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Apramycin and gentamicin resistance in *Escherichia coli* and salmonellas isolated from farm animals

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SUMMARY

Since the aminoglycoside antibiotic apramycin was licensed for veterinary use in 1980, all isolates of *Escherichia coli* and salmonellas received at the Central Veterinary Laboratory have been monitored for resistance to apramycin and the related antibiotic gentamicin. During the period 1982–4, the incidence of resistance in *E. coli* to apramycin increased from 0·6 % in 1982 to 2·6 % in 1984. In salmonellas the incidence of resistance to apramycin increased from 0·1 % in 1982 to 1·4 % in 1984. Resistance to both apramycin and gentamicin was detected in six different salmonella serotypes, although an isolate of *Salmonella thompson* from poultry was resistant to gentamicin but not apramycin. Most of the cultures were isolated from pigs, although the incidence of apramycin resistance in *S. typhimurium* (DT 204C) from calves has shown a recent dramatic increase. All the isolates with one exception produced the enzyme aminoglycoside 3-N-acetyltransferase IV (ACC(3)IV). The resistance was transferable by conjugation in most of the strains examined, and the plasmids specifying the resistance have been found to belong to a number of different incompatibility groups. Plasmids from three *E. coli* strains were compatible with all the reference plasmids and belonged to a previously undescribed group which was investigated further.

It is suggested that bacteria from humans should be examined for resistance to apramycin and gentamicin to determine the possibility of the antibiotic-resistance bacteria, and their genes, spreading from animals to humans.

INTRODUCTION

Apramycin, an aminoglycoside antibiotic which has not been used in human medicine, was licensed for veterinary use in the United Kingdom in 1980. Resistance to this compound is rare in enterobacteria (Ryden & Moore, 1977) and the frequency of mutations to high-level resistance is extremely low (Bowen *et al.* 1976; Davies & O'Connor, 1978), but resistant strains are detectable in nature, especially after treatment of farm animals with apramycin (Bowen *et al.* 1976).

Transmissible plasmids conferring apramycin resistance have been identified in two of five apramycin-resistant *Escherichia coli* examined by Hedges & Shannon (1984). One of these strains produced the enzyme aminoglycoside 3-N-acetyltransferase IV (AAC(3)IV), and the other a novel aminoglycoside-modifying enzyme.

Since the introduction of gentamicin, tobramycin and other aminoglycoside antibiotics into hospital medicine, disease outbreaks caused by Gram-negative bacteria resistant to these agents have been reported in many different parts of the world. Some of these earlier disease outbreaks are described by Datta *et al.* (1980) and Witchitz (1981). Resistance is usually determined by transmissible plasmids, and several different enzymological mechanisms have been described (Davies, 1980). One of the mechanisms, acetylation, is determined by several different enzymes.

The genes for the enzyme group 3-N-acetyltransferase (AAC(3)) occur on plasmids in a number of different enterobacterial species and there are at least four distinct AAC(3), each with their own substrate specificity (Davies & O'Connor, 1978). Thus, AAC(3)I confers resistance to gentamicin but not to tobramycin whilst AAC(3)II and AAC(3)III confer resistance to both drugs (Davies, 1980). AAC(3)IV differs in conferring resistance by acetylation to apramycin as well as to both gentamicin and tobramycin (Davies & O'Connor, 1978). The enzymes responsible for gentamicin resistance among bacteria from disease outbreaks in hospitals have been determined in many cases (for example, Datta *et al.* 1980; Witchitz, 1981). The most comprehensive enzymological study was that of the 'Mechanism of Resistance Service' at Bristol Laboratories, New York (Price *et al.* 1981). They studied 1349 aminoglycoside-resistant isolates from 12 countries (on 4 continents). Despite these investigations, not a single isolate resistant to aminoglycosides by production of AAC(3)IV has been reported from a human source. Ose, Ryden & Muenster (1976) studied the frequency of AAC(3)IV-producing (apramycin-resistant) *E. coli* from farm animals in the USA. Although such strains were a small minority ($< 1\%$), in the absence of selection by the agent they were sufficiently common to be detected in 8 of 10 pigs after a course of treatment with apramycin (Bowen *et al.* 1976).

The purpose of our investigation was to discover to what extent this enzyme was present in bacteria from animals in Great Britain, to characterize the plasmids involved and to determine whether a relationship exists between gentamicin-resistant strains from farm animals and humans.

MATERIALS AND METHODS

Bacteriological procedures

All the *E. coli* and salmonella cultures examined were isolated from disease outbreaks in pigs, cattle, sheep and poultry and were submitted to the Central Veterinary Laboratory during the period 1982–4 for serological identification. During 1982, 1983 and 1984 the numbers of *E. coli* isolates examined were 1293, 1159 and 1233 respectively and the number of salmonella isolates were 4043, 4923 and 4722 respectively. The antimicrobial resistance pattern of all isolates was determined by the diffusion method using multodiscs (Sojka, Wray & McLaren,

1984). The two multodiscs used were 3866E (*Oxoid Limited, Wade Road, Basingstoke, Hants RG24 0PW), which contained the following antibiotics: compound sulphonamide (Su), 50 μg; streptomycin (S), 10 μg; chlortetracycline (T), 10 μg; chloramphenicol (C), 10 μg; ampicillin (Am), 10 μg; neomycin (N), 10 μg; trimethoprim-sulphamethoxazole (Tm), 25 μg and furazolidone (F) 15 μg; and 7923E (Oxoid), which contained colistin (CT), 25 μg; amikacin (Ak), 10 μg; amoxycillin (AML), 25 μg; gentamicin (G), 20 μg; streptomycin, 25 μg; compound sulphonamide, 500 μg; carbenicillin (Py), 10 μg and apramycin (Apr), 15 μg.

A selected number of cultures which showed either apramycin or gentamicin resistance were subjected to further investigation. In addition, six gentamicin-resistant strains of salmonella from poultry reared in West Germany were examined. Minimum inhibitory concentrations (MIC) of aminoglycosides were determined by agar dilutions in Diagnostic Sensitivity Test Agar (Oxide – CM 261) with an inoculum of about 10^3–10^4 organisms. The MIC was taken as the lowest concentration that completely suppressed growth after incubation at 37 °C for 18 h. The aminoglycosides tested were apramycin, gentamicin, neomycin, kanamycin, tobramycin and amikacin, whose concentrations ranged from 0·5 to 512 mg l^{-1}. The type of aminoglycoside-modifying enzyme was determined in 38 *E. coli* isolates and 29 salmonellas by the cellulose phosphate paper binding method of Ozanne *et al.* (1969) as described by Hedges & Shannon (1984).

GENETIC STUDIES

Bacterial strains, plasmids and bacteriophages

E. coli K-12 strain JE2571 (*leu*, *thr*, *str*, *fla*, *pil*) was used as a plasmid host (Bradley, 1980). The filamentous phage fd was reviewed by Marvin & Hohn (1969). The transfer-derepressed version of the plasmid *Folac* (Falkow & Baron, 1962) designated EDP208, was constructed and supplied by N. Willetts. The plasmid-specific RNA containing phage *Folac* was supplied by J. N. Coetzee (personal communication).

Agarose gel electrophoresis for molecular weight determination

The method of Kado & Liu (1981) was used for plasmid DNA preparation. The horizontal gel electrophoresis system of Bethesda Research Laboratories was employed using their brand of agarose at 0·7 % concentration. Calibration was obtained by comparison of the migration of DNA from a variety of reference plasmids with known molecular weights.

Electron microscopy

The preparation of conjugative pili for transmission electron microscopy (normal and immune) and the raising of antisera to pili, were as described by Bradley (1980).

Conjugal transfer of apramycin resistance

Isolates of 25 *E. coli* and 15 salmonellas were tested for their ability to transfer apramycin resistance by conjugation, and the incompatibility group of the plasmids was determined by the methods of Hedges & Shannon (1984).

Table 1. *Characterization of apramycin-resistant isolates*

Strain number	Serotype	Source	Resistance determinants	Transfer of apramycin resistance*	Co-transferred resistances	Plasmid incompatibility group
	E.coli					
M185	0149:K91:K88	Pig	S Su T Agt†	+	T Agt	New group
M186	0149:K91:K88	Pig	S Su T Agt	+	T Agt	New group
M162	08:K?:K88	Pig	S Su N Am Agt	++	S Am Agt	II
M484	0?:K?:K88	Pig	Su T Tm Agt	+	T Tm Agt	New group
M136	UT‡	Pig	S Su T Am Agt	++	S T Agt	II
M384	UT	Pig	S Su N Am Tm Agt	−	S Su N Tm Agt	II
M437	UT	Pig	S Su N T Agt	+	S Su N T Agt	U
M462	0149:K91:K88	Pig	S Su N Am Agt	++	Agt	
M482	0141:K85	Pig	S Su Agt	++	S Agt	
M100	0141:K85	Pig	Su T C F Agt	++	T Agt	
M102	0141:K85	Pig	S Su T Agt	++	T Agt	
M125	UT‡	Pig	S Su T Agt	++	Agt	
M434	UT	Pig	S Su T N Agt	++	T Agt	
M455	0147:K89	Pig	S Su T Tm Agt	++	T Tm Agt	
M145	0157:K?:K88	Pig	S Su T Am F Tm Agt	++	Tm Agt	
M191	0157:K?:K88	Pig	S Su T Am C Tm Agt	++	S C Tm Agt	
M193		Pig				
M375	UT	Pig	S Su Am Agt	++	Am Agt	Not tested
M376	UT	Pig	S Su Am Agt	+	Am Agt	
M407	UT	Pig	S Su T Am Agt	−		
M20	UT	Pig	S Su T N Am Agt	−		
M21	0149:K91:K88	Pig	S Su T N Am Agt	−		
X61	UT	Cattle	S Su T N F C Tm Agt	−		
M103	045:K?:K88	Pig	Su Agt	−		
X172	0101:K30	Cattle	S Su T N Am C Agt	+	S Su Agt	

	Salmonella					
S1237	*typhimurium* (DT49)	Pig	S Su T N Am C Tm Agt	++	Su Am Agt	Il
S3444	*give*	Pig	Agt	++	Agt	?
S2206	*typhimurium* (DT204C)	Cattle	S Su T Am N C Tm Agt	++	Agt	Not tested
S2457	*typhimurium* (DT204C)	Cattle		+	Agt S Tm	
S3020	*typhimurium* (DT204C)	Cattle		++	Agt ST Tm N Am	
S2138	*typhimurium* (DT204C)	Cattle		++	S T Agt	
S3001	*bredeney*	Pig	S Su T Agt	++	S Agt	
S3471	*derby*	Pig	S Su Agt	++	S Agt	
S2984	*kedougou*	Pig	S Su Tm Agt	++	Agt	
S4211	04,12:d–	Cattle	Agt	++	Agt	
S878	*london*	Pig	S Su Am Agt	not tested		
S454	*thompson*	Chicken	S Su N G**			

* Transfer of apramycin resistance. † Apramycin, gentamicin, tobramycin. ‡ UT, Untypable.
++ Transfer > 10^{-4} per donor overnight. + Transfer > 10^{-8} per donor overnight. − Transfer < 10^{10} per donor overnight.
** Sensitive to apramycin.

RESULTS

Apramycin/gentamicin resistance in E. coli *and salmonellas*

During the period 1982–4, the incidence of apramycin resistance in *E. coli* increased from 0·6 % in 1982 to 2·6 % in 1984. Fifty-three of the 72 *E. coli* cultures examined were isolated from pigs and the remainder from calves. Forty-nine of the *E. coli* cultures belonged to serogroups associated with disease and 20 of the porcine isolates possessed the K88 antigen. Most of the cultures showed multiple antibiotic resistance, which also included gentamicin. The number of R determinants varied from 2 to 12, with 81 % of the strains being resistant to at least 5 of the 14 antibacterial agents used for sensitivity testing. Table 1 shows the antibiotic resistance patterns of 25 isolates of *E. coli* which were investigated for resistance transfer.

During the same period the incidence of apramycin/gentamicin resistance in salmonella cultures increased from 0·1 % in 1982 to 1·4 % in 1984. Six salmonella serotypes (*S. typhimurium* (DT49 and 204C), *S. london*, *S. kedougou*, *S. bredeney*, *S. give*, *S. derby* and *Salmonella* 4,12:d) showed apramycin resistance. An isolate of *S. thompson* from poultry was found to be resistant to gentamicin but not apramycin. The *S. typhimurium* (DT204C) strains and *Salmonella* 4,12:d were from cattle and the remainder from pigs. During 1983, 20 of the 37 apramycin-resistant salmonellas were *S. typhimurium*, and their numbers increased so that during the following year they constituted 59 of the 60 apramycin-resistant salmonella. Almost all of the *S. typhimurium* were of phage type 204C. The resistance patterns of the different salmonella serotypes are shown in Table 1.

Enzyme production by apramycin- or gentamicin-resistant organisms

All the apramycin-resistant organisms which produced AAC(3)IV were, with one exception, resistant also to gentamicin ($\geqslant 8$ mg l^{-1}) and tobramycin ($\geqslant 32$ mg l^{-1}) (Table 2). Although kanamycin and neomycin were substrates of the enzyme, MICs of these compounds were mostly relatively low ($\leqslant 16$ mg l^{-1}). For this reason we inferred that those strains with neomycin and kanamycin MICs ($\geqslant 64$ l^{-1}) also produced aminoglycoside 3-*O*-phosphotransferase [APH(3′)] or an enzyme with a comparable spectrum of activity. Seven apramycin-sensitive, gentamicin-, tobramycin- and kanamycin-resistant strains of salmonella, which included the six West German strains isolated from poultry, were found to produce aminoglycoside 2-*O*-adenyltransferase (AAD(2″)].

Conjugal transfer of apramycin resistance

Table 1 shows the results of experiments to transfer the apramycin resistance from *E. coli* and salmonella strains to *E. coli* K12. The *E. coli* strains fall into three groups: 13 strains were able to transfer their resistance efficiently ($> 10^{-4}$ transconjugants per donor overnight), 5 strains which transferred their resistance at low efficiency ($> 10^{-8}$ transconjugants per donor overnight) and 5 strains unable to transfer resistance at detectable frequencies ($< 10^{-10}$ transconjugants per donor overnight). All the salmonella cultures tested transferred their resistance efficiently.

Table 2. *Susceptibility pattern and enzyme production by apramycin- or gentamicin-resistant organisms*

Organism	No. of isolates	MICs (mg/l)						Aminoglycoside-modifying enzymes
		Apramycin	Tobramycin	Gentamicin	Amikacin	Kanamycin	Neomycin	
E.coli	23	>128	32–>128	8–128	1–8	4–16	2–8	AAC(3)IV
E.coli	14	>128	32–>128	8–64	1–8	128–>128	8–>128	AAC(3)IV APH(3′)*
E.coli	1	4	1	0·5	2	2	1	AAC(3)IV
Salmonella sp.	14	>128	32–>128	16–128	2–4	4–16	2–8	AAC(3)IV
Salmonella sp.	8	>128	128–>128	32–>128	2–4	128–>128	>128	ACC(3)IV APH(3′)*
Salmomella sp.	6	4	8–128	32–128	2–4	64–>128	1–2	AAD(2″)
Salmonella sp.	1	4	32	32	4	64	128	AAD(2″)+APH (3′)*

*APH(3′) production inferred from resistance to neomycin and kanamycin.

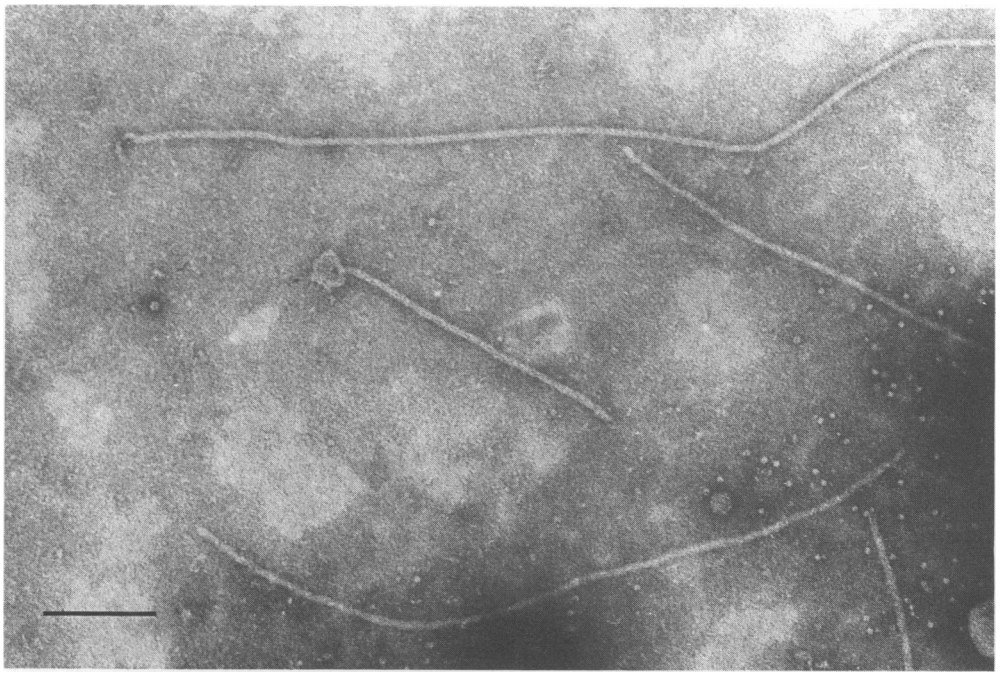

Fig. 1. R1545b, showing that they belong to the morphological type which is thick, flexible and non-aggregating (Bradley, 1980). Bar 100 nm.

Strains of the first group transfer apramycin resistance as part of a conjugative plasmid and include members of the I and U incompatibility groups (Table 1). *S. give* possessed apramycin-resistance plasmids which appeared to be compatible with all groups examined and could not be placed in any incompatibility group.

In three of the *E. coli* strains (M185, M186 and M484, Table 1) the plasmids were compatible with all the reference plasmids but mutually incompatible with each other, and belonged to a new group or groups, which were investigated in greater detail. These strains also carried tetracycline resistance in addition to apramycin resistance, and both determinants could be transferred conjugally at low efficiency.

The level was not influenced by mutations abolishing restriction in the recipient, and transfer of either plasmid to secondary recipients occurs with a frequency of between 10^{-3} and 10^{-2} transconjugants per donor per hour.

Further investigations of the new plasmid incompatibility group (plasmids R1545b and R1545c)

Plasmids R1545b and R1545c were investigated further and found to have molecular weights of 46 and 53 MDa respectively. They were incompatible with one another and determined numerous thick flexible conjugative pili that did not aggregate (Fig. 1; see Bradley, 1980). Immune electron microscopy (not illustrated) revealed that R1545b and R1545c pili were serologically identical, and also that they bound antibodies to the serologically related pili for four single plasmids (forming their own incompatibility groups): F*olac* (Falkow & Baron, 1962), R71 (Inc9, see Bradley, 1980), TP224 (unclassified, from strain E7476 in Scotland *et al.* 1979)

and pPLS (unclassified, Bradley, 1985). Pili of these four plasmids have been found to act as receptors for the RNA-containing bacteriophage F*olac* (Coetzee *et al.* in preparation), showing that they are virtually identical. The bacteriophage also attached to R1545b and R1545c pili, although it did not cause lysis of the cells. The identity of their pili placed the plasmids in the same complex of compatible plasmids as F*olac*, R71, TP224, and the pPLS (Coetzee *et al.* in preparation).

The three isolates M185, M186 and M484 (Table 1) were found to possess numerous conjugative pili that were heavily labelled with antisera to both F*olac* and R1545b pili. All three isolates possessed the K88 adhesin (Table 1) and they were also able to utilize raffinose as a sole carbon source (Raf^+). These characterisitics are identical with those of the naturally occurring isolates carrying pPLS (Bradley, 1985).

DISCUSSION

The results show that although only a small proportion of *E. coli* isolates from farm animals produce the enzyme AAC(3)IV, such strains are widely distributed and the total number of such cells is probably very great. Most of the apramycin-resistant *E. coli* and salmonellas, with the exception of *S. typhimurium* (DT204C) and *Salmonella* 4,12 : d :—, were isolated from pigs, and since most pig farms are self-contained it is unlikely that epidemic spread of apramycin-resistant porcine strains is occurring. In contrast, all the apramycin-resistant *S. typhimurium*, isolated from calves, belonged to phage type 204C, the incidence of which has increased considerably in recent years (Wray, 1985). Thus given a mobile, susceptible host population, such as calves, spread of pathogenic organisms and associated resistance plasmids may occur rapidly. Consequently, apramycin-resistant strains of *S. typhimurium*, which were first isolated from calves in the west of England, have now been isolated from calves in many areas of the country. The gentamicin-resistant strains of *S. thompson*, and the six salmonellas from West Germany which were isolated from poultry, did not show cross-resistance to apramycin, and differed from the other isolates by producing the enzyme AAD(2″).

Helmuth (personal communication) found that the plasmid profile patterns of the British and German isolates differed, which suggested that the isolates were not related. He believed that the German isolates acquired resistance as a result of the prophylactic use of gentamicin in the poultry industry (Helmuth *et al.* 1984).

The level of gentamicin resistance demonstrated among the isolates which were also resistant to apramycin varied, but all the strains produced AAC(3)IV enzymes with indistinguishable substrate profiles. In cases where the resistance could be transferred, all recipients expressed comparable levels of gentamicin resistance. Indeed, the gentamicin MIC was as high, or higher, than those conferred by the enzyme ACC(3)I.

Some of the apramycin-resistance plasmids belong to a new complex of plasmid incompatibility groups which have been found to be associated with the presence of the K88 adhesin. Indeed 28% of the isolates carried this antigen, which is determined by a non-self-transmissible plasmid which also carries Raf^+ genes (Shipley, Gyles & Falkow, 1978). The K88+ Raf^+ plasmid, however, can be mobilized by many drug-resistance plasmids which have no apparent connexions

with them (Bradley, 1985). It may be significant that three of the six K88-mobilizing plasmids whose transfer systems have been examined are similar. Those plasmids of the new group carrying K88+ Raf^+ are naturally derepressed for transfer, which appears to enable them to mobilize these determinants very efficiently (Bradley, 1985); this may be of importance in the evolution of new pathogenic serotypes.

The lack of correlation of AAC(3)IV production with a particular plasmid group suggests that the genes for this enzyme may be carried on a transposable element. The earlier studies of Hedges & Shannon (1984) indicated that the transposable element was carried on a non-transmissible replicon, possibly the transposon, and that it must be transposed before it can be transferred conjugally. Strains which produce AAC(3)IV but do not transfer it probably cannot effect transposition either because the mechanism is inoperative or because there is no suitable recipient plasmid available. Although the enzyme AAC(3)IV has been demonstrated in bacteria from farm animals both in the United Kingdom and in France (Chaslus-Dancla & Lafont, 1985) the only record of its occurrence in human isolates (*S. typhimurium* DT204C) is that of Threlfall *et al.* (1985). Since the production of AAC(3)IV confers resistance to gentamicin and tobramycin, it appears surprising that this enzyme has not been widely reported in bacteria isolated from humans. The resistance plasmids belonged to a number of incompatibility groups well represented in isolates from humans, and there is no evidence that the carriage of these plasmids is a barrier to the colonization of the human intestine. Even if the orginal strain was ill adapted to humans, the transmissible nature of many of the plasmids should favour their transfer to better-adapted clones. For these reasons, Hedges & Shannon (1984) suggested that the failure to observe AAC(3)IV in hospital isolates is ecological separation of the bacteria of farm animals from the microflora of hospital patients. That such a barrier may exist seems contrary to the conclusions of many workers, who have emphasized the hazards of antobiotic-resistance transfer from bacteria of farm animals to the human microflora (Levy, Fitzgerald & Macone, 1976; Rowe & Threlfall, 1981; O'Brien *et al.* 1982).

A possible explanation for this apparent contradiction may lie in the nature of the antibiotic resistance determinants. Thus, unlike antibacterial drugs that are absorbed after oral administration, gentamicin and tobramycin are, in most countries, used only in hospitals. It is, therefore, unlikely that a former patient would retain sufficient antibiotic to select in favour of resistant bacteria should he come into contact with farm animals or their products. Where an antibiotic is in general use among the population people may acquire resistant bacteria, support their selective growth and transmit them to new sites of multiplication. The absence of such selective carriers may explain the absence of *E. coli* producing AAC(3)IV from the hospital population.

Salmonella are more likely, however, to transfer through animal products from farm animals to man, and the recent report by Threlfall *el al.* (1985) would suggest the possible entry of the AAC(3)IV resistance plasmid into the human population. In view of the continuing debate regarding the contribution of bacteria from farm animals to the bacterial resistance pool in humans, the opportunity should now be taken to determine whether AAC(3)IV-producing organisms are present, and if so, their extent and the speed at which spread is occurring.

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REFERENCES

BOWEN, R. E., DAVIES, J., WALTON, J. R. & BENNETT, T. H. (1976). Apramycin, a new aminoglycoside antibiotic: microbiological safety. In *Proceedings of the Fourth International Pig Veterinary Society Congress*, p. B.3. Ames, Iowa: Iowa State University Press.

BRADLEY, D. E. (1980). Morphological and serological relationship of conjugative pili. *Plasmid* **4**, 155–169.

BRADLEY, D. E. (1985). Transfer systems of K88 and K99 plasmids. *Plasmid* **13**, 118–128.

CHASLUS-DANCLA, E. & LAFONT, J. P. (1985). Resistance to gentamicin and apramycin in *Escherichia coli* from calves in France. *Veterinary Record* **117**, 90–91.

DATTA, N., DACEY, S., HUGHES, V., KNIGHT, S., RICHARDS, H., WILLIAMS, G., CASEWELL, M. & SHANNON, K. P. (1980). Distribution of genes for trimethoprim and gentamicin resistance in bacteria and their plasmids in a general hospital. *Journal of General Microbiology* **118**, 495–508.

DAVIES, J. (1980). *Mechanisms of Antibiotic Resistance. Current Concepts.* Sussex: UpJohn.

DAVIES, J. & O'CONNOR, S. (1978). Enzymatic modification of aminoglycoside antibiotics: 3-N-acetyltransferase with broad specificity that determines resistance to the novel aminoglycoside apramycin. *Antimicrobial Agents and Chemotherapy* **14**, 69–72.

FALKOW, S. & BARON, L. S. (1962). Episomic element in a strain of *Salmonella typhosa*. *Journal of Bacteriology* **84**, 581–589.

HEDGES, R. W. & SHANNON, K. P. (1984). Resistance of apramycin in *Escherichia coli* isolated from animals; detection of a novel aminoglycoside-modifying enzyme. *Journal of General Microbiology* **130**, 473–482.

HELMUTH, R., PIETZSCH, O., STEPHAN, R., CHAKRABORTY, T. & BULLING, E. (1984). Antimicrobials and agriculture. In *Proceeding of the 4th International Symposium on Antibiotics in Agriculture; Benefits and Malefits* (ed. M. Woodbine) London; Butterworth. pp. 237–242.

KADO, C. I. & LIU, S. T. (1981). Rapid procedure for the detection and isolation of large and small plasmids. *Journal of Bacteriology* **145**, 1365–1373.

LEVY, S. B., FITZGERALD, G. B. & MACONE, A. B. (1976). Spread of antibiotic-resistance plasmids from chicken to chicken and from chicken to man. *Nature* (London) **260**, 40–42.

MARVIN, D. A. & HOHN, B. (1969). Filamentous bacterial viruses. *Bacteriological Reviews* **33**, 172–209.

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., CHRISTERSON, E., BISSETT, M. L. & MORSE, E. V. (1982). Molecular epidemiology of antibiotic resistance in *Salmonella* from animals and human beings in the United States. *The New England Journal of Medicine* **307**, 1–6.

OSE, E. E., RYDEN, R. & MUENSTER, O. A. (1976). Apramycin, a new aminocyclitol antibiotic. 1. *In vitro* evaluation. In *Proceedings of the Fourth International Pig Veterinary Society Congress*, p. B.2. Ames, Iowa: Iowa State Univerisity Press.

OZANNE, R., BENVENISTE, R., TIPPER, D. & DAVIES, J. (1969). Aminoglycoside antibiotics: inactivation by phosphorylation in *Escherichia coli* carrying R factors. *Journal of Bacteriology* **100**, 1144–1146.

PRICE, K. E., KRESSEL, P. A., FARCHIONE, L. A., SISKIN, S. B. & KARPOW, S. A. (1981). Epidemiological studies of aminoglycoside resistance in the U.S.A. *Journal of Antimicrobial Chemotherapy* **8** (suppl. A), 89–105.

ROWE, B. & THRELFALL, E. J. (1981). Multiply-resistant clones of *Salmonella typhimurium* in Britain: epidemiological and laboratory aspects. In *Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids* (eds. S. B. Levy, R. C. Clowes and E. L. Loering) London and New York: Plenum Press. pp. 567–573.

RYDEN, R. & MOORE, B. J. (1977). The *in-vitro* activity of apramycin, a new aminocyclitol antibiotic. *Journal of Antimicrobial Chemotherapy* **3**, 609–613.

SCOTLAND, S. M., GROSS, R. J., CHEASTY, T. & RAVE, B. (1979). The occurrence of plasmids carrying genes for both enterotoxin production and drug resistance in *Escherichia coli* of human orgin. *Journal of Hygiene* **83**, 531–538.

SHIPLEY, P. L., GYLES, C. L. & FALKOW, S. (1978). Characterization of plasmids that encode for the K88 colonization antigen. *Infection and Immunity* **20**, 559–566.

SOJKA, W. J., WRAY, C. & MCLAREN, I. (1984). A survey of drug resistance in salmonellae isolated from animals in England and Wales in 1982 and 1983. *British Veterinary Journal* **140**, 576–591.

THRELFALL, E. J., ROWE, B., FERGUSON, J. L. & WARD, L. R. (1982). Increasing incidence of resistance to gentamicin and related aminoglycosides in *Salmonella typhimurium* phage type 204C in England, Wales and Scotland. *Veterinary Record* **117**, 355–357.

WITCHITZ, J. L. (1981). Epidemiological aspects of aminoglycoside resistance in France. *Journal of Antimicrobial Chemotherapy* **8** (suppl. A), 71–81.

WRAY, C. (1985). Is salmonellosis still a serious problem in veterinary practice? *Veterinary Record* **116**, 485–489.