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Transmission of infectious drug resistance from animals to man

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SUMMARY

The antibiotic resistance patterns of coliforms in faecal specimens from pigs and their human contacts were studied. The ability of the resistant coliforms to transfer their resistance *in vitro* to antibiotic-sensitive recipients was examined. The results showed that pigs which had received antibiotics carried more multiply-resistant, R-factor bearing coliforms than pigs which had not been given antibiotics. Human contacts of the antibiotic-treated pigs had a higher incidence of antibiotic-resistant coliforms with R-factors than human contacts of pigs which had not been given antibiotics. It is concluded that antibiotic treatment of farm animals may lead to acquisition of antibiotic resistance by gut coliforms of man.

INTRODUCTION

Infectious drug resistance was first noted by the Japanese (Ochiai, Yamanaka, Kimura & Sawada, 1959) who observed the transfer of antibiotic resistance from *Escherichia* to *Shigella* in patients with dysentery. Infectious drug resistance is of widespread occurrence (Datta, 1962; Maré & Coetzee, 1965; Mann & Gebedou, 1966; Schroeder, Terry & Bennett, 1968; Anderson & Lewis, 1965*a*; Anderson, 1968) and is mediated by R-factors which are extra-chromosomal elements consisting of deoxyribonucleic acid (Falkow, Citarella, Wohlhieter & Watanabe, 1966). R-factors may be transferred from antibiotic-resistant bacteria to antibiotic-sensitive bacteria during conjugation (Ochiai *et al.* 1959); the recipients of R-factors become potential donors so that these factors may spread rapidly. The origin of R-factors is not known but their selection and spread appear to be promoted by the widespread use of antibiotics in human and veterinary medicine (Mann & Gebedou, 1966; Smith, 1966). Walton (1966) found a high incidence of infectious drug resistance in strains of *E. coli* isolated from the faeces of healthy pigs and calves and was able to correlate this with antibiotics given in the feeds.

The indiscriminate use of antibiotics as feed additives and for prophylaxis in animal farming has come under scrutiny and criticism by Anderson & Lewis (1965*a*), who suggested that normally harmless bacteria of animal origin could develop drug resistance as a result of exposure to antibiotics and transfer this resistance to normal gut commensals in humans. The mechanical transmission of organisms from animals to man could result from direct contact with the animals

or from eating animal products, e.g. sausages (Moorhouse, O'Grady & O'Connor, 1969). These resistant gut commensals could then act as reservoirs capable of transferring their drug resistance to bacterial pathogens during subsequent infections.

This investigation was an attempt to study the transmission of drug-resistant bacteria from pigs to man by ascertaining:

(1) the incidence of antibiotic-resistant bacteria in the gut coliforms of pigs and their human contacts;

(2) any similarity in the antibiotic resistance patterns of the coliforms from pigs and their human contacts;

(3) whether these antibiotic-resistant bacteria could transfer their resistances *in vitro*.

MATERIALS AND METHODS

Specimens

Rectal swabs were taken from 110 pigs known to have received antibiotics as injections or with their feeds. The antibiotics were penicillin, chlor- and oxy-tetracyclines, dihydrostreptomycin and sulphamethazine. Faecal samples were also taken from 42 human contacts of the pigs (handlers and their close relatives).

A control group consisted of faecal samples from 72 pigs, not known to have received any antibiotics, and from 28 human contacts of these pigs. All of these contacts had at some time handled the pigs so that there is no distinction between handlers and their relatives in this group.

Processing of specimens

These were plated on MacConkey agar and their sensitivity to the following antibiotics determined by the Bauer–Kirby technique (Bauer, Kirby, Sherris & Turck, 1966); ampicillin (A) 25 μg., streptomycin (S) 25 μg., tetracycline (T) 50 μg., chloramphenicol (C) 50 μg., sulphatriad (Su) 300 μg. and nalidixic acid (NA) 30 μg. per disk. Resistant colonies were identified by conventional procedures and picked to Mueller–Hinton broth to be used as donors in mating experiments. All donor strains were sensitive to nalidixic acid while the recipient was a multiply-sensitive strain of *E. coli*, resistant to nalidixic acid only.

Equal volumes of 0·5 ml. of 18 hr. broth cultures of donor and recipient were mixed in tubes and 4·0 ml. tryptose broth added. The mixture was then incubated for 3 hr. at 37° C., after which approximately 0·2 ml. was plated on MacConkey agar plates containing nalidixic acid (25 μg./ml.) plus streptomycin (10 μg./ml.) or tetracycline (10 μg./ml.). On these plates neither the donor nor the recipient could grow, so that organisms recovered from the mating mixture on these plates were considered recombinants. These were picked to fresh Mueller–Hinton broth and their antibiotic sensitivity determined. All antibiotic resistances acquired by the recipient strain were considered to have been transferred from the antibiotic-resistant donor.

Table 1. *Multiple drug resistance of coliforms isolated from pigs and their human contacts*

No. of antibiotics to which coliforms resistant	CP 72	TP 110	CH 28	TH 42	EM 14	RE 28
3 or more	18 (25)	106 (96)	0	24 (57)	12 (86)	12 (43)
2	20 (28)	4 (4)	10 (36)	8 (18)	2 (14)	6 (21)
1	30 (41)	0	10 (36)	4 (9)	0	4 (14)
0	4 (6)	0	8 (28)	6 (15)	0	6 (22)

Figures at top of table indicate total number of individuals in each group; figures in parentheses are approximate percentages.
See text for explanation of abbreviations.

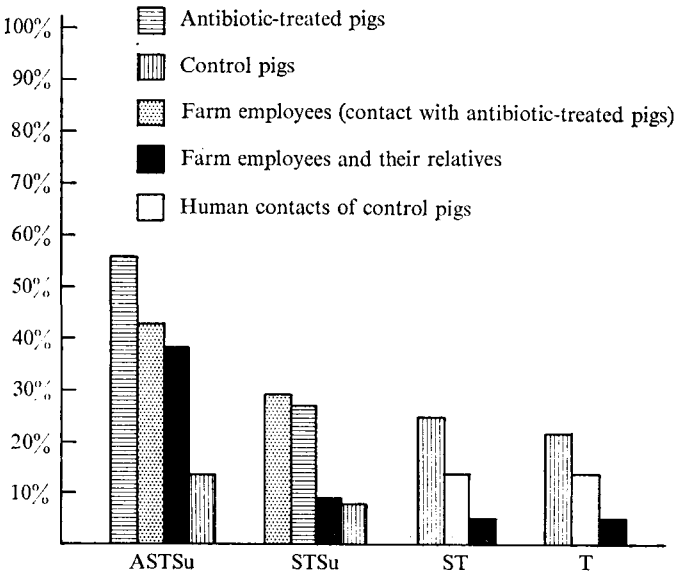

Fig. 1. Patterns of antibiotic resistance.

RESULTS

Resistance patterns of the strains

Of the specimens from the antibiotic-treated pigs (TP), 96 % carried coliforms resistant to more than three antibiotics as against only 25 % from the control pigs (CP).

Of the human contacts of the antibiotic-treated pigs (TH), 57 % carried coliforms resistant to more than three antibiotics as against none of the contacts of the control pigs (CH). Of the farm employees who had direct contact with the antibiotic-treated pigs (EM) 86 % carried coliforms resistant to more than three antibiotics while of their relatives, who had only indirect contact with the pigs (RE), 43 % carried coliforms resistant to more than three antibiotics. These results are summarized in Table 1.

Table 2. *Antibiotic resistance to individual drugs in test and control groups*

Resistant to:	CP 72	TP 110	CH 28	TH 42
Ampicillin	10 (14)	78 (71)	2 (7)	20 (48)
Streptomycin	36 (50)	104 (95)	8 (29)	30 (72)
Tetracycline	52 (72)	100 (91)	12 (43)	26 (62)
Sulphonamide	36 (50)	102 (91)	8 (29)	32 (76)
Chloramphenicol	2 (3)	0	0	0
Sensitive to all	4 (6)	0	8 (29)	6 (14)

Figures at top of table indicate total number of individuals in each group; figures in parentheses are approximate percentages.

See text for explanation of abbreviations.

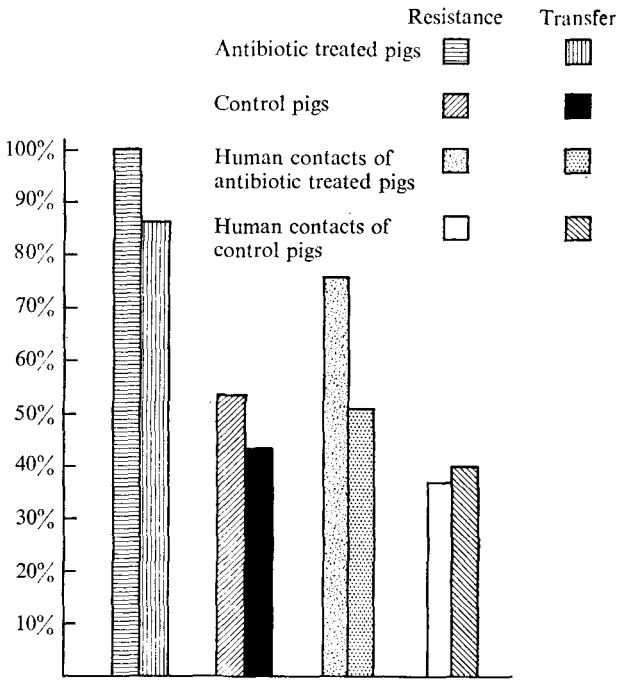

Fig. 2. Antibiotic resistances and transfer of two or more drugs. The left-hand column in each pair shows the percentage of resistant strains, the right-hand column the percentage of these resistant strains which had transferable drug resistance.

The commonest combinations of antibiotics to which resistance was noted were ASTSu, STSu, ST and T only. Antibiotic-treated pigs and their human contacts had a higher incidence of the ASTSu combination than the control pigs and their human contacts. These results are shown in Fig. 1.

The commonest resistance pattern was STSu, and the incidence of this pattern was higher in the antibiotic-treated group than in the controls. These results are summarized in Table 2.

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Transferability of resistances

Of the resistant strains from the antibiotic-treated pigs, 86 % transferred resistance to two or more antibiotics. By contrast, only 42 % of the resistant isolates from the control pigs transferred resistance to two or more antibiotics. Of the resistant isolates from contacts of the antibiotic-treated pigs, 50 % transferred resistance to two or more antibiotics as against 40 % of the resistant isolates from contacts of the control pigs. The results are represented in Fig. 2.

DISCUSSION

All the antibiotic-treated pigs carried coliforms with resistance to two or more antibiotics as against 53 % in the control pigs. This confirms the effect of antibiotic exposure on the selection of antibiotic-resistant bacteria. Resistance was commonest to those antibiotics to which the animals had been exposed. It was interesting to note that although none of the antibiotic-treated pigs had received ampicillin (they received penicillin) yet resistance to ampicillin was frequent in the antibiotic-treated pigs and their human contacts. A possible explanation of the relatively high incidence of antibiotic resistance in the gut flora of the control pigs (53 % resistant to two or more drugs) is that some of the pigs may have received antibiotics unknown to their owner, e.g. before purchase. It is also possible that animals may acquire R-factors by ingestion of vegetation containing antibiotic-producing organisms.

All the handlers of the antibiotic-treated pigs carried coliforms resistant to two or more antibiotics; 64 % of the relatives of these pig-handlers also carried coliforms resistant to two or more antibiotics as against 36 % of the human contacts of the control pigs. Thus, a lesser degree of contact with antibiotic-treated pigs was paralleled by a lower incidence of gut coliforms with multiple drug resistance. The similarity of the patterns of antibiotic resistances (ASTSu, STSu) between the antibiotic-treated pigs and their contacts is impressive and these results suggest that multiply-drug resistant organisms are transmitted from the animals to man. All of the handlers of the antibiotic-treated pigs were healthy young men, none of whom had received antibiotics within the previous 2 years and most of whom denied ever having received any antibiotics to their knowledge.

Strains from 78 % of the control pigs and 79 % of their human contacts did not exhibit infectious drug resistance. This contrasted with 14 % of the strains from the antibiotic-treated pigs and 53 % from their human contacts. Although the resistance patterns were similar in the antibiotic-treated pigs and their human contacts, yet the coliforms from the latter transferred their resistances at a much lower rate than those of the pigs (47 % against 86 %). This may point to some relative instability in the human gut of the Resistance Transfer Factor (RTF) from animal gut flora, a possibility supported by the findings of Smith (1969*a*, *b*). Without the RTF, an antibiotic resistance may be expressed but not transferred; for transfer of drug resistance both the resistance determinants and a transfer factor must be present (Anderson & Lewis, 1965*b*).

Another interesting observation made during this study was that as the pigs got

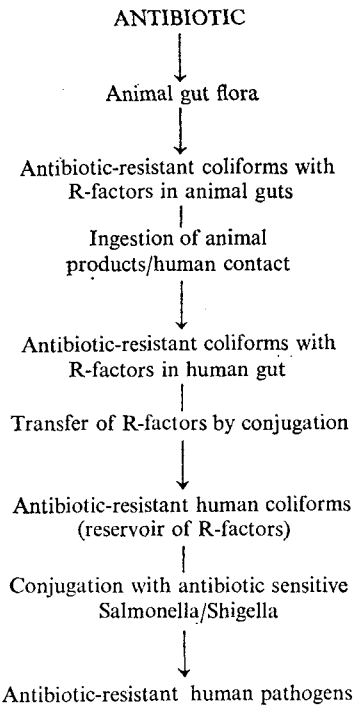

Fig. 3. Possible mode of transfer of multiple drug resistance from animals to man.

older and were moved to a different section of the farm (from 'nursery' – where they all received antibiotics, to 'finishing' to 'gestation' to 'farrowing' – where they infrequently received antibiotics) the incidence of multiple drug resistance diminished slightly, while the ability to transfer the resistances diminished much more. This was some indication of reduced antibiotic exposure being paralleled by reduced incidence of multiple infectious drug resistance.

Our findings suggest that widespread use of antibiotics on animal farms can lead to the development of highly drug-resistant bacteria in the gut flora of the animals. These bacteria may be transmitted to human contacts who will then harbour resistant coliforms in the gut. Some of these may retain the transfer factor and be capable of passing on their resistance to initially sensitive bacterial pathogens with which the subject may become infected. The possible course of events is shown in Fig. 3.

It is recommended that antibiotics be used only when absolutely necessary, for therapeutic rather than prophylactic purposes. When the circumstances dictate, they should be given in adequate dosage for an adequate length of time. By these means one can minimize the emergence of antibiotic-resistant organisms which so often destroy the efficacy of antibiotics in the treatment of infection.

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