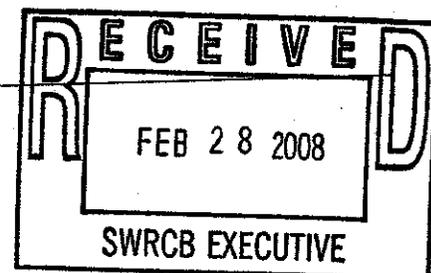


Jeanine Townsend - Comments on revised recycled water policy

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Date: Thursday, February 28, 2008 8:05 AM
Subject: Comments on revised recycled water policy



To: California State Water Resources Control Board via Jeanine Townsend,
 Clerk to the Board---jtownsend@waterboards.ca.gov

Fm: Dr Edo McGowan

Re: Comments on revised reclaimed water policy staff report and environmental document

Please confirm receipt of this email.

I had discussed the following omissions in the environmental document previously but I see that those comments were not addressed. Thus again I wish to comment on the deficiency of the staff's environmental analysis.

I am an expert in these matters, having been the environmental advisor to the U.S. Department of State and UN for 22 nations in Africa on water quality issues and public health impacts. I believe that the Board has my CV. Additionally, I was just on a national WERF/U.S. EPA panel brought together to review the public health and illness impacts related to wastewater industry byproducts, including pathogens and antibiotic resistance. I have a PhD in water quality and a degree in medicine. Thus the following comments are made as an expert.

When compared to NEPA, CEQA is not merely a procedural exercise. CEQA contains an impressive directive to public agencies that they refrain from approving projects with significant adverse environmental effects if there are feasible alternatives. If the agency has not even explored these adverse impacts, it cannot thus entertain alternatives. CEQA first compels a governmental agency to identify the significant adverse environmental effects and then to mitigate those impacts through feasible mitigation measures or feasible alternatives. The environmental review process has also become a process whereby the public interacts. The California Supreme Court has commented on this aspect, indicating that CEQA and its process "protects not only the environment but also informed government". For informed self government to function, the process "ensures that members of [the governmental decisionmaking body] fully consider information necessary to render decisions that fully consider the information necessary to render decisions that intelligently take into account the environmental consequences. That concept thus includes acknowledgement of an underlying policy of citizen input under CEQA

By withholding critical information from the policy maker, it is possible to purposefully skew the policy determination and shift perspective. Without a well rounded analysis from which to gain perspective, the people of this state are deprived of an adequate appraisal of impacts. Because recycled water, while meeting state standards, has been shown to contain serious pathogens and many of those pathogens carry antimicrobial and chlorine resistance, misguiding the Board in its duty is tantamount to a criminal act when considering the rapid advancement of antibiotic resistance and its impact on public health.

In several areas, the staff report has neglected to discuss the existence and probability for potentially serious adverse impacts. Pathogens and their genetic material may be contained within the produced recycled water and thus there is the potential for newly emerging infectious diseases to accompany or be influenced by recycled water. Additionally, newly emerging contaminants of concern such as pharmaceuticals, endocrine disrupters, flame retardants, industrial chemicals, and personal care products are contained within the recycled water; these can bioaccumulate, and are not discussed yet will have a potentially adverse impact on the environment.

On page 12 of the staff report, agriculture is discussed. The impacts on agriculture from irrigation with recycled water warrant considerably more discussion. In an independent analyses of recycled water we demonstrated that water meeting state recycled water standards did contain multi-antibiotic and chlorine resistant bacteria. Our findings parallel the report by WERF as conducted by Joan B Rose, et al (2004) which demonstrated that significant levels of pathogens were noted within recycled water that met state standards. Rose, et al also noted that the indicators used for recycled water bore little relationship to the actual numbers of pathogens contained in that water. This was reflected also within the subsequent report by Harwood, et al. Further Chad Kinney has published on the levels of pharmaceuticals noted in recycled water and their bioaccumulation in soils. Thus there is the opportunity for development of resistance post irrigation. Previous submissions by me had discussed these issues and presented ample scientific data to demonstrate the validity of these statements. Those previous comments are hereby incorporated by reference. None of these issues are discussed within the current staff report and yet they have the potential to produce significant adverse impacts on agriculture, the environment, and mankind.

These constituents within recycled water pose a potentially significant adverse impact on agricultural systems as well as the crops produced. Multiple illnesses associated with agricultural produce can and have adversely impacted the economy of this sector and thus the economy of the state. Since this recycled water is capable of transmitting antibiotic resistant genes, these genes may interact with soil microbes and thus the crop can be contaminated by secondary means. The ability of bacteria to enter crops and track into the inner tissues means that surface washing will have no effect, thence for crops consumed raw, the genetic information and potential pathogens can enter the human gut and exchange with the commensals and flora of the gut. Sjolund has demonstrated that this exchanged genetic information may remain within the gut flora for up to four years and is thus available to incoming pathogens. Additionally because pharmaceuticals and other emerging contaminants can bioaccumulate, the soil microbes can be adversely impacted. Again these potentially significantly adverse impacts are not discussed. Thus the decision maker and the public are deprived of critical information.

The staff report at page #6, et seq, discusses salt and states that salt can move to the ground water. Thus at least here the mechanism of movement to ground water is acknowledged for soluble materials. This analysis of soluble materials stops there but also within the soluble materials category are several of the emerging contaminants of concern—such as antibiotic resistant genes (ARGs), pharmaceuticals, endocrine disrupters, personal care products, all of which can and will reach the ground water just as salts but there is no discussion of these nor their potential impacts on ground water, hence public health.

Of interest here when speaking of ARGs, Pruden demonstrated that these genetic fragments could pass through drinking water treatment plants, unaffected by filters and chlorine, and be found in the potable water supply.

Item (d) of that that heading notes that the regional board has apparently an independent capacity to find and correct issues that may impact public health, for salt. Thus there must be some mechanism that allows this absent a direction from the state's public health arm, i.e., an ability to independently consider impacts on public health. The anti degradation policy seems to be at odds here if only salts are considered. Additionally, it is possible for numerous materials found within recycled water to bioaccumulate in crops. In fact the phytoremediation processes depend on the ability of plants (read crops here) to accumulate numerous materials and do so at several times the concentration found in the soil. Again, none of this is considered. In the staff considered alternatives, staff notes at (d), p.7, that there might be a need to regulate discharge from industrial users. Thus the staff admits that industrial users discharge to sewers and that recycled water contains industrial discharges. Staff, however, limit's the discussion to salt, yet there are myriad industrial materials that are discharged that make it into recycled water.

The staff document also notes that one proposed mitigation would be to monitor ground water and if the cumulative input was starting to exceed some limit controls would be imposed. But if the intervening material between the surface is saturated to the extent that inputs to ground water are exceeded, the continued

percolation of materials into the ground water would continue for some time depending on overlying soils, their ability to transmit pollutants and the limits on surface input. While the staff may feel this is adequate for salts, this is hardly adequate for other pollutants and those pollutants are not even discussed. Thus the scope of the discussion on pollutants is highly and artificially limited, thus failing to be an adequate discussion to inform either the public or the decision makers.

On page 13 of the staff report, biological resources are discussed. The treatment here is deficient. The policy as originally considered included allowances for incidental runoff, notwithstanding PL92-500, the Clean Water Act. Runoff and downwind drift may see the contained pathogens, including their genetic material and pharmaceuticals move off site, thus potentially affecting off site soil and aquatic systems. These potentially adverse impacts are not discussed. The use of subsurface drain tile as a mode of transport for drain water and salts to distant areas is not discussed. The issue of pathogens to accompany this drain water also is not discussed. The potentially adverse impacts from the movement of pathogens, their genetic material and pharmaceuticals is not discussed in the environmental document but is well discussed within the literature. The policy excludes impoundments. This is shortsighted. Large impoundments are capable of becoming aerosol generators and thus have the ability to carry pathogens down wind. Anything in excess of 8m/sec wind run will see micro-chop at the surface and these, upon breaking up, will produce droplet separation with down-wind drift. Thus impoundments, from a public health perspective warrant more discussion. These issues all need to be incorporated into the document and presented to the public and the Board so a rational and open appraisal can be undertaken. By withholding this information, the state board's analysis and hence decision will be skewed. A skewed policy thus deprives the citizens of this state the proper protection they are assured within statute.

On page 14 of the draft staff report and certified regulatory program, there is a discussion demonstrating that the state board and regional boards actually have independent capacity to consider public health impacts. This states that if the state's health arm has not developed MCLs or provided recommendations, the regional boards are thus capable of independent action. Thus contrary to statements that the state board and regional boards are helpless to deal with issues impacting public health absent direction from CDPH, this inserted language seems to give lie to that statement. In fact, there are several areas in statute that support this and these will be touched upon momentarily. The draft staff report/environmental analysis at p. 14 discusses 4 items that must come together for this ability to act independently to come to fruition (items a through d). These include a requirement that the constituent be found in the recycled water; the constituent is likely to be found in the ground water recharge area; adequate information is available to determine the toxicity and thus determine limits (in this case when discussing pathogens and transfer of genetic material, a de minimis amount is capable of producing disease); and approved analytical procedures are available. These requirements are all able to be met. Pathogens and their genetic material as well as pharmaceuticals should not be found in recycled water, especially if that water has contact with the public, including contact through consumption of crops.

Recycled water is part of an overall train of processes coming from the treatment of sewage. To fully appreciate this, the state and regional boards need to look at the broader impacts, considerably beyond salt and nitrate, especially those other constituents that impact upon public health. The state board has, within its capacity, the where-with-all to address some of this and certainly is charged with protecting the public health. Thus to attain that goal of protecting public health it needs to appreciate the following. It can not merely defer to the state's health arm and thus blindly forge ahead absent some understanding of its impact on public health. Presumably this eventuality has been considered and is reflected in the language on page 14 of the draft staff report/environmental analysis.

There are several issues, however, that are not covered within the currently proposed policy statements and the environmental analysis. This skews the ability of the state board and public to adequately appraise impacts and thus has the potential to create a weak policy, one that may well have a seriously adverse impact on public health and the economy of the state. The policy is very weak on public health impacts stemming from recycled water as currently produced. These impacts include disease transmission and this stems from the pass-through and synergistic effects of what enters the treatment works. That is further dependent on how those inputs are processed. This will affect the exchange of genetic information that enhances antimicrobial (antibiotic) resistance as well as virulence, and thus what is left over to become part of the recycled/reclaimed water. This water is then spread into the environment in various ways. As I have indicated, we ran Title 22 water in the lab and it contained multi-drug resistant and chlorine bacteria. Pruden, et al have demonstrated the pass-through of genetic material and Rose, et al confirmed the carriage of numerous pathogens within reclaimed water. Thus, in spite of what one may wish to believe, Title 22, as currently produced, is not protective of public health---in fact it is a major transmission vehicle for multi-drug resistant pathogens. Since these microbes are placed into large

areas where the public has considerable contact, this allows transmission to the public, hence enhancing the risk of disease and the advancement of drug-resistance. Thus we have what, at least I would consider, contamination, pollution and nuisance, all clearly defined within statute. To the extent that there is incidental movement off-site, this impacts other water bodies via transport routes and thus the capacity to establish terrestrial and aquatic niches that then act as lending libraries. Again these issues are not discussed within the environmental analysis.

When we consider disease transmission, we need to look at epidemiology possibilities and thus human health risks. There are no such human health risk assessment studies on reclaimed water of which I am aware. Thus, as far as I am concerned, the state board is punting down the field, but the stadium lights are out and no one knows where the ball will go. It will be reckless to progress unless the entirety of the interconnecting parts are well understood, and the staff guiding the state board seems not to appreciate that aspect.

Above, agriculture was discussed. Pruden, et al note that ARGs pass through filters used in water treatment and are not affected by chlorine. Thus when we consider percolation into ground water, that is an issue that warrants more discussion, but is apparently not discussed within any document prepared by staff as presented to the state board. Additionally, the ability for viruses to reach ground water is well validated. Thus there is much missing here that may impact public health that warrants discussion, but such discussion is absent within the environmental analysis.

For example, when speaking of viruses, there is no valid scientific evidence that turbidity and viral numbers actually correlate. Yet it is assumed that a surrogate measure operating on assumptions of a technology based train that looks at indicator bacteria will demonstrate adequate viral reductions. This lack of scientific evidence has been confirmed by the state's public health arm. Further, the 2.2 MPN/100ml fails to appreciate several important aspects that do impact disease transmission. One of these is viable but non-culturable (VBNC) which is not considered by current lab tests. Neither are similar states such as persisters. Additionally, since the current indicators are vegetative bacteria that only require low-level disinfection, this protocol completely ignores the pathogens that would require high-level disinfection. Additionally since antibiotic resistant genes and virulence islands are not "alive", current levels of chlorine have no impact on them, yet these bits of genetic information are clearly capable of moving into other organisms and thus rendering them resistant or more virulent. None of this is considered within the standards or for that matter within the instruction and licensing of sewer plant operators who will be producing reclaimed/recycled water. Again, considering the reports by Rose and Harwood, it is obvious that the indicators dictated by state criteria and non functional in predicting pathogen loads.

One of the issues that warrants clarity is how the state board and regional boards interpret the legislative directives. For example, one of the operative Water Code sections is 13550 (a)(3), which makes a pronouncement that the use of recycled water will not be detrimental to public health. How that is interpreted by the state and regional boards will have ripple effects on public health. If the state and regional boards do not appreciate the interconnections between the pass through of pathogens and genetic material as found within reclaimed/recycled water (see for example: Pruden, et al; Rose, et al; Harwood, et al, Kinney, et al; Kummerer, et al; Firl, et al; McGowan; Higgins & Murthy) and the impacts on public health, it will be unable to connect the dots. In short Title 22 needs a thorough review as the standards as presently written and applied, fail to protect public health. It is evident that there are no CDPH established MCLs here, thus this falls directly back onto the state and regional boards.

WC 13521 requires protection of public health, it is the moving section relating to reclaimed water. That is followed by 13522 and requires abatement of contamination.

Then we have health and Safety Code provisions. 5410---contamination means impairment of the quality of the waters of the state by waste to a degree which creates a hazard to the public health through poisoning or through the spread of disease. Waters of the state is defined via 5410 (c) to mean any water, surface or underground including saline waters within the boundaries of the state.

5410(d) further defines contamination.

5411â€”no person shall discharge sewage or other waste, or the effluent of treated sewage or other waste, in any manner which will result in contamination, pollution, or nuisance. Nuisance is defined via 5410(f) as anything which: 1, is injurious to health and occurs during or as a result of the treatment or disposal of wastes.

Taking the above together, it would seem that the state board by ignoring these code sections is violating its own laws. Consider the above discussion of the ability of the state board and regional boards to step into areas where there are no MCLs. Certainly, as currently produced and scientifically verified, Title 22 water does contain numerous pathogens and multi-drug resistant pathogens, pharmaceuticals that enhance the opportunity for resistance and myriad other contaminants. These are potentially a direct risk to public health. These pathogens are by definition contaminants and thus fall under several of the code sections that direct the state board and regional boards. The CDPH via H&SC 100125, is to examine and prevent water pollution, but where and how does that tie back to Title 22 and thus what the state board and regional boards do? If there are no MCLs in this area, then again this falls to the state board and regional boards. Further, H&SC 120125 would require an examination into the causes of communicable disease. Again where is this with respect to genetic material conferring both resistance and virulence? As an academic question, for which I would like an answer, are the water boards precluded from enforcement of the provisions of H&SC 100125?

If an activity that meets all applicable standards is still a threat to the public health, then that activity by definition is an ultrahazardous activity.

On page 17 of the staff report, hazardous materials are discussed. To the extent that pathogens and their toxins are hazardous, the impact to health warrants discussion which is now missing in the environmental analysis and ancillary discussions presented by staff. Hypochlorite is, however discussed. The immune system's leukocytes utilize hypochlorite to kill pathogens. Use of hypochlorite or chlorine in water treatment renders pathogens rapidly resistant to these materials. Hence that resistance impacts the human immune system. On the other hand antibiotic resistant genes (ARGs) are not affected by current chlorine levels or typical filter systems used in water treatment. Thus there is an adverse synergistic impact created by inability to control ARGs and at the same time a diminution of capacity in leukocytes because of chlorine resistant pathogens. As an example in our tests of recycled water post chlorination, we noted bacteria within two separate pathogen groups that were at the same time resistant to chlorine as well to 11 of the 12 challenge antibiotics. One of those antibiotics was vancomycin. Thus we had a multi-drug resistant pathogen that was also resistant to chlorine. This adverse situation is exacerbated by the use of bacteriostatic rather than bactericidal antibiotics. The former merely reduce the number of pathogens and thus rely on the immune system to control pathogens at some reduced level. If the immune system is compromised, this may not work.

Additionally the industrial chemicals and pharmaceuticals contained within recycled water may bioaccumulate within soils or aquatic systems as well as ground water, but are not discussed within the environmental document. These accumulated materials can augment resistance in soil microbes as well as introduced pathogens. These issues are potentially significantly adverse, are absent from discussion within the environmental analyses but need to be discussed within the environmental document. Absent such a discussion, the state board and the public are deprived of that input. Again, the policy is skewed by withholding information.

On page 18 of the staff report, hydrology and water quality are discussed. More is warranted on the impacts on water quality. The absence of any discussion of pharmaceuticals and pathogens needs to be corrected. The USGS has an abundance of good papers on the impacts of pharmaceuticals and emerging contaminants on water quality. Additionally the impact of other contaminants found in recycled water on the receiving waters (surface and ground) warrants more discussion. Absent also is any discussion on impacts from pathogens and ARGs. Without that information, the Board is hindered in its duty to evaluate recycled water and the citizens are deprived of a complete picture of impacts and mitigations. The main thrust of the environmental document is on salts but ignores other potential and serious adverse impacts and issues.

On page 20 of the staff report, the issue of land use and planning is discussed. There are several potentially adverse impacts that accrue to this area that are not discussed. The off-setting of potable water demand by recycled water allows for the development of and expansion of communities. That will create impacts on public services, create traffic that in turn will impact air quality and noise, all of which are potentially significantly adverse and cumulative but are not discussed under this heading nor for that matter under later headings such as noise, traffic, utilities, or public services. Again, this lack of foresight and well rounded planning deprives the Board and citizens of needed information upon which to judge the project. These increases will impact local planning staff in the areas utilizing this water. All these issues are potentially significantly adverse but receive no discussion within the document.

Accordingly the mandatory findings noted within the document are in error as there are several areas that are

potentially significantly adverse, yet the document contends that there are no potentially significant impacts. The statement that there are no significant impacts is thus a fiction visited on the Board and the public.

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Abstract

There is huge potential for genetic exchange to occur within the dense, diverse anaerobic microbial population inhabiting the gastrointestinal tract (GIT) of humans and animals. However, the incidence of conjugative transposons (CTns) and the antibiotic resistance genes they carry has not been well studied among this population. Since any incoming bacteria, including pathogens, can access this reservoir of genes, this oversight would appear to be an important one. Recent evidence has shown that anaerobic bacteria native to the rumen or hindgut harbour both novel antibiotic resistance genes and novel conjugative transposons. These CTns, and previously characterized CTns, can be transferred to a wide range of commensal bacteria under laboratory and in vivo conditions. The main evidence that gene transfer occurs widely in vivo between GIT bacteria, and between GIT bacteria and pathogenic bacteria, is that identical resistance genes are present in diverse bacterial species from different hosts.

Keywords

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Research

Unexpected Occurrence of Plasmid-Mediated Quinolone Resistance Determinants in Environmental *Aeromonas* spp.

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Suggested citation for this article

Abstract

We searched for plasmid-mediated quinolone resistance determinants of the Qnr type in several water samples collected at diverse locations from the Seine River (Paris, France). The *qnrS2* genes were identified from *Aeromonas punctata* subsp. *punctata* and *A. media*. The *qnrS2* gene was located on IncU-type plasmids in both isolates, which resulted in increased MIC values of quinolones and fluoroquinolones, once they were transferred into *Escherichia coli*. The *qnrS2* gene identified in *A. punctata* was part of novel genetic structure corresponding to a mobile insertion cassette element. This identification of plasmid-mediated *qnr* genes outside Enterobacteriaceae underlines a possible diffusion of those resistance determinants within gram-negative rods. Quinolones are broad-spectrum antibacterial agents used in human and veterinary medicine. Their extensive use has been associated with a rising level of quinolone resistance (1). The 2 main mechanisms of quinolone resistance are chromosomally encoded, either a modification of the quinolone targets with changes of DNA gyrase (*gyrA*) and/or of topoisomerase IV (*parC*) genes; or a decreased intracellular concentration due to impermeability of the membrane or to overexpression of efflux pump systems (2). Plasmid-mediated quinolone resistance was first identified in a *Klebsiella pneumoniae* clinical isolate from the United States (3). It is mediated by a 218-aa protein, Qnr (lately termed QnrA), which belongs to the pentapeptide repeat family of proteins that protects DNA from quinolone binding to topoisomerases (4,5). QnrA confers resistance to quinolones such as nalidixic acid and increases MICs of fluoroquinolones up to 32-fold in *Escherichia coli* (6). In addition, it enhances selection of associated chromosome-encoded quinolone resistance determinants that confer additional resistance to fluoroquinolones (7). The QnrA determinants have been reported worldwide in many enterobacterial species, and 6 of them are known so far (QnrA1 to QnrA6) (8). Other plasmid-mediated quinolone resistance determinants, QnrB (QnrB1 to QnrB10) and QnrS (QnrS1 and QnrS2), have been identified in enterobacterial species, sharing 41% and 60% amino acid identity with QnrA, respectively (8â€10). The plasmid-mediated *qnr* genes have been identified so far only in Enterobacteriaceae (6,8). Recent findings indicated that those genes originate from environmental gram-negative bacterial species, such as *Shewanella* algae, the progenitor of the *qnrA* genes (11), and *Vibrio splendidus*, the progenitor of *qnrS* genes (12). We have shown that many Vibrionaceae species may harbor chromosome-encoded *qnr*-type genes (13). To further evaluate the spread of plasmid-mediated resistance determinants in the environment, we have

searched for those genes in water samples drawn from the Seine River in Paris, France. We identified QnrS determinants in *Aeromonas* species in uncommon genetic environments.

Our study identified plasmid-mediated quinolone resistance QnrS determinants from water samples collected in different sites in a Paris river. To our knowledge, this is the first identification of plasmid-mediated QnrS determinants in nonenterobacterial species. Previous studies did not identify such qnr genes from tested gram-negative isolates that represented *Campylobacter jejuni* (27), *Aeromonadaceae*, *Pseudomonadaceae*, *Xanthomonadaceae*, *Moraxellaceae*, and *Shewanellaceae* (11). Identification of QnrS-positive isolates at 2 collection sites in different water samples may highlight their relative persistence in the environment, at least in this area at that time.

The high-level resistance to quinolones and fluoroquinolones might be due to mutations in type II topoisomerase genes because the mutations described in type II topoisomerases in *A. media* and *A. punctata* subsp. *punctata* have already been associated with resistance in *Aeromonas* spp (18). QnrS2 may confer low-level resistance to quinolones, as known in *E. coli*.

The qnrS1 gene has been identified now from several enterobacterial isolates from Japan (9), Germany (28), the United Kingdom (29), the United States (25), France (17), Vietnam (30), Taiwan (31,32), and Denmark (33). The qnrS2 gene (92% amino acid identity with QnrS1) was identified from a transferable IncQ-related plasmid (pGNB2) isolated from an activated sludge bacterial community of a wastewater treatment plant in Germany (24) and in a single non-Typhi *Salmonella* clinical isolate from the United States (25). Identification of QnrS determinants in *Aeromonas* spp. indicates that those bacterial species may play a role as a reservoir of the qnrS genes in an aquatic environment, as already evidenced for tet genes (34,35). However, whether *Aeromonas* species are a main or an accessory reservoir of plasmid-mediated quinolone resistance determinants in regard to Enterobacteriaceae remains to be determined. For *Aeromonas* spp. to act as a reservoir of qnr genes it must be capable of acquiring these resistance genes from their progenitors (36) and transferring this genetic information to Enterobacteriaceae. QnrS2-positive plasmids p37 and p42 were not able to be transferred by conjugation to an *E. coli* host in vitro, but they were able to replicate in *E. coli*, indicating their broad host spectrum. In addition, our study demonstrated that this IncU-type plasmid-mediated qnrS2 gene was expressed and able to confer reduced susceptibility to quinolones, at least in *E. coli*. *Aeromonas* species and IncU plasmids, which are ubiquitous in a wide range of environments, might therefore act as important vectors for transfer of plasmid-mediated quinolone resistance determinants (23).

As opposed to most qnrA and qnrB genes, qnrS genes have never been reported to be associated with sul1-type class 1 integrons (6,8). The qnrS1 gene has been identified either upstream of Tn3-like transposon (9,28) or upstream of the insertion sequence ISEcl2 (17,37). In IncQ-related plasmid pGNB2 and in pMG308 from a *Salmonella* isolate, surrounding genetic structures of the qnrS2 genes were similar, with 2 open reading frames located immediately downstream of qnrS2, similar to repC and repA genes involved in plasmid replication (24,25) (Figure 2). In plasmid p37 from *A. punctata* 37, the genetic structure was different since the qnrS2 was part of a transposon-like structure and inserted in an open reading frame coding for a zinc Mpr.

We have shown that plasmid integration of the qnrS2 gene may result from a peculiar transposition process that likely corresponds to trans-transposition. The qnrS2 gene was inserted in a peculiar mic. Such mic elements have been identified rarely, e.g., mic231-like elements in *Bacillus cereus*, carrying in only 1 instance an antibiotic resistance gene, the fos gene encoding resistance to fosfomycin (26). This finding may indicate that mic elements might be clinically relevant and the origin of an additional gene plasticity in a bacterial species. These elements containing genetic features involved in gene dissemination (with a transposase likely acting in-trans) and expression may be also vehicles for antibiotic resistance genes. This structure type may be added to the list of genetic tools at the origin of dissemination and expression of antibiotic resistance determinants.

As previously described for the spread of a carbapenemase gene (blaIMI-2) in US rivers (20), this report underlines that the aquatic environment is an important reservoir of novel antibiotic resistance determinants. Quinolones are antimicrobial agents extensively used in aquaculture and are stable molecules in water (as opposed to β -lactams) (38). Thus, they may be the source of an important driving force for selection of quinolone resistance, which explains why QnrS2-positive plasmids did not possess any additional resistance determinants. Further studies might focus on the particular effect of quinolone use for inducing the qnrS2 gene mobility because it is known those molecules may induce bacterial repair systems and antibiotic resistance gene transfer (39).

We have previously shown that the qnrA and qnrS genes originate from water-borne bacterial species, *S. algae* and *Vibrio splendidus*, respectively. This identification of a qnrS gene in another water-borne species, *Aeromonas*, further strengthens the role of water as a vehicle for spread of those resistance determinants.

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High frequency of antimicrobial resistance in human fecal flora.

Levy, SB; Marshall, B; Schluederberg, S; Rowse, D; Davis, J
 Antimicrobial Agents & Chemotherapy [ANTIMICROB. AGENTS CHEMOTHER.]. Vol. 32, no. 12, pp. 1801-1806.
 1988.

The frequency of resistance to seven different antimicrobial agents was examined in the aerobic gram-negative gut flora of over 600 individuals from hospitals, from laboratories where antibiotics were used, and from urban and rural communities. In a majority (62.5%) of fecal samples from people without a recent history of taking antibiotics, 10% or more of the total organisms were resistant to at least one of the antibiotics. In about 40% of the samples, resistance to more than one drug was present at this level. More than one-third of the samples contained resistant organisms comprising 50% or more of the total flora examined. This extensive study revealed a high prevalence of resistant bacteria in the gut flora of ambulatory and hospitalized individuals whether or not they were taking antibiotics.

The impact of antibiotic use on resistance development and persistence - all 2 versions Â»
 TM Barbosa, SB Levy - Drug Resistance Updates, 2000 - public.asu.edu
 ... consistently has resulted in the survival and spread ... an illustrative example of how
 antibiotic use in ... hospitals and community affects the resistance levels in ...
 Cited by 41 - Related Articles - Web Search

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Survival, and Protection against Chlorination, of Human Enteric Pathogens in Free-Living Nematodes Isolated from Water Supplies

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The findings gathered in this investigation are summarized as follows:

- a) The *D. nudicapitatus* and *C. quadrilabiatu*s readily ingested *Salmonella* and *Shigella* organisms on plate cultures and could even ingest small amounts of Coxsackie and Echo viruses in a fluid suspension.
- b) About 5 to 6% of the ingested *S. typhosa* or *Sh. sonnei* and about 12 to 16% of the ingested *S. paratyphi* or Coxsackie A9 virus survived for 24 hours; but the survivals of these were reduced to about 0.1% and 1% respectively after 48 hours. There was no evidence of excretion of viable pathogens.
- c) These nematodes are so highly resistant to the destructive action of free chlorine in water that they were not affected by 2.5-3.0 ppm of chlorine in a 120-minute exposure or by 15 to 45 ppm of chlorine in a 1-minute exposure when the water temperature was 25°C, pH 6.6-7.2, and the chlorine residuals were only slightly lower than initials. Even with an initial chlorine as high as 95-100 ppm, 50-60% of these nematodes survived a 5-minute contact and 10-20% survived a 15-minute contact. The *C. quadrilabiatu*s appeared to be somewhat more resistant than the *D. nudicapitatus*, but the difference was reduced as the chlorine dosage was increased from 15 to 95-100 ppm.
- d) The ingested pathogens were protected by the carrier nematodes to the extent that they had a complete survival even when about 90% of the worms were immobilized by the free chlorine. *Am. J. Trop. Med. Hyg.*, 9(2), 1960, pp. 136-142

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Occurrence of the New Tetracycline Resistance Gene *tet(W)* in Bacteria from the Human Gut
 Karen P. Scott,*
 Claire M. Melville, Teresa M. Barbosa, and Harry J. Flint

Members of our group recently identified a new tetracycline resistance gene, *tet(W)*, in three genera of rumen obligate anaerobes. Here, we show that *tet(W)* is also present in bacteria isolated from human feces. The *tet(W)* genes found in human *Fusobacterium prausnitzii* and *Bifidobacterium longum* isolates were more than 99.9% identical to those from a rumen isolate of *Butyrivibrio fibrisolvens*.

The rapid increase in antibiotic resistance in human pathogenic bacteria is a major problem, particularly for nosocomial infections (5). In the past, antibiotic resistance genes have primarily been described either in clinical pathogens or in antibiotic-producing microorganisms, and comparatively little work has been done on the incidence of antibiotic resistance in the commensal gut flora, either of humans or of animals. A new ribosome-protection-type tetracycline resistance (*Tcr*) gene, *tet(W)*, (GenBank accession no. AJ222769), was recently identified in the rumen anaerobe *Butyrivibrio fibrisolvens* and was also found in rumen isolates of *Selenomonas* spp. and *Mitsuokella* spp. and in one *Mitsuokella* isolate from a Japanese pig (1). The high degree of homology between all of these *tet(W)* genes suggested that recent gene transfer events had resulted in the spread of the gene. *tet(W)* was shown to be chromosomally located in *B. fibrisolvens* and to transfer at frequencies of 10⁻³ to

10-5 per recipient between genotypically diverse *B. fibrisolvens* strains in vitro (10). The translated product of tet(W) shares only 68% amino acid homology with Tet(O) and Tet(M) proteins (1). Here, we describe for the first time the identification of tet(W) in anaerobic bacteria recovered from human feces.

Human fecal samples were resuspended in anaerobic 0.1 M sodium phosphate buffer (pH 7.2), and dilutions were plated out anaerobically either on M2GCS agar plates (6) containing 5 or 10 μg of tetracycline per ml or in M2GCS roll tubes (2) containing 10 μg of tetracycline per ml. Plates were inoculated in an anaerobic cabinet (55% CO₂, 40% N₂, and 5% H₂; Coy Laboratory Products Inc., Grass Lake, Mich.), and roll tubes were prepared under 100% CO₂ (2). Cultures were incubated at 37°C.

For one sample from a middle-aged male receiving daily tetracycline treatment over a 10-year period, more than 99% of the 8.3 $\times 10^{10}$ colonies growing anaerobically were Tcr. Random colonies were picked from roll tubes and regrown in the presence of 10 μg of tetracycline per ml. Total genomic DNA was purified (10) and amplified by PCR, either using degenerate primers which identify all ribosome-protection-type Tcr genes (1) or using a primer combination specific for tet(W) (tetW for [5' AAGCGGCAGTCACTTCCTTCC 3'] and tet2 [see reference 1]). All 14 of the colonies tested yielded a product with the degenerate Tcr primer set, while only one, isolate K10, yielded a product with primers specific for tet(W). Culturing of two additional samples from 25-year-old individuals who had not taken antibiotics for at least 10 years showed that less than 0.01% of the total anaerobic bacterial count was Tcr. Total genomic DNA purified from 3 of 20 Tcr colonies (F5, F8, and F10) from one individual yielded a PCR product when the primer set specific for tet(W) was used.

The PCR products obtained as described above were sequenced using the ABI 377 automated sequencing system and confirmed to be tet(W) products using a basic local alignment search tool search for database comparisons. This initial sequence analysis demonstrated that the tet(W) gene from the human isolates was very closely related to tet(W) genes from the rumen isolates (1). An extended region of the new tet(W) genes was amplified using primers corresponding to positions 165 to 185 and 2096 to 2113 in the database sequence AJ222769. Subsequent sequence analysis showed that the genes from K10 and F5 differed by a single nucleotide and, furthermore, differed by 0 or 1 nucleotides (nt), respectively, over 1,864 nt of the 1,917-nt coding sequence of the *B. fibrisolvens* tet(W) gene. Table 1 indicates the sequence divergence between the tet(W) genes we have identified so far. The degree of homology observed for tet(W) genes of diverse origin is much higher than that observed for other ribosome-protection-type Tcr genes and indicates that the gene has not evolved greatly following acquisition by the divergent host bacteria, which therefore implies that transfer events resulting in the spread of tet(W) have been recent. A survey done to compare tet(Q) genes from *Bacteroides* or *Prevotella* isolates of animal and human origin indicated that an internal 407-nt segment differed by up to 59 nt between different isolates (8). Although this survey found that human isolates of *Prevotella intermedia* and *Bacteroides fragilis* contained tet(Q) genes which were identical across the region analyzed, the closest homology between genes from different hosts was 98%.

The occurrence of almost identical Tcr genes in commensal bacteria from the animal and human gut is evidence of recent gene flow between these populations and leads to the important conclusion that obligate anaerobiosis is not a barrier to genetic exchange. The most likely route for transfer between hosts may be via intermediary facultative anaerobes that are capable of colonizing animals and man. Alternatively, it is also likely that transfer of obligately anaerobic gut bacteria between hosts occurs with sufficient frequency to mediate gene transfer events. It is of course impossible to conclude from the present evidence whether transfer of tet(W) has been predominantly to or from the human gut flora. This question is clearly central to the debate over the use of antibiotics as growth promoters in agriculture and the impact such use has on the clinical use of antibiotics in the treatment of human disease. Tetracyclines continue to be important as therapeutic antibiotics, but they are still employed in agriculture in many countries (3), making them overall the second most used group of antibiotics worldwide (9).

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Commensal Host-Bacterial Relationships in the Gut

Lora V. Hooper, Jeffrey I. Gordon*

One potential outcome of the adaptive coevolution of humans and bacteria is the development of commensal relationships, where neither partner is harmed, or symbiotic relationships, where unique metabolic traits or other benefits are provided. Our gastrointestinal tract is colonized by a vast community of symbionts and commensals that have important effects on immune function, nutrient processing, and a broad range of other host activities. The current genomic revolution offers an unprecedented opportunity to identify the molecular foundations of these relationships so that we can understand how they contribute to our normal physiology and how they can be exploited to develop new therapeutic strategies.

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Evidence for Extensive Resistance Gene Transfer among *Bacteroides* spp. and among *Bacteroides* and Other Genera in the Human Colon

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Transfer of antibiotic resistance genes by conjugation is thought to play an important role in the spread of resistance. Yet virtually no information is available about the extent to which such horizontal transfers occur in natural settings. In this paper, we show that conjugal gene transfer has made a major contribution to increased antibiotic resistance in *Bacteroides* species, a numerically predominant group of human colonic bacteria. Over the past 3 decades, carriage of the tetracycline resistance gene, *tetQ*, has increased from about 30% to more than 80% of strains. Alleles of *tetQ* in different *Bacteroides* species, with one exception, were 96 to 100% identical at the DNA sequence level, as expected if horizontal gene transfer was responsible for their spread. Southern blot analyses showed further that transfer of *tetQ* was mediated by a conjugative transposon (CTn) of the CTnDOT type. Carriage of two erythromycin resistance genes, *ermF* and *ermG*, rose from <2 to 23% and accounted for about 70% of the total erythromycin resistances observed. Carriage of *tetQ* and the *erm* genes was the same in isolates taken from healthy people with no recent history of antibiotic use as in isolates obtained from patients with *Bacteroides* infections. This finding indicates that resistance transfer is occurring in the community and not just in clinical environments. The high percentage of strains that are carrying these resistance genes in people who are not taking antibiotics is consistent with the hypothesis that once acquired, these resistance genes are stably maintained in the absence of antibiotic selection. Six recently isolated strains carried *ermB* genes. Two were identical to *erm(B)-P* from *Clostridium perfringens*, and the other four had only one to three mismatches. The nine strains with *ermG* genes had DNA sequences that were more than 99% identical to the *ermG* of *Bacillus sphaericus*. Evidently, there is a genetic conduit open between gram-positive bacteria, including bacteria that only pass through the human colon, and the gram-negative *Bacteroides* species. Our results support the hypothesis that extensive gene transfer occurs among bacteria in the human colon, both within the genus *Bacteroides* and among *Bacteroides* species and gram-positive bacteria.

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Multiple Antibiotic Resistance Gene Transfer from Animal to Human Enterococci in the Digestive Tract of Gnotobiotic Mice. Moubareck,1 N. Bourgeois,1 P. Courvalin,2 and F. Doucet-Populaire1*

It has been proposed that food animals represent the source of glycopeptide resistance genes present in enterococci from humans. We demonstrated the transfer of *vanA* and of other resistance genes from porcine to human *Enterococcus faecium* at high frequency in the digestive tract of gnotobiotic mice. Tylosin in the drinking water favored colonization by transconjugants.

The acquisition and spread of glycopeptide-resistant enterococci (GRE) is a global problem, although the selective pressure that has led to dissemination differs between geographical areas (19). In the United States, where antibiotics which represent high-level risk factors for colonization or infection by GRE are extensively prescribed, such strains are isolated from hospitalized patients but not from community-based volunteers without hospital exposure or from the environment or animals (4, 10). By contrast, a low incidence of clinical GRE infection (2.2%) is observed in most European countries (8) but the strains are found in the healthy human population and in animals (1). As opposed to practices in the United States, the glycopeptide avoparcin was used as a growth promoter in animal husbandry in Europe until 1997 and is associated with the dissemination (in poultry and pigs in particular) of enterococci that are cross-resistant to avoparcin and vancomycin (9). The finding of GRE in nonhospitalized humans and in meat eaters but not in vegetarians further suggests a food-associated spread of vancomycin-resistant enterococci from animals to humans (17). A study after ingestion of GRE of animal origin by healthy volunteers revealed the presence of such strains in human stools for prolonged periods of time (18). The entry of GRE of animal origin into the human food chain not only allows these strains to become established in the human gut but can also favor transfer of their resistance genes to human commensals (21). It has been shown that in 20 GRE isolates taken in Germany from infections in patients, from nonhospitalized humans, from sewage and animal feces, and from meat products, the *vanA* operon was structurally conserved, which suggests gene spread among these various ecosystems (22). Transfer of genes encoding glycopeptide resistance between animal and human strains in nature is still controversial (15), even though transfer of the *satA* gene (encoding resistance to streptogramin A) has been demonstrated for *Enterococcus faecium* in the gastrointestinal tract of gnotobiotic rats (11). The purpose of this study was to examine the possibility of the transfer of antibiotic resistance genes between *E. faecium* strains of animal and human origins in vitro and in vivo in the digestive tract of gnotobiotic mice.

Human fecal isolate *E. faecium* 64/3 (resistant to rifampin and fusidic acid) was used as a recipient. Four *E. faecium* isolates of porcine origin (UW4, UW7, UW261, and UW262), harboring the *vanA* and *ermB* genes (conferring resistance to vancomycin and erythromycin, respectively), the *tet(L)* and *ant(6)* genes (mediating resistance to tetracycline and streptomycin, respectively, in UW262), and the *tet(M)* tetracycline determinant (in

UW7), were used as donors. Transposon Tn1546, which carries the *vanA* gene cluster in all animal strains, was characterized by PCR as previously described (12).

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Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. H Kruse and H SÃrum

Plasmids harboring multiple antimicrobial-resistance determinants (R plasmids) were transferred in simulated natural microenvironments from various bacterial pathogens of human, animal, or fish origin to susceptible strains isolated from a different ecological niche. R plasmids in a strain of the human pathogen *Vibrio cholerae* O1 E1 Tor and a bovine *Escherichia coli* strain were conjugated to a susceptible strain of the fish pathogenic bacterium *Aeromonas salmonicida* subsp. *salmonicida* in marine water. Conjugations of R plasmids between a resistant bovine pathogenic *E. coli* strain and a susceptible *E. coli* strain of human origin were performed on a hand towel contaminated with milk from a cow with mastitis. A similar conjugation event between a resistant porcine pathogenic *E. coli* strain of human origin was studied in minced meat on a cutting board. Conjugation of R plasmids between a resistant strain of the fish pathogenic bacterium *A. salmonicida* subsp. *salmonicida* and a susceptible *E. coli* strain of human origin was performed in raw salmon on a cutting board. R plasmids in a strain of *A. salmonicida* subsp. *salmonicida* and a human pathogenic *E. coli* strain were conjugated to a susceptible porcine *E. coli* strain in porcine feces. Transfer of the different R plasmids was confirmed by plasmid profile analyses and determination of the resistance pattern of the transconjugants. The different R plasmids were transferred equally well under simulated natural conditions and under controlled laboratory conditions, with median conjugation frequencies ranging from 3×10^{-6} to 8×10^{-3} . The present study demonstrates that conjugation and transfer of R plasmids is a phenomenon that belongs to the environment and can occur between bacterial strains of human, animal, and fish origins that are unrelated either evolutionarily or ecologically even in the absence of antibiotics. Consequently, the contamination of the environment with bacterial pathogens resistant to antimicrobial agents is a real threat not only as a source of disease but also as a source from which R plasmids can easily spread to other pathogens of diverse origins.

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Horizontal Transfer of a Multi-Drug Resistance Plasmid between Coliform Bacteria of Human and Bovine Origin in a Farm Environment Hanne Oppegaard,* Terje M. Steinum, and Yngvild Wasteson

Multi-drug-resistant coliform bacteria were isolated from feces of cattle exposed to antimicrobial agents and humans associated with the animals. Isolates from both cattle and humans harbored an R plasmid of 65 kb (pTMS1) that may have been transferred between them due to selective antibiotic pressure in the farm environment.

The amount of antimicrobial agents used for therapeutic and nontherapeutic purposes in agriculture far exceeds what is used for humans in many parts of the world (11). Since exposure to antimicrobial agents is the most important factor with regard to development of antimicrobial resistance, animals and animal products could thus be significant sources of resistant bacteria for the human population (1, 5, 12, 17, 18). Nonpathogenic, multiple-drug-resistant *Escherichia coli* in the intestine is probably an important reservoir of resistance genes (3, 10, 13, 15, 21), and drug-resistant, intestinal *E. coli* of animal origin may colonize the human intestine, at least temporarily (14, 15, 20). However, the ease with which bacteria acquire new resistance genes by self-transmissible and mobilizable plasmids and conjugative transposons may represent a more significant contribution to the increasing incidence of resistant strains (19, 23, 24).

In this study, farm inhabitants were investigated for the occurrence of multi-drug-resistant intestinal *E. coli*. On the farm studied here, various antimicrobials had been used extensively, primarily to treat recurrent *Staphylococcus aureus* mastitis in dairy cattle (L. SÃrverÃ, personal communication). One family lived on the farm, and one veterinarian had served the animals. During the spring of 1996, fecal swabs (Culturette; Becton Dickinson Europe, Meyland, France) were collected from 13 cattle, three family members, and the local veterinarian. One year later, sampling of the family members and the veterinarian was repeated, and fecal swabs from four other veterinarians operating sporadically in the area were also included. For a primary screening of the total aerobic fecal flora, each swab was plated onto blood agar (blood agar base [Difco Laboratories, Detroit, Mich.] containing 5% citrated bovine blood) and Mueller-Hinton agar (Difco) with Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark) containing 33 μg of ampicillin (AMP), 80 μg of tetracycline (TET), 100 μg of streptomycin (STR), 5.2 μg of trimethoprim (TMP), 240 μg of sulfonamides (SUL), 60 μg of chloramphenicol, and 10 μg of enrofloxacin. Agar plates were incubated at 37°C for 24 h. A total of six cattle and five human samples exhibited multiple-drug-resistant patterns (Ampr Tetr Strr Tmpr Sulr). From these plates, three colonies were picked from the zones close to the Neo-Sensitabs containing AMP, TET, and SUL, respectively. Colonies were subcultivated on blood agar and BTB-lactose agar plates to assure pure cultures and retested for

susceptibility to the above-mentioned antimicrobials. MICs of AMP, TET, STR, TMP, and SUL were determined (16). A total of 39 of 90 lactose-fermenting (coliform) bovine and human isolates expressed resistance to more than one antimicrobial and were selected for further studies. Species identification was performed by using Enterotube (Becton Dickinson). A selection of three bovine and three human *E. coli* isolates with the phenotype Ampr Tetr Strr Tmpr Sulr was serotyped at Statens Serum Institut, Copenhagen, Denmark. Plasmid DNA was isolated by using the Maxi kit (Qiagen GmbH, Hilden, Germany), digested with EcoRI, HindIII, and BamHI (Gibco BRL, Gaithersburg, Md.), and hybridized with probes for various resistance determinants (Table 1) by using the AlkPhos Direct Nucleic Acid Labelling and Detection system (Amersham Life Science). Class 1 integrons, commonly carried by large conjugative plasmids, are of particular importance in development of multiple-drug resistance in gram-negative bacteria (7, 8) and were detected by using the Gene Amp PCR Reagent kit (Perkin-Elmer Cetus, Norwalk, Conn.) with plasmid DNA as a template, primers corresponding to the 5' end of the integrase gene *intI* (5'-TGATATTATGGAGCAGCAGCAACGATG-3') (EMBL accession no. M73819), and an internal region of the *dfrI* gene cassette (5'-GTATCTACTTGATCGATCAGGC-3') (EMBL accession no. X00926). The multi-drug-resistant human and bovine coliforms were used as donors in broth mating experiments with nalidixic acid (NAL)-resistant, plasmid-free *E. coli* DH5 (MIC_{NAL} > 20 µg/ml) as a recipient. Transconjugants were selected on Mueller-Hinton agar containing 20 µg of NAL/ml and 40 µg of sulfadiazine/ml.

TABLE 1

Resistance gene probes used in hybridization experiments			
Resistance determinant ^a	Probe description ^b	Reference	
<i>sulI</i>	660-bp SacII-BglII fragment of plasmid pGS72	27	
<i>sulIII</i>	780-bp HincII fragment of plasmid pSUL204	22	
<i>dfrI</i>	500-bp HpaI fragment of plasmid pFE872	6	
<i>dfrIV</i>	1,600-bp ClaI fragment of plasmid pUK1148	28	
<i>dfrXII</i>	500-bp BamHI-HindIII fragment of plasmid pBEM155	25	
<i>tetA</i>	750-bp SmaI fragment of plasmid RP4	4	
<i>tetB</i>	2,800-bp BglII fragment of plasmid R222	9	
<i>tetC</i>	1,400-bp AvaI-HindIII fragment of plasmid pBR322	2	
<i>strA-strB</i>	538 bp PCR product of plasmid RSF1010	26	

atetA, -B, and -C are classes of tetracycline resistance genes; *sulI* and *sulIII* are classes of sulfonamide-resistant dihydropteroate synthases; *dfrI*, *dfrIV*, and *dfrXII* are different types of trimethoprim-resistant dihydrofolate reductases. ^bAll plasmids were propagated in *E. coli* host strains.

Of 39 multi-drug-resistant coliform isolates, 30 carried the phenotype Ampr Tetr Strr Tmpr Sulr and the MICs of AMP, TET, STR, TMP, and SUL were >256 µg/ml. Table 2 shows characteristics of selected isolates. These were all *E. coli*, except one human isolate of *Citrobacter freundii*, and the data support the view that *E. coli* is a major carrier of resistance traits in the coliform flora of both humans and animals. Multi-drug-resistant commensal strains within the *Citrobacter* species are only occasionally described (21).

TABLE 2

Characteristics of selected multi-drug-resistant coliform bacteria isolated from cattle and humans in a farm environment

Strain and isolate	Origin (date) ^a	Serotype ^b	Resistance pattern ³	Plasmid size (kb)	Resistance genotype
<i>E. coli</i>					
1/4 F5-96	Cow 129	O100:H-	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F10-96	Cow 125	O100:H-	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F37-96	Cow 124	O100:H-	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F1-96	Human 1 (1996)	ND ^c	Tetr Strr ND ND		
1/4 F2-97	Human 1 (1997)	Oru:H+	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F14-96	Human 3 (1996)	Oru:K1:H-	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F15-97	Human 3 (1997)	ND ^c 1/4	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F60-96	Human 4 (vet 1) (1996)	O20:H+	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F14-97	Human 4 (vet 1) (1997)	ND ^c 1/4	Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>
1/4 F18-97	Human 5 (vet 2) (1997)	ND ^c 1/4	Ampr Tetr Strr Tmpr Sulr	62, 6.5	<i>strAB sulII</i>
1/4 F22-97	Human 6 (vet 3) (1997)	ND ^c 1/4	Ampr Tetr Strr Tmpr Sulr	60, 8	<i>strAB sulII</i>
1/4 <i>C. freundii</i> F20-96	Human 3 (1996)		Ampr Tetr Strr Tmpr Sulr	65	<i>tetB strAB dfrI sulI sulII</i>

^aThe year of isolation is indicated when relevant. vet, veterinarian. ^bO, carbohydrate antigen; H, flagellar antigen; K, capsular antigen; Oru, O antigen could not be determined. ^cND, not done.

A single plasmid of approximately 65 kb, designated pTMS1, was found in eight bovine and five human multi-drug-resistant isolates that were studied in detail (Table 2). Restriction analysis of pTMS1 from both sources showed identical profiles. Hybridization studies showed that *sulI*, *sulII*, *dfcI*, *strA-strB*, and *tetB* resistance determinants were associated with pTMS1. The hybridizing patterns were identical for all probes, suggesting a similar organization of resistance genes, and the presence of *sulI* was evidence of a class 1 integron. Furthermore, PCR results indicated that pTMS1 contained the *dfcI* gene downstream of and adjacent to the *intI* gene of the class 1 integron. Despite identical restriction and hybridization patterns, no PCR product was obtained when the 65-kb plasmid of *C. freundii* was used as template DNA, indicating a different organization of gene cassettes in this isolate.

No identical serotypes were found when comparing bovine and human *E. coli* carrying pTMS1 that were O100:H- and O20:H+ or nontypable, respectively. Although only six strains were serotyped, this result indicates that resistance was disseminated through horizontal transfer of resistance genes, rather than by transfer of the resistant isolates themselves.

Transconjugants expressing resistance to NAL, AMP, TET, STR, TMP, and SUL were obtained at moderate frequencies (approximately 5×10^{-5}) only when bovine *E. coli* strains were donors. This could be because conjugation was dependent of host factors that were not present in the human strains. Transfer of a plasmid of approximately 65 kb, with a restriction pattern identical to that of pTMS1, was confirmed by isolation of the plasmid from donor, recipient, and transconjugants.

The results indicate persistence of pTMS1 in *E. coli* in the intestine of one farm inhabitant and the local veterinarian (Table 2). Also, a second farm inhabitant seemed to have acquired *E. coli* harboring pTMS1 between the first and second sampling. The persistence of antibiotic resistance is not fully understood, but one hypothesis is that acquisition of resistance genes results in gain of fitness and colonizing ability (23). However, we cannot exclude the possibility that multi-drug-resistant fecal flora were established due to other factors before or during this study.

The penicillin- and tetracycline-resistant *S. aureus* causing mastitis in the herd shared a single plasmid of approximately 20 kb harboring the *tetA(K)* determinant, while the *blaZ* gene is chromosomally located (29). *S. aureus* and *E. coli* differ with regard to resistance genes, and genetic exchange between staphylococci and coliform bacteria is unlikely to occur. The results of this study do, however, demonstrate how antibacterial treatment targeted at a pathogenic organism in one organ system may affect the endogenous flora of other organ systems in the exposed individual and in its environment.

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Transferable Plasmid-Mediated Resistance to Streptomycin in Clinical Isolate of *Yersinia pestis*

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Plasmid-mediated high-level resistance to multiple antibiotics was reported in a clinical isolate of *Yersinia pestis* in Madagascar in 1997. We describe a second *Y. pestis* strain with high-level resistance to streptomycin, isolated from a human case of bubonic plague in Madagascar. The resistance determinants were carried by a self-transferable plasmid that could conjugate at high frequencies to other *Y. pestis* isolates. The plasmid and the host bacterium were different from those previously associated with multiple-drug resistance, indicating that acquisition of resistance plasmids is occurring in this bacterial species. Emergence of resistance to streptomycin in *Y. pestis* represents a critical public health problem since this antibiotic is used as the first-line treatment against plague in many countries.

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Persistence of Resistant *Staphylococcus epidermidis* after Single Course of Clarithromycin

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Suggested citation for this article

We examined how a common therapy that includes clarithromycin affects normally colonizing *Staphylococcus epidermidis*. Samples from the nostrils of 5 patients receiving therapy were collected before, immediately after, 1 year after, and 4 years after treatment. From each patient and sample, *S. epidermidis* strains were isolated and analyzed for clarithromycin susceptibility and presence of the *erm(C)* gene. We show that macrolide-resistant

strains of *S. epidermidis* were selected during therapy and that the same resistant strain may persist for 4 years, in the absence of further antimicrobial treatment.

The emergence and spread of drug-resistant bacteria pose a serious threat to global public health (1,2), and the normal biota constitutes a potential reservoir of resistance genes that can spread to invading pathogens (3,4). A gene (*aphA-3*) that confers resistance to amikacin and kanamycin in *Campylobacter* spp. may have originated from the gram-positive *Enterococcus*, *Streptococcus*, or *Staphylococcus* spp. (4). Similarly, *aadE* and *tet(O)*, which encode streptomycin and tetracycline resistance, respectively, have been found in *Campylobacter* spp. but are considered to have been transferred from gram-positive bacteria (4). Moreover, parts of the mosaic penicillin-binding protein genes of *Streptococcus pneumoniae* that confer penicillin resistance are likely to originate from *viridans* streptococci, which tend to be more resistant (5), and the *mecA* gene that renders *Staphylococcus aureus* resistant to all β -lactams likely originated in coagulase-negative staphylococci (6).

Staphylococcus epidermidis, a coagulase-negative staphylococcus, is a major component of the normal human biota (7). Large populations (10^3 – 10^6 CFU/cm²) of *S. epidermidis* are commonly found in the anterior nares and the axillae (7). Coagulase-negative staphylococci have been increasingly recognized as important nosocomial pathogens (8), affecting immunocompromised patients or those with indwelling devices, such as joint prostheses, prosthetic heart valves, and central venous catheters (8,9). Since the infections associated with *S. epidermidis* are chiefly acquired during hospitalization, it is not surprising that they are increasingly resistant to antimicrobial drugs (10). Macrolide resistance in *S. epidermidis* is commonly caused by *erm* genes (10), whose products dimethylate a 23S rRNA adenine residue, preventing macrolide binding to the 50S ribosomal subunit (11,12). In *S. epidermidis*, *erm(C)*, which induces high-level macrolide resistance, predominates (13,14).

In this study, we have assessed how a commonly used therapy that includes clarithromycin affects the normal microbiota of *S. epidermidis*. We show that a 1-week course of clarithromycin selects for macrolide-resistant *S. epidermidis* that may persist up to 4 years after treatment.

Discussion

Since antimicrobial drugs do not distinguish between pathogenic and colonizing bacteria, our indigenous biota is affected every time a drug is given (3,15). Resistance development in staphylococci that normally colonize the skin has previously been observed after antimicrobial prophylaxis or treatment (16–18). Depending on mechanism, resistance can be selected *de novo*, exist in the pretreatment biota, or be acquired, especially in hospital environments.

In this study of the effect of a 1-week course of clarithromycin on indigenous *S. epidermidis* populations, we show that macrolide-resistant *S. epidermidis* strains are selected during therapy and that, without further selection, resistant clones can persist for up to 4 years. This finding is important for several reasons. First, although *S. epidermidis* belongs to the normal cutaneous microbiota, it may be a pathogen, especially in hospitalized patients (8); stably resistant populations increase the risk for treatment failure. Second, resistance in the normal microbiota might contribute to increased resistance in higher-grade pathogens by interspecies genetic transfer. Since the population size of the normal microbiota is large, multiple and different resistant variants can develop, which increases the risk for spread to populations of pathogens. Persisting populations of resistant microbiota further enhance transfer risk, especially if the selecting agent is used for treatment. Third, antimicrobial drugs may affect the stability of residential populations.

Whether a resistant population persists is mostly determined by the fitness and transmission costs of resistance (19,20). Most resistance involves a cost (21–24), but resistance may occur without detectable cost (25). If most resistance is costly for bacteria, resistant populations should decline once the selective antimicrobial pressure is removed. However, mutations may arise that compensate for the fitness cost, restoring the bacteria's fitness without reversion of the resistant phenotype. This phenomenon, compensatory evolution, is considered to be relevant to stabilizing resistant populations (26). Other important mechanisms that could stabilize resistant populations are no-cost resistance mutations (25) and genetic linkage with adjacent genes. Despite substantially decreased sulfonamide use in the United Kingdom from 1991 to 1999, *Escherichia coli* resistance to sulfonamides remained high (39.7% in 1991, 46.0% in 1999) because sulfonamide resistance was linked to other resistance genes that continued to be under selective pressure (27). In poultry, since *vanA* can be co-selected with *erm(B)* in *Enterococcus hirae* isolates (28), vancomycin resistance can be maintained by using macrolides, despite excluding avoparcin from animal feed. Thus, the stability and maintenance of antimicrobial drug resistance depends on the magnitude of selective pressure, compensatory evolution, no-cost associated resistance, and genetic linkage with co-selected resistance genes.

In our study, resistant isolates persisted long after drug treatment was completed. However, a variation in length of persistence between the patients was observed. Whether this variation is related to different costs associated with *erm(C)* carriage or different extents of genetic compensation for an initial cost cannot be concluded from current data. The observed variation in persistence of resistance could further be affected by the degree of recolonization and transient colonization of new strains during the 4-year study period. Although recolonization

with *S. epidermidis* is presumably low, it can be enhanced by, for example, nosocomial spread during hospital stays (29). That indigenous *S. epidermidis* populations may naturally change in composition over time was reflected in the control group. According to the PFGE profiles from controls 1 and 2, populations of *S. epidermidis* can either remain stable for 4 years or show a more dynamic state, with new strains appearing over time. A change in the composition of the flora was also observed in control 5, in whom 5 resistant isolates appeared in the susceptible flora after 4 years. Since this patient did not receive any antimicrobial drugs during the study period, this finding is likely due to recolonization or transient colonization of a strain from the environment. However, most importantly, although a few resistant isolates were detected among the controls, no selection of resistant *S. epidermidis* occurred over the 4-year study period, as was observed in the treated patients. In conclusion, antimicrobial drug treatment affects our indigenous microbiota and can give rise to long-term colonization with resistant populations. Our results show that as part of a combination therapy, a 7-day course of clarithromycin resulted in macrolide-resistant *S. epidermidis* that persisted up to 4 years without any further selection. In total, these observations suggest that selection of resistance in our microbiota after short antimicrobial drug courses may not be a rare phenomenon. However, the extent, to which other antimicrobial treatment regimens select for resistant *S. epidermidis* remains to be investigated.

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