%) R plasmid carrying strains were resistant to at least 6 antibiotics. In addition, R plasmids are capable of transferring all or part of their resistances to sensitive recipients. In our conjugation experiments, the whole resistance pattern was transferred to the sensitive *E. coli* in 15 strains.

Outbreaks of infection caused by antibiotic-resistant *Enterobacteriaceae* are an increasing problem in many parts of the world. In Mexico there was an outbreak of typhoid fever due to chloramphenicol-resistant *S. typhi* in 1972-1973 [12] and in Bangladesh, an extensive outbreak of shigellosis due to antibiotic-resistant *Shigella dysenteriae* 1 occurred in 1973. [13] In addition, an equally serious problem is that of nosocomial infections with antibiotic-resistant Gram-negative bacilli. Nosocomial infections with multi-resistant *Salmonella* have become increasingly common, causing severe morbidity and mortality in paediatric units and nurseries. In the United States of America, there is an estimated 5 cases of nosocomial infections per 100 patients discharged and 51% of such infections are caused by *E. coli, Proteus, Klebsiella, Enterobacter* and *Pseudomonas*. [14]

There is thus a need to enforce strict control of antibiotic usage to reduce the selection pressure of antibiotics and to implement good infection control

TABLE IV
R PLASMIDS IN CLINICAL ISOLATES OF SALMONELLAE

Serotype	Source	Resistance Pattern	Patterns Transferred
S.stanley	Stool	ACGKNSSu	ACGKNSu/AKNSSu
S.stanley	Stool	AGKNSSu	AGKNSSu*
S.stanley	Stool	AGKNSSu	AGKNSSu*
S.stanley	Stool	ACGKNSu	ACGKNSu*/Su
S.stanley	Blood	ACGKNSSu	ACGKNSSu*/ACGKSu/ACGKNSu
S.stanley	Stool	ACKSuT	AKSu/ACKSu/KSu
S.krefeld	Stool	ACKNSSu	AKNSSu
S.krefeld	Stool	ACGKNSSu	KNSSu
S.krefeld	Stool	ACGKNSSuT	ACK/AK/AC
S.krefeld	Stool	AGKNSSuT	AKSSu/AK/A
S.lexington	Stool	ACKNSSuT	ACKNSSuT*
S.lexington	Stool	AGK	AK/A
S.infantis	Stool	AGKNSSu	AGKNSSu*/AGKNSu
S.paratyphi B	Stool	ACKNSSuT	ACKNSSuT*/CSSuT
S.typhimurium	Stool	ACGKNSSu	ACGKNSSu*/ ACGKNSu

* Complete pattern transferred.

procedures in hospitals to prevent the spread of resistant organisms.

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