Ciba Foundation Symposium 207

ANTIBIOTIC RESISTANCE: ORIGINS, EVOLUTION, SELECTION AND SPREAD

1997

JOHN WILEY & SONS

Chichester · New York · Weinheim · Brisbane · Singapore · Toronto

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Antibiotic resistance: an ecological imbalance

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Abstract. Antibiotic resistance thwarts the treatment of infectious diseases worldwide. Although a number of factors can be identified which contribute to the problem, clearly the *antibiotic* as a selective agent and the *resistance gene* as the vehicle of resistance are the two most important, making up a 'drug resistance equation'. Both are needed in order for a clinical problem to arise. Given sufficient time and quantity of antibiotic, drug resistance will eventually appear. But a public health problem is not inevitable if the two components of the drug resistance equation are kept in check. Enhancing the emergence of resistance is the ease by which resistance determinants and resistant bacteria can spread locally and globally, selected by widespread use of the same antibiotics in people, animal husbandry and agriculture. Antibiotics are societal drugs. Each individual use contributes to the sum total of society's antibiotic exposure. In a broader sense, the resistance problem is ecological. In the framework of natural competition between susceptible and resistant bacteria, antibiotic use has encouraged growth of the resistant strains, leading to an imbalance in prior relationships between susceptible and resistant bacteria. To restore efficacy to earlier antibiotics and to maintain the success of new antibiotics that are introduced, we need to use antibiotics in a way which assures an ecological balance that favours the predominance of susceptible bacterial flora.

1997 Antibiotic resistance: origins, evolution, selection and spread. Wiley, Chichester (Ciba Foundation Symposium 207) p 1–14

In large part, bacteria live in harmony with other inhabitants of the earth. Although some infections are caused by bacteria for which humans are a specific host, in most instances the infections follow entry of bacteria into the body by chance. Over the past 50 years, the classic treatment of bacterial infectious diseases has been antibiotics, the discovery of which vastly changed the relationship between bacteria and people. Today we are witnessing another change, that is, among the bacteria themselves.

While diversity characterizes the microbial flora, antibiotic use has led to a further subgrouping into those bacteria that are susceptible and those that are resistant to antibiotics. Prior to antibiotic introduction, the large majority of commensal and

infectious bacteria associated with people were susceptible to these agents. Over the ensuing five decades the mounting increase in the use of antibiotics, not only in people, but also in animals and in agriculture, has delivered a selection unprecedented in the history of evolution (Levy 1992). The powerful killing and growth inhibitory effects of antibiotics have reduced the numbers of susceptible strains, leading to the propagation of resistant variants. These have eventually evolved into prominent members of the microbial flora. The antibiotic susceptibility profile of bacteria on the skin of people today, and in the environments of hospitals and homes, is very different from what it was in the pre-antibiotic era, and even 10 years ago. Multidrug resistance is commonly found in bacteria which cause infections as well as in commensal organisms which colonize our intestinal tract, skin and upper respiratory tracts. The resistant bacteria are the survivors of the antibiotic selection which has been taking place within various segments of society.

Microbes circulate everywhere, and there is a continual exchange among the different human, animal and agricultural hosts. We do not know which bacteria are resistant and which are susceptible. As has been suggested, it would be very helpful if we had a system by which we could see resistant bacteria in different colours, distinguishing them from susceptible bacterial populations (O'Brien & Stelling 1995). We could then determine the environments needing remediation, i.e. a return of susceptible flora.

Antibiotics are unique therapeutics. They treat more than just the individual. They treat the environment and in that way they affect society. This characteristic of antibiotics is why today's society is facing one of its gravest public health problems — numerous infectious bacteria with resistance to many, and in some cases to all, available antibiotics. Antibiotic resistance exemplifies *par excellence* Darwinism: surviving strains have emerged under the protection and selection by the antibiotic. Use of the same antibiotics in all parts of the world has led to the emergence of resistant bacteria that find ready havens for propagation wherever they move.

Antibiotics have also revealed the genetic fluidity of bacteria in terms of their ability to exchange genetic traits among genera and species which are evolutionarily millennia apart. Antibiotic resistance genes on plasmids and transposons flow to and from Gram-positive and Gram-negative bacteria, and among bacteria which inhabit vastly different ecological niches.

In assessing the antibiotic resistance problem, we can identify a number of factors which have contributed and continue to impact on the emergence of resistance. The leading two are the antibiotic itself and the resistance determinant. They make up what I have called the 'drug resistance equation.' (Fig. 1) (Levy 1994) The two entities ebb and flow to affect the magnitude of the clinical drug resistance problem. If either is

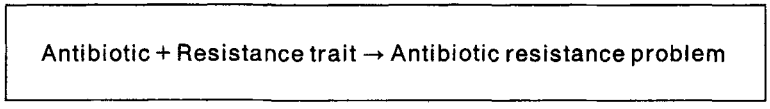

FIG. 1. The drug resistance equation.

absent, a drug resistance problem will not emerge; but given the presence of both the antibiotic and a resistance trait, drug resistant bacteria will be selected and propagated. To these two factors, we can add spread of resistant bacteria themselves and the cell to cell spread of the resistance traits. It is no wonder that an environment can become rapidly populated with different kinds of resistant bacteria.

Antibiotics and the emergence of resistance: the selection density

Antibiotics were initially developed for the treatment of infectious diseases in people. Their miraculous effects led to their being solicited and used for the treatment of animals and eventually plants. The same ones are being used in all three areas. Thus, an enormous worldwide selective pressure has occurred. Antibiotics are used both internally and externally to control bacterial problems for society, maintaining the health of people, animals and agricultural crops. If different antibiotics had been chosen for animals and agriculture than those used in people, we might be witnessing a lower level of resistance today. But, in fact, with each ensuing year, 4–5% more antibiotics have been produced, developed and used. In the USA alone, an estimated 160 million prescriptions for antibiotics were written last year and over 50 million pounds were produced for use in people, animals and agriculture.

There are two major effects of an antibiotic: therapeutically, it treats the invading infectious organism, but it also eliminates other, or non-disease producing, bacteria in its wake. The latter do, in fact, contribute to the diversity of the ecosystem and the natural balance between susceptible and resistant strains. The consequence of antibiotic use is, therefore, the disruption of the natural microbial ecology. This alteration may be revealed in the emergence of types of bacteria which are very different from those previously found there, or drug resistant variants of the same ones that were already present. The dominance acquired by these new strains in the treated environment is directly linked to the intrinsic or acquired resistance to the antibiotics being used.

To a large extent, the reversibility of the selection process is dependent on repopulation by the original susceptible bacteria. Their residual numbers will be related to the total amount of selective drug used in that environment. This relationship suggests that it is the density of the antibiotic, i.e. the total quantity applied, the number of individuals (people, animals, plants) treated, and the size of the geographic area affected, which quantitatively and qualitatively affects microbial ecology. This concept translates directly into a 'density' selection process which affects that ecology (Fig. 2). The introduction of an antibiotic into an environment has the eventual effect of killing-off most, if not all, of the resident susceptible strains. Any resistant survivors will then have a chance to propagate and take over. But adjacent to that selective environment, and encroaching on it, are untreated, susceptible strains which are still potential competitors for the treated area, if given the opportunity. The size of the area selected for resistance will be related to the total amount of antibiotic used and the geographical extent of its influence. It further relies on the potential for susceptible strains to return after the selective event. One would not expect the same ecological

Amount of Antibiotic	*per*	Individual	*per*	Geographic Area

FIG. 2. Selection density.

effect if a hundred pounds of antibiotic were distributed among 20 animals as compared to 20 000 animals. As long as the dosage of antibiotic is above its growth-inhibitory concentration, a greater effect will be seen in the larger numbers of animals being treated—as they will be propagating a thousand times more resistant bacteria. Likewise, the ecological effect of two individuals treated in one room will be different from two being treated in two different rooms or homes. The selection of antibiotic resistance is, therefore, greatly affected by the numbers being treated as well as the size of the treatment area and the numbers of susceptible bacteria surviving the treatment.

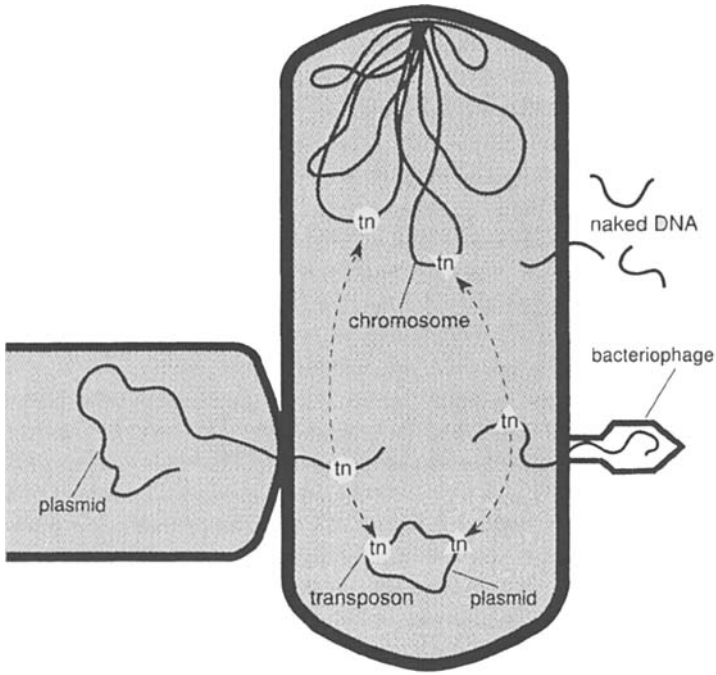

FIG. 3. Bacteria exchange resistance genes through a variety of mechanisms. Extrachromosomal plasmids can deliver genes they bear from one bacterium to other bacteria of different types. Small DNA elements, called transposons (tn), can move among various DNAs: plasmids, chromosomes and bacteriophages. DNA can enter the cell on plasmids through cell to cell contact (conjugation) between two cells, by bacteriophage introduction, or by cellular uptake of naked DNA. (Adapted from Fig. 4.3 in Levy 1992.)

Genetics of drug resistance and spread

The emergence of resistant bacteria raises concern about the bacteria and their progeny and also the extent that they can spread to other environments. The bacterium itself is the focus, if the resistance trait is linked solely to that bacterium and cannot be shared by others. This is, however, not the case with most resistance traits in the majority of bacteria. They have evolved extrachromosomal replicating genes called plasmids and their associated transposons which allow rapid and very broad dissemination of genes (Fig. 3). Gene transfer crosses species and genus barriers (DeFlaun & Levy 1989). Thus, resistant enterococci selected in one environment can pass resistance genes not only to other members of their own genus and species but also to other organisms in other genera. Staphylococci share their plasmids with *Listeria; Escherichia coli* can share genes with other members of the *Enterobacteriaceae* as well as the pseudomonads and *Neisseria*, just to mention a few. In fact, the same tetracycline resistance determinants can be found among Gram-positive and Gram-negative bacteria as well as in the mycobacterium (Roberts 1997, this volume). The genetic flexibility and versatility of bacteria have therefore contributed largely to the efficiency by which antibiotic resistance has spread among bacteria and among environments globally. However, it is equally evident that the transfer event has no consequence unless the antibiotic selection is there. Thus, the emergence and maintenance of bacterial resistance relies on the interrelationship between the resistance determinant and the antibiotic.

Reversal of resistance

Data on the reversal of the resistance selection offer further insights into the selection process. The faecal flora of a volunteer, myself, taking tetracycline for five days was examined. Initially tetracycline resistance was present at a low level; it peaked within two days of tetracycline use. After five days, tetracycline was stopped, but the resistance frequency declined very slowly. The rate of loss did not mimic the rate of gain of resistance: it took 15 days to return to the initial pre-antibiotic level (Levy 1986). Antibiotics are so powerful that they provide rapid selection for a new resistant breed, but when you remove the antibiotic, a reversal is slow in coming. The resistant bacteria selected by tetracycline are no less 'fit' than the susceptible flora; hence they continue to propagate and persist.

We did similar studies among chickens excreting *E. coli* with multi-resistance plasmids. They did not lose the *E. coli*, despite multiple cleanings of the cage over several months (Levy 1986). However, this was a closed environment, and there was no easy route of entry for susceptible strains. Moreover, the resistant bacteria were clearly not disadvantaged by bearing resistance. When the cages were relocated to different sites around the barn, the surrounding environment was altered and the chickens' flora slowly returned to a more susceptible one (Levy 1986). In another study, we added four chickens excreting a resistant flora to 10 other chickens excreting a susceptible flora. Resistance was lost; the susceptible flora won out. For an immediate change in resistance frequency, the result relies on numbers, not large

differences in bacterial fitness. Moreover, there is no active counter-selective force which propels repopulation with susceptible strains.

In the short term, the resistant bacteria were not less fit than the susceptible ones, so we did not observe a rapid shift from resistance to susceptibility. However, in the long term such changes have been documented in hospitals (Giamarellou & Antoniadou 1997, this volume) and on farms (Levy et al 1976) when antibiotics have been removed. But it takes time. In some instances, a newly gained plasmid is not stably kept in its new host. Early on, this instability will help in reversing the resistance. However, with time, the plasmid and bacteria may develop a synergistic relationship whereby both are needed for growth, demonstrating a phenomenon to be discussed later in this symposium (Lenski 1997, this volume). Still, the evidence suggests that, given a 'ready and willing' susceptible flora, a resistance predominance can be overturned if antibiotics are removed.

The resistance reservoir

Resistance genes reside not only in disease-causing organisms, but in commensal organisms as well. These normally harmless bacteria, such as *E. coli* or enterococcus, can cause a fatal illness if the person is immunocompromised. Moreover, these bacteria harbour resistance genes which can spread to the bacterial strains that do cause infection. Unfortunately, these reservoirs are not being examined very much.

People today harbour many multidrug resistant bacteria. In a study of faecal flora from an ambulatory community, we found that 40% of people on antibiotics carried two or more resistances in 10% of their *E. coli*; 25% had three or more resistances, and 10% had four or more (Levy et al 1988). People excrete resistant *E. coli* at the 50% level, even when not consuming antibiotics (Levy et al 1988). High carriage levels of resistant faecal flora have been reported from Holland (Bonten et al 1992), and elsewhere (Calva et al 1992, Leistevuo et al 1996). Resistant bacteria are plentiful in the environment, providing evidence for an environment in a state of imbalance. While not necessarily inflicting harm, they certainly reflect a significant selection process.

One source of resistant bacteria is food. A large number of drug resistant Gram-negative bacteria are asssociated with uncooked foods (Levy 1984). In the great majority of instances these bacteria pose no health problem. But they too tell us a lot about the environmental imbalance. A study from France assessed the contribution of food bacteria to the intestinal flora by examining the same volunteers when eating normal or sterilized food (Corpet 1988). Tetracycline resistance in the faecal flora was high when the volunteers were eating normal, non-sterilized food for 21 days, but dropped dramatically when the diet was shifted to sterilized food for 17 days (Table 1).

Besides selecting resistant variants, antibiotics can affect the ecology by changing the types of organisms there. New opportunistic infectious disease agents, intrinsically resistant to the antibiotic in use, can emerge and predominate. For instance, the use of second and third generation cephalosporins in hospitals, introduced for Gram-negative bacteria, selected the normally harmless enterococcus, which is intrinsically resistant to these antibiotics. The enterococci, selected by these drugs, have now become prominent members of the

TABLE 1 Log number of total and tetracycline-resistant lactose-fermenting enteric bacilli from six volunteers on a sterile diet

Control diet (21 d)		*Sterile diet (17 d)*	
Total	*Tet*^{R}	*Total*	*Tet*^{R}
7.4±0.7	5.2±1.3	6.9±1.0	2.5±1.4

Data for the control and sterile diets are means ±SD for 21 and 17 daily counts, respectively. Tet^R denotes tetracycline resistant. Data from Corpet (1988).

hospital acquired flora. Moreover, the organism has emerged with its own multiplicity of resistances, e.g. to aminoglycosides and vancomycin. It is a likely potential donor of vancomycin resistance to the staphylococcus. Replacement of an endogenous flora with a new flora as a consequence of antibiotic use is an important concept that is too often disregarded. It has a significant impact to our health.

If one is thinking about using an antibiotic to target the disease-causing organism, which, of course, is the magic of these drugs, one has to think about the other bacteria as well. If the antibiotic's sphere of influence is large, then its ecological effect will be large. As we widen antibiotic usage from the individual to the hospitals and the community, we see more and more effect on the susceptible strains. Some have talked about spraying hospital rooms with susceptible commensal organisms to replace and compete with the disease agents. It is an approach worth considering.

Overall, let's focus not just on the antibiotic, but also on the susceptible flora. Susceptible bacteria should be our teammates in confronting and reversing the resistance problem.

Why all the current publicity?

Why has so much recent attention been given to a field that some of us in this room have been working in for decades? Many journalists writing about it are directed by a personal experience. Many of these writers, or their editors, have children who have, or have had, ear infections or other infections that did not respond to antibiotics. The pneumococcus, whether the real culprit or not, has clearly brought the drug resistance issue to public awareness. Not just the kids are suffering, but the parents, as well, because they cannot fulfil their job obligations having to stay home with a sick child. Besides the pneumococcus, there are other resistant bacteria confronting society at large. The tubercle bacillus, which causes tuberculosis, is multidrug resistant and, in some patients, incurable. The gonococcus, the agent of gonorrhoea and a community acquired infection, is now resistant to penicillin, tetracycline, quinolones and some strains show early signs of resistance to cephalosporins. Few if any options remain after the cephalosporins. This is a societal problem. Imagine what's going to happen when we lose our ability to rapidly treat this organism. The staphylococcus can only reliably be treated with vancomycin. To these can be added *Pseudomonas aeruginosa*, *Acinetobacter*

and other bacterial disease agents, all thwarting therapy by resistance. The decade of the 1990s is unique. Resistance is no longer confined to hospital environments, but is now common in community populations worldwide. As important, this crisis is heightened by a lack of new antibiotics developed during the decade.

Approaches to the problem

No novel antibiotic is expected to appear soon, and an increasing number of bacterial infectious agents bear resistance to many if not all antibiotics. We must somehow find a means to reverse the ecological imbalance that has occurred in terms of resistant and susceptible strains. One way is to remove or adjust the selection process so as to allow the susceptible strains to regain their former dominance. As demonstrated above, such reversals are possible and provide the necessary optimism. There still are sufficient susceptible bacteria in our environment which, when given a chance, can return and re-establish the susceptible flora. The crux for reversing and curbing the resistance problem lies in restoring the susceptible microbial flora, whether this is in the intestinal tract, the skin, or elsewhere in the environment. To do this, antibiotic use needs to be more rational. The misconceptions and misunderstandings of antibiotics as miracle drugs without adverse consequences have led to their inappropriate use and prescription. Education of the prescriber and the consumer is critical.

In previous decades the pharmaceutical industry has been able to identify and produce newer and more potent antibacterial agents. However, experience in the present decade indicates that this is no longer true. Discovery has diminished, although encouraging signs are appearing once more (Service 1995). There are now renewed efforts in large pharmaceutical houses and smaller biotechnology companies to discover truly novel drugs. These drugs would be those with no structural relationship to prior antibiotics and thus not intrinsically subject to already existing resistances. This offers one approach towards a solution. Another is to define sufficiently the resistance mechanism and use it to identify novel drugs which can poison or inactivate resistance mechanisms and allow the effective antibiotic to work. This is the basis for the success of the combination of β-lactamase blockers and an effective β-lactam drug, initially introduced as clavulanate and amoxicillin by Beecham Pharmaceuticals. It is this same approach which we are using to restore efficacy to the tetracycline family. Here we are using a semi-synthetic tetracycline to block a drug efflux, allowing a classical tetracycline to enter and stop growth (Nelson et al 1993, 1994).

The control of the antibiotic resistance problem lies in a better understanding of how we use antibiotics. Conditions can be envisioned whereby we encourage the re-emergence of susceptible strains following treatments and the maintenance of the normal susceptible microbial flora between treatments. We need to restore the original microbial balance between susceptible and resistant bacteria — a balance which has been devastatingly altered by the inappropriate and continued application of antibiotics to our environments.

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DISCUSSION

Lenski: The goal of shifting the ecological balance from resistant to susceptible strains of bacteria is clearly an attractive idea. However, how strong is the evidence that when antibiotic usage is relaxed the resistant flora decline? What kinds of rates

are we talking about? You gave the example that when you treated yourself with tetracycline it took about three times longer for the resistance to be lost than it did to appear. That struck me as a rather fast rate of disappearance: it suggests there may have been a high cost to resistance for the bacteria.

Bush: In the β-lactamase situation, although ceftazidime resistance can be diminished if you take away the drug, resistance plasmids are maintained in colonizing flora. If the use of ceftazidime is reduced, the number of ceftazidime-resistant isolates will diminish and seem to disappear within a hospital. But it has been demonstrated in Chicago nursing home patients that the plasmids continue to survive in colonizing flora (Wiener et al 1992).

Levy: Thus, although the resistant bacteria are no longer seen as a nosocomial infection, they're still present in the hospital.

Bush: Yes.

Davies: In the tetracycline ingestion experiment, you only analysed tetracycline-resistant organisms of one particular type. You have no idea of the reservoir of tetracycline resistance in the gut flora. Thus it seems to me that the experiment is incomplete and you should do it again using PCR amplification with *tet*-specific primers to find out how long the tetracycline-resistance gene stays around in the gut. I believe that the gene for resistance is going to persist longer than you have shown.

Levy: Tetracycline resistance did not go away. The total tetracycline-resistant *E. coli* flora went back down to what it was before, but it was clearly detectable.

Levin: I once did a similar experiment to that of Stuart, by sampling my own faeces and plating them on antibiotic-free lactose minimal agar and lactose minimal agar containing antibiotics such as streptomycin, ampicillin, kanamycin and tetracycline. In that way it was possible to monitor the frequency of resistant bacteria, even when they were quite rare, in the order of 10^{-5} or less. Before taking tetracycline, the frequency of resistance to the antibiotic was between 10^{-3} and 10^{-2}. One day after I started taking tetracycline, virtually all the bacteria recovered were tetracycline resistant. Moreover, the frequency of resistance to the other antibiotics also increased. Following the termination of treatment, the frequency of resistance to tetracycline and the other antibiotics waned, but continued to oscillate at levels in excess of 10^{-3} for the month or so I sampled for (B. R. Levin, unpublished results).

Levy: There are clearly many other bacteria entering the intestinal flora from the food we eat. Thus there is a lot of mixing with the external flora. A better way to do that experiment would be for me to go on a sterile diet, so I would just be looking at what was happening within my intestinal flora.

Roberts: If you look at the opposite end — the oral cavity — virtually all of us carry tetracycline-resistant α-streptococci, regardless of whether we have had tetracycline or not. Many children who routinely never take tetracycline have tetracycline resistant α-streptococci. One has to go back to the 1960s to find α-streptococci that are susceptible. Many people make the wrong assumption that they are innately resistant, but they have all acquired the *tet* genes. So there are other organisms where you may not get the waxing and waning. We studied bacterial vaginosis in pregnant women, again a group who do not receive tetracycline. Virtually every patient had Tet resistant

streptococci and peptostreptococci (Roberts & Hillier 1990). I'm proposing that in streptococci you actually get less fluctuation from susceptible to resistant then you do with *E. coli* in the gut.

Levy: So there's a resident oral flora that persists?

Roberts: Yes. In some of the dental literature, people mistakenly say that the α-streptococci in the oral cavity are intrinsically resistant which is not true; all of them have acquired *tet* genes.

Baquero: That is correct, but the real problem is that it may be too late to react, in the sense that our normal flora is now the normal resistant flora. They have adopted the resistance determinants, perhaps taking advantage of these mechanisms for other functions. One of the key points from your discussion is the concept of a 'resistance gene'. This is a somewhat controversial issue, because many bacteria are normally physiologically insusceptible, or intrinsically resistant. I'm worried by the fate of these intrinsically resistant bacteria in the face of antibiotic pressure. Imagine that we are just looking at potentially pathogenic bacteria: the problem with the multiple antibiotics we are taking to control pathogens is that by their use we are altering our normal bacterial environment. For instance, we are eliminating some of our old lactobacilli and these are replaced by other less 'human adapted' lactobacilli intrinsically resistant to the antibiotics we are using. Perhaps we are changing our normal gut physiology, replacing the bacteria that have been co-selected with us during evolution. Eventually, we are doing something even worse than modifying the pathogenic bacteria—modifying the normal saprophytic bacteria in alliance with human beings through evolution. Who knows what the implications of such changes are for human health?

Summers: Has anybody done any prospective studies on hospital admissions in checking the level of resistance on entry to see what level of resistance indeed does involve subsequent clinical compromise?

Huovinen: We have some results from a study in geriatric units (Leistevuo et al 1996). When someone is treated with an antimicrobial drug, resistant strains will be enriched in this subject and he or she will then excrete these strains to the surroundings.

Levy: You come into hospital with a trace of resistant bacteria. You receive an antibiotic, and resistance frequency rises. Anne Summers is asking: if a patient comes into hospital with 10% resistance, does that level of resistance get them into trouble? I don't know that anyone has done such a prospective study, although many of us have found resistant organisms in the faeces. The frequency probably doesn't matter, because as soon as you start using the antibiotic the numbers rise.

Huovinen: In long-term treatment the patients are colonized in the hospital. We can show that although the level of antimicrobial usage in the ward is very low, patients are still colonized with resistant strains. Antimicrobial treatment is not the only factor.

Cohen: This is a reflection of the underlying problem, which is that we look at only a subset of the microbial flora. Within the greater ecosystem, we're examining just a small part: often just a single pathogen. We often don't know what other species are out there. It is difficult to look at this small microcosm and understand everything that's occurring.

Bush: If we look at the Chicago experience with β-lactamases, the first ceftazidime-resistant β-lactamases appeared in two *Klebsiella* isolates in January 1988. They were not identified again until November 1990 when nursing home patients were then admitted to Chicago hospitals. Resistant *Klebsiella* strains in the hospital were apparently taken back to nursing homes where patients were eventually colonized with *E. coli* with the same plasmids. Species to species transmission was occurring (Wiener et al 1992).

Davies: It's my understanding that we cannot culture all of the organisms in the gastrointestinal tract. The whole point about resistance transfer is that we really don't know where resistance genes are going within the bacterial population. In many cases we don't know what the reservoir is. While we are looking at the bugs that we can grow, we are missing a lot of the microbial ecology of drug resistance.

Noble: It's going to be slightly naïve to look just at one genus, because within a genus you can get quite diverse results. In patients with peritoneal dialysis, the coagulase-negative staphylococci are quite often resistant to three or four antibiotics. In contrast, the coagulase-positive staphylococci are usually sensitive to everything except perhaps penicillin and tetracycline. It may be the case that what is normal on the skin is able to cope with lots of resistant determinants, but what is abnormal on skin can't, unless it's under antibiotic pressure.

Some years ago we showed there are differences between what skin patients have in their nose and what they have on their skin (Noble 1977). What they have on their skin tends to be much more antibiotic resistant even if it's apparently the same strain. There are other factors. For example, many of the penicillinase-producing strains are somewhat more resistant to lipid. We thought this was a rather tidy way of looking at it. It may be that it is actually the skin lipid that is selecting for penicillinase-producing strains, and not the penicillin. We're talking about a resistance determinant as though there's no other DNA on that plasmid or transposon.

Levy: This echoes what Fernando Baquero was saying: can you really call this a 'resistance determinant'? What else might it be doing?

Levin: The basic premise of Stuart Levy's paper is that there is a genetically diverse population containing both bacteria that are resistant and those that are susceptible, and that we should encourage the reservoir of susceptible strains to replace the resistant. On a broader scale, each time antibiotics are used we are increasing the relative frequency of resistance, so that this pristine population you want to replace is going to decline if there is no cost of resistance. Consequently, this reservoir of wonderfully naïve sensitive bacteria would be in a continual state of decline. It seems that already, among the commensal bacteria such as *E. coli*, this sensitive reservoir is already lower than we would like.

Last spring, an undergraduate working in our laboratory, Bassam Tomeh, did a survey of the frequency of resistant bacteria in the faeces of children younger than 30 months in a local day-care centre. He used the lactose minimal-selective plating procedure described above for the egoistic excursion into the comings and goings of my own enteric flora. Bassam's selecting agars contained ampicillin, streptomycin, kanamycin, chloramphenicol and tetracycline. The results, which we are now just

analysing, are not optimistic. 15 of the 25 children in the survey were treated with at least one antibiotic during the 3 month sampling period and/or the 3 months before, with one infant being treated with as many as five different drugs during this interval. On average, nearly 50% of the bacteria isolated from these 'treated' infants were resistant to ampicillin, with the entire sample taken from one child being resistant to this antibiotic. Of the total of 13 treated children for whom we had sampled bacteria, resistance to ampicillin was observed in 11. On average, about 25% of the bacteria isolated from the children in this treated subset were resistant to 20 μg/ml streptomycin. The next most common resistances among the bacteria isolated from the treated children were to kanamycin and tetracycline, about 10% on average. For a few children in the treated sample, the frequency of resistance to these two antibiotics in the bacteria recovered from the faecal samples was substantially higher, as great as 50% and 30%, respectively. Kanamycin and tetracycline resistance, however, was not observed in the flora of the majority of the treated children. On average, the frequency of bacteria resistant to ampicillin and streptomycin in seven untreated children for which we had bacterial samples was no different from that in the treated.

Levy: At the core of the clinician's decision to use antibiotics are sick patients who are not getting better. The attention of the microbiologist is directed to the source of the resistance. Quite clearly some way of combining these aims is needed.

Lerner: We've had an interesting experience in Detroit with MRSA. We are in an unusual situation in that we have a major reservoir of the organisms in the community among intravenous drug users. For over a decade, until recently, the majority of community *Staphylococcus aureus* isolates from intravenous drug-abusing patients as they came into hospital were methicillin-resistant. In the past year or two, the incidence of MRSA in this patient population appears to have declined dramatically, for reasons which are obscure. This decline in resistance is welcome, but we wish we understood it.

Levy: Echoing some of the themes that we've discussed, the change could be the removal of a selective force, or the encouragement of some other organisms which interfere with the resistant organism you have selected.

Baquero: It seems that in several instances where we have a problem with MRSA, things have been getting better, just spontaneously. My impression is that some very clonal bacteria have just got tired of being on top! This may have some genetic background. For instance, one given serotype may be substituted by another in the human population, either because of herd immunity or because the new serotype is resistant to another antibiotic which is also being consumed heavily.

Giamarellou: We have seen the same thing with MRSA: percentage resistance is fluctuating. For instance, after a mean resistance rate of 50% in 1990 the rate went down to 12% in 1992, and then doubled by 1995. I guess that it is resistance in the community that transfers to the hospital, and vice versa. Physicians in the community are concerned about staphylococci and they are aware of the MRSA problem. Therefore they do not use empirically any anti-staphylococcal penicillin in the community for long periods. This fact may explain the fluctuation in MRSA.

Another point that concerns me is that although in Greece people in the community use tons of β-lactams, we have a low rate of *Streptococcus pneumoniae* resistance: only 8% of the intermediate type with a fairly low MIC of 0.25 to 0.5 μg/ml. I cannot explain it.

Witte: Is there real evidence that MRSA has already become part of the colonizing flora in the community? It was my impression that whenever this has been reported there has always been some link back to the hospital. In Germany, the frequency of MRSA doubled from 1990 to 1995. We now have 5% in nosocomial infections. Our community studies have shown that MRSA is still rare among carriers outside hospitals and that we cannot always exclude previous hospital stays.

Roberts: We are currently working with the native population in Alaska, where there is some evidence to suggest that antibiotic resistance has developed *in situ* primarily because they are small communities. For the first 10–12 years there was multidrug resistant *Streptococcus pneumoniae* 6B only in one region of Alaska; now it has been spread all over the state. You will occasionally get another serotype with the same pattern, but this does not seem to spread. There are some factors in the 6B which allow it to be maintained in the community and other factors where it perhaps isn't as good a pathogen, and therefore it may have a little cluster effect but you don't see it maintained for long periods.

Levy: Antibiotics not only select the resistant form of the organism you are trying to treat, but also wreak havoc in the environment. We don't know how large that domino effect is. You cause the resistant organisms to emerge, but they are now in an environment which has also changed. Thus a bacterium that might have been a minor participant in the previous environment, now finds an environment so changed that it can become a major participant.

The antibiotic certainly is a player in the resistance imbalance, but so are the non-target organisms in the environment, many of which we do not know about. We can only look at those things we know. One of the recommendations that I propose should come out of this Symposium is that greater attention should be given to the other organisms being affected by antibiotic use, as well as the factors which cause a change in the levels of resistance which are not linked to antibiotic usage.

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Origins, acquisition and dissemination of antibiotic resistance determinants

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Abstract. Since the introduction of antibiotics in the late 1940s there has been an inexorable propagation of antibiotic resistance genes in bacterial pathogens (and their relatives). This survival phenomenon was first characterized as the appearance of point mutations that altered drug targets, but in the mid-1950s transmissible antibiotic resistance genes were reported in Japan. Since this time both resistance strategies have been used, often in concert. For some types of antibiotic, only resistance by mutation has been identified, for others only resistance by plasmid acquisition. There is conflicting evidence with respect to the presence of antibiotic resistance in bacterial pathogens in the 'pre-antibiotic' era; however, it is likely that the evolution of antibiotic resistance occurred over short periods. Thus, antibiotic resistance genes must be common in the environment, but their derivation remains to be established conclusively. This paper examines the proposals that antibiotic resistance genes originated in the bacterial population, either as *bona fide* resistance genes or genes encoding metabolic functions. In addition, the acquisition of heterologous resistance determinants by different genetic elements, their intergeneric exchange mechanisms, and the possible roles of antibiotics in these processes are discussed. Are there prospects for drug intervention that eliminate or retard these natural evolutionary processes?

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The phenomenon of antibiotic resistance presents an unusual aspect of microbial ecology and diversity which has evolved recently, entirely the result of human activity. About 50 years ago, antibiotics were introduced for the treatment of microbial diseases with the expectation that their use would end, once and for all, the threat of infectious diseases. For the microbial population the use of antibiotics created a situation that should have been catastrophic; however, the (then unsuspected) genetic flexibility of bacteria allowed them to survive and even thrive in this hostile environment. What actually happened to the dynamics of the microbial population will never be known, since it is not possible to grow all of the bacterial species and genera that inhabit a given environment; fewer than 1% of microbes are culturable.