The Presence and Persistence of Transmissible Drug Resistance Factors in the Coliform Intestinal Flora of Calves Reared in an Intensive System

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Introduction

A bacterium may be fully sensitive to a given antimicrobial drug on initial isolation, but resistant when re-isolated later from the same patient. The emergence of such antibiotic resistance could be related to the ease with which mutation might take place, to the exchange of genetic information in bacteria by, conjugation, transformation and transduction, and to the selective pressure exerted by the large scale usage of antimicrobial agents in the biosphere. Bacterial resistance is the principal obstacle to the therapeutic use of many antibiotics. When resistance develops during the course of treatment, the proper therapeutic effect cannot be achieved in the patient. A more important long-term effect is the elimination of sensitive and the dissemination of resistant strains in the environment of associated animal and human populations.

Antimicrobial agents and antibiotics are administered to livestock in both therapeutic and subtherapeutic amounts for treatment and prophylaxis of diarrhoeal disease by the oral and parenteral routes. Although of advantage to the livestock producer such usage of antibiotics is commonly questioned because of the possible detrimental effects on human and animal health. The main concern being that pervasive use of antibiotics in food animals may increase the pool of resistant bacteria to the point where a serious human health hazard could develop. Oral administration of antimicrobials for both therapeutic and prophylactic purposes to persons and animals have previously been shown to result in an increased prevalence of resistant intestinal bacteria.

In the present study the prevalence of drug resistance in the coliform intestinal flora of intensively reared dairy calves was determined, with particular reference to transferable resistance. The persistence of drug resistence was also determined after a period of two months during which no antimicrobials were administered to any calf.

Materials and Methods

Animals

Rectal swabs were taken from apparently normal healthy calves 4 weeks after they entered an intensive calf rearing unit for a feeding trial. At the time of sampling they were more or less 5-6 weeks old. All the calves received tetracycline per os for the first 4 days as prophylaxis against diarrhoea. Following this, most of the calves received a proprietary antidiarrhoeal mixture, containing neomycin, chloramphenicol, furazolidone, phtalylsulphathiazole, vit. A, kaolin and electrolytes (Furanicol, Centaur laboratories), on one or more occasions whenever they manifested signs of diarrhoea. On occasion individual calves also received streptomycin per os. All the calves were individually tied up and kept on slatted floors. Contact between neighbouring calves were possible. Two months after the first sampling, rectal swabs were again collected from all the calves. The standard of hygiene and the general management of the unit were excellent during the whole course of the trial.

Bacterial strains

Coliform bacteria were isolated from the rectal swabs and were confirmed to be strains of *Escherichia coli* following accepted bacteriological procedures (3).

Resistance Spectra

A technique that was standardized according to the guidelines given in a report of an international collaborative study on the testing for antimicrobial sensitivity (4) was used to determine the resistance pattern of each strain to the following drugs: streptomycin (10 ug), neomycin (10 ug), kanamycin (30 ug), gentamycin (10 ug), tetracycline (25 ug), chloramphenicol (25 ug), sulphonamides (200 ug), furazolidone (200 ug), ampicillin (10 ug) and trimethoprim (2.5 ug). To evaluate the sensitivity of resistance of each strain (6), their respective inhibition zones to the various

drugs were compared with that of an internationally accepted standard sensitive reference strain of *E. coli* NCTC 10148.

Transfer of Resistance

Transferable resistance was determined by conjugation experiments (9) with an *E. coli* K12 F recipient strain that was resistant to nalidixic acid. All the potential donor resistant strains were sensitive to nalidixic acid. All the potential donor strains as well as the known recipient strain were separately grown in nutrient broth (Difco) to more than 10^8 cells/ml. One millilitre of the broth culture of each resistant strain and 1 ml of the broth culture of the recipient strain were both inoculated into 5 ml nutrient broth. Following overnight incubation of the mixed culture at 37°C, some of it was used as inoculum to perform a sensitivity test on Mueller-Hinton agar (Oxoid) into which nalidixic acid was incorporated.

Results

Drug resistance was encountered in all the strains of *E. coli* isolated from the calves during the time that they were still exposed to antimicrobial drugs (series A), whereas the prevalence of drug resistance in strains isolated from the same calves 2 months after the last occasion on which they received any antimicrobial drug (series B) was only 30%. Resistance patterns to each drug and the prevalence of transferable resistance are shown in Table 1. None of the series B strains manifested transferable resistance.

Resistance to a single antibiotic was observed only in 4 strains. Two series A strains manifested resistance to tetracycline and ampicillin respectively and 2 series B strains showed similar resistance. All the other resistant strains of both series manifested multiple drug resistance.

TABLE 1. Drug resistance pattern and prevalence of transferable resistance.

Drug*	Series A strains (n=52)				Series B strains (n=20)	
	Resistant strains		Transferable resistance		Resistant strains n=6)	
	No.	%	No.	%	No.	%
SF	50	96,2	40	80,0	4	20
T	50	96,2	26	52,0	4	20
K	27	51,9	13	48,1	1	5
NE	27	51,9	11	40,7	1	5
S	44	84,6	16	36,4	3	15
C	37	71,2	13	35,1	1	5
AP	38	73,1	10	26,3	3	15
TM	5	9,6	1	20,0	0	0
FZ	25	48,0	0	0	0	0
G	0	0	0	0	0	0

*SF = Sulphonamide; T = Tetracycline; K = Kanamycin; NE = Neomycin; S = Streptomycin; C = Chloramphenicol; AP = Ampicillin; TM = Trimethoprim; FZ = Furazolidone; G = Gentamycin.

TABLE 2. Multiplicity of antimicrobial resistance.

Multiplicity of resistance*	Strains	%
Series A:		
S, NE, K, T, C, SF, FZ, AP	18	34,6
T, SF	6	11,5
S, T, C, SF, AP	4	7,7
S, T, SF, AP	4	7,7
S, T, C, SF, FZ, AP	3	5,8
S, T, C, SF	2	3,8
S, NE, K, TM, T, C, SF, FZ, AP	2	3,8
S, NE, K, T, C, SF, AP	2	3,8
S, NE, K, TM, T, C, SF, AP	2	3,8
S, T, SF	2	3,8
S, NE, K, T, C, SF, FZ	2	3,8
S, TM, T, C, SF, AP	1	1,9
S, NE, K, T, C, SF	1	1,9
S, SF, AP	1	1,9
Series B:		
T, SF	1	16,7
S, T, SF, AP	1	16,7
S, NE, K, SF	1	16,7
S, T, C, SF, AP	1	16,7

* See footnote Table 1

Discussion

A previously published leading article of a medical journal (1) entitled, "Why has Swann failed?" highlighted much confused thinking on the use of antibiotics in animals and their consequences. It suggested that the only way to avoid further episodes arising in zoonotic pathogens would be by more stringent restriction on the use of antibiotics in animals.

The original predictions regarding development of antibiotic resistance erred by not realizing to what extent antimicrobial agents would be used. The amount is steadily increasing with much of this usage concentrated in specific areas such as medical hospitals and farms. The results from the present study confirmed that of previous studies, where a greater use of antibiotics resulted in an increased number of resistant strains of *E. coli* (7, 8). It also confirmed that multiple resistance could increase with the use of one antibiotic where resistance to it and to other antibiotics are on the same plasmid. Because of the increase in antibiotic resistant organisms arising by clonal selection on the local and international scale one can argue strongly for the increased restrictions in the use of antimicrobial agents. This restriction would apply to both human and animal medicine.

Whatever the cause of this increased resistance, there is a growing body of evidence of the spread of antibiotic-resistant organisms that is disquieting in its implications for both human and animal health (2). Human infections with resistant organisms may present difficulties in therapy and therefore the resistant flora in man must be restricted as far as possible. The question therefore arises as to the

importance of the animal reservoir for man.

Resistance problems in humans seem to arise principally from the administratin of antimicrobials by human physicians as a result of the increased selection pressure. Evidence for this is supplied by the direct relationship between selection pressure and resistance which is present in nosocomial infections (5). Evidence for resistance in human infections resulting from transferable drug resistance in strains derived from animals appears to be insignificant in relation to the overall use of antimicrobials in animals. The blame sometimes cast by the medical profession on the use of antimicrobial drugs in animals appears to be an excuse for its own mass prescribing of antimicrobials.

Despite this, the need for an improvement in the attempts of the veterinary profession to contain resistant organisms will always remain, because of the extremely disturbing possibility that a large reservoir of antibiotic resistant organisms can be selected for in our farm animals. However, the results obtained from the population of calves under the conditions of this trial, show that the high degree of resistance and transferable resistance found in their faecal strains of *E. coli* did not persist once the exposure to antimicrobials came to an end. The calf unit was under optimal management and the possibility of faecal cross-contamination between calves was extremely limited. Although the resistant strains originated in the intestine, they were not able to persist in the calves, once the selective pressure was removed.

A previous report indicated that it was rather an unhygienic neighbourhood that would serve as a reservoir for resistant strains in human populations. Their results illustrated that the undesirable effects of antimicrobial usage rather arose from the uncontrolled use of drugs among an overcrowded, poor and uneducated population with inadequate domestic hygiene facilities and sewage treatment (10). They concluded that when drugs are used with educated affluent populations, the incidence of resistance need not rise above that found in untreated populations.

The present and previous results therefore underlines the importance of responsible management and sound environmental hygiene whenever use is made of antimicrobial drugs, be it in the human or animal populations.

References

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