REVIEWS IN MEDICAL MICROBIOLOGY (2002), 13(1), 1–13

Occurrence and reservoirs of antibiotic resistance genes in the environment

Nadine Seveno, Kornelia Smalla*, Jan Dirk van Elsas†, Jean-Claude Collard‡, Amalia Karagouni§, Dimitris Kallifidas and Elizabeth M. H. Wellington

*Department of Biological Sciences University of Warwick, Coventry, UK, *Federal Biological Research Centre BBA, Braunschweig Germany, †Plant Research International, Wageningen, the Netherlands, ‡Institute of Hygiene and Epidemiology, Federal Ministry of Health and Environment, Brussels, Belgium, §Institute of General Botany, University of Athens, Greece*

Antibiotic resistance genes have become highly mobile since the development of antibiotic chemotherapy. A considerable body of evidence exists proving the link between antibiotic use and the significant increase in drug-resistant human bacterial pathogens. The application of molecular detection and tracking techniques in microbial ecological studies has allowed the reservoirs of antibiotic resistance genes to be investigated. It is clear that the transfer of resistance genes has occurred on a global scale and in all natural environments where man is active. The considerable diversity of bacteria and mobile elements in soils has meant that the spread of resistance genes has occurred by all currently known mechanisms for bacterial gene transfer. Even trans-kingdom transfers from plants to bacteria have been detected in soil. Hot spots for gene transfer in the soil/plant environment have been described and colonized niches such as the rhizosphere and other nutrient enriched sites, for example manured soil, have been identified as reservoirs of resistance genes. Although exposure and selection for tolerance of antibiotics is considerable in clinical environments there is increasing evidence that selection for resistant phenotypes is occurring in natural environments. Antibiotic-producing bacteria are abundant in soil and there is evidence that they are actively producing antibiotics in nutrient-enriched environments in soil. In addition there is clear evidence that the self-resistance genes found within antibiotic gene clusters of the producers have transferred to other non-producing bacteria. Perhaps most important of all is the use of antibiotics in agriculture as growth promotants and for treatment of disease in intensively reared farm animals. These treatments have resulted in gut commensal and pathogenic bacteria acquiring resistance genes under selection and then, due to the way in which farm slurries are disposed of, the spread of these genes to the soil bacterial community. Multi-resistance gene cassettes have been selected and disseminated in this way; many of these cassettes carry other genes such as those conferring heavy metal and disinfectant resistance which have been co-selected in bacteria surviving in effluents and contaminated soils, further maintaining and spreading the antibiotic resistance genes.

Address for correspondence: Elizabeth M. H. Wellington, Department of Biological Sciences University of Warwick, Coventry CV4 7AL, UK. E-mail: ewellington@bio.warwick.ac.uk

0954–139X © 2002 Lippincott Williams & Wilkins

Article number = 404

Keywords: Antibiotic resistance, environmental resistance reservoirs, gene transfer in nature, horizontal gene transfer, antibiotics in agriculture, environmental microbiology.

INTRODUCTION

Resistance to antimicrobials is a natural consequence of bacterial cell adaptation following exposure to antibiotics and is a direct result of the competitive advantage conferred by the resistance phenotype. Multiple uses and misuses of antimicrobial agents in medicine and agriculture have increased the selective pressure for resistance in a wide range of bacterial groups. The widespread use of disinfectants in household products may also be contributing to the development of resistance [1]. It has been argued that antibiotic resistance genes have an environmental origin, and have evolved as part of defence mechanisms against toxic compounds in the environment such as plant and microbial metabolites often encountered in soil [2]. There is clear evidence that the multi-drug resistance determinants occurring in opportunist pathogens from soil, such as the multi-drug resistance efflux pumps found in *Pseudomonas aeruginosa*, have a key role in nature functioning in the export of cell signalling compounds. As both the drug and signal were quinolones this could explain the resistance of these pathogens to clinically useful fluoroquinolones such as ciprofloxacin.

However, the appearance of multi-drug resistance plasmids and integrons is a direct result of strong antibiotic selective pressure resulting from antibiotic therapy forcing the persistence and dissemination of resistance gene cassettes in plasmids predating the discovery of antibiotics [3,4]. The origins of some of these genes is not clear and although theories persist that antibiotic producers may be the source of resistance genes to natural product antibiotics [5,6], further more recent evidence of horizontal gene transfer is needed to confirm this. Genome analysis has revealed that a considerable amount of horizontal gene transfer has occurred during bacterial speciation and in the case of *Escherichia coli* it has been argued that more than 3 megabases of novel DNA has been acquired and lost since divergence from *Salmonella enterica* [7].

The evidence for the development of multiply resistant bacterial pathogens is fast accumulating in various databases. PROTEKT (Prospective Resistance Organism Tracking and Epidimiology for the Ketolide Telithromycin) indicated a high prevalence of global bacterial resistance to antibiotics. The data was derived from testing the susceptibility of seven common and atypical community acquired respiratory tract infection-causing bacteria to most current treatments including penicillins (such as amoxycillin), cephalosporins and commonly used macrolides (such as azithromycin and clarithromycin) [8]. The availability of molecular techniques for plasmid detection and characterization and recent efforts in tracking specific genes in clinical populations has allowed clinical microbiologists to identify antibiotic resistance gene flow [9].

This review is concerned with the evidence for environmental origins of antibiotic resistance and how reservoirs for drug resistance in the environment have been detected using molecular methods.

TRANSFER OF GENES ENCODING ANTIBIOTIC RESISTANCE

Mechanisms of transfer of resistance genes

Multi-resistance plasmids found in bacterial pathogens must have arisen in the past five decades. The use of antibiotics possibly has not increased the spread of bacterial clones carrying such plasmids but encouraged the dissemination of antibiotic resistance genes [4]. Conjugation is thought to be the primary route of broad host range DNA transfer between different genera of bacteria (horizontal transfer). In principle, it requires only the addition of a short DNA sequence – the origin of transfer (*oriT*) – to a replicative element to render it mobilizable by conjugation, by trans-action of the transfer functions of another plasmid [10]. Many different bacterial species have been shown to participate in 'sex factor-directed mating', hence, conjugation can be viewed as a 'non-species-specific process' [10,11]. There are now numerous example of horizontal gene transfer between bacterial species, genera or families and even between bacteria and eukaryotes [12]. Many catabolic functions and antibiotic pathways are associated

with plasmids or larger linear plasmids (Table 1). These can be subject to gene transfer by conjugation and be integrated into the recipient's chromosome by homologous or illegitimate recombination at specific genomic sites, such as the chromosomal ends of the *Streptomyces* linear chromosome. Recent reports of transformation of an acinetobacter strain by transgenic plant DNA in soil prove that other mechanisms for horizontal gene transfer will be important in transkingdom transfers [24].

Plasmids

Traditionally, plasmids were obtained by endogenous isolation techniques. Environmental samples were plated on selective media containing antibiotics or heavy metals. Colonies grown were screened for the presence of plasmids by the transfer of the resistance marker to a sensitive recipient bacterium in a biparental mating. The process was followed by plasmid isolation [25]. The disadvantage of this method is that only the plasmids with a selectable phenotype are recovered, and plasmids that belong to the majority of bacteria that are not culturable are not recovered. However, this method has the advantage that it enables the identification of the bacterial host. Recently, exogenous plasmid isolation techniques have been developed to overcome cultivation biases [26]. The main characteristic of exogenous plasmid isolation is that the bacterial fraction recovered directly from an environmental sample is applied as a potential plasmid donor in matings with selectable recipient bacteria. The recipient needs an appropriate selectable marker, and because of the commonly low levels of natural background, chromosomal rifampicin resistance has been used frequently for matings. To increase plasmid transfer frequencies, an enrichment of the environment sample can be made using nutrient broth for example and, in addition, antibiotics or heavy metals to which the recipient is sensitive. Smalla *et al.* [27] reported the use of the antibiotics tetracycline, streptomycin, gentamicin or nourseothricin to select recipients which obtained resistances to these antibiotics from manure bacteria. By using this method, not only conjugative plasmids were isolated from manure bacteria, but also mobilizable plasmids. To obtain the latter the presence of mobilizing helper plasmids is required, either in the same cell or in another one, in a triparental mating. Plasmid isolation techniques are dependent on their transfer efficiency under the mating conditions chosen as well as their ability to replicate and express selectable marker genes in the recipient. A major disadvantage, different from the endogenous isolation technique, is that the identity of the host remains unknown. To discover the host identity, DNA probes targeting specific genes of the identified plasmid can be designed to chase back the specific plasmid in indigenous bacteria. The exogenous isolation of plasmids can also be performed in triparental matings [28]. In this case, two donors, i.e., the environmental bacterial fraction and a strain carrying a non-self-transferable but mobilizable (Tra^-Mob^+) plasmid are used together with a

Table 1. Transferable phenotypes attributed to linear and other types of plasmids.

Organism	Plasmid	Phenotype attributed	Reference
Rhodococcus erythropolis	pBD2	Isopropylbenzene and trichlorethene catabolism, arsenite and mercury resistance	[13]
Rhodococcus spp.		Autotrophy	
		Tallium resistance	[14,15]
	pHG201	Autotrophy	
	pHG204		
	pHG205	Methylenomycin synthesis	
Streptomyces coelicolor	SCP1	Tylosin synthesis	[16]
S. fradiae		Lasalocid A synthesis	[17]
S. lasaliensis	pKSL	Actinomycin D synthesis	[16]
S. parvulus		Cryptic	[18]
S. rimosus	pPZG101	Chloraphenicol synthesis	[19]
S. venezuelae		Mercury resistance	[18]
Streptomyces spp	pRJ3L	2,4-D metabolism	[20,21]
	pRJ28		
Alkaligenes eutrophus	JMP134	Napthalene degradation	[22]
Pseudomonas putida			[23]

recipient. Therefore, triparental matings provide information on the gene mobilizing capacity of environmental bacteria. Most frequently, mobilization of IncQ (broad-host range) plasmids is exploited in triparental matings [29,30]. This method is dependent on the culturability of the plasmid host and the ability of the plasmid to replicate in recipients. Another approach to detecting plasmids in the environment, which is independent from these factors, is the PCR-based detection of replicon-specific sequences from DNA extracted directly from environmental samples [31]. However, using this approach, only plasmids with published sequence data may be detected.

Several studies have reported *in situ* monitoring of gene transfer by using different non-disruptive methods employing the green fluorescent protein [32,33]. In addition a novel non-disruptive approach was proposed by Dahlberg and co-workers who were able to study the dissemination of plasmids in different systems *in situ* without limiting the detection of gene transfer to the culturable fraction of the bacteria [34].

Transposable elements

Bacteria also possess other mobile elements, i.e., transposable elements, which can transfer within and/or between bacterial cells. Transposable elements can associate with other elements such as chromosomes, plasmids or bacteriophages and may allow the establishment of antibiotic resistance in bacterial hosts in which a plasmid can not replicate [35]. Thus, transposable elements can be transferred horizontally to other bacterial cells (intercellular mobility) either by transduction, in which case the vehicle is a phage, or by conjugation via a conjugative plasmid or a conjugative transposon. [36]. Phage-mediated transfer of genetic elements in natural environments is thought to occur at low frequency although it might be comparable, with respect to its frequency, to conjugation and/or transformation encountered in soil [37]. The stability and the abundance of the phage suggest that they can mediate horizontal gene transfer in ecosystems such as the marine environment [38].

Transformation

Natural transformation in soil has been underestimated because of the belief that released DNA is rapidly degraded. Contrary to this, DNA bound to mineral surfaces has been shown to retain its transforming ability [39,40] (Table 2). Bacterial transformation is also likely to be significant for resistance gene transfer process in nature, as most bacterial genera are competent for transformation under some conditions [48]. Even *E. coli* is transformable under natural conditions [49]. Webb and Davies speculated that antibiotic resistance genes were disseminated from DNA of the antibiotic-producing microorganisms which contaminated the commercial preparations of antibiotics [50]. Antibiotic gene transfer could occur through natural transformation of bacterial cells in the human or animal intestine. Orpin *et al.* reported the natural transformation of a ruminal bacterium *Selenomonas ruminatium* [51].

However, little is known about gene transfer via transformation compared with conjugative transfer and the importance and frequencies of transformation in natural environments remain to be elucidated. Lorenz and Wachernagel showed that DNA absorbed on soil particles could naturally transform soil bacteria [52]. However, non-conjugative mechanisms of horizontal gene transfer might play an important role under specific conditions. For example, transformation may be important in the transfer of non-conjugative plasmids which are common in marine bacteria [53]. Unlike transduction and conjugation, transformation allows for the uptake of huge blocks of heterologous DNA. *Streptococcus pneumoniae* and *Neisseria meningitidis* can take up foreign DNA by transformation and incorporate it into their chromosomes.

Table 2. Persistence of DNA in various environments.

Location	DNA persistence	Method	Reference
Freshwater	8–10 h	CDD	[41]
Marine water	6–11 h	CDD	[42]
Ocean surface	1 day	CDD	[41]
Soil			
Loamy sand	20 h	Trans.	[43]
Silty clay	30 h	Trans.	[43]
Clay soil	2 days	Trans.	[43]
Soil	60 days	PCR	[44]
Soil	40 days	PCR	[45]
Soil	15 days	Trans.	[46]
Soil	n.d.	Trans.	[47]

CDD, Colorimetric DNA determination; Trans., loss of transformation activity of plasmid DNA; PCR, polymerase chain reaction with specific primers; n.d., not determined.

This mechanism has accounted for the creation of mosaic penicillin-binding protein genes which have a reduced affinity to penicillin [54]. It is likely that in the environment more than one mechanism is involved in the dissemination of resistance determinants within a bacterial population.

Origins of genes encoding antibiotic resistance

A traditional hypothesis that a primary source of some resistance genes may have been bacteria producing antibiotics in nature has been widely accepted but few studies have been done to further elucidate this route of transfer. Actinomycetes, especially streptomycetes, are the producers of most clinically used aminoglycosides and have evolved antibiotic modification as one method of self-protection [5]. At least four ways of modifying aminoglycosides are found in actinomycetes. Nucleic acid and protein sequence comparisons from producing organisms and clinical isolates have provided support for the origin of such genes in the producers [55,56]. Confirmation of transfer of resistance genes from producers to pathogens is illustrated by the finding that mycobacteria possess tetracycline-resistance determinants that are identical to those found in the tetracycline-producer *Streptomyces rimosus* [57]. Selection for antibiotic resistance imposed by antibiotic-producing bacteria can also be demonstrated in laboratory studies [58,59]. However, antibiotic-producing microorganisms are not the only potential source of antibiotic resistance genes. Studies by Udou *et al.* [56], Shaw *et al.* [60] and Rather *et al.* [61] indicate that 'housekeeping' genes such as the sugar kinases and acetyltransferases may have evolved to also modify by phosphorylation or adenylation aminoglycoside antibiotics. According to Salyers and Shoemaker [62] resistance genes may have originated via mutations of genes important for significant functions of the bacterial cell. For example a tetracycline resistance gene showed high homology to microbial elongation factors and this gene may have evolved from these factors [62]. However, despite these plausible hypotheses the reservoirs for the original antibiotic-resistant determinants remain obscure. Many of the studies so far have concentrated on plasmids obtained from culturable microorganisms and especially so on those from clinical isolates. This might have led to a large underestimation in the degree of plasmid diversity present in nature.

Resistance is often the result of a single or multiple genetic mutation, for example the development of resistance in *Mycobacterium tuberculosis* to streptomycin, the extended spectrum β-lactamases in Gram-negative bacteria, all fluoroquinolone resistance, and rifampicin resistance [63,64]. Resistance due to adaptive mutations is an important mechanism for generating resistance, especially for those pathogenic bacteria which remain intracellular for much of their life cycle such as *Mycobacterium* species. Adaptive mutations produce only those phenotypes that allow cells to grow and occur in non-dividing cells that are under selection pressure for particular phenotypes. There is *in vitro* evidence for adaptive mutation to produce resistance to ciprofloxacin [65].

TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN THE SOIL/ PLANT ENVIRONMENT

Bacterial gene transfer is now known to occur not only in the human and animal intestine but throughout the biosphere, especially in nutrient-rich sites such as aquatic systems, sediments, soils, in the vicinity of plant roots and in the sludge of the biological sewage treatment plants [66–68]. These environments contain a multitude of bacterial species and gene transfer is likely to proceed through multiple steps involving many different genera. The European Commission has reported that 'Four ecological compartments may be considered as important for the transfer of antimicrobial resistance; humans, animals, plants and soil-water. The common factors between the four ecological compartments are the antimicrobials, the bacteria and the genes that code for the resistance'. Some resistance genes can move between bacteria in each compartment, it is possible for bacteria to move between the compartments, and antibiotics have been applied or found in each ecological compartment.

Transfer of resistance gene in soil and agricultural practices

In the European Union in 1997, an estimated 10 493 tons of active antimicrobial substance was used: 52% for humans, 48% for therapeutic use and as feed additives for animal growth promotion use (Table 3 and Table 4). Antimicrobials used as feed additives are administered to a larger number of animals than would be needed

Table 3. Estimated annual usage of antimicrobials in humans and animals in the EU (1997). Data from information provided at the 1998 FEDESA ('European Animal Health'), Copenhagen, 1998.

	Tons of active ingredient at 100% purity	Per cent
Human usage (hospital and general practise)	5400	52
Veterinary (therapeutic) usage	3494	33
Animal feed additives	1599	15
Total	10493	100

for disease control alone. The period of administration is often life-long and, as noted by the European Scientific Steering Committee (1999), 'recipient animals enter directly the human food chain thereby providing a greater opportunity for transmission of bacterial antibiotic resistance directly to bacteria that associate with humans'. The largest amount of antimicrobials is administered to animal flocks and herds through feed or water, and run-off from farms is introducing antibiotics and antibiotic-resistant bacteria into groundwater and other water sources. The ban in 1998 of four growth-promoting antibiotic compounds by the EU highlighted the need for justification for the continued use of such feed additives. It has been observed that antibiotics are most effective for promotion of growth on farms with poorer animal health and hygiene records. In Sweden, where all antibiotics were withdrawn from use in growth promotion in 1986, the growth of animals is not less than in countries using antibiotics. Most of the antibiotics used for human or animal medicine are not allowed for use as growth promoters; however, drugs of the same class, to which cross-resistance can occur, are still in use. Furthermore, many studies have shown that the introduction of specific antimicrobials into veterinary practice is followed by detection of resistance to those antimicrobials. The prevalence of resistance tends to fall once the drugs cease to be used. Although the incidence of antibiotic-resistant strains decreases, it can stabilize at 6–10% of the number of isolates. For example, in the UK, after the ban of tetracycline as a feed additive, tetracycline-resistant *S. typhimurium* isolates from calves fell from 60% in 1970 to 8% in 1977. However, this level can be sufficient to prevent future effective use of the antibiotic.

Table 4. Estimated sales volume[a] in the EU for each antimicrobial group (1997). Data from information provided at the 1998 FEDESA ('European Animal Health'), Copenhagen, 1998. Coccidiostats (ionophores and others) are excluded.

	Tons of active ingredient at 100% purity	Per cent
Penicillins	322	6.3
Tetracyclines	2294	45.0
Macrolides	424	8.3
Aminoglycosides	154	3.0
Fluoroquinolones	43	0.8
Trimethoprim/sulphas	75	1.5
Other therapeutics	182	3.6
Animal feed additives	1599	31.4
Total	5093	100

Antimicrobial resistance is now widespread in the environment and should therefore be seen as a phenomenon of global genetic ecology. Resistant bacteria have been isolated from continental and coastal waters and from sediments, from soils and in the sludge of sewage treatment systems [69]. As the percentage of soil bacteria that can be cultured is low (1–10%) [70], an accurate estimate of the percentage of natural antibiotic resistance in soil microorganisms is not possible. In both soil and water, gene transfer frequencies are thought to be low, and the main factor limiting gene transfer in these environments seems to be nutrient availability as a factor controlling bacterial density and activity [71]. In soil, the amount and diversity of nutrients is especially high around plant roots and these sites are privileged bacterial 'hot spots' for gene transfer but transfer is affected also by other factors such as soil moisture, pH and temperature [72,73].

Bacterial survival and activity is enhanced in manure slurries and, together with the selective pressure resulting from the presence of antibiotic in animal faeces, horizontal gene transfer may be favoured among manure bacteria [74]. Manure slurries are applied as fertilizers in agriculture because of their high content of organic substances. However, although manuring is a traditional agricultural practice, intensive animal factory farming has led to a dramatic increase in its use, and consequently to an increase in environmental pollution such as the well-recognized problems of nitrate-contaminated groundwaters and eutrophized water surfaces. Furthermore, manure slurries

can serve as a reservoir of pathogenic microorganisms such as salmonellae [75]. Another public health issue is the contribution of manure to the environmental spread of antibiotic resistance genes and plasmids. Most studies in manure have been based on traditional microbiological techniques, that is on cultivation techniques. This approach provides resistance quotients, as well as data on plasmids in culturable bacteria, but these studies have only focussed on a selected group of culturable bacteria, mainly coliforms and faecal enterococci. Before the application of nourseothricin (streptothricin antibiotic) as a feed additive, resistance quotients for coliforms in manure were approximately 0.1–1%, whereas after the application of nourseothricin, resistance quotients of up to 80% were observed. Furthermore, plasmid-borne resistance was detected among strains isolated from pigs, and subsequently in isolates from manure, river water, food, and human beings [76]. Specific probes and PCR-primer systems have been developed for the detection of specific antibiotic resistance genes in resistant bacteria isolated from the environment or for the direct detection of resistance genes in DNA extracted from the environment. Probes for the detection of streptothricin resistance genes were derived from a set of different streptothricin acetyltransferase (*sat*)-genes, notably the resistance gene probes for the determinants *sat1* and *sat2* (both transposon encoded) and for the resistance determinant *sat3* of the IncQ R-plasmid pIE639 [77]. Streptothricin resistance genes have been detected in streptothricin-resistant bacteria isolated from pig manure, sewage and surface water [78]. The *sat1* and *sat2* genes both occur as gene cassettes together with the streptomycin resistance gene *aphA* in Tn7-like integron structures and this explains why the streptothricin resistance phenotype is always coupled with the streptomycin resistance [79]. PCR-amplification of specific target in total DNA extracts offers a highly sensitive technique, more sensitive than DNA : DNA hybridization, for the detection of resistance genes in the environment [78]. Using this approach, Götz and Smalla [74] in comparing manure and non-manure soils showed that *sat2* genes enter into soil via manure. They also showed, using exogenous plasmid isolation in the same field study, that manurring enhances the gene-mobilizing capacity of soils. Mobilization of selectable IncQ plasmids from an introduced *E. coli* strain was observed in manured soils, whereas mobilization was not detectable in non-manured soils. Epidemiological studies using endogenous plasmid isolation, which allow the identification of the resistant host, can be coupled with PCR-based identification of a specific plasmid or resistance gene. The emergence and spread of streptothricin resistance in enteric bacteria as a consequence of the application of nourseothricin as a feed additive in piggeries was traced in this manner. Most of the resistance plasmids isolated by endogenous plasmid isolation from manure were assigned to the known Inc groups by incompatibility and phage testing. Streptothricin-resistance plasmids found in manure belonged mainly to IncW3, IncI1, IncI2, IncN, IncH2, IncX and IncFII [76]. However, using biparental exogenous plasmid isolation, PCR fingerprinting techniques, and incompatibility studies, Götz and Smalla [74] found much more diversity among IncQ palsmids isolated from manure samples. These IncQ-plasmids were all mobilizable by mobilizing plasmids present in manure into the *Pseudomonas putida* recipient in a biparental mating, and the majority of plasmids obtained by exogenous isolation could not be assigned to any of the known Inc groups by means of inc/rep probes. Therefore, the endogenous and exogenous techniques yielded a different range of plasmids. With the endogenous isolation technique, only the most abundant plasmids are retrieved whereas the exogenous approach recovers the most efficiently transferring plasmids and therefore those capable of spreading antibiotic resistance genes. Hence, the exogenous isolation technique, which allows the study of plasmids independently of the culturability of their original host and the expression of resistance genes, provides new insights in the diversity of plasmids present in the environment.

Plant protection and antibiotic resistance

Some drugs that have not been used for treatments of human diseases for years are still used in animal husbandry or to treat plant diseases, and resistance to these drugs remains high. For example, drugs in the chloramphenicol family and many other antibiotics are used to treat farm animals. Streptomycin and oxytetracycline are still regularly used in agriculture by commercial fruit growers and home gardeners to prevent bacteria-induced rotting of apples, pears, nectarines and other commercially valuable

fruits [80]. Streptomycin resistance (first reported in 1955; [81]) has been found in the phytopathogens *Erwinia amylovora, Pseudomonas cichorii, Pseudomonas syringae, Xanthomonas campestris, Pantonea agglomerans* [82–85]. Fire blight, caused by *Eewinia amylovora*, is the most destructive disease of pear and apple orchards worldwide. The application of antibiotics to plants is markedly different from clinical use as it happens in the open environment. Growers, however, defend their practice as being so limited in scope as to be inconsequential to human and environmental health. Although antibiotic use on plants is minor compared with the total use, application of antibiotics in the agro-ecosystem might influence the build-up and persistence of antibiotic resistance genes in the environment. In regions of dense apple and pear production, antibiotics are applied over hundreds of acres of nearly contiguous orchards. The genes involved in streptomycin resistance found in *Pseudomonas syringae* pv. *papulans* and *Erwinia amylovora, strA* and *strB*, are localized on a new class II, Tn3-like transposons (Tn*5393*), which in turn is present on a plasmid (pEa34) in *Erwinia amylovora* [82]. The resistance genes shared high homology with those found on the well-known clinically isolated streptomycin/sulfonomide resistance plasmid RSF1010. Non-hospital use of antibiotics may provide a continuous selection pressure in the environment that creates and maintains resistant bacteria [86,87].

Antibiotic use in plant protection poses two different risks: development of antibiotic resistance in plant pathogens and in human pathogens. No antimicrobial residues have yet been found on or in crops. However, antimicrobials used in plant protection are washed into soil and ground water where they may select for resistance. Therefore, there is a risk that exposure to antimicrobials of possible mammalian commensals or even pathogens present in the environment induces the selection of resistant forms as well as the transfer of resistance genes to human pathogens. There is also a possibility that when crops are fertilized with animal manure, pathogenic bacteria, such as *E. coli* 0157:H7 that can transmit direct infection to consumers and may develop resistance to antibiotics such as streptomycin used in plant protection. Antimicrobials used in plant protection may provide an advantage over chemical pesticides as they reduce pesticide use by one-tenth to one-hundredth and antimicrobials may degrade faster and accumulate less in soil. Quantitative data on the persistence and spread of antibiotic resistance genes in orchards are still lacking as well as knowledge of the environmental fate and ecological effect of streptomycin. Good cultural practices (integrated pest management strategies) may help to minimize the problems caused by plant pathogens without an excessive use of antimicrobials. Biocontrol agents which allow control of pathogens using saprophytic bacteria antagonistic to pathogenic bacteria, have shown promising results in the prevention of bacterial diseases. Antibiosis (antagonism via production of antibiotics) is an important factor in the efficacy of bacteria in biological control [88,89]. However, microorganisms used for biocontrol are likely to produce *in situ* minute amounts of antibiotic, and microbes deliver the antibiotic to the exact location needed, as opposed to the blanket approach used with fungicides.

Antibiotic resistance gene transfer via zoonotic and commensal bacteria

An important question is to what extent the increasing prevalence of antimicrobial resistance in microbes associated with animals contributes to the increasing prevalence of resistance among human pathogens. There is evidence of a spread of bacteria bearing determinants for antimicrobial resistance from animals to man, transmission to man of zoonotic agents such as *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp. is of particular interest to assess this relationship. It now clear that, for example, the use of fluoroquinolones in food animals has led to the emergence of fluoroquinolone-resistant campylobacter and salmonellae. Resistant bacteria and salmonellae are generally present in the intestinal tract of a large proportion of food-producing animals [90] and it is difficult to prevent the contamination of carcasses by the intestinal microflora during slaughtering and subsequent steps in the food chain. Thus, humans may directly acquire both pathogenic and non-pathogenic antibiotic-resistant organisms from food-producing animals [90]. Before the introduction of antimicrobials, salmonellae were fully susceptible to most agents [91]. Study of serotypes from isolated asymptotic human carriers in a meat packing plant gave the first evidence for passage of salmonellae from animal products to man as the serotypes were shown to correspond with those isolated from raw meat. Calves are the

primary reservoir of *Salmonella typhimurium* but sheep, goats, pigs, poultry and horses can also become infected. To avoid the spread of the epidemics, animals are treated with antimicrobials and, as a result of selection pressure, multiresistance emerges. There have been several sporadic epidemics due to the emergence of clones with enhanced pathogenicity for animals and humans such as *Salmonella typhimurium* DT104 which has been the cause of an epidemic in animals since 1994. This strain is resistant to most of the antimicrobials normally used to treat enteric infections for example ampicillin, tetracycline, streptomycin, chloramphenicol and sulphonamides. It has also acquired resistance against trimethoprim and fluoroquinolones most probably from animals that could be treated only with these compounds.

Exposure to antimicrobials is also associated with an increased prevalence of resistance among bacteria of the normal flora of both humans and animals. Commensal bacteria may be a good indicator for the selection pressure due to antimicrobials and may be used to predict the appearance of resistance in pathogens. Commensal bacteria may be playing a critical role as reservoirs of resistance genes. Human commensals that become resistant to antibiotics may eventually cause opportunistic infections for instance in wounded or immunocompromised individuals. Many life-threatening, post-surgical infections are caused by a member of the patient's own microflora. Transfer of resistance genes from commensal to pathogenic bacteria has been shown [92,93]. However, no systematic survey of the impact of such transfers on human health has been made. The possibility of gene transfer from commensal to pathogenic bacteria implies that antibiotic resistance is not a problem confined to hospitals; it is an environmental problem. It implies that decisions made about agricultural feeding practices, food safety monitoring or water treatment standards may have important consequences for the effective treatment of human disease. It also means that the development of resistance in non-clinical bacteria and habitats such as those involved in the food chain should be monitored.

Transfer of antibiotic resistance genes in aquatic habitats

In the aquatic environment, bacterial activity and gene transfer is enhanced in sediments and water surfaces that provide higher nutrient input and favourable temperatures [94]. The presence of nutrients as well as colonizable surfaces is particularly important as these factors can promote the occurrence of large densities of metabolically active microorganisms [95]. Sediments, which tend to be rich in organic material, can support up to 1000-fold higher bacterial populations than bulk water. Nutritionally rich, complex, active communities can also be found in epilithon (biofilms) on stones in rivers or lakes [96]. In these solid–water interfaces, bacteria are embedded within a protective polysaccharide matrix, which absorbs dissolved and particulate organic matter from the overlying water. Sewage systems and water treatment facilities (filterbeds and others) also constitute nutrient-rich environments that can support high bacterial population densities. These sites can therefore offer an adequate environment for high gene transfer frequency. Thus, the main factors affecting conjugative transfer in aquatic environments are: the cellular activity of the microbial communities which is itself related to nutrient availability; the water temperature; the density (and maturity for biofilms) of the microbial communities; the presence of a selective pressure; the donor and recipient bacteria, frequencies being generally higher for taxonomically related species [66].

The majority of studies on plasmid transfer in aquatic environments have been performed in microcosms which confer the advantage of simplicity, reproducibility, and flexibility, and give an opportunity for control and adjustment of chosen conditions such as pH, temperature, etc. However, gene transfer experiments performed in microcosms and *in situ* (directly in the environment) have given consistent results. Plasmid transfer has been unequivocally demonstrated in many different aquatic habitats. Hill *et al.* [28] recovered the mobilizing plasmid pQKH33 by exogenous isolation and triparental matings using river epilithon bacteria as donors, and this plasmid conferred tetracycline, streptomycin and mercury resistance to the *Pseudomonas putida* recipient. Several studies have shown the prevalence of antibiotic resistance determinants and antibiotic resistance transfer in bacteria isolated from wastewater treatment plant [97]. These studies provided indirect evidence that mobilization of non-self-transferable plasmids is favoured in this nutrient-rich environment. Goni-Urriza *et al.* [98] showed that an

urban effluent can increase the levels of resistance to antibiotics in *Enterobacteriaceae* (representative of the human and animal commensal flora) and aeromonad (typically waterborne bacteria) populations. They showed high frequencies of occurrence of resistance to nalidixic acid (for both populations), to tetracycline and β-lactams (for the *Enterobacteriaceae*) and to tetracycline and co-trimoxazole (for the aeromonad population). Resistance of *Pseudomonas aeruginosa* strains isolated from freshwater and seawater to different antimicrobial agents was studied and it was found that resistance in these environments was correlated with the level of faecal pollution.

CONCLUSIONS

A range of different studies performed in soil and aquatic environments have suggested that plasmid transfer is enhanced in nutrient-rich environments, most probably by promoting cellular activity and cell-to-cell contacts [66]. Antibiotic resistance determinants are often located on self-transmissible or mobilizable plasmids or on conjugative transposons. Therefore it is plausible to suggest that the dissemination of these genes follows the same pattern as that of the vectors. So far, most data have been obtained from microcosms rather than from field experiments but microcosms are set up in optimal conditions and tend to give maximal frequency values for gene transfer. Clearly more information is also needed about prevalence of antibiotic resistance in the environment, about the amounts of antimicrobials used, and the fate of antimicrobials in the environment. Although some data on human consumption of antibiotics are available, governments have so far not requested that data on antimicrobial consumption by animals be supplied by industry, suppliers or pharmacists. There is only limited information on the amounts of antimicrobials used for plant protection in the EU. The quality of information available on antibiotic use is poor and is inadequate as a basis from which to draw useful scientific conclusions regarding the relationship between antimicrobial resistance and the amounts of antimicrobials used. Quantitative and qualitative monitoring data will also enable the detection of changes in the prevalence of antibiotic resistance over time following changes or cessation in the consumption of a specific antibiotic. Further knowledge about the factors (biological, chemical and physical) that influence gene transfer *in situ* is needed, as novel strategies and medical products aimed at reducing antimicrobial resistance may be discovered. There is no scientific information about the ecological consequences on higher organisms (other than human) of the introduction of antimicrobial resistance genes into aquatic and terrestrial systems. The instance of reversibility (the time for resistance to be lost) needs to be addressed in a more systematic manner. Similarly, a better understanding of the genetic basis of antimicrobial resistance may help to circumvent the emergence of resistance mechanisms in bacteria.

ACKNOWLEDGEMENTS

We acknowledge financial support from the European Union for the research project RESERVOIR B104-CT98-0053 (DG 12-SSMI), and for the concerted action MECBAD BIO4-CT-0099. E.M.W. was also supported by Biotechnology and Biological Sciences Research Council grant 88/GM114200 and N.S. was in receipt of a BBSRC studentship.

REFERENCES

1. Moken MC, McMurry M, Levy SB: Selection of multiple antibiotic-resistant (Mar) mutants of *Escherichia coli* by using the disinfectant pine oil. *Antimicrob Agents Chemother* 1998, 41:2770–2772.
2. Alonso A, Sanchez P, Martinez JL: Environmental selection of antibiotic resistance genes. *Environ Microbiol* 2001, 3:1–9.
3. Datta N, Hughes VM: Plasmids of the same Inc groups in Enterobacteriaceae before and after the medical use of antibiotics. *Nature* 1983, 306:616–617.
4. Hughes VM, Datta N: Conjugative plasmids in bacteria of the 'pre-antibiotic' era. *Nature* 1983, 302:725–726.
5. Benveniste R, Davies J: Aminoglycoside antibiotic-activating enzymes in actinomycetes similar to these present in clinical isolates of antibiotic-resistant bacteria. *Proc Natl Acad Sci USA* 1973, 70:2276–2280.
6. Webb V, Davies J: Antibiotic preparations contain DNA: a source of drug resistance genes? *Antimicrob Agents Chemother* 1993, 37:2379–2384.
7. Ochman H, Lawrence JG, Groisman EA: Lateral gene transfer and the nature of bacterial innovation. *Nature* 2000, 405:299–304.
8. MacKay D: Antibiotic resistance. *Trends Biotechnol* 2001, 19:42.
9. Hawkey P: The enemy within-hospital-acquired, antibiotic-resistant baceria. *Microbiol Today* 2001, 28:7–9.
10. Davies JE: Inactivation of antibiotics and the dissemination of resistance genes. *Science* 1994, 264:375–382.
11. Mazodier P, Davies J: Gene transfer between distantly

related bacteria. *Annu Rev Genet* 1991, 25:147–171.
12. Amabile-Cuevas CF, Chicurel ME: Bacterial plasmids and gene flux. *Cell* 1992, 70:189–199.
13. Kesseler M, Dabbs ER, Averhoff B, Gottschalk G: Studies on the isopropylbenzene 2,3-dioxygenase and the 3-isopropylcatechol 2,3-dioxygenase genes encoded by the linear plasmid of *Rhodococcus erythropolis* BD2. *Microbiology* 1996, 142:3241–3251.
14. Kalkus J, Reh M, Schegel HG: Hydrogen autotrophy of *Nocardia opaca* strains is encoded by linear megaplasmids. *J Gen Microbiol* 1990, 136:1145–1151.
15. Kalkus J, Dörrie C, Fisher D, Reh M, Schegel HG: The giant plasmid pHG207 from *Rhodococcus* sp. encoding hydrogen autotrophy: characterization of the plasmid and its termini. *J Gen Microbiol* 1993, 139:2055–2060.
16. Kinashi H, Shimaji M, Sakai A: Giant linear plasmids in *Streptomyces* which code for antibiotic biosynthesis genes. *Nature* 1987, 328:454–456.
17. Stonesifer J, Matsushima P, Baltz RH: High frequency conjugal transfer of tylosin genes and amplifiable DNA in *Streptomuces fradiae*. *Mol Gen Genet* 1986, 202:348–355.
18. Kinashi H, Shimaji M: Detection of giant linear plasmids in antibiotic producing strains of *Streptomyces* by the ofage technique. *J Antibiotics* 1987, XL(6):913–916.
19. Gravius B, Glocker D, Pigac J, Pandža K, Hranueli D, Cullum J: The 387 kb linear plasmid pPZG101 of *Streptomyces rimosus* and its interactions with the chromosome. *Microbiology* 1994, 140:2271–2277.
20. Ravel J, Schrempf H, Hill RT: Mercury resistance is encoded by transferable giant linear plasmids in two Chesapeake Bay *Streptomyces* strains. *Appl Environ Microbiol* 1998, 64:3383–3388.
21. Ravel J, Wellington EMH, Hill RT: Interspecific transfer of *Streptomyces* giant linear plasmids in sterile amended soil microcosms. *Appl Environ Microbiol* 2000, 66:529–534.
22. Top EM, Maltsever OV, Forney LJ: Capture of a catabolic plasmid that encodes only 2,4-dichlorophenoxyacetic acid:alpha ketoglutaric acid dioxygenase (TfdA) by genetic complementation. *Appl Environ Microbiol* 1996, 62:2470–2476.
23. Herrick JB, Stuart-Keil KG, Ghiorse WC, Madsen EL: Natural horizontal transfer of a naphthalene dioxygenase gene between bacteria native to a coaltar-contaminated field site. *Appl Environ Microbiol* 1997, 63:2330–2337.
24. Nielsen KM, van Elsas JD, Smalla K: Transformation of *Acinetobacter* sp. strain BD413 (pFG4Δ*nptII*) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Appl Environ Microbiol* 2000, 66:1237–1242.
25. Smalla K, Osborn M, Wellington EMH: Isolation and characterisation of plasmids from bacteria. In *The Horizontal Gene Pool*. Edited by Thomas CM. Amsterdam: Harwood Academic; 2000:207–248.
26. Bale MJ, Day MJ, Fry JC: Novel method for studying plasmid transfer in undisturbed river epilithon. *Appl Environ Microbiol* 1988, 54:2756–2758.
27. Smalla K, Heuer H, Götz A, Niemeyer D, Krögerrecklenfort E, Tietze E: Exogenous isolation of antibiotic resistance plasmids from piggery manure slurries reveals a high prevalence and diversity of IncQ-like plasmids. *Appl Environ Microbiol* 2000, 66:4854–4862.
28. Hill KE, Weightman AJ, Fry JC: Isolation and screening of plasmids from the epilithon which mobilise recombinant plasmid pD10. *Appl Environ Microbiol* 1992, 58:1292–1300.
29. Top E, De Smet I, Verstraete W, Dijkmans R, Mergeay M: Exogenous isolation of mobilizing plasmids from polluted soils and sludges. *Appl Environ Microbiol* 1994, 60:831–839.
30. van Elsas JD, McSpadden Gardener BB, Wolters AC, Smit E: Isolation, characterisation, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. *Appl Environ Microbiol* 1998, 64:880–889.
31. Thomas CM, Thorsted P: PCR probes for promiscuous plasmids. *Microbiology* 1994, 140:1.
32. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC: Green fluorescent protein as a marker for gene expression. *Science* 1994, 263:802–804.
33. Christensen BB, Sternberg C, Molin S: Bacterial plasmid conjugation on semi-solid syrfaces monitored with the green fluorescent protein (GFP) from *Aequorea victoria* as a marker. *Gene* 1996, 173:59–65.
34. Dahlberg C, Bergstrom M, Hermansson M: *In situ* detection of high levels of horizontal plasmid transfer in marine bacterial communities. *Appl Environ Microbiol* 1998, 64:2670–2675.
35. Top EM, Moënne-Loccoz Y, Pembroke T, Thomas CM: Phenotypic traits conferred by plasmids. In *The Horizontal Gene Pool*. Edited by Thomas CM. Amsterdam: Harwood Academic; 2000:249–276.
36. Merlin C, Mahillon J, Neövera J, Toussaint A: Gene recruiters and transporters: The modular structure of bacterial mobile elements. In *The Horizontal Gene Pool*. Edited by Thomas CM. Amsterdam: Harwood Academic; 2000:363–401.
37. Marsh P, Wellington EMH: Phage-host interactions in soil. *FEMS Microbiol Ecol* 1994, 15:99–108.
38. Jiang SC, Paul JH: Gene transfer by transduction in the marine environment. *Appl Environ Microbiol* 1998, 64:2780–2787.
39. Romanowski G, Lorenz MG, Wackernagel W: Use of polymerase chain reaction and electroporation of *Escherichia coli* to monitor the persistence of extracellular plasmid DNA introduced into natural soils. *Appl Environ Microbiol* 1993, 59:3438–3446.
40. Nielsen KM, Bones AM, van Elsas JD: Induced natural transformation of *Acinetobacter calcoaceticus* in soil microcosms. *Appl Environ Microbiol* 1997, 63:3972–3977.
41. Paul JH, David AW: Production of extracellular nucleic acids by genetically altered bacteria in aquatic-environment microcosms. *Appl Environ Microbiol* 1989, 55: 1865–1869.
42. Paul JH, Jeffrey WH, DeFlaun MF: Dynamics of extracellular DNA in the marine environment. *Appl Environ Microbiol* 1987, 53:170–179.
43. Romanowski G, Lorenz MG, Salyer G, Wackernagel W: Persistence of free plasmid DNA in soil monitored by various methods, included a transformation assay. *Appl Environ Microbiol* 1992, 58:3012–3019.
44. Romanowski G, Lorenz MG, Wackernagel W: Use of polymerase chain reaction and electroporation of Escherichia coli to monitor the persistence of extracellular plasmid DNA introduced into natural soils. *Appl Environ Microbiol* 1993, 59:3438–3446.
45. Recorbet G, Picard C, Normand P, Simonet P: Kinetics of the persistence of chromosomal DNA from genetically engineered Escherichia coli introduced into soil. *Appl Environ Microbiol* 1993, 59:4289–4294.
46. Gallori E, Bazzicalupo M, Dal Canto L, *et al.*: Transformation of *Bacillus subtilis* by DNA bound on clay in non

sterile soil. *FEMS Microbiol Ecol* 1994, 15:119–126.
47. Khana M, Stotzky G: Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. *Appl Environ Microbiol* 1992, 58:1930–1939.
48. Lorenz MG, Wackernagel W: Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol Rev* 1994, 58:563–602.
49. Baur B, Hanselmann K, Schlimme W, Jenni B: Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. *Appl Environ Microbiol* 1996, 62:3673–3678.
50. Webb V, Davies J: Accidental release of antibiotic-resistance genes. *Tibteck* 1994, 123:74–75.
51. Orpin CG, Jordan DJ, Hazlewood GP, Mann SP: Genetic transformation of the ruminal bacterium *Seleromonas ruminatium*. *J Appl Bacteriol* 1986, 61:xvi.
52. Lorenz MG, Wachernagel W: Absorption of DNA to sand and variable degradation rates of absorbed DNA. *Appl Environ Microbiol* 1987, 53:2948–2952.
53. Paul JH, Thurmond JM, Frischer ME: Gene transfer in marine water column and sediment microcosms by natural plasmid transformation. *Appl Environ Microbiol* 1991, 57:1509–1515.
54. Coffey TJ, Enright MC, Daniels M *et al.*: Serotype 19A variants of the Spanish serotype 23F multiresistant clone of *Streptococcus pneumoniae*. *Microb Drug Resist* 1998, 4: 51–55.
55. Davies J: Another look at antibiotic resistance. *J Gen Microbiol* 1992, 138:1553–1559.
56. Udou T, MizuguchiY, Wallace RJ: Does aminoglycoside-acetyltransferase in rapidly growing mycobacteria have a metabolic function in addition to aminoglycoside inactivation? *FEMS Microbiol Lett* 1989, 57:227–230.
57. Pang Y, Brown BA, Steingrube VA, Wallace RJ Jr, Roberts MC: Tetracycline resistance determinants in *Mycobacterium* and *Streptomyces* species. *Antimicrob Agents Chemother* 1994, 38:1408–1412.
58. Li DM, Alexander M: Factors affecting co-inoculation with antibiotic-producing bacteria to enhance rhizobial colonisation and nodulation. *Plant and Soil* 1990, 129:195–201.
59. Weiner P, Egan S, Wellington EMH: Evidence for transfer of antibiotic-resistance genes in soil populations of streptomycetes. *Mol Ecol* 1998, 7:1205–1216.
60. Shaw KJ, Rather PN, Hare RS, Miller GH: Molecular genetics of aminoglycoside resistance genes and familial relationships of aminoglycoside-modifying enzymes. *Microbiol Rev* 1993, 57:138–163.
61. Rather PN, Munayyer H, Mann PA, Hare RS, Miller GH, Shaw KJ: Genetic analysis of bacterial acetyltransferases: identification of amino acids determining the specificities of the aminoglycoside 6'-N-acetyltransferase Ib and IIa proteins. *J Bacteriol* 1992, 174:3196–3203.
62. Salyers AA, Shoemaker NB: Resistance gene transfer in anaerobes: New insights, new problems. *Clin Infect Dis* 1996, 23:S36–S43.
63. Rasheed JK, Jay C, Metchock B, *et al.*: Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. *Antimicrob Agents Chemother* 1997, 41: 647–653.
64. Deplano A, Zekhnini A, Allali N, Couturier M, Struelens MJ: Association of mutations in *grlA* and *gyrA* topoisomerase genes with the resistance to ciprofloxacin in epidemic and sporadic isolates of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1997, 41:2023–2025.
65. Riesenfeld C, Everett M, Piddock LJ, Hall BG: Adaptive mutations produce resistance to ciprofloxacin. *Antimicrob Agents Chemother* 1997, 41:2059–2060.
66. van Elsas JD, Fry J, Hirsch P, Molin S: Ecology of plasmid transfer and spread. In *The Horizontal Gene Pool*. Edited by Thomas CM. Amsterdam: Harwood Academic; 2000:175–199.
67. Pearce DA, Bazin MJ, Lynch JM: Substrate concentration and plasmid transfer frequency between bacteria in a model rhizosphere. *Microbiol Ecol* 2000, 41:57–63.
68. Droge M, Puhler A, Selbitschka W: Phenotypic and molecular characterization of conjugative antibiotic resistance plasmids isolated from bacterial communities of activated sludge. *Mol Gen Genet* 2000, 263:471–482.
69. Dahlberg C, Bergstrom M, Hermansson M: *In situ* detection of high levels of horizontal plasmid transfer in marine bacterial communities. *Appl Environ Microbiol* 1998, 64:2670–2675.
70. Amann RI, Ludwig W, Schleifer KH: Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 1995, 59: 143–169.
71. Smit E, Wolters A, van Elsas JD: Self-transmissible mercury plasmids with gene-mobilizing capacity in soil bacterial populations: Influence of wheat roots and mercury addition. *Appl Environ Microbiol* 1998, 64: 1210–1219.
72. van Elsas JD, Trvors JT, Stardub ME: Bacterial conjugation between Pseudomonads in the rhizosphere of wheat. *FEMS Microbiol Ecol* 1988, 53:299–306.
73. van Elsas JD, Gardener BB, Wolters AC, Smit E: Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. *Appl Environ Microbiol* 1998, 64:880–889.
74. Götz A, Smalla K: Manure enhances plasmid mobilization and survival of *Pseudomonas putida* introduced into field soil. *Appl Environ Microbiol* 1997, 63:1980–1986.
75. Turpin PE, Maycroft KA, Rowlands CL, Wellington EMH: An ion-exchange based extraction method for the detection of salmonellas in soil. *J Appl Bacteriol* 1993, 74:181–190.
76. Tschäpe H: The spread of plasmids as a function of bacterial adaptability. *FEMS Microbiol Ecol* 1994, 15: 23–32.
77. Tietze E, Tschape H, Golubev AV: DNA probes for studying streptothricin resistance evolution in enteric bacteria. *J Basic Microbiol* 1990, 30:279–287.
78. Smalla K, van Elsas JD: Application of the PCR for detection of antibiotic resistance genes in environmental samples. In *Nucleic Acids in the Environment*. Edited by Trevors JT, van Elsas JD. Berlin, Heidelberg, New York: Springer-Verlag; 1995:241–256.
79. Tietze E, Brevet J: The trimethoprim resistance transposon Tn7 contains a cryptic streptothricin resistance gene. Plasmid 1991, 25:217–220.
80. Schnabel EL, Jones AL: Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. *Appl Environ Microbiol* 1999, 65:4898–4907.
81. Misato T, Ko K, Yamaguchi I: Use of antibiotics in agriculture. *Adv Appl Microbiol* 1977, 21:53–88.
82. Chiou CS, Jones AL: Nucleotide sequence analysis of a

transposon Tn5393 carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. *J Bacteriol* 1993, 175:723–740.
83. Sundin GW, Bender CL: Expression of the *strA-strB* streptomycin resistance genes in *Pseudomonas syringae* and *Xanthomonas campestris* and characterization of *IS6100* in *X. campestris*. *Appl Environ Microbiol* 1995, 61:2891–2897.
84. Sundin GW, Monks DE, Bender CL: Distribution of the streptomycin-resistance transposon Tn5393 among phylloplane and soil bacteria from managed agricultural habitats. *Can J Microbiol* 1996, 41:792–799.
85. Huang TC, Burr TJ: Characterization of plasmids that encode streptomycin-resistance in bacterial epiphytes of apple. *J Appl Microbiol* 1999, 86:741–751.
86. Huddleston AS, Cresswell N, Neves MCP *et al.*: Molecular detection of streptomycin-producing streptomycetes in Brazilian soils. *Appl Environ Microbiol* 1997, 63:1288–1297.
87. Egan S, Wiener P, Kallifidas D, Wellington EMH: Transfer of streptomycin biosynthesis gene clusters within streptomycetes isolated from soil. *Appl Environ Microbiol* 1999, 64:5061–5063.
88. Thomashow LS, Weller DM: Role of a phenazine antibiotic from Pseudomonas fluorescens in biological control of *Gaeumannomyces graminis var. tritici*. *J Bacteriol* 1988, 170:3499–3508.
89. Seveno NA, Mprgan JAW, Wellington EMH: Growth of *Pseudomonas aureofaciens* PGS12 and the dynamics of HHL and phenazine production in liquid culture, on nutrient agar, and on plant roots. *Microb Ecol* 2001, 41:314–324.
90. Wierup M, Engstrom B, Engvall A, Wahlstrom H: Control of Salmonella enteritidis in Sweden. *Int J Food Microbiol* 1995, 25:219–226.
91. Datta N: Bacterial resistance to antibiotics. *Ciba Found Symp* 1984, 102:204–218.
92. Nikolich MP, Hong G, Shoemaker NB, Salyers AA: Evidence for natural horizontal transfer of tetQ between bacteria that normally colonize humans and bacteria that normally colonize livestock. *Appl Environ Microbiol* 1994, 60:3255–3260.
93. Sundin GW, Bender CL: Dissemination of the *strA-strB* streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals and plants. *Mol Ecol* 1996, 5:133–143.
94. Wellington EMH, van Elsas JD: *Gene Transfer Between Microorganisms in the Natural Environment*. London, Pergamon Press; 1992.
95. Hill KE, Fry JC, Weightman AJ: Gene transfer in the aquatic environment: persistence and mobilization of the catabolic recombinant plasmid pD10 in the epilithon. *Microbiology* 1994, 140:1555–1563.
96. Hill KE, Marchesi JR, Fry JC: Conjugation and mobilization in the epilithon. In *Molecular Microbial Ecology Manual*. Edited by Akkermans DL, van Elsas JD, de Bruijn FJ. Dordrecht: Kluwer Academic; 1996: 5.2.2/1–5.2.2/28.
97. Baya AM, Brayton PR, Brown VL, Grimes DJ, Russek-Cohen E, Colwell RR: Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples. *Appl Environ Microbiol* 1986, 51:1285–1292.
98. Goni-Urriza M, Pineau L, Capdepuy M, Roques C, Caumette P, Quentin C: Antimicrobial resistance of mesophilic Aeromonas spp. isolated from two European rivers. *J Antimicrob Chemother* 2000, 46:297–301.

AQ1 Please check alignment of the data in Table 1 and repeat entries if necessary to make clear.