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The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals

By H. WILLIAMS SMITH
Animal Health Trust, Stock, Essex

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Following upon its discovery in Japan in 1959, infective resistance has been shown to be important in the spread of drug resistance among members of the Enterobacteriaceae; most of the available evidence has been reviewed by Watanabe (1963). The principal feature distinguishing infective resistance from other forms of drug resistance is that it can be transmitted from an organism of one species to an organism of the same or of a different species by contact. Recently, Smith & Halls (1966*a*) showed that most of the drug resistance in non-pathogenic *Escherichia coli* isolated from the faeces of healthy human beings, calves, pigs and fowls in Britain was infective and transmissible to the principal *Salmonella* serotypes and to some of the *E. coli* serotypes pathogenic for these species. Since non-pathogenic *E. coli* form the bulk of the enterobacteriaceal flora of the alimentary tract of man and domestic animals it is probable that these bacteria are the reservoir from which the numerically inferior pathogenic members of the group, e.g. *Salmonella*, *Shigella* and certain serotypes of *E. coli*, may acquire infective drug resistance.

As far as salmonellas are concerned, Datta (1962), Anderson & Datta (1965) Anderson & Lewis (1965) and Anderson (1965) have reported on the incidence of infective resistance in *Salmonella typhimurium* in man and domestic animals in Britain. Anderson & Lewis (1965), for example, found that 61% of 450 strains examined by them between December 1964 and February 1965 were resistant to one or more drugs; in a high proportion of these strains the resistance was of the infective type.

It is generally accepted that certain serotypes of *E. coli* are implicated in neonatal diarrhoea in human beings (Taylor, 1961), neonatal and post-weaning diarrhoea and bowel oedema in pigs and bacteraemia in fowls, calves and lambs (Sojka, 1965) There is not agreement as to the part *E. coli* plays in diarrhoea in calves and lambs. Antibacterial drugs are commonly used in the treatment of all these diseases. It seemed important, therefore, to determine the incidence of infective drug resistance in strains of *E. coli* isolated from babies and domestic animals suffering from these diseases; the results are reported in this paper.

MATERIALS AND METHODS

Escherichia coli *strains*

All the strains of *E. coli* were, as far as could be determined, epidemiologically unrelated. The human strains had been isolated from the faeces of babies suffering from neonatal diarrhoea in 1966. They had been sent by laboratories in Britain to Dr Joan Taylor of the Central Public Health Laboratory, Colindale, London, N.W.9, for serotyping; they belonged to serotypes considered enteropathogenic for human beings (Taylor, 1961). The strains from pigs had been isolated in 1960–62 or in 1965 from the small intestine or faeces of cases of neonatal or post-weaning diarrhoea or bowel oedema under conditions strongly suggesting that they were playing a pathogenic role. The fowl strains had been cultured from the internal organs of intensively reared broiler fowls that had died from 'coli-septicaemia', mainly in 1966. Of the 100 calf strains 14 had been isolated in 1962–65 from the internal organs of calves that had died from bacteraemia and the remainder from the small intestine or faeces of calves that had died from or were suffering from diarrhoea that could not be attributed to causes such as salmonella infection. Ten of the twenty-five lamb strains had been cultured from lambs that had died from bacteraemia and the remainder, under similar conditions as the calf strains, from cases of diarrhoea. The lambs, like the calves, were under 10 days old and were examined in 1962–65. The strains from the pigs, the fowls and those from the bacteraemic calves and lambs belonged to serotypes considered pathogenic for these species (Sojka, 1965).

Drug sensitivity tests

Approximately 0·15 ml. of a 1/50 dilution of a 24 hr. broth culture of the strain of *E. coli* to be tested was spread evenly over the surface of a dried MacConkey agar plate and disks containing different drugs were then applied at approximately equal distances apart. The amounts of drug used in the disks were 50 μg. of streptomycin, oxytetracylcine, chlortetracycline, chloramphenicol, neomycin and nalidixic acid, 25 μg. of ampicillin, cephalosporin C and polymixin, 15 μg. of furazolidone and 150 μg. of sulphadimidine. The plates were incubated at 37° C. for 24 hr. and read. Except in the case of sulphadimidine, no difficulty was experienced in making readings; the zones of inhibition surrounding the disks were either wide, very narrow or absent. All strains that appeared to be sulphadimidine-resistant were retested on nutrient agar in which the sulphonamide-inhibitors had been neutralized by the addition of lysed horse erythrocytes (Smith & Crabb, 1957). Similar results were obtained with oxytetracycline and chlortetracycline; in the results they are referred to collectively as tetracyclines. The results for cephalosporin C are not quoted because they were the same as those for ampicillin. Some tests were also performed with kanamycin and framycetin. They were not used as a routine because the results obtained with them were the same as those with neomycin.

Minimum inhibitory concentration of drugs for Escherichia coli

Nutrient agar plates containing twofold falling concentrations of the drug under test were inoculated lightly with 24 hr. broth cultures of strains of *E. coli* and incubated at 37° C. for 24 hr. The lowest concentration of drug that prevented growth was recorded as the minimum inhibitory concentration (MIC). Lysed horse erythrocytes were incorporated into the medium when sulphadimidine was studied and the cultures diluted so as to yield a growth consisting of discrete colonies on drug-free medium.

Transfer of drug resistance

Nutrient broth (Oxoid no. 2) in 10 ml. amounts was seeded with 0·02 ml. of a 24 hr. broth culture of the prospective donor strain of *E. coli*, i.e. one of those shown in sensitivity tests to be resistant to one or more drugs, and 0·1 ml. of a similar culture of the prospective recipient strain. In all the experiments in which the prospective donor strain was ampicillin-sensitive, an ampicillin-resistant chromosome mutant of a human enteropathogenic strain of *E. coli*, H 1, antigenic formula 026:K 60:H −, was used as recipient. This mutant was sensitive to all the other drugs studied. It had been obtained by heavily inoculating nutrient agar containing 30 μg./ml. of ampicillin with the fully sensitive H 1 and selecting one of the few colonies that grew thereon. The mixed culture of prospective donor and recipient strain was incubated at 37° C. for 24 hr. and then centrifuged. The deposit was inoculated on to a plate of MacConkey medium containing ampicillin and one of the drugs to which the prospective donor was resistant and the prospective recipient was sensitive (referred to as selection medium). The plate was incubated at 37° C. for 24 hr. Any colonies that grew on the medium were provisionally accepted as being those of the recipient strain to which drug resistance had been transferred from the donor strain; growth of the donor strain was suppressed by the ampicillin in the medium. One of the colonies was purified by plating on blood agar and a single colony picked into broth after it had been checked serologically to be of the recipient strain. The drug resistance of the resulting culture was then determined. Controls consisting of nutrient broth that had been inoculated with the recipient strain only were always kept. Whenever failure was encountered in attempts to transfer resistance to strain H 1, the relevant mixed culture experiment was repeated using an ampicillin-resistant mutant, prepared in the laboratory, from a drug-sensitive enteropathogenic pig strain of *E. coli*, P 11, antigenic formula 0141:K 85ab; 88ab, as recipient. This strain was also used in mixed culture experiments instead of H 1 when the prospective donor possessed an antigenic structure similar to that of H 1.

When the prospective donor was ampicillin-resistant, a nalidixic acid-resistant chromosome mutant of H 1, obtained in a similar manner as the ampicillin-resistant mutant, was employed as the prospective recipient, nalidixic acid being incorporated in the selection medium as well as one of the drugs to which the prospective donor was resistant. Finally the transmissible nature of the resistance of strains detected by the above methods was confirmed using a nalidixic acid-resistant

Table 1. *Incidence of drug-resistance in strains of* Escherichia coli *isolated from diseased human beings and domestic animals*

	Source					
		Pigs*				
Strains	Human beings	1960–62	1965	Calves	Lambs	Fowls
No. examined	70	70	70	100	25	35
Percentage resistant to one or more drugs	42·9	28·6	61·4	65·0	12·0	28·8
Percentage resistant to						
Tetracyclines	36·6	28·6	38·6	50·0	8·0	17·1
Streptomycin	35·3	11·4	40·0	52·0	8·0	2·9
Sulphonamides	33·0	8·6	22·9	57·0	12·0	17·1
Chloramphenicol	14·3	7·1	7·1	15·0	0	0
Ampicillin	18·6	0	0	14·0	4·0	0
Neomycin	4·3	0	7·1	5·0	0	0
Furazolidone	1·4	0	7·1	7·0	0	0
Polymixin	0	0	0	0	0	0
Nalidixic acid	0	0	0	0	0	0
Percentage with resistance pattern						
A S T C N Su	2·8	0	0	0	0	0
A S T C Su F	0	0	0	4·0	0	0
A S T C Su	5·7	0	0	1·0	0	0
S T C N Su	1·4	0	0	0	0	0
A S T Su	5·7	0	0	5·0	0	0
A S C Su	0	0	0	1·0	0	0
S T C Su	4·2	7·1	5·7	8·0	0	0
S T Su F	1·4	0	0	3·0	0	0
S T N Su	0	0	1·4	3·0	0	0
A S N	0	0	0	1·0	0	0
A S Su	1·4	0	0	0	0	0
A T Su	1·4	0	0	1·0	0	0
S T Su	4·2	1·4	5·7	16·0	8	2·8
S T N	0	0	2·8	1·0	0	0
S C Su	0	0	1·4	1·0	0	0
S Su F	0	0	1·4	0	0	0
S N F	0	0	1·4	0	0	0
T Su F	0	0	1·4	0	0	0
A Su	0	0	0	1·0	4·0	0
S T	5·7	2·8	2·8	2·0	0	0
S Su	2·8	0	5·7	5·0	0	0
S N	0	0	1·4	0	0	0
T Su	0	0	0	3·0	0	2·8
T F	0	0	1·4	0	0	0
A	1·4	0	0	0	0	0
S	0	0	10·0	1·0	0	0
T	4·2	17·1	17·1	3·0	0	11·4
Su	0	0	0	5·0	0	11·4
F	0	0	1·4	0	0	0

* The pig strains were classified according to the period of time in which they were isolated.

A, Ampicillin; S, streptomycin; T, tetracyclines; C, chloramphenicol; N, neomycin; Su, sulphonamide; F, furazolidone.

The results for cephalosporin C were the same as those for ampicillin. All the neomycin-resistant strains were resistant to kanamycin and framycetin.

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chromosome mutant of *E. coli* K12F$^-$ as a recipient. In all studies employing sulphadimidine in the selection medium, the basal medium was nutrient agar in which the sulphonamide inhibitors had been neutralized in the manner described previously.

Normally, the drug levels used in the selection medium were 25 μg./ml. of streptomycin, oxytetracycline, chloramphenicol and neomycin, 30 μg./ml. of ampicillin and nalidixic acid, 10–15 μg./ml. of furazolidone and 1000 μg./ml. of sulphadimidine.

RESULTS

Incidence of drug-resistance amongst strains of Escherichia coli *isolated from diseased human beings and domestic animals*

The drug-resistance found amongst strains of *E. coli* isolated from human beings suffering from neonatal diarrhoea, calves and lambs suffering from neonatal diarrhoea or bacteraemia, pigs suffering from neonatal or post-weaning diarrhoea or bowel oedema and from fowls suffering from 'coli-septicaemia' is illustrated in Table 1.

The highest incidence of drug resistance was found amongst the strains from calves and the lowest incidence amongst those from lambs. The most complex resistance patterns were found amongst those from calves and human beings, some of the strains from these sources being resistant to as many as six drugs. In general, the incidence of resistance to any drug amongst the strains from any species appeared to be directly related to the extent that drug has been used in that species. The absence of neomycin and furazolidone resistance from the pig strains isolated in 1960–62 and their presence amongst those isolated in 1965 is probably a reflexion of the much greater usage of those drugs in pigs after 1962. It is noteworthy that the incidence of drug resistance amongst the 1965 pig strains was more than twice that in the 1960–62 pig strains. Of all the drugs studied, only two, polymixin and nalidixic acid, were active against all the strains of *E. coli* tested; both are drugs that have been used very little in human beings and not at all in domestic animals.

The incidence of infective resistance amongst the strains of Escherichia coli *isolated from diseased human beings and domestic animals*

The results of experiments designed to determine the incidence of infective resistance amongst a representative selection of the strains of *E. coli* from human beings and domestic animals referred to in Table 1 are summarized in Table 2. The resistance of most of the strains examined from all five species was of the infective type and was easily demonstrated to be so. The whole resistance pattern was usually transferable. In a minority of cases one or, very occasionally, two of the drug resistances involved in the complete resistance pattern of a strain was not transferred. Furazolidone resistance was exceptional; it was not transferred, despite repeated tests, from the eight furazolidone-resistant strains.

The ability with which resistance to different drugs could be transferred from mutliply and singly drug-resistant strains isolated in the present study is illustrated

in Table 3. Practically all the tetracycline, chloramphenicol and neomycin resistance was transmissible. This was so in the case of tetracycline resistance whether the donor was singly or multiply resistant; no strains resistant to chloramphenicol only or to neomycin only were found in this survey. Ampicillin resistance was

Table 2. *Incidence of infective resistance among strains of* Escherichia coli *from diseased human beings and domestic animals*

	No. of strains infectively resistant/no. of strains tested from:					
		Pigs				
Resistance pattern	Human beings	1960–62	1965	Calves	Lambs	Fowls
A S T C N Su	2/2	—	—	—	—	—
A S T C Su F	—	—	—	1/1 (A)	—	—
A S T C Su	4/4	—	—	1/1	—	—
S T C N Su	1/1	—	—	—	—	—
A S T Su	2/2	—	—	2/2	—	—
S T C Su	2/2	3/3	3/3	3/3	—	—
S T Su F	1/1	—	—	2/2	—	—
ST N Su	—	—	1/1 (S)	2/2 (S Su, S)	—	—
A S N	—	—	—	1/1 (A)	—	—
A S Su	1/1	—	—	—	—	—
S T Su	2/2	1/1	1/2	5/5 (T)	2/2	0/1
S T N	—	—	1/1	1/1	—	—
S C Su	—	—	1/1	—	—	—
S Su F	—	—	0/1	—	—	—
S N F	—	—	1/1	—	—	—
T Su F	—	1/1	—	—	—	—
A S	—	—	—	—	0/1	—
S T	3/3	1/1	—	—	—	—
S Su	0/1	1/1 (Su)	2/2	2/2 (Su)	—	—
T Su	—	—	—	—	—	1/1 (Su)
A	0/1	—	—	—	—	—
S	—	—	0/2	0/1	—	—
T	1/1	5/5	5/5	1/1	—	4/4
Su	—	—	—	1/2	—	1/3
F	—	—	0/1	—	—	—
Total	19/21	12/12	15/20	22/24	2/3	6/9

A, Ampicillin; S, streptomycin; T, tetracyclines; C, chloramphenicol; N, neomycin; Su, sulphonamide; F, furazolidone.

Furazolidone resistance was not transferred from any of the strains examined. The symbol of a drug, in parentheses, indicates that resistance to that drug was not transferred from *one* of the strains of the same resistance pattern that were examined, e.g. 5/5 (T) indicates that tetracycline resistance was not transferred from one of the five strains tested. Apart from these exceptions, the whole resistance pattern was transferred.

transferred from two-thirds of the strains tested. Infective resistance to streptomycin and sulphonamide was detected more commonly in multiply resistant strains than in singly resistant strains or in strains resistant to both these drugs only. As mentioned previously, none of the furazolidone resistance was transmitted.

To determine whether bacterial virus might be playing a mediating part in the transfer of drug resistance in the studies reported above, all the strains used as

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donors were examined to see whether they were latently infected with bacterial virus active on the recipient strains. Only one donor strain was so infected. Its use did not result in the transference of drug resistance. To establish that the strains used as recipients were not unique or specially suited for the purpose, mixed culture experiments were performed using one tetracycline-resistant strain of *E. coli* as prospective donor and ampicillin-resistant or streptomycin-resistant chromosome

Table 3. *The ability to transfer single drug resistance from multiply and singly resistant strains of* Escherichia coli

	Kind of resistance possessed by prospective donor strains					
	Multiple		Streptomycin and sulphonamide resistance only		Single	
Drug resistance	No. tested	No. transferable*	No. tested	No. transferable	No. tested	No. transferable
Tetracycline	35	32	—	—	22	22
Chloramphenicol	12	12	—	—	—	—
Neomycin	7	7	—	—	—	—
Streptomycin	39	32	14	6	7	0
Sulphonamide	35	25	9	3	9	2
Furazolidone	7	0	—	—	1	0
Ampicillin	23	16	—	—	1	0

* No. of strains from which the particular resistance under study was shown to be transferable.

Table 4. *The minimum inhibitory concentration (MIC) of drugs for strains of* E. coli *classified as sensitive or resistant by disk tests*

	MIC (μg./ml.) for strains classified as					
			Resistant*			
	Sensitive		Natural		Transmitted	
Drug	Median	Range	Median	Range	Median	Range
Ampicillin	8	2–16 (10)	256	64–1024 (16)	512	64–1024 (11)
Cephalosporin C	4	4–8 (10)	256	128–256 (12)	—	—
Streptomycin	2	2–4 (20)	64	64–256 (20)	128	64–256 (20)
			> 2000	> 2000–> 2000 (9)	> 2000	> 2000 (4)
Oxytetracycline	4	4–8 (20)	512	64–512 (20)	512	64–1024 (17)
Chloramphenicol	4	4–8 (20)	512	256–1024 (20)	512	512–1024 (8)
Neomycin	2	1–2 (10)	256	128–1024 (9)	256	64–512 (7)
Sulphadimidine	20	10–40 (20)	> 5000	> 5000–> 5000 (20)	> 5000	> 5000–> 5000 (12)
Furazolidone	4	1–4 (20)	32	16–64 (7)	—	—
Polymixin	2	1–2 (20)	—	—	—	—
Nalidixic acid	4	2–4 (24)	—	—	—	—

* Most of the resistant strains classified as 'natural' were used as donors to produce from strain H 1 those classified as 'transmitted'.

The figures in parentheses refer to the number of strains tested.

Two levels of streptomycin resistance were encountered; the resistant strains are classified accordingly into two groups.

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mutants of thirty-eight different strains of *E. coli* as prospective recipients. The selection medium employed contained oxytetracycline and ampicillin or streptomycin, depending on the mutant used as recipient. Each mixed culture experiment was performed once only. Tetracycline resistance was transferred to thirty-seven of the thirty-eight prospective recipient strains.

The minimum inhibitory concentration of drugs for Escherichia coli *strains classified as sensitive or resistant by disk tests*

The results of determining the minimum inhibitory concentration (MIC) of drugs for strains of *E. coli* that had been classified as sensitive or resistant by disk tests and of strains to which resistance had been transferred is summarized in Table 4. A great difference was noted between the MIC for strains that had been classified as resistant and for those that had been classified as sensitive; it was least in the case of furazolidone. The MIC of drugs for strains to which resistance had been transferred closely resembled those for the corresponding donor strains. Two levels of streptomycin resistance existed, both of which were transmissible.

DISCUSSION

The results show that most of the drug resistance possessed by the strains of *E. coli* isolated from human beings and domestic animals suffering from diseases of the alimentary tract or bacteraemia was of the infective type. This was also a feature of the non-pathogenic *E. coli* organisms that form the bulk of the enterobacteriaceal flora of the alimentary tract of these species (Smith & Halls, 1966*a*). The few failures to transmit resistance, other than those with furazolidone, were usually with ampicillin, streptomycin or sulphadimidine. Since chromosome mutants resistant to these three drugs could be obtained relatively easily from sensitive strains of *E. coli* by laboratory procedures, it is probable that the strains from which it was not possible to transmit resistance to these drugs were themselves mutants that had emerged as a result of selection pressure provided by drug-containing environments.

The high incidence of resistant strains to a large number of drugs and the complex resistance patterns of some of the strains was a disquieting feature of this survey, particularly as the diseases caused are acute and severe to the extent that they may terminate fatally if the drug with which they are first treated is not active against the infecting strain; the result of sensitivity tests cannot be awaited before commencing treatment. Only two drugs were found that were active on all the strains of *E. coli* tested. These were nalidixic acid and polymixin, drugs that are not commonly used in the treatment of *E. coli* infections. Since nalidixic acid-resistant mutants were easily obtained in the present work from cultures of *E. coli* by laboratory procedures, it is unlikely that nalidixic acid would have an extensive therapeutic life if brought into general use; the prospects of polymixin are better in this respect.

The results for the pig strains present a good illustration of the general situation that is developing as a consequence of the continued use of anti-bacterial drugs—

a doubling of the proportion of resistant strains between 1960–62 and 1965 with the presence in the 1965 collection of strains resistant to two drugs, neomycin and furazolidone, to which all the strains in the 1960–62 collection were sensitive. Since many of the multiply resistant strains from pigs and from the other species included tetracyclines in their resistance patterns, the incidence of these strains will be increased not only by the use in the treatment of clinical disease of any one of the drugs involved in the resistance patterns but also by such procedures as the continuous feeding with diets containing low levels of tetracyclines as growth promoters. The 'blanket' use of antibacterial drugs in flocks or herds in attempting to control non-specific 'stress' conditions or to stimulate egg production, policies which are powerfully advertised, is another potent stimulus to the emergence of drug-resistant bacterial populations. Nevertheless, it must be appreciated that a high incidence of drug resistance can occur when antibacterial drugs are used in a species solely for the treatment and prevention of clinical disease. This is well illustrated by the results for the human strains.

The broiler-fowl industry provides a good example of the influence the widespread use of antibiotic feeding has on the pathogenic *E. coli* in the animal population. From 1957 to 1960 the practice of feeding broiler fowls on diets containing tetracyclines increased greatly and progressively, and during that time Sojka & Carnaghan (1961) recorded the incidence of tetracycline-resistant strains amongst the pathogenic serotypes they isolated from broiler fowls suffering from 'coli-septicaemia' as 3·5 % in 1957, 20·5 % in 1958, 40·9 % in 1959 and 63·2 % in 1960. Since 1961 the three major animal food compounders, amongst others, have ceased adding tetracyclines to their broiler foods. In 1962 the incidence of tetracycline-resistant strains causing 'coli-septicaemia' had decreased to 30 % (K. M. Smith quoted by Smith, 1962) and in the present survey to 17·1 %.

The high incidence of drug resistance amongst the strains of *E. coli* examined from calves stems mainly from the frequent use of drugs in attempting to control diarrhoea in these animals. As mentioned previously, doubt arises as to the part *E. coli* plays in calf diarrhoea. Recent studies (Smith & Halls, 1966*b*) indicate that in only a small proportion of cases do these organisms play an aetiological role, most calf diarrhoea probably being non-infective in origin and the consequence of the harsh present-day methods of calf-rearing. The common practice of using antibacterial drugs in treating *all* cases of calf diarrhoea or in trying to prevent it, therefore, probably has little beneficial effect on calf diarrhoea as a whole but it gives rise to infectively-resistant non-pathogenic *E. coli* populations whose resistance may be transferred to strains that are truly enteropathogenic for the calf and to other pathogens such as *Salmonella*.

SUMMARY

A high incidence of drug resistance, mostly of the infective type, was found amongst strains of *Escherichia coli* isolated from human beings suffering from neonatal diarrhoea, calves and lambs suffering from neonatal diarrhoea or bacteraemia, pigs suffering from neonatal or post-weaning diarrhoea or bowel oedema and from fowls suffering from 'coli-septicaemia'. The strains from the

human beings, pigs and fowls, and those from calves and lambs suffering from bacteraemia, belonged to serotypes generally accepted as being pathogenic for these species.

Complex drug-resistance patterns were a common feature, particularly amongst the human and calf strains, some being resistant to six drugs. Only two drugs, polymyixin and nalidixic acid, were active on all the strains.

The incidence of drug resistance amongst pig strains was twice as great in those isolated in 1965 as in those isolated in 1960–62; neomycin and furazolidone resistance was found amongst the 1965 strains but not amongst the 1960–62 strains.

Infective resistance was easily demonstrated; tetracycline resistance was transferred from one tetracycline-resistant strain of *E. coli* to 37 of 38 tetracycline-sensitive strains at the first attempt.

Most ampicillin, streptomycin, tetracycline, chloramphenicol, neomycin and sulphonamide resistance was of the infective type; the furazolidone resistance in all of eight strains studied was not shown to be infective.

In the experiments to demonstrate the presence of infective resistance the level of drug resistance was usually found to be the same in donor and recipient strains.

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