PAPERS AND ORIGINALS

Antibiotic resistance and survival of *E coli* in the alimentary tract

C L HARTLEY, M H RICHMOND

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Summary

Some antibiotics tend to select for R-factor-carrying *Escherichia coli* in the human gut, with complex long-term consequences. Some resistant strains disappear rapidly when treatment ends, while others persist for months in the absence of obvious antibiotic selection pressure, and the performance of individual resistant strains seems to depend more on the nature of the strain than on the plasmid carried. R plasmids are relatively rare in those *E coli* that colonize well in the gut and resistant bacteria therefore tend to disappear when treatment ends, but this situation could change dramatically if R plasmids became prevalent among those strains of *E coli* that colonize effectively.

Introduction

There has been much discussion about the threat to health posed by resistant coliform bacteria in the gut and the part played by antibiotics in maintaining this reservoir of resistant organisms.¹⁻¹² Undoubtedly treatment of man and animals with many different antibiotics (particularly ampicillin and tetracycline¹³⁻¹⁴) selects effectively for a predominantly resistant gut coliform flora, but even the flora of those not receiving antibiotics usually contains a few resistant organisms.⁶⁻¹⁰ Resistant bacteria may even be found in people unlikely ever to have been given antibiotics.¹⁵⁻¹⁶ This low prevalence of resistant organisms in those not receiving antibiotics has been taken to be a long-term and indirect consequence of antibiotic treatment in the population at large,³ while others have argued that it may reflect a continuing selection pressure imposed by low levels of antibiotics in the environment.¹⁰ This second view is usually based on the argument that some such selection pressure must exist otherwise R-positive bacteria, which in some experiments seem

less able to survive in the human gut than their R-negative counterparts,¹⁷ would disappear. One source of contamination is thought to be the indiscriminate use of antibiotics, mainly in animal husbandry.^{3 5 10}

The belief that R-positive strains of *Escherichia coli* survive less well than R-negative ones is based on two main types of observation. Firstly, there are studies in which a mixture of R-positive and R-negative *E coli* of the same strain are fed and their survivals followed by monitoring faecal elimination.¹⁷ Such experiments do, indeed, tend to support the view that R-factor carriage impairs the persistence of *E coli* strains in the human gut in the absence of selection pressure, but the number of pairs of strains so studied is still small. Furthermore, the practical relevance of such studies is doubtful, since the competition between resistant and sensitive bacteria that occurs in the alimentary tract is not normally between R-positive and R-negative variants of the same strain but between resistant and sensitive bacteria not otherwise isogenic.

Persistence of resistant strains in the gut

The other observation that tends to suggest that R-positive bacteria survive less well in general than their R-negative counterparts comes from the sequence of events that follows the end of antibiotic use in individual patients. Often the coliform flora of the gut returns to a predominantly sensitive state within 10 days or so of the end of treatment. Fig 1 shows two typical examples, one after the use of tetracycline and the other after the use of ampicillin. In both cases resistant strains had disappeared by the tenth day after treatment. But this pattern of events is by no means universal. The table shows the response in a patient after the end of tetracycline treatment. A serotype O18 strain of *E coli* harbouring a plasmid conferring resistance to streptomycin, tetracycline, and sulphonamides persisted for at least eight months after the end of treatment, and for much of this time the resistant strain remained predominant among faecal coliforms. Another example (fig 2) involved the persistence of a chloramphenicol-resistant strain of *E coli* in a person for more than three months even though that person had never knowingly received this antibiotic, and again the resistant organisms frequently constituted more than 10% of the coliform flora of the faeces.

Department of Bacteriology, University of Bristol, Bristol BS8 1TD
C L HARTLEY, B SC, PH D, MRC postdoctoral research assistant
M H RICHMOND, PH D, SC D, professor of bacteriology

*T = Tetracycline. Su = Sulphonamides. S = Streptomycin. A = Ampicillin. C = Chloramphenicol. NT = not typable with available sera.
†In instances where number is under 10, the remaining isolates proved not to be *E. coli*.
‡Culture on selective agar showed presence of strain as a substantial minority component of flora.

FIG 2—Survival of chloramphenicol-resistant O6 strain of *E. coli* in faecal flora of person never knowingly having been given this antibiotic and not receiving any antibiotic at time of study. H = Holiday period during which no samples were taken.

coliforms, whether sensitive or resistant, in the gut under various conditions. When antibiotic treatment is in progress it is clearly the antibiotic action itself that is of prime importance, but why do some resistant strains persist in people not undergoing treatment, and what factors are important for the survival of resistant strains during the transition from an antibiotic-rich to an antibiotic-poor (or antibiotic-free) environment, as occurs when treatment ends?

We have begun to investigate this problem by studying the survival of individual *E. coli* O types, resistant and sensitive, in people not known to be receiving antibiotics. Faecal samples were taken on a routine basis at intervals of a few days from

volunteers.^{18 19} Each specimen was weighed and suspended in a known volume of standard sterile physiological saline. Dilutions of these suspensions were then prepared and plated on MacConkey's bile lactose agar to determine the total coliform count. Ten colonies were then picked from these plates, tested with discs for their resistance to ampicillin (25 µg/disc), tetracycline (50 µg), streptomycin (25 µg), and chloramphenicol (50 µg), and O-typed by methods already described.^{19 20} When the isolates so obtained were resistant to antibiotics the transfer of their resistance determinants to an R-negative *E. coli* recipient was tested by a standard mating procedure.¹⁹ This series of operations therefore allows one to decide which *E. coli* O types are predominant in the flora at any time. The survival of the individual O types was scored as follows: when a particular type persisted from one sample to the next it was assumed to have been present at that level throughout the period between the samples. A given O type that appeared sporadically in samples from a given person—that is, where some samples in a series did not show the presence of the strain—was scored as having appeared repeatedly rather than continuously. The method of scoring therefore measures the ability of the various O types to become a predominant part of the coliform flora but not their ability to remain in the gut as minority components.

So far the incidence of both resistant and sensitive *E. coli* O types in the predominant part of the coliform flora has been followed in 33 people for a total of 2578 days. In all, 95 different sensitive and 65 different resistant O types were found. Calculation of the mean residence time of all sensitive O types in the samples gave a value of 5.7 days, while a similar calculation for resistant strains gave 6.1 days. When this second average was recalculated for those coliforms carrying R factors alone (as shown either by resistance transfer in the standard test or by the presence of a tetracycline resistance determinant in the strain^{19 21}) the mean residence time for R-positive O types was 6.6 days. Overall, therefore, R-factor carriage does not seem to have a major effect on the survival of *E. coli* strains as identified by O-typing in people not receiving antibiotics. Moreover, the data show that, on average, individual O types turn over fairly rapidly in the human gut, at least when measured in terms of their ability to reach the predominant status in the coliform flora.

Effect of antibiotics on bacterial survival

Though over 2500 man-days have been examined during this survey, it is important to stress that this period of study is still relatively short and that the conclusions must be considered tentative until confirmed by more-prolonged surveys. Nevertheless, the observations made so far have many implications for our understanding of the factors affecting R-factor survival in the absence of antibiotic treatment, since they suggest that the survival of resistant bacteria in the absence of antibiotic selection pressure depends on the characteristics of the *E. coli* host rather than on those of the plasmid that is carried. Thus the survival of the plasmids in the coliform flora shown in the table probably had little to do with the resistance characters carried by the plasmids but depended on the properties of the *E. coli* strains that carried them. Similarly the low but perceptible prevalence of R-factor-carrying *E. coli* in the faecal flora of people not receiving antibiotics probably reflects the survival properties of the *E. coli* strains that happen to carry plasmids rather than any selective effect of low levels of antibiotics in the environment.

If these conclusions are correct it would be possible to predict, to some extent at least, how the faecal flora would respond to the completion of a therapeutic course of an antibiotic that selects for R-factor-mediated resistance. At the end of such a course the coliform flora is normally resistant to the antibiotic and commonly consists of a single strain harbouring a single type of R factor. Removal of the selection pressure allows any sensitive bacteria that may have survived in small numbers, or

any such bacteria coming in with food, to compete with the resistant population. In most cases this competition results in the reduction of the resistant population and an increase in sensitive strains; and within 10 days or so the resistant line has commonly disappeared below the level of detection. But in some cases the resistant strain seems able to compete successfully with any surviving or superinfecting bacteria and remains a predominant component of the flora even though the direct pressure for the survival of the plasmid in the strain through its resistance determinants has ended. Indeed, if the competitive ability of the resistant strain is good enough the R factor may be maintained for long periods even though no antibiotics are administered.

There are probably major differences between the circumstances that lead to a strain becoming the predominant component of the coliform flora and those that allow survival as a minority strain. Certain R-factor-carrying strains may not, therefore, disappear completely from the faecal flora even though their abundance decreases rapidly after the end of treatment. As is the case with strains that remain common, however, their final level may have nothing directly to do with the survival of the strain in the presence of antibiotics. More likely it will reflect the ability of the organisms to adhere to the wall of the gut or some similar property of immediate survival rate.

R factors and individual *E. coli* O-antigen types

The abundance of the various O types of *E. coli* in the faecal flora of man has been studied for many years and certain O types are known to be more abundant than others.^{22 23} Types O1, O2, O4, O5, O18, O20, O25, and O75 are particularly notable in this respect and their prevalence is normally taken as a measure of their ability to persist in the human intestinal tract.^{22 23} When the abundance of the various O types among R-factor-carrying strains is examined the pattern is found to be very different: O8, O9, O86, and O101 strains are particularly common, and O1, O2, O4, O5, and O75 strains are relatively rare.^{18 19} At present, therefore, R factors are less widespread than they might be in those *E. coli* O types known to persist well in the human gut, and this may account for the fact that R factors are still relatively rare in the faecal flora of those not receiving antibiotics. It also may explain why the removal of antibiotic selection pressure tends to result in the replacement of a predominantly R-factor-carrying flora by a sensitive one, but not to the point that all R-positive strains disappear from the gut completely. Were R-factor-carriage in *E. coli* serotypes O1 and O2 to become more common, the prevalence of R factors in the human faecal flora might be expected to rise, and this might well occur without any increases in the use of antibiotics.

Long-term consequences of antibiotic use

Though these arguments stress the importance of selective influences other than the use of antibacterials on the long-term survival of R-positive bacteria in man, this should not be taken to deny the impact of treatment and other forms of antibiotic administration. Indeed, the reservoir is likely to be maintained by a combination of the effect of the R factors in protecting the bacteria in the presence of antibiotics and the ability of the bacterial host to survive in man for long periods regardless of whether R factors are carried or not. In this way the use of an antibiotic at one point in time may significantly influence events in a person long after the antibiotic has ceased to have any direct action. And since many R-positive coliforms may themselves transfer their resistance plasmids to sensitive bacteria of other species and genera, antibiotic use even for a limited period may well select indirectly for the appearance of resistance in unexpected organisms some time later. Furthermore, bacterial cross-infection may lead one group of resistant organisms which has become abundant in a patient or group of patients because of treatment to colonize others. Indeed, this may be the source

of the resistant organisms found in human populations that have never been treated with antibiotics.⁶⁻¹⁰ Visitors may have brought R-factor-carrying bacteria from localities where antibiotic use was widespread.

The long-term effect of antibiotics on the *E coli* flora of the population as a whole seems therefore to be insidious rather than dramatic, unlike the case with *Salmonella* species.³⁻²⁴ But its implications are none the less important. Nor should one be lulled into a sense of false security by the apparent inferiority of some R-factor-carrying strains at surviving in the gut when compared with the survival of their sensitive counterparts. The relative rarity of R factors in those strains of *E coli* notably effective at colonizing the gut of man in the absence of selection pressure means that the potential reservoir in the human population is not yet as full as it might be. But this is no excuse for using antibiotics with anything but scrupulous care.

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Intravenous diazoxide in treatment of hypertension associated with recent myocardial infarction

K P O'BRIEN, R R GRIGOR, P M TAYLOR

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Summary

Twenty patients with blood pressure over 180/110 mm Hg one hour after admission to a coronary care unit with recent acute myocardial infarction were given intravenous diazoxide in a bolus of 300 mg. The average blood pressure before diazoxide was 194/122 mm Hg. Blood pressure fell considerably in all patients, though six patients required two injections. The average fall was 58 mm Hg systolic and 40 mm Hg diastolic. No patient became severely hypotensive. The heart rate increased by an average of 10 beats/min. In nine patients the electrocardiographic changes immediately after the administration of diazoxide suggested an increase in myocardial injury. Though none of the patients seemed to deteriorate clinically from the diazoxide the electrocardiographic changes suggested that the use of intravenous diazoxide to lower blood pressure in patients with acute myocardial infarction might possibly be deleterious.

Cardiology Department, Auckland Hospital, Auckland, New Zealand
K P O'BRIEN, FRACP, MRCP, senior cardiologist
R R GRIGOR, MB, MRACP, cardiology registrar
P M TAYLOR, NZRN, sister-in-charge, coronary care unit

Introduction

Much recent discussion about the treatment of acute myocardial infarction has centred on possible methods for limiting infarct size, and it has been suggested that certain manipulations carried out after the patient's arrival in hospital might salvage quantities of myocardium.

Because blood pressure is one of the main determinants of myocardial oxygen demand it has been suggested¹ that a reduction in abnormally raised blood pressure in patients with acute myocardial infarction might reduce infarct size and thus mortality. Shell and Sobel² have reported beneficial results from reducing blood pressure with trimethaphan camsylate, and others³⁻⁴ have shown benefit from the use of vasodilators such as nitroprusside and phentolamine. Cardiac rupture after myocardial infarction⁵ occurs more often in patients who are hypertensive than in those who are normotensive, which also suggests that it might be advantageous to lower abnormally raised blood pressures in patients with acute infarction.

To our knowledge there have been no previous reports on the use of intravenous diazoxide in patients with hypertension associated with acute myocardial infarction. Intravenous diazoxide was considered a suitable agent for use in acute myocardial infarction because of its well-known speed of action and efficacy and also because it has been shown to increase coronary blood flow and cardiac output.⁶⁻⁸ Furthermore, the use of intravenous diazoxide has not been associated with the pronounced increase in heart rate that occurs with some other intravenous blood pressure lowering agents such as hydralazine.

Thus we undertook a trial to assess the effects of intravenous diazoxide in 20 patients with proved recent myocardial infarction and associated hypertension.