ISSN : 2321-9602

Indo-American Journal of Agricultural and Veterinary Sciences

editor@iajavs.com
iajavs.editor@gmail.com

Indo-Am. J. Agric. & Vet. Sci., 2013 **VOL .2 ,ISSUSE.1 Feb , 2014**

ISSN 2321–9602 www.iajavs.com

Indo-American Journal of Agricultural and Veterinary Sciences

Conflict with the Natural World

K. Kümerin *

Abstract

Environments have been sampled and found to include bacteria resistant to antibiotics and disinfectants in waste water, surface water, ground water, sediments, and soils. Antibiotics find their way into the environment after being employed for medical reasons, for agricultural and animal growth, and for industrial uses such as in aquaculture. There has been a recent uptick in worries about the ecotoxic effects of infections and their rising resistance to treatment. The environmental impact of antibiotic resistance is increasingly being acknowledged. This requires knowledge of the ecology of both resistant bacteria and the genes that give resistance. Little is known regarding the effects of subinhibitory concentrations of antibiotics and disinfectants on environmental microorganisms, especially with relation to resistance. Based on what we know now, the impact of antibacterials in the environment on the spread of resistance is questionable at best. The introduction of resistant microbes seems to be a major source of resistance in the environment. The potential consequences for ecosystems of bacterial resistance are still unclear. More research is required on these worries.

Keywords: environmental microbiology, gene transfer, ecotoxicity, xenobiotics

Introduction

Antibiotics are very important in both human and animal medicine for the treatment and prevention of bacterial and fungal illnesses. They aid animal growth and development and are utilized in aquaculture.

Most pharmaceutical compounds are only partially metabolized in patients before they are flushed down the toilet or washed down the drain and sent to the hospital's sewage treatment facility or the municipal waste water treatment plant. They go to the STP in the sewage together with the rest of the human waste. They may potentially spread across the ecosystem through the water supply and the sewage system. Antibiotics used on cattle get up in the environment through manure. These chemicals might contaminate the land, water, and sediment.

High local quantities of antimicrobial medicines are found in the water compartment and nearby sediments because to their use in intensive fish farming. Antibiotics like streptomycins are used in fruit orchards, whereas a separate family of antibiotics is used by beekeepers. Disinfectants find use in several sectors, including farming, medicine, the food and glue industries, and the animal care sector. Humans and animals alike 1-4 emit bacteria that can survive in the presence of antimicrobials and disinfectants, which then spread across the environment through sewers, animal waste, and other routes.Since their inception, antimicrobials have been utilized to lessen the impact of microbial overpopulation. It has also long been known that different species of animals are susceptible to these poisons to varying degrees. Results from susceptibility tests and resistance assessments are very sensitive to the specific mechanisms of action and evaluation methods used.5 The word "resistance" is used to indicate the degree to which a microbe is protected against a certain treatment under particular conditions. In light of this caveat, the reader is referred to the aforementioned works for more explanation. Antibiotic resistance is typically quantified as the lowest effective concentration (IC50) against a given cell population. An organism that has developed resistance to a medicine is one whose sensitivity to the medication has changed over time. Many species have never been sensitive to a chemical because of natural resistance built into their physiology or biochemistry. A vulnerable organism may become immune after mutation or introduction of the resistance gene.

This short analysis sums up current knowledge regarding antibiotic resistance across ecosystems. The structure looks like this: Environmental resistance and antimicrobials are introduced with some background information. Possible entrance points for antibiotic- and bacteria-resistant strains into the environment are discussed, including hospital waste water, municipal sewage water, and other sewage treatment facilities. There is a short discussion of other elements of the aquatic environment, such as surface water, ground water, sea water, and sediments. First, the existing knowledge gaps on environmental microorganisms and antimicrobials are presented.

Applied Environmental Research Section, Institute of Environmental Medicine and Hospital Epidemiology,Freiburg University Hospital, Hugstetter Straße 55, D-79106 Freiburg, Germany

ISSN 2321–9602 www.iajavs.com
Indo-American Journal of Agricultural and Veterinary Sciences

Antibiotics, bacteria, and resistancein the environment

Antibiotics in the environment

Although antibiotics have been applied in large quantities for some decades, until recently the existence of these sub- stances in the environment was accorded very little attention. Studies conducted in various countries have detected a num- ber of antibiotics in the low microgram per litre or the nano- gram per litre (Table 1) range in different environmental compartments, i.e. hospital effluent, municipal waste water, effluent from sewage treatment plants, surface water and in some cases ground water.[1–4,6–9] The compounds detected are from different important antibiotic classes such as macrolides, tetracyclines, sulphonamides, quinolones and others as far as

The main processes of substance elimination in the environ- ment, especially in waste water, sediments, and soil are due to bacteria. The concentration of antibiotics may be much higher if the active compounds are persistent and accumulate, e.g. by sorption to solid surfaces in certain environmental

Bacteria in the environment

Apart from the issue of resistance (see below), it has to be keptin mind that bacteria form one of the most important groups of organisms in soil and in other environmental compartments as well as in natural or technical sewage treatment. Without bac- teria, water would not be clarified. Bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulphur and phosphorous cycle. Without bacteria, soil would not be

Antimicrobial resistance in the environment

The most common response of the cell to antibiotics is to cease growing (bacteriostasis), but for certain classes of compounds such as b-lactams, continued growth is permitted, with inhibition of the target in the organism leading indirectly to cell death. Inthe treatment of an infection, bacteriostasis is often effective because the killing and elimination of the pathogen are mediated through host immune defences. Such augmentation is typically absent in the environment. In this respect, there is little know- ledge of environments such as waste water, sludge, surface water, and soil compared with the medical use and effectiveness of antibacterials. Furthermore, in waste water, surface water, sediments, sludge and soil a cocktail of different active com- pounds may be present in contrast to the medical and veterinary application of antibiotics and disinfectants. Concentrations are normally some orders of magnitude lower in the environment than for therapeutic use.[1–4,6–10]

There has been growing concern about antimicrobial resist- ance for some years now. In a report by the UK House of Lords,it is stated: 'Resistance to antibiotics and other anti- infectiveagents constitutes a major threat to public health and ought to be recognized as such more widely than it is at present.'[15] There isa vast amount of literature related to use of antimicrobials and resistance against them in medicine, veterinary medicine and animal husbandry.[16–25] Resistance genes as well as resistant bac- teria in the environment are increasingly seen as an ecological

analytical methods are available. b-Lactams have not been detected yet despite the fact that b-lactams are used in the highest amounts.[10] Obviously, most of the atibiotics are not fully eliminated during the sewage purification process. The results of investigations using test systems indicate that a number of antibiotics and disinfectants are not biodegradable in the aquatic environment.[11,12] In soil, tetracycline

con- centrations in the range of several hundred micrograms per kilogram have been detected some months after manure application.[13,14]

compartments such as sewage sludge, sediments or soil. In these cases, the role of antimicrobial concentration could be different from that inwater. It is not known how strongly the antibiotics are sorbedand under what circumstances they are still available and active after sorption.

fertileand organic matter such as straw or leaves would accumulate within a short time. In soil, naturally occur- ring antibiotics from bacteria and fungi amongst others control the dynamics of bacterial populations. In contrast to these, most of the compounds used nowadays are semi-synthetic or syn- thetic. They are often much more stable and are not biodegrad- able by bacteria. They may therefore persist in the environment. Furthermore, they often have a different, e.g. broader, activity spectrum.

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

Table 1. Concentrations (ng/L) of antibiotics and disinfectants measured in the aqueous environment (for references see text)

Class	Compound	Hospital effluent	Sewage water	Surface water	Ground water
Penicillins	ampicillin	up to 80			
Tetracyclines[a]			1		
Macrolides	erythromycin–H_2O (metabolite)		up to 6.0	0.1 – 1.7	0.05
	clarithromycin and roxithromycin		<1.0	0.1 – 0.6	0.03
Quinolones	ciprofloxacin	up to 124.5	0.4	0.1	
Sulphonamides[a]	sulfamethoxazole		up to 1.0	0.1 – 0.2	0.4
	trimethoprim and others		<0.2	up to 0.03	
Disinfectants	quaternary ammonium compounds	up to 5000			

[a]Also found in soils, tetracyclines have been detected in concentrations up to 0.3 mg per kg in soils.

problem.[25,26] The most prominent medical examples are vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and multi-resistant pseudomo- nads. With respect to the causes of resistance, the focus is on the use of antimicrobials in hospitals, by medical practitioners andin animal husbandry.

The selection pressure of antibiotics present above a certain concentration against the microbial biocoenosis is an important factor in the selection and spread of resistant bacteria. The trans- fer of resistance genes as well as the already resistant bacteria themselves is favoured particularly by the presence of antibiotics over a long period and at subtherapeutic concentrations. Theyplay a role in the stimulation of resistance and its transfer by genetic material in bacteria. Exposure of bacteria to such sub- therapeutic antimicrobial concentrations is thought to increasethe speed with which resistant bacterial strains are selected, e.g.if antibiotics are used as growth promoters[16–18] or by improperuse in veterinary medicine and medicine.[19,23] In general, the emergence of resistance is a highly complex process, which isnot yet fully understood with respect to the significance of the interaction of bacterial populations and antibiotics,[24–26] even in a medicinal environment.[24,25]

Smith and co-workers[27] identified a number of limitations in the current understanding of antibacterial resistance and difficult- ies in interpreting resistance data from environmental samples such as sediments under fish ponds. Westen makes three main points:[28] (i) the use of one antibacterial agent can increase levels of resistance not only to that specific drug but to many others, even those using very different modes of antibacterial action (cross resistance); (ii) antibacterial resistance does not always respond in a predictable fashion correlating with the amount of drugs used or with the concentrations of residues in the environ- ment; (iii) often, existing data which are used for the environ- mental effects of antibacterials are not adequate to establish how long bacteria maintain antibacterial resistance in the absence of continued selective pressure for that resistance.

On the one hand, the knowledge of subinhibitory concen- trations of antimicrobials and their effects on environmental bac- teria is scarce and contradictory, especially with respect to resistance. On the other hand, there is a huge volume of evi-dence that antibiotic resistance is already present in natural environments and that it is exchanged between bacteria.[26]

According to Murray,[29] the transfer as well as the emergence of new combinations of resistance genes will happen most frequently in compartments with high bacterial density, i.e. bio- films. Such biofilms are not only found in a medical context. Bacterial density is very high both in aerobic and anaerobic septic tanks of sewage treatment works, and in biofilms, e.g. in drinking water pipes, in sediments, and soils. Biofilms are no taxonomic barrier to this horizontal genetic transfer. Bacteria are stable enough for transfer to the new environment, e.g. fromthe human body to surface water which is colder and much poorer in nutrients or the transfer from plants into animals. Therefore, the question is whether the input of antibiotics intothe environment is an important source for resistant bacteria in the environment, i.e. was the concentration of the antibiotic and the bacterial density high enough, was the exposure long enough to promote resistance or to select resistant bacteria? Or is the transfer of resistance from already resistant bacteria following improper use of antibiotics much more important than the inputof antibiotic compounds themselves?

Identification of resistance and resistant bacteriain the environment

It has been known for quite some time that cultivable bacteria represent only a small portion of the vast number of bacteria present in the environment and that the number that can be enumerated is higher than can be cultivated. The known and cultivable environmental bacteria add up to only 5 – 10% of the total number assumed to be present in waste water and waste water treatment plants.[30] For soil, only 1% of the bacteria present are cultivable and can be identified by classical microbiological methods.[31] Enrichment, cultivation and differentiation of bacteria are the most important steps. Within the last few decades, techniques have been developed to improve the situation.Methods relying on biomarkers such

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

as chemotaxonomy or genetically based methods are now widely employed. Most of the recent work dealing with resistance in the environment found in the literature uses both approaches. Frequently, bacteria from a sample are cultivated first. Nutrients typical for known (pathogenic) bacteria or groups of bacteria with similar nutrient requirements are used for this purpose. That is, bacteria which are able to grow on the nutrient used are selected and then isolated. Other bacteria present are not monitored. Enterococci, Enterobactaericeae, *Streptomyces* spp., coliform bacteria, *Escherichia coli* and *Acinetobacter* spp., are quite typical (Table 2). In a second step, these isolates are classified either resistant by nature or they may have become resistant bythe use of antibiotics as well as in the environment by uptake of genetic material encoding resistance (e.g. in hospital effluent or manure) before they reach a sewage treatment plant or the soil. It is under discussion whether they may also become resistant in the sewage treatment plant or soil itself. The

Source	Resistant bacteria/resistance genes detected
Hospital effluent	*vanA*, *mecA*
Waste water	*vanA*, *ampC*, gentamicin resistance genes, *P. aeruginosa, Acinetobacter* spp., *E. coli*, *Campylobacter* spp., *Pseudomonas* spp., Enterococci
Sediments	*E. coli*
Surface water	*vanA*, *ampC*
Ground water	*Mycobacterium* spp., *Streptomyces* spp.

bacteria are able to survive or at least the genetic material is

according to their susceptibility to a certain antibiotic or a mix- ture of antibiotics to detect resistant bacteria or multi-resistant ones. For this purpose, antibiotic concentrations up to the milli- gram per litre range are used. These concentrations are orders of magnitude higher than the environmental ones. The bacteria identified as resistant are investigated further by monitoring

able 2. Examples of resistant bacteria and genetic material associated with resistance found in different environmental compartments (references see text)

transfer of resistant bacteria to humans may occur via water or food if plants are watered with surface water or sewage sludge, if manure is usedas a fertilizer or if resistant bacteria are present in meat.[19,22,23]A prerequisite for a direct transfer of resistance is that the

the genetic material encoding the resistance found. Resistance genes in samples can be detected if they are extractable from the environmental matrix and if they can be amplified. The link between the presence of antimicrobials and the favouring of resistant bacteria as well as the transfer of resistance at concen-trations as low as found for the antimicrobials in the environ-ment is not yet established. Preliminary results indicate that the transfer of resistance and the selection of resistant bacteriaare not favoured at antibiotic concentrations found in the environment.

Results of clinical trials are contradictionary.[5] At present, nodata are available for hospital effluents.

Municipal sewage and activated sludge of STPs

Antibiotics and disinfectants have been detected in sewage water (Table 1) at concentrations of a few micrograms per litre. Resistant bacteria are present in municipal sewage as well as in aeration tanks and the anaerobic digestion process of[36,37,42 – 50]STPs. Reinthaler and co-workers investigated the resist-

Sources of antibiotics and resistancein the environment

Hospital effluent

Antibiotics used in medicine for the treatment of infections and prophylactically are mainly released non-metabolized into the aquatic environment via waste water.

This can only have an effect if an active compound is present. Unused therapeutic drugs are sometimes disposed of down drains. Amongst other active compounds used, anti-biotics and disinfectants are present in the effluent of hospi-tals.[33] Ciprofloxacin, for example, was found in concentrationsof between 0.7 and 124.5 mg/L in hospital effluent and was assumed to be the main source of genotoxic effects measured with the umuC test in hospital effluent.[34] Ampicillin was found in concentrations of between 20 and 80

mg/L in the effluent of a large German hospital.[35] Concentrations as high as 5 mg/L were found for benzalkonium chloride, a quatern- ary ammonium compound, in the effluent of European hospi-tals.[9] The IC_{50}s for nitrifying bacteria have been found to be

ance of *E. coli* in three Austrian sewage treatment plants against 24 antibiotics by classical means, i.e. isolation, cultivation and resistance testing. *E. coli* were resistant to several antibiotics such as penicillins (ampicillin, piperacillin), cephalosporins (cefalothin, cefuroxime), quinolones (nalidixic acid), tetracycline and sulfamethoxazole/trimethoprim. Resistance rates were

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

highest for tetracycline (57%).[51]

Bacteria carrying the *vanA* gene have been detected in waste water in Europe as well as inthe USA.[26,48,49] Also the b-lactamase gene encoding AmpC was amplified by PCR in waste water.[36] Gentamicin resistance genes have been found in *Acinetobacter*, *Pseudomonas*, and Enterobacteriaceae and in phylogenetically distant bacteria, such as members of alpha and beta-proteobacteriain municipal sewage.[37] Tetracycline-resistant bacteria have also been identified in waste water[44,46] as well as resistant *P. aeruginosa.*[50]

Resistant and multi-resistant pathogenic bacteria such as *Acinetobacter* spp.[37,42] have been detected in waste water and STPs as well as transfer of resistance.[37,45,47] Exchange of genes encoding for resistance between pseudomonads and *E. coli* in sewage sludge[36] has been reported.

Up to 99% of *Campylobacter* spp. were eliminated from sew-

in the order of 1 – 2 mg/L.[9] Antibiotic concentrations calculated and measured in hospital effluents are in the same order of magnitude as the MICs for sensitive pathogenic bacteria.[35] Resistant bacteria may be selected or favoured by antibiotic substances in hospital effluent. For single compounds, concentrations calculated and measured in hospital effluents are below MIC_{50} values. They may reach this rangeor even exceed MIC_{50} values in hospital effluent if not onlysingle compounds but groups of compounds acting via age water by treatment in an STP. A similar elimination rate

was found for imipenem-resistant *P. aeruginosa*, ciprofloxacin- resistant *E. coli* and VRE.[53] Elimination rates were 95 – 99% for

E. coli, *Pseudomonas* spp. and *Enterococcus* spp. For resistant bacteria, the elimination rate was 93.5 – 100%. There was no difference between resistant and non-resistant bacteria. These results apply in winter and spring time. Jones found a seasonal pattern in the elimination rate of *Campylobacter*

in an STP.[52] Thus, studies should be conducted over a periodof at least 1 year and take into consideration the treatmentthe same mechanism are considered. Schwartz *et al.*[36] found

bacteria carrying *vanA* genes in hospital effluent. The *mecA* gene encoding resistance against methicillin in staphylococci was only found in bacteria in hospital waste water, but not in bacteria in municipal waste water.[37] Gentamicin resistance genes were found in *Acinetobacter*, *Pseudomonas* and Entero- bacteriaceae in hospital sewage.[37] An important source of the resistance in hospital effluents is also the input of bacteria already resistant because of the use of antibiotics in medicaltreatment. There have been reports that the widespread use of biocides such as triclosan and quaternary ammonium com- pounds used in hospitals and homes could select for anti-biotic-resistant bacteria.[38] Triclosan for example has been shown to select for low-level antibiotic resistance in *E. coli*[39] and high-level ciprofloxacin resistance in triclosan-sensitive *Pseudomonas aeruginosa* mutants.[40] Others have suggestedthat the introduction of cationic biocides into clinical practice has been associated with the selection of *S. aureus* strains containing *qacA/qacB* genes under clinical conditions applied.

The prevalence of bacteria with reduced susceptibility to benzalkonium chloride was elevated in the effluent of a muni-cipal STP.[54] A strong selecting effect of benzalkonium chloride was found in biodegradability testing.[55,56] In such tests, the benzalkonium chloride concentration was at least 100-fold higher than in hospital effluents or municipal sewage. Benzalkonium chloride and other quaternary ammonium compounds are sorbed by sewage sludge whereas pyridiniumsalts are not.[57]

Input of resistant bacteria into municipal sewage

It is often assumed that hospitals are the most important sourcefor the input of resistant bacteria into municipal waste water.The numbers of resistant bacteria found in the effluent of an intensive care unit (ICU) of a hospital with maximum medical service spectrum were in the same range as those found for

the influent of municipal STPs.[53] Taking into consideration that the dilution of hospital effluent by municipal sewage is normally more than 100-fold,[35] and that in municipal sewage without hos- pital effluent resistant bacteria are also present[53] because of the use of antibiotics at home, the conclusion is that it is probably the general community which is responsible for the main inputof resistant bacteria into STPs. Another point is that in Germany for example only one-quarter of the total consumption of anti- biotics can be attributed to hospitals.[35]

The input into and the elimination of resistant bacteria in three different sewage treatment plants (a municipal one and two located in the countryside) were monitored.[53] The STPs weredifferent with respect to hospitals and old peoples' homes pas- sing their waste water into the sewage as well as the technology used for water treatment and size (7500 – 600 000 population equivalents). No correlation between input, i.e. size and number of hospitals passing waste water and the STPs was found. Taking into consideration that hospital effluents contribute less than 1% of the total amount of municipal sewage, it is plausible that hospitals are not the main source of resistant bacteria in municipal sewage. The situation might be different for multi- resistant bacteria.

It is assumed that multi-resistant bacteria are selected mainlyin hospitals and passed into waste water.[50] The number of multi- resistant bacteria in sewage correlated with the size and the number of hospitals connected to an STP. The numbers and types of resistant bacteria found in the effluent of the ICU of the hospital offering maximum medical service showed that the number in the ICU effluent and in the influent of the STP are in the same range.[53] Reinthaler *et al.* found the highest resistance rates in an STP receiving municipal sewage

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

which also con-tained hospital effluent.[51] More detailed studies are necessary to assess the different sources of input of resistant bacteria into STPs. Separate treatment of hospital effluent to reduce the input of resistant bacteria into the aquatic environment is not recom- mendable from the present state of knowledge.

Surface water

Concentrations of antibiotics in surface waters, i.e. in rivers and lakes, are in the low microgram per litre range for most com- pounds (Table 1). In a study using ciprofloxacin and ceftazidime, it was concluded that the average concentrations of these com- pounds actually found in surface water will be clearly below concentrations able to change bacterial populations.[58] This was monitored by classical microbiological methods such as Gram- staining, aminopeptidase and catalase test as well as metabolic fingerprints using the Biolog system. However, some restrictions imposed by the methodology have to be taken into account inthis study.

Bacteria resistant to antibiotics are present in surface water.[36,43,59] Goni-Urizza *et al.*[60] found a correlation between resistant bacteria in rivers and urban water input. Schwartz *et al.*[36] were able to amplify *AmpC* b-lactamase gene sequences by PCR in surface water. Genetic transformations have for instance been reported for *E. coli.*[61] Antimicrobial resistance was also found in marine bacteria and bacteria living in estuaries.[62,63] Gentamicin resistance genes were found in *Acinetobacter*, *Pseudomonas*, Enterobacteriaceae, and in phylogenetically distant bacteria such as members of alpha and beta proteobac- teria in coastal water polluted with sewage water.[37]Ground water

Antibiotics are rarely found in ground water and if they do occur, they are far below the microgram per litre range (Table 1). Leaching from fields fertilized with animal slurry or passing through sediments into the ground water might be a source of antibiotics in ground water. However, the volume loadof antibacterial agents in ground water in rural areas with high concentrations of livestock has proved to be small.[8] Antibiotic-resistant *E. coli* have been found with a surprisingly high incidence in rural ground water.[64] The authors do not specu- late much on the origin of this resistance but manure runofffrom farms or leakage from septic tanks are clear possibilitiesfor the input of resistant bacteria into ground water as well as broken sewage pipes.

Drinking water

Antibiotic-resistant bacteria were detected in drinking water as early as the 1980s and later in the 1990s.[65,66] These authors found that resistant bacteria identified using classical microbiolo- gical methods, i.e. standard plate counting, occurred within the distribution network of drinking water supply systems. They concluded that the treatment of raw water and its subsequent dis- tribution selects for antibiotic-resistant bacteria. In agreement with these data, increased phenotypic resistance rates were also detected at the drinking water sampling points in the study by Schwartz *et al.*[36]

These authors also found *vanA* and *ampC* genes in heterotrophic bacteria in drinking water biofilms. Enterococci were not detected. The authors concluded that this is an indication of the possible resistance transfer to autochthonous bacteria.

Sediments

Resistant bacteria may be present because of the application of antibiotics in fish or because of selection by the antibiotics pre- sent in sediments. High antibiotic load in sediments in concen- trations sufficiently potent to inhibit the growth of bacteria were reported for aquaculture. The fact that the exposure is highly locally concentrated has to be considered critical. The substances used in fish farming can enter sediments directly from the water without undergoing any kind of purification process. Some investigations have demonstrated the presence and persistence of antibiotics applied extensively in fish farming in sediments beneath fish farms.[67–72]

Quinolones, sulphonamides and tetracyclines are sorbed byorganic matter. Therefore, they can accumulate. It is not yet known to what degree and under what circumstances the com- pounds are effective after sorption or whether they are released and may contribute to resistance. Antimicrobials may have qualitative and quantitative effects upon the resident microbial community of the sediments.[73] Bacteria resistant against these compounds have been detected in sediments. Anderson & Sandaa[72] and Samuelson *et al.*,[73] for example, isolated tetra- cycline-resistant Gram-negative bacteria from polluted and unpolluted marine sediments. An increased antibacterial resist- ance in sedimentary bacteria is often the most sensitive environ- mental indicator of past antibacterial use.

Soil

Antibiotics used for veterinary purposes or as growth promoters are excreted by the animals and end up in manure as well as in disinfectants used in livestock. The manure is applied to agricul- tural land as a fertilizer. If antibiotics are used in animal husban- dry, they pass from manure into the soil. Tetracyclines havebeen detected in soil in concentrations up to 0.3 mg/kg.[13] Tetra- cyclines, some sulphonamides, and quinolones are strongly sor- bed by soils.[13,14,74–78] Sulfachloropyridazine is not sorbed very much.[79] Compounds from different antibiotic groups such as vir- giniamycin, sarafloxacin, tetracycline, oxytetracycline, chlor- tetracycline and cyclosporine A were only slowly biodegraded in soils.[13,14,74,76,78,80] Tylosin disappeared soon after the application of manure.[14] Enrofloxacin was degraded in laboratory tests by a white-rot fungus[81] which may be present in soils but not in sew- age or sewage sludge. Thus, the antibiotics may accumulate and reach concentrations in the MIC range. If they are still effective after sorption, resistant bacteria may be selected by antibiotic substances due to their application in animals, their use as growth promoters and in soil.

Sengelov *et al.* found that some soil microbial populations are affected by applying manure containing antibiotics.[82] The

Indo-American Journal of Agricultural and Veterinary Sciences ISSN 2321–9602 www.iajavs.com

bac- terial composition returns to the original one some weeks after application of the manure. It was not investigated whether the change was due to antibiotics or to other constituents of the manure. Pang *et al.*[83] reported the acquisition of Gram-positive tetracycline resistance genes in *Mycobacterium* and *Strepto-myces* species in a laboratory test. This is one of the few reports of possible gene transfer between soil bacteria and human intes- tinal bacteria. Lorenz & Wackernagel also reported the exchange of genetic material between soil bacteria.[84] Amino acid vari- ations in the *GyrA* subunit of bacteria are potentially associated with natural resistance to fluoroquinolone antibiotics.[85] A high incidence of bacteria resistant to fluoroquinolones was found in soil isolates. The origin of this resistance is not clear as enro- floxacin is widely used in agriculture. Resistance could be aresult of the input of already resistant bacteria into soils follow- ing the application of the antibiotic pressure. It has been suggested that the development of new antibiotics should takeinto account the pattern of resistance in naturally occurring isolates.[85]

The spread of resistant bacteria and resistance genes by man- ure and sewage sludge used as fertilizer in agriculture or for land amendment has not been sufficiently investigated so far. Furthermore, antibiotics occur naturally in soils. Resistance against these antibiotics plays an important role in the population dynamics in soils. Antibiotics are a natural mechanism used by microbes in their natural ecology. The dynamics of soil micro- biology and antibiotics has played out for millions of years. The abundance of natural antibiotics seems to be low on average and to be restricted to the nearest surroundings, i.e. the micro- environment of the bacteria. Tetracycline, for example, is pro- duced by bacteria occurring naturally in soils. To the author'sbest knowledge, there are no findings of tetracycline in soilswhich had not been fertilized with manure containing tetra- cycline. In soils used as control when studying the input and fate of tetracycline in soils, tetracycline concentration was always below the detection limit.[13] This situation may be different in tropical soils as the bacteria producing tetracycline naturally occur in such soils in higher density.Most of the compounds used nowadays are synthetic or at least semi-synthetic. The speed of their depletion will probably be lower than that of naturally occurring compounds, but to the best of the author's knowledge, no information is available. Summarizing, at present there is insufficient information avail- able on the impact of antibiotics on the structural and functional changes of bacterial populations in the environment which would allow for the assessment of potential risks related, forinstance, to soil fertility. Input of resistant bacteria as well as of antibiotics could disturb the established well-balanced and important interdependencies.

Understanding the interaction of bacteriaand antimicrobials in the environment

Antibiotics and resistant bacteria are present in the environment. Antibiotics could favour resistant bacteria. As in other environ- ments, the significance of this process depends on the antibiotic concentration, its bioavailability and other constraints. This var- ies in water, sewage sludge, soils, and sediments, because the concentration of antibiotics, the physicochemical constraints and the mobility of bacteria as well as their resistance genes vary. The ability to take up DNA from the environment is widespread among natural isolates. To understand the interaction of antimi- crobial compounds and bacteria in the environment and for a sound risk assessment, the use of test systems and field studies is crucial.

A broad range of unknown bacterial species is present in the environment.[30,31] When checking the toxicity of the antibiotics and the size of the biomass in the test guidelines of the Inter- national Standards Organization (ISO) or the Organization of Economic Cooperation and Development (OECD), it often appears that the bacteria are unaffected in the presence of anti- biotics. But what does 'unaffected' mean? All bacteria present? And if so, are all the organisms affected in the same way? Doesit mean that resistant bacteria are selected or that others or the same ones produce enzymes to degrade a compound which isnot toxic to them? In other words, one has to take account of microbial ecology.[25,86] It is known that antibiotics at subinhibi- tory concentrations can have an impact on cell functions and change the expression of virulence factors or the transfer of anti- biotic resistance.[87,88] In *in vitro* experiments, it was shown that gentamicin at a concentration of 100 mg/L improved the transfer rate in staphylococci. Other such as macrolides, quinolones or vancomycin did not have such an impact.[32]

When a complex mixture of bacteria is used, in some cases increased activity can be observed.[89,90] Do the results depend on the test system and the inocula used? What is the role of (natu- ral) resistance against bacteria?[86] In other words, what do we mean by resistance? Even from a medical standpoint, this ques- tion is not unimportant.[24–26,29,91] Accordingly, several definitions of resistance can be found in the literature. Is it a matter of the ability of bacteria to ignore the toxic properties of the antibiotic, or do they cope with it despite its adverse effects?

Regarding the MICs for pathogenic bacteria the individual susceptibility of every species is well known and documented.[92] In the environment the vast majority of individual bacteria are unknown and so are their MICs. Furthermore, even when resist- ance is encoded in the genes, it is sometimes not expressed.Why does resistance occur in the environment and what is its ecological significance? Are the environmental bacteria resistant by nature or is the resistance achieved through exposure to anti- microbials or other xenobiotics such as heavy metals? The nature and significance of resistance and the impact of the use of antibacterials on natural habitats such as soil are not yet understood.

What is the role of the concentration and type of antibacterial compound?[24–26] In some instances, the substance concentrationis reduced by sorption. It is unclear whether elimination by sorp- tion is an irreversible process. Some of the compounds may be deactivated or reduced in their activity by sorption. Antibiotics present in soil and sediment can lose their antimicrobial activityas a result of binding to sediment particles or complex formation with ions, which and

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

for a few substances, this has been demon- strated. However, contradictory results concerning the reduced antibacterial activity and bioavailability due to binding or com- plex formation have been reported for one and the same sub- stance.[66,67,93,94] The reason could be the differences in sediment composition, which seem to play a key role in the effects of sub- stances upon the resident population.

Future developments

The volume of waste water will drop in future as water-saving measures are introduced and the growing quantity of antibiotics used will probably increase antibiotic concentration in urban waste water. This will depend on demographic developmentsand changes in standards of living. According to the presentlevel of our knowledge, concentrations necessary to favour the spread of resistance will not be reached. We have to understand this issue much better, not only with regard to resistance butalso in relation to the functions and services which bacteria in the environment offer us. Frequently, it is unknown whether resistance is natural or acquired, because of the lack of identifi- cation methods regarding environmental bacteria and a lack of investigation of environmental samples from areas where no antibiotics are present due to human use.

Conclusions and work to be done

There are enough gaps in the existing body of scientific evidence to make it a risky response to increasing public concern to deny that there is a problem with respect to resistance in the environ- ment. On the basis of our present knowledge, an increased direct impact of antibiotics on bacteria in the aquatic environment andin soils is questionable. The input of bacteria already resistant fol-lowing the use of antibiotics in human and veterinary medicine seems to be the more important source of resistant bacteria in STPs and the environment. What has been learned so far is that it is critical to prevent the selection of resistant strains in the first place both in human and veterinary medicine. The opportunities and routes whereby this may be achieved are different in both fields. According to present knowledge, the prudent use of anti- biotics in all areas seems to be the key to coping successfullywith resistance in the environment. Therefore, the proper use of antibiotics and disinfectants in human medicine and livestockfarming will significantly reduce the risk for the general public and for the environment. This not only includes limiting the dura-tion of the selective pressure by reducing the treatment period

and the continuous use of subtherapeutic concentrations. It also includes controlling the dissemination of antibiotics being used.

The use of antibiotics as growth promoters in fattening ani- mals will diminish in Europe because of the complete ban onuse of these substances by 2005.

The use of antibiotics and disinfectants in veterinary medicine for prophylactic and therapeutic reasons must be monitored to make sure that there are no excessive compensatory effects. Using proper hygienic procedures and housing for animals can successfully compensate for the abandonment of growth promo- ters as shown by examples from Sweden and Denmark. Experi- ence gained in Norway shows that the use of vaccines can significantly reduce the use of antimicrobials in fish farming. It isalso important to use the possibilities to reduce properly the consumption in medicine.[15,21] Among other things, this will resultin reducing the input of both resistant bacteria and antibioticsinto the environment. These measures will also reduce costs. Therefore, the issue of resistance as a whole should be incorporated into the curricula of doctors and pharmacists. Pub- lic awareness has to be raised.

Knowledge about how antibiotic resistance arises, how resist- ant strains and resistance genes spread in nature and the signifi- cance of this for humans and nature is far from complete. There are not enough data available to draw a final conclusion espec- ially with respect to the input of already resistant bacteria into the environment. This topic needs further consideration and investigation. An assessment of the different pathways also hasto take into consideration the ingestion of resistant bacteria with food (e.g. poultry, pork).

The importance of the different sources of resistance found in the environment, i.e. the presence of antibiotics in the environ- ment and the importance of resistant bacteria resulting from the use of antibiotics in the various fields has to be measured. For this purpose, it is important to make a more detailed assessment of the significance of culture-dependent and laboratory-based methods in relation to conditions found in the environment. This also applies to the concentrations of antibiotics applied in the identification of resistant bacteria in laboratory testing compared to the concentrations of antibiotics in the environment. Thres- holds favouring selection and transfer of resistance genes bet- ween different species under environmental conditions should be established. For this purpose, the significance of the availability and activity of the antibiotics in the environment, i.e. the extent and importance of their sorption to sludge, particles in surface water, sediment, and soil should be determined. The significance of the semi-synthetic and synthetic compounds used compared to naturally occurring compounds needs to be known for a final risk assessment. The conditions and time scales under whichantibiotics and resistance are lost in the environment are also of importance in relation to the input of antibiotics and resistant bacteria.

References

1. Kümmerer, K. (2003). The significance of antibiotics in the environment. *Journal of Antimicrobial Chemotherapy* 52, 5-7.

2. Golet, E. M., Alder, A. C., Hartmann, A. *et al.* (2001). Trace determination of fluoroquinolone antibacterial agents in urban waste- water by solid-phase extraction and liquid chromatography with fluorescence detection. *Analytical Chemistry* 73, 3632-8.

3. Zuccato, E., Calamari, D., Natangelo, M. *et al.* (2000). Presenceof therapeutic drugs in the environment. *Lancet* 335, 1789 -90.

4. Richardson, M. L. & Bowron, J. M. (1985). The fate of pharmaceutical chemicals in the aquatic environment. *Journal of Pharmacy and Pharmacology* 37, 1-12.

Indo-American Journal of Agricultural and Veterinary Sciences

ISSN 2321–9602 www.iajavs.com

5. Gilbert, P. & McBain, A. J. (2003). Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. *Clinical Microbiology Reviews* 16, 189-208.

6. Kolpin, D., Furlong, E. T., Meyer, M. T. *et al.* (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A National Reconnaissance. *Environmental Science and Technology* 36, 1202-11.

7. Sacher, F., Lange, F. T., Brauch, H. J. *et al.* (2001). Pharmaceuticals in groundwaters analytical methods and results of a monitoring program in Baden-Wurttemberg, Germany. *Journal of Chromatography A* 938, 199-210.

8. Hirsch, R., Ternes, T., Haberer, K. *et al.* (1999). Occurrence of antibiotics in the aquatic environment. *Science of the Total Environ- ment* 225, 109-18.

9. Kü mmerer, K. (2004). *Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks*. 2nd edn. Springer, Berlin.

10. Mölstad, S., Lundborg, C. S., Karlsson, A. K. *et al.* (2002). Antibiotic prescription rates vary markedly between 13 European countries. *Scandinavian Journal of Infectious Diseases* 34, 366-71.

11. Al-Ahmad, A., Daschner, F. D. & Kümmerer, K. (1999). Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. *Archives of Environmental Contamination and Toxicology* 37, 158-63.

12. Kü mmerer, K., Al-Ahmad, A. & Mersch-Sundermann, V. (2000). Biodegradability of some antibiotics, elimination of their genotoxicity and affection of waste water bacteria in a simple test. *Chemosphere* 40, 701-10.

13. Hamscher, G., Sczesny, S., Höper, H. *et al.* (2002). Determi- nation of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Analytical Chemistry* 74, 1509-18.

14. De Liguroro, M., Cipin, V., Capolongo, F. *et al.* (2003). Use of oxytetracyline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. *Chemosphere* 52, 203-12.

15. House of Lords (UK) (1998). *House of Lords Select Committee on Science and Technology. 7th Report*. The Stationery Office, London.

16. Witte, W., Klare, I. & Werner, G. (1999). Selective pressure by antibiotics as feed additives. *Infection* 27, 35-7.

17. Aarestrup, F. M., Seyfarth, A. M., Embrog, H.-D. *et al.* (2001). Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in faecal enter- ococci from food animals in Denmark. *Antimicrobial Agents and Chemotherapy* 45, 2054-9.

18. Khachatourians, G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *CanadianMedical Association Journal* 159, 1129-36.

19. Salyers, A. A. (2002). An overview of the genetic basis of antibiotic resistance in bacteria and its implications for agriculture. *Animal Biotechnology* 13, 1-5.

20. Wise, R., Hart, T., Cars, O. *et al.* (1998). Antimicrobial resistance is a major threat to public health. *British Medical Journal* 317, 609 -10.Harrison, P. F. & Lederberg, J. Eds (1998). *Antimicrobial Resistance, Issues and Options*. National Academic Press, Washing- ton, DC, USA.

21. Perretin, V., Schwarz, F., Cresta, L. *et al.* (1997). Antibiotic resistance spread in food. *Nature* 389, 801-2.

22. Teubner, M. (1999). Spread of antibiotic resistance with food- borne pathogens. *Cellular and Molecular Life Sciences* 56, 755-63.

23. Martinez, J. L. (2000). Mutation frequencies and antibiotic resistance. Minireview. *Antimicrobial Agents and Chemotherapy* 44, 1771-7.

24. Björkman, J., Nagaev, I., Berg, O. G. *et al.* (2000). Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* 287, 1479-82.

25. Davison, J. (1999). Genetic exchange between bacteria in the environment. *Plasmid* 42, 73-91.

26. Smith, P., Hiney, M. & Samuelson, O. (1994). Bacterial resistance to antimicrobial agents used in fish farming; a critical evaluation of method and meaning. *Annual Review of Fish Diseases* 4,273-313.

27. Westen, D. P. (1996). Environmental considerations in the useof antibacterial drugs in aquaculture. In *Aquaculture and Water Resource Management*. (Baird, D. J., Beveridge, M. C. M., Kelly, L. A.,*et al.* Eds). Blackwell Science, Oxford.

28. Murray, B. E. (1997). Antibiotic resistance. *Advances in Internal Medicine* 42, 339-67.

29. Hiraishi, A. (1998). Respiratory quinone profiles as tools for identifying different bacterial populations in activated sludge. *Journal ofGeneral and Applied Microbiology* 34, 39-56.

30. Zelles, L. (1996). Fatty acid patterns of microbial phospholi- pids and lipopolysaccharides. In *Methods in Soil Biology*. (Schinner, F., Öhlinger, R., Kandeler, E., *et al.,* Eds). Springer Verlag, Heidelberg.

31. Ohlsen, K., Werner, G., Ternes, T., *et al.* (2001). *Untersuchun- gen zum Einfluss von Antibiotikaeinträgen in die Umwelt auf die zunehmende Verbreitung von Antibiotikaresistenzen* (Investigations into the impact of antibiotics' input into the environment on the increasing spread of antibiotic resistance). Grant No. 29761127. German Environmental Agency, Berlin.

32. Kümmerer, K. (2001). Drugs in the environment: emission of drugs, diagnostic aids, and disinfectants into wastewater by hospitalsin relation to other sources–a review. *Chemosphere* 45, 957-69.

33. Hartmann, A., Alder, A. C., Koller, T. *et al.* (1998). Identification of fluoroquinolone antibiotics as the main source of umuC genotoxicity in native hospital water. *Environmental Toxicology and Chemistry* 17, 377-82.

34. Kü mmerer, K. & Henninger, A. (2004). Promoting resistance by the emission of antibiotics from hospitals and households into effluents. *European Journal of Clinical Microbiology and Infection* 9, 1203-14.

35. Schwartz, T., Kohnen, T., Jansen, B. *et al.* (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology*43, 325-35.

36. Heuer, H., Krogerrecklenfort, E., Wellington, E. M. H. *et al.* (2002). Gentamicin resistance genes in environmental bacteria: prevalence and transfer. *FEMS Microbiology Ecology* 42, 289-302.

37. Russell, A. D. (2000). Do biocides select for antibiotic resistance? *Journal of Pharmacy and Pharmacology* 52, 227-33.

38. McMurry, L. M., Oethinger, M. & Levy, S. B. (1998). Over- expression of marA, soxS or acrAB produces resistance to triclosan in laboratory and clinical strains of *Escherichia coli*. *FEMS Microbiology Letters* 166, 305-9.

39. Chuanchen, R., Beilich, K., Hoang, T. T. *et al.* (2001). Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects *nfxB* mutants over- expressing MexCD-OprJ. *Antimicrobial Agents and Chemotherapy* 45, 428-32.

Indo-American Journal of Agricultural and Veterinary Sciences ISSN 2321–9602 www.iajavs.com

40. Paulsen, I. T., Brwon, H. & Skurray, R. A. (1998). Characterisation of the earliest known *Staphylococcus aureus* plasmid encoding a multidrug efflux system. *Journal of Bacteriology* 180, 3477-9.

41. Feuerpfeil, I., López-Pila, J., Schmidt, R. *et al.* (1999). Antibiotic resistance and antibiotics in the environment. *Bundesgesundheitsblatt- Gesundheitsforschung-Gesundheitsschutz* 42, 37-50.

42. Guardabassi, L., Dalsgaard, A. & Olsen, J. E. (1999). Phenotypic characterization and antibiotic resistance of *Acinetobacter* spp. isolatedfrom aquatic sources. *Journal of Applied Microbiology* 87, 659-67.

43. Guardabassi, L., Dijkshoorn, L., Olsen, J. E. *et al.* (2000). Distribution of tetracycline resistance determinants A to E and transfer *in vitro* of tetracycline resistance in clinical and aquatic *Acinetobacter* strains. *Journal of Medical Microbiology* 49, 929-36.

44. Pü hler, A. (1999). Horizontaler Gentransfer von Antibiotikaresis- tenzgenen — Diskussion und Erkenntnisse. *Nachrichten Chemie Technik Labor* 47, 1088-92.

45. Guillaume, G., Verbrugge, D., Chasseru-Libotte, M.-L. *et al.* (2000). PCR typing of tetracycline resistance determinants (Tet A to E) in Salmonella enterica serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiology Ecology* 32, 77-85.

46. Marcinek, H., Wirth, R., Muscholl-Silberhorn, A. *et al.* (1998). *Enterococcus faecalis* gene transfer under natural conditions in municipal sewage treatment plants. *Applied and Environmental Microbiology* 64, 626-32.

47. Harwood, V., Brownell, M., Perusek, W. *et al.* (2001). Vancomycin-resistant *Enterococcus* spp. isolated from wastewater andchicken faeces in the United States. *Applied and Environmental Microbiology* 67, 4930-3.

48. Iversen, A., Kuhn, I., Franklin, A. *et al.* (2002). High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology* 68, 2838–42.

49. Römling, U., Wingender, J., Müller, H. *et al.* (1994). A major *Pseudomonas aeruginosa* clone common to patients and aquatic habitats. *Applied and Environmental Microbiology* 60, 1734 –8.

50. Reinthaler, F. F., Posch, J., Feierl, G. *et al.* (2003). Antibiotic resistance of *E. coli* in sewage and sludge. *Water Research* 37, 1685-90.

51. Jones, K. (2001). Campylobacter in water, sewage and the environment. *Journal of Applied Microbiology* 90, 68-79.

52. Wiethan, J., Unger, J., Brunswik-Titze, A. *et al.* (2001). Occurrence and reduction of antibiotic resistant (pathogenic) bacteriain municipal sewage treatment plants. In *Proc. International Water Association 2nd World Water Congress,* Berlin. 15 -19th October. Abstract P0009, p 227. International Water Association, Berlin.

53. Hingst, V., Klippel, K. M. & Sonntag, H. G. (1995). Untersuchun- gen zur Epidemiologie mikrobieller Biozidresistenzen (Investigations ofthe epidemiology of microbial resistance to biocides). *Zentralblatt für Hygiene* 197, 232-51.

54. Kümmerer, K., Al-Ahmad, A. & Henninger, A. (2002). Use of chemotaxonomy to study the influence of benzalkonium chloride on bacterial populations in biodegradation testing. *Acta hydrochimica et hydrobiologica* 30, 171-8.

55. Al-Ahmad, A., Wiedmann-Al-Ahmad, A., Schön, G. *et al.* (2000). Role of *Acinetobacter* for biodegradability of quaternary ammonium compounds. *Bulletin of Environmental Contamination and Toxicology* 64, 764-70.

56. Merino, F., Rubio, S. & Perez-Bendito, D. (2003). Mixed aggregate-based acid-induced cloud-point extraction and ion-trap liquidchromatography-mass spectrometry for the determination of cationic surfactants in sewage sludge. *Journal of Chromatography A* 998, 143-54.

57. Wiethan, J., Al-Ahmad, A., Henninger, A. *et al.* (2000). Simulation des Selektionsdrucks der Antibiotika Ciprofloxacin und Ceftazidim in Oberflächengewässern mittels klassischer Methoden (Simulation of the selection pressure of ciprofloxacin and ceftazidime insurface water). *Vom Wasser* 95, 107-18.Muela, A., Pocino, I., Arana, J. *et al.* (1994). Effects of growth phase and parental cell survival in river water on plasmid transferbetween *Escherichia coli* strains. *Applied and Environmental Micro- biology* 60, 4273–8.

58. Goni-Urriza, M., Capdepuy, M., Arpin, C. *et al.* (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp. *Applied and Environmental Microbiology* 66, 125-32.

59. Baur, B., Hanselmann, K., Schlimme, W. *et al.* (1996). Genetic transformation in freshwater: *Escherichia coli* is able to develop natural competence. *Applied and Environmental Microbiology* 62, 3673-8.

60. Cohen, E. & Colwell, R. (1986). Coincident plasmids and antimicrobial resistance in marine bacteria isolated from polluted and unpolluted Atlantic Ocean samples. *Applied and Environmental Microbiology* 51, 1285-92.

61. Barkay, T., Kroer, N., Rasmussen, L. D. *et al.* (1995). Conjugal gene transfer at natural population densities in a microcosmos simulating an estuarine environment. *FEMS Microbiology Ecology* 16, 43-54.

62. McKeon, D. M., Calabrese, J. P. & Bissonnette, G. K. (1995). Antibiotic resistant Gram-negative bacteria in rural groundwatersupplies. *Water Research* 29, 1902-8.

63. Armstrong, J., Shigeno, D., Calomiris, J. *et al.* (1981). Antibiotic- resistant bacteria in drinking water. *Applied and Environmental Microbiology* 42, 277-83.

64. Kolwzan, B., Traczewska, T. & Pawlaczyk-Szipilowa, M. (1991). Examination of resistance of bacteria isolated from drinking water to antibacterial agents. *Environmental Protection Engineering* 17, 53-60.

65. Nygaard, K., Lunestad, B. T., Hektoern, H. *et al.* (1992). Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteriafrom marine sediments. *Aquaculture* 104, 21-36.

66. Coyne, R., Hiney, M., O'Conner, B. *et al.* (1994). Concentration and persistence of oxytetracycline in sediments under a marine salmonfarm. *Aquaculture* 123, 31–42.

67. Jacobsen, B. & Berglind, L. (1988). Persistence of oxytetracy- cline in sediments from fish farms. *Aquaculture* 70, 365-70.

68. Hektoen, H., Berge, J. A., Hormazabal, V. *et al.* (1995). Persistence of antibacterial agents in marine sediments. *Aquaculture* 133, 175-84.

69. Björklund, H., Råbergh, C. M. I. & Bylund, G. (1991). Residues of oxytetracycline in wild fish and sediments from fish farms.*Aquaculture* 86, 359-67.

70. Andersen, S. R. & Sandaa, R. A. (1994). Distribution of tetracycline resistance determinants among Gram-negative bacteriaisolated from polluted and unpolluted marine sediments. *Applied and Environmental Microbiology* 60, 908-12.

71. Samuelson, O. B., Torsvik, V. & Ervik, A. (1992). Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Science of the Total Environment* 114, 25-36.

72. Weerasinghe, C. A. & Towner, D. (1997). Aerobic biodegrada- tion of virginiamycin in soil. *Environmental Toxicology and Chemistry* 16, 1873-6.

73. Marengo, J. R., Kok, R. A., Velagaleti, R. *et al.* (1997).

Indo-American Journal of Agricultural and Veterinary Sciences ISSN 2321–9602 www.iajavs.com

Aerobic degradation of ^{14}C-sarafloxacin hydrochloride in soil. *Environmental Toxicology and Chemistry* 16, 462-71.

74. Gavalchin, J. & Katz, S. E. (1994). The persistence of faecal- borne antibiotics in soil. *Journal of the Association of Official Analytical Chemists International* 77, 481-5.

75. Tolls, J. (2001). Sorption of veterinary pharmaceuticals in soils:a review. *Environmental Science and Technology* 35, 3397-406.

76. Golet, E. M., Strehler, A., Alder, A. C. *et al.* (2002). Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followedby solid-phase extraction. *Analytical Chemistry* 74, 5455-62.

77. Boxall, A. B., Blackwell, P., Cavallo, R. *et al.* (2003). The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters* 131, 19-28.

78. Hübener, B., Dornberger, K., Zielke, R. *et al.* (1992). Microbial degradation of cyclosporin A. *Umweltwissenschaften Schadstof- forschung-Zeitschrift für Umweltchemie und Ö kotoxikologie* 4, 227-30.

79. Martens, R., Wetzstein, H. G., Zadrazil, F. *et al.* (1996). Degradation of the fluoroquinolone enrofloxacin by wood-rotting fungi. *Applied and Environmental Microbiology* 62, 4206-9.

80. Sengelov, G., Agerso, Y., Halling-Sørensen, B. *et al.* (2001). Bacterial antibiotic resistance levels in farmland as a result of treatmentwith pig slurry. *Environment International* 28, 587-95.

81. Pang, Y., Brown, B. A., Steingrube, B. A. *et al.* (1994). Acquisition of Gram-positive tetracycline resistance genes in *Mycobac-terium* and *Streptomyces* species. *Antimicrobial Agents and Che- motherapy* 38, 1408-12.

82. Lorenz, M. G. & Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiological Reviews* 58, 563-602.

83. Waters, B. & Davies, J. (1997). Amino acid variation in the *GyrA* subunit of bacteria potentially associated with natural resistance to fluoroquinolone antibiotics. *Antimicrobial Agents and Chemotherapy* 41, 2766-9.

84. Ingerslev, F., Unger, J., Wiethan, J., *et al.* (2001). Testing biodegradability and bacterial toxicity of pharmaceuticals in standar-dized methods–is there a conceptual problem? *Proc. 11th Annual Meeting of SETAC Europe*. Abstract M/EH 064, p114. SETAC Europe, Madrid.

95. 07-12.

85.

86.

87. Salyers, A. A., Shoemaker, N. B., Stevens, A. M. *et al.* (1995). Conjugative transposons: an unusual and diverse set of integratedgene transfer elements. *Microbiological Reviews* 59, 579-90.

88. Ohlsen, K., Ziebuhr, W., Koller, K. *et al.* (1998). Effects of sub inhibitory concentrations of antibiotics on alpha-toxin (hla) gene expression of methicillin-sensitive and methicillin-resistant *Staphylococ- cus aureus* isolates. *Antimicrobial Agents and Chemotherapy* 42, 2817-23.

89. Halling-Sørensen, B. (2000). Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. *Archives of Environmental Contamination and Toxicology* 40, 451-60.

90. Alexy, R., Kümpel, T., Dörner, M., *et al.* (2001). Effects of antibiotics against environmental bacteria studied with simple tests.In *Proc. 11th Annual Meeting of SETAC Europe*. Abstract M/EH 045, p110. SETAC Europe, Madrid.

91. Cooper, A. J., Shoemaker, N. B. & Salyers, A. A. (1996). The erythromycin resistance gene from the Bacteroides conjugative transposon TcrEmr 7853 is nearly identical to ermG from *Bacillussphaericus*. *Antimicrobial Agents and Chemotherapy* 40, 506-8.

92. Lorian, V. (1999). *Antibiotics in Laboratory Medicine*, 3rd edn (Lorian, V., Ed.). Williams & Wilkins, Baltimore, MD, USA.

93. Lunestad, B. T. & Goksøyr, J. (1990). Reduction in the antibacterial effect of oxytetracycline in sea water by complex formationwith magnesium and calcium. *Diseases of Aquatic Organisms* 9, 67-72.

94. Hansen, P. K., Lunestad, B. T. & Samuelsen, O. B. (1992). Effects of oxytetracycline, oxolinic acid and flumequine on bacteria in an artificial marine fish farm sediment. *Canadian Journal of Micro- biology* 38, 13