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ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

EUR/ICP/CWS 199g53
ENGLISH ONLY
UNEDITED

BACTERIA RESISTANT TO ANTIBIOTICS IN WATER AND WASTE WATER AND THEIR PUBLIC HEALTH IMPLICATIONS

by

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1989

EUR/HFA Target 20

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Water pollution

By 1990, all people of the Region should have adequate supplies of safe drinking-water, and by the year 1995 pollution of rivers, lakes and seas should no longer pose a threat to human health.

WHO Collaborating Centre for Environmental Problems and Health Aspects,
Research Institute for Hygiene and Microbiology (FHM),
Bad Elster, German Democratic Republic in cooperation with the Institute of Experimental Epidemiology, Wernigerode, German Democratic Republic

Bacteria resistant to antibiotics in water and waste water and their public health implications

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1. Introduction

In this century, both the introduction of public water supplies and the application of antibiotics have significantly contributed to the control of infectious diseases in developed, industrialized countries. The medical and agricultural usage of antibiotics solved numerous problems(6) but the difficulties in controlling bacterial infectious diseases reappear by the evolution of microbial resistance to antibiotics.

The findings of similar antibiotic resistance determinants in different microorganisms and ecosystems have revealed on a worldwide scale a previously unappreciated extent of microbial genetic exchange (8). The molecular basis of this spread relates to the presence of antibiotic resistance genes on plasmids and transposons(8). Prevalence and spread of antibiotic resistance was forced by selective pressure of antibiotics upon a gene pool characterized by a remarkable flexibility (19). Both, transposons and plasmids, are vehicles of interspecies and intergenus exchange of antibiotic resistance genes (horizontal evolution) by conjugation, transduction and possibly by transformation in the environment. Presumably, other genes are being exchanged in a similar way, but their phenotypes usually do not permit an easy identification (18). The extensive usage of antibiotics lead to a further enhancement of the dissemination of antibiotic resistance determinants in the environment. Therefore, efforts in policies, laws and regulations pertaining to antibiotics are indicated (13).

Sewage, sewage sludge and contaminated surface waters play a significant role in the transmission of antibiotic resistant bacteria (R^+ bacteria) in the environment. Therefore, surveillance of the circulation of the type of plasmids and their host bacteria in sewage and surface water is to be regarded as an essential part of a surveillance programme (11). Obviously, it is an almost impossible task to define the relative contribution of water to the distribution of R^+ bacteria in the environment.

This report characterizes (i) the occurrence of antibiotic resistant coliforms in the fecal flora of healthy persons, (ii) the occurrence and circulation of drug resistant plasmids among fecal strains in sewage and waste waters, (iii) the behaviour of antibiotic resistant coliforms in conventional sewage treatment processes, (iv) the key position of sewage to study the dissemination and ecology of R plasmids and (v) the extent of R plasmid transfer in sewage and contaminated surface waters. The results are restricted to the coliform group well known both as a reservoir of R plasmids in the enterobacteriaceae and as indicator organisms of water quality standards. Recommendations are given concerning the inclusion of water studies into a nation-wide drug surveillance programme.

2. Antibiotic resistant coliforms in the fecal flora of healthy persons

Gut flora of humans and animals represents a reservoir of antibiotic resistant microorganisms. In a recent study of the fecal flora of healthy individuals in the Boston area over 60 % of fecal samples contained 10 % of total aerobic gramnegative flora resistant to one of seven antibiotics tested (7). In 20 - 40 % of these samples the organisms were resistant to ampicillin, tetracycline, streptomycin or kanamycin (8). Fecal samples of healthy persons from different continents contained from 81.5 to 100 % tetracycline resistant E. coli strains (10), indicating that human feaces are a large reservoir of resistance genes and resistant bacteria all over the world. We studied fecal samples of 77 healthy persons (14). Total coliform counts were found to range from 100.000 - 10.000.000.000/g faeces (figure 1). In 62 (80.5 %) out of 77 fecal samples resistant coliforms were found. Tetracycline, chloramphenicol and kanamycin resistant coliforms were detected in 58, 39, and 34 out of these samples, respectively (table 1).

In total, about 50 % of fecal samples of healthy individuals contained $\geq$1.000 chloramphenicol and kanamycin resistant coliforms/g faeces and in about 75 % of all fecal samples the contents of tetracycline resistant coliforms ranged from 1.000 to 10.000.000.000/g faeces (figure 1). In 11 (14.3 %), 3 (3.9 %), and 2 (2.6 %) of the fecal samples 10 % of total coliforms were resistant to tetracycline, chloramphenicol and kanamycin, respectively (table 1).

Among the 915 E. coli strains isolated 35 different resistance patterns were identified. The most frequently resistance patterns identified are summarized in table 2.

It is interesting to note the striking difference between the percentages of resistance patterns identified in this study and the resistance patterns encoded by plasmids from fecal coliforms of sewage in a ten year survey study (see 4., table 8).

It is suggested that fecal sampling will not give representative information about the overall drug resistance situation unless a large number of samples can be examined. From this point of view it seems much more useful to survey drug resistance among fecal coliforms in sewage or waste water which might reflect in a more general way, the overall antibiotic resistance development in a given area. Nevertheless, as summarized in table 3, the antibiotic resistant coliforms in faeces indicate the antibiotic selection pressure in a given population (22).

3. Antibiotic resistant coliforms in sewage and surface waters

3.1 Antibiotic resistant coliforms in hospital sewage

Several studies outline the rapid selection of antibiotic resistant bacteria in humans and animals under selective pressure exercised by antibiotics (5,6,8). This has particularly shown in hospitals where in routine work antibiotics are used on a large scale and quantity. Therefore, the selection of multiple antibiotic resistant bacteria under such conditions must be expected. Additionally, the selection of multiple drug resistance by distinct antibiotics such as gentamicin and trimethoprim is to be taken into consideration. A selection of multiple resistance by single antibiotic usage is frequently found (4). This is outlined in table 4. These data underline that antibiotics such as chloramphenicol, kanamycin, gentamicin or trimethoprim select mainly for multiple drug resistance phenotypes among bacteria.

The monitoring of hospital sewages for drug resistant coliforms showed a clear correlation of resistance quotients for the distinct antibiotics and the drug consumption in the respective hospitals (table 5).

For the purpose of comparison similar results were gained from examinations of sewage effluents of an antibiotica manufacturing plant and from effluents discharges of animal farms where antibiotics are used as food additives (figure 2).

3.2. Antibiotic resistant coliforms in municipal waste waters and river waters

As pointed out, human and animal faeces are the major sources of antibiotic resistant bacteria in sewage and surface waters. Long-term studies of sewage purification systems showed at average 100 - 10.000 antibiotic resistant coliforms/ml of raw sewage as indicated for tetracycline, chloramphenicol and kanamycin (3,4,14,15).

Figure 3 illustrates an example in detail. The investigated municipal sewage plant with a sewage flow of about 30.000 m^3/day includes primary treatment (grit separating tank, sedimentation tank), biological purification with a completely mixed activated sludge process and final sedimentation. Samples were collected from raw sewage after the grit separating tank, from the activated sludge tank effluent and from the sewage plant effluent. The raw sewage samples contained at average colony counts of 24.000.000/ml total coliforms of 800.000/ml, 1.300 tetracycline resistant coliforms/ml, 900 chloramphenicol resistant coliforms/ml, 1000 kanamycin resistant coliforms/ml and 65 gentamicin resistant coliforms/ml. Primary treatment and the activated sludge

process have not shown any reduction of the microbial pollution (figure 3). Only the final sedimentation eliminated between 70 and 90 % of the antibiotic resistant coliforms (figure 3). Nevertheless, the sewage plant effluent discharges about 10^{13} antibiotic resistant coliforms per day into the receiving water. The final effluents of all studied sewage treatment plants with activated sludge process discharged antibiotic resistant coliforms as revealed for tetracycline, kanamycin and chloramphenicol in the range from 10 to 1.000 per ml (4,14).

The high fluctuations in the bacterial loading over three log's (figure 3) are to be noted. Therefore, it is difficult to obtain exact trends in the development of the general resistance situation from sewage samples only. Nevertheless, sewage investigations represent basic information about the resistance situation in the area. The results summarized in table 6 underline that most of the clinically significant resistance patterns could be isolated from municipal sewage samples, too.

A few resistance types are confined to a narrow host range (table 6). Investigations of municipal sewage and waste waters of additional plants demonstrated similar results. The resistance quotients for tetracycline, chloramphenicol, and kanamycin ranged up to 7 %, whereas the quotients for antibiotics such as gentamicin and trimethoprim were established with not more than 0.15 % and 1.7 %, respectively. The very high resistance values for ampicillin are caused by species specific ampicillin resistance of Klebsiella and Enterobacter.

In low-rate oxydation pond systems stable elimination rates of antibiotic resistant coliforms of 95 % were obtained (data not shown). From above it can be concluded that the prevalence of R^+ bacteria in surface waters depends on the sewage pollution. Alltogether, resistance quotients of antibiotic resistant coliforms similar to the results achieved in sewage investigations were found in river water samples (table 7).

4. Plasmids in antibiotic resistant coliforms from sewage and surface waters

Antibiotic resistant bacteria can be further characterized by the plasmids which encode the corresponding antibiotica resistance functions. Plasmid typing gives more detailed information on the character and the prevalence of antibiotica resistance among bacteria common in humans, animals, and the environment.

Antibiotica resistance plasmids can be isolated from their natural host bacteria what is due to their transferability to plasmid-free E. coli test strains. Nearly 80 % of the naturally resistant coliform isolates were shown to carry auto-transferable (conjugative) plasmids. The remaining 20 % can be characterized to harbour non-transferable plasmids, but rarely chromosomally integrated plasmids or transposons. The plasmids isolated from wild-type strains into E. coli test strains can be subtyped by means of a set of genetic and molecular methods. (16) This kind of plasmid typing is based on the nature of plasmid species and enables a clear-cut identification and distinction of plasmids irrespective of their bacterial, geographical, or temporal origin.

Therefore, a direct comparison between the spread of plasmids among bacteria in human populations and among bacteria of environmental populations can be made with interlockings between the corresponding populations being analyzed. A large number of plasmid species (plasmid incompatibility groups) encoding a broad range of different drug resistance phenotypes were detected in such bacteria from sewage and surface waters (tables 8 - 11). In a ten-year-survey-study of a municipal sewage plant E. coli bacteria carrying multiple drug resistance plasmids were isolated regularly. The prevalence of plasmids, the type of plasmid species, and the drug resistance patterns encoded by the isolated plasmids (including the mode of resistance mechanism such as aminoglycosid-acetyl-transferase AAC-3, or β-lactamase TEM or SH1 etc.) are summarized in tables 8 - 11.

As expected, the vast majority of plasmids identified belong to the very common incompatibility groups such as IncFI, IncFII, IncI1, IncH1 etc. Irrespective of some distinct resistant patterns represented by only one plasmid species such as the drug resistance pattern CmSmSuTcApKmGm encoded by IncM plasmids exclusively, the antibiotic resistance patterns identified were mainly due to a broad range of different plasmid species. Moreover, identical resistance patterns could be related to different plasmid species, and vice versa, identical plasmid species can code for different resistance pattern.

In conclusion, the broad range of plasmid species encoding a broad spectrum of different drug resistance phenotypes which were detected in bacteria from sewage and surface waters is nearly identical to that of the isolates from the human and animal gut flora, or from pathogenic bacteria. Wild strains often harbour several plasmids (plasmid profile). In this manner definite strains can be traced in the environment by the help of plasmid profiles and distinct plasmid-encoded markers as recently shown for gentamicin encoding plasmids in sewage treatment processes (15).

5. Key position of water in surveying antibiotic resistance of bacteria

The close correlation between the extent of antibiotica usage and the incidence of antibiotic resistant bacteria in the intestinal flora of man and animals has been well documented in the past. As pointed out, the gut flora of each individual is different in respect to the occurrence of antibiotic resistant bacteria and the surveillance of drug resistance including the plasmids might be hampered unless a large number of individuals would be examined. Therefore, the surveillance of drug resistance and the corresponding plasmids from bacteria in the sewage and surface waters is an indication for the overall circulation of plasmids and the resistance development. In this context it reflects the current selection pressure created by antibiotica use in a given area. This will become particularly obvious, if the respective antibiotics are newly introduced for therapeutic or nutritive purposes as occurred in the GDR within the last decade for trimethoprim (1975), gentamicin (1979), amikacin (1985), cefotiam (1985) and nourseothricin (1982). The quantitative correlations between the rise of antibiotic resistant bacteria and the consumption of trimethoprim and gentamicin are demonstrated in figures 4 and 5, respectively.

By analysing the plasmid encoding resistance to newly introduced antibiotics a more clear-cut picture is achieved. Before the introduction of the new drugs for therapeutic or nutritive purposes the corresponding plasmids were not to be detected in enteric bacteria, even if isolated by selection on drug containing media. However 2 - 4 years later (depending on the quantity of drug use) several plasmids responsible for the corresponding drug resistance functions emerged more and more frequently.

By means of determination of plasmid types (tables 9 - 11), particular plasmids with the corresponding drug resistance determinants could be traced first among bacteria from sewage and surface waters, but later in bacteria from clinical specimens, too. This is particularly remarkable for the gentamicin resistance encoding plasmids of the IncOF type (pIE585, pIE613), which were not reported from other countries. The clonal nature of these IncOF isolates from environmental as well as clinical sources was additionally underlined by DNA fingerprinting. Therefore, it is rather unlikely, that these IncOF plasmids are imported from other countries.

Particular is the fact that their spread among bacteria from sewage and surface waters occurs earlier than among enteric bacteria from clinical origins what might reflect the natural way of drug resistance development.

However, large variations of the incidence of the IncOF plasmids, such as pIE584, among bacteria from different hospitals located in different geographic regions were found. In some hospitals located in different units, this type is rather frequently observed, in other ones and among municipal populations a rather low-level incidence was noted (table 12).

When following up the trimethoprim resistance development it was detected that plasmids (pIE415, see table 10) encoding DHFR were different from the plasmids occuring in other countries. Transposons from these isolated plasmids (designated Tn1824) were shown to be identical to Tn7, isolated in England (17). However, the import of plasmids can be noted at first by monitoring sewage and surface waters for drug resistant coliforms, too. This was demonstrated with observations on the spread of IncM plasmid pIE459 type (table 9) among fecal coliforms in the GDR, at first detected among coliforms in sewage and two years later from pathogenic bacteria such as E. coli from UTI ward, meningitis/neonatal E. coli or enteropathogenic E. coli. Such an influx is also very likely for the amikacin resistance plasmid pIE866 encoding an AAC-6 acetyltransferase enzyme for complex detoxification of gentamicin, amikacin, kanamycin, neomycin, tobramycin or sisomycin (21).

These results support the hypothesis established somewhere else that sewage and surface waters can be regarded as a pool for plasmid- carrying bacteria. Therefore, investigations on R^+ bacteria from sewage and surface waters provide an early information on the development of plasmid-induced resistance in pathogenic bacteria.

Plasmids encoding gentamicin and trimethoprim resistance in Gram negative bacteria have been surveyed world-wide, but streptothricin resistance plasmids were unknown up to now (table 11). Such plasmids did not occur in enteric bacteria prior to the application of nourseothricins in animal husbandry. Originally, the development of this kind of resistance was also detected among bacteria from slurry and surface water, later on, the same plasmid species were found in pathogenic E. coli strains, too (table 13).

6. Behaviour of antibiotic resistant bacteria in sewage and surface waters

R plasmids represent one of the most important vehicles of foreign DNA sequences in genetic engineering experiments. Therefore, R plasmid-bearing bacteria simulate to some extent the fate of genetically modified organisms in the environment. Long-term survival of R plasmid- bearing organisms and

gene transfer processes are considered to be important factors in the maintenance of resistance genes in the environment even without any selective pressure. R plasmids were found to influence the survival and the growth rate of their bacterial hosts only to a moderate extent.

Some data demonstrate a better survival of R plasmid-bearing strains in the environment (2). Unknown properties of R plasmids are suggested to increase the "fitness" of bacterial hosts by enhancing their survival under natural conditions. Even non essential eucariotic DNA sequences, when inserted into plasmid DNA, have low effects on the survival of the bacterial host in soil, and the maintenance of the vector (1). Another ecological aspect concerns the gene transfer to indigenous flora under natural conditions. Plasmid and gene exchanges may occur readily in nature, but neither the extent nor the sources are well known (8).

In model experiments we have failed to observe any R plasmid transfer of bacterial densities below 100.000 cells/ml (figure 6). It was proved that temperature is an additional factor influencing the plasmid transfer rate (figure 7). The close relationship of R plasmid transfer to bacterial biomass and temperature confirms that gene exchange may occur first of all in sewage sludge, wastewater, contaminated surface waters, and sediments.

To quantify these results under simulated natural conditions we used dialysis membrane bags filled with 10 ml of sterilized waste water or contaminated river water containing 100.000.000 cells/ml of test strains. These dialysis membrane bags were incubated at room temperature in untreated waste water or river water, respectively. The control experiments in nutrient broth resulted in transfer frequencies in the range from 0,1 - 0,0000001 per donor of all the strains involved (figure 8). In waste water more than 80 % of mating pairs transferred antibiotic resistance in the range of 0,000005 /donor cell or less. Similar results were obtained from river water experiments (figure 8). Mating experiments with sterilized (20 min boiled) sewage samples demonstrated the R plasmid transfer in sewage without antibiotic pressure (figures 9 and 10). Transfer frequencies about one log lower were found at 10°C in comparison with to experiments at room temperature (figure 10).

To conduct in situ R plasmid transfer studies we have constructed a plasmid free E. coli recipient strain with chromosomal resistance to rifampicin and nalidixic acid (14) resistance pattern, which is unlikely to be found originally in water habitats. This strain picked up different R plasmids from the indigenuous flora of waste water with transfer frequencies, of 0,000001/recipient cell. However, a stable maintenance of these R plasmids in the new host was not observed (table 14).

One may conclude, that gene transfer occurs under natural conditions obviously at a low rate, but the recipient will fail to reach a detectable population size in natural waters, if the new genes do not confer any selective advantage to the host.

All studied sewage treatment plants did not show any detectable selective advantage for the survival of R^+ bacteria as demonstrated by Grabow et al (2).

On the other hand, a rapid dissemination of R plasmids under selective pressure has been shown. These findings have led to the concept of "epidemic plasmids" (8).

Furthermore, when R plasmids have once evolved, they may exhibit a high degree of environmental stability. Plasmids well adapted to their hosts are hardly to be lost in the absence of selective pressure. In fact, loss appears to be related to dilution with susceptible strains in the environment and not to any selective disadvantage confered by R plasmids (8). Gene transfer at a low rate by transduction and transformation is discribed in the aquatic environments too (18).

It is interesting to note, that immediately after mixing the donor and recipient bacteria in sewage and plating on selective agar transconjugants can be seen (figure 11). Comparable results were found by direct plating of donor and recipient bacteria on selective agar plates ("plate conjugation") (14) (data not shown). Increasing donor and recipient bacteria populations and vice versa enhanced the "plate conjugation" phenomenon (figure 11).

7. Natural dissemination of drug resistant bacteria and their plasmids

The natural dissemination of antibiotic resistant bacterial strains is not fully understood up to now. Foods, foodstuff, soil, and water are known as a potential source. Resistant bacterial strains from food, food-stuff and water are capable of to passing trough the gastrointestinal tract and of being discharged to sewage by human and animal faeces (figure 12 A).

The efficiency of the sewage treatment can be assessed by the results as followed. Obviously, primary treatment of sewage is ineffective in the elimination of antibiotic resistant bacteria. In conventional biological sewage treatment plants approximately a one log reduction of R^+ bacteria can be found. A further reduction of the microbial pollution of about 90 % by tertiary sewage treatment is possible. Stable elimination rates of more than 95 % have been reproduced in low rate oxydation pond systems, which are

to be recommended in rural areas and as a further treatment step of biological sewage plant effluents. Completely conventional sewage treatment systems are able to provide a reduction of microbial pollution of 2 to 3 log's. Nevertheless, it must be emphasized, that with effluents of conventional sewage treatment systems large amounts of R^+ bacteria are discharged into the receiving waters. With the increasing intensity of water and sludge usage in agriculture the question arose, whether it could contribute to further dissemination (figure 12 B, C). Modern molecular techniques are necessary to label the bacteria and plasmids involved in such studies. Moreover, clear-cut modells are required for following up the main routes of such distributions from the water and sludge back to animals and man. Nevertheless, in one of our studies (14) it was shown that there is a relative contribution of irrigation to the environmental dissemination of antibiotic resistant bacteria.

The investigated sewage plant with a sewage flow of about 7.000m^3/day includes a primary treatment (grit separating tank, sedimentation tank), only. About 1.200 ha of pastures and forage fields are irrigated by sewage plant effluent water during the vegetation period from april to october. Forage is directly fed to cattles without pasteurization. In the sewage effluent median values of colony counts of 8.000.000/ml and total coliforms of 1.100.000/ml were estimated. 0.3 %, 0.5 %, 1.6 %, 17.6 %, 1.8 % and 0.03 % of the total coliforms showed resistance to the antibiotics tetracycline, chloramphenicol, kanamycin, ampicillin, trimethoprim and gentamicin, respectively. After a fortnight waiting period 40 % of the foliage samples contained antibiotic resistant coliforms. In some cases more than 1.000 resistant coliforms/g fresh weight were detected. A variety of resistance patterns was observed among resistant coliforms (table 15). The percentage of multiple resistant strains of coliforms slightly decreased during the waiting period (data not shown). It can be concluded that land utilization of sewage sludge, waste water and contaminated surface waters play a key role in the environmental dissemination of resistance genes. Although there is no direct influence on human health, an increasing human health risk might arise from the fact that an increase in environmental contamination with R^+ bacteria enhances the possibility of the rapid selection of multiple resistant strains by plasmid transfer. However, the main point of this problem remains open for discussion because appropriate studies to trace R^+ bacteria distribution from water to land are not available up to now.

Another problem concerns the significance of plasmid transfer in the spread of drug resistant bacteria. This question was researched by analysing the streptothricin resistance development. As mentioned, the antibiotic agent nourseothricin, a mixture of streptothricin F and D, has been used on a commercial scale since 1982. At that time extensive

studies to detect natural streptothricin resistance among fecal coliforms demonstrated that plasmid encoded resistance was not detectable. However, about one year later, streptothricin resistance plasmids, encoding a novel streptothricin/acetyltransferase, emerged. These plasmids occurred in bacteria which were at first isolated from the gut flora of pigs fed with the drug (table 13). Later, identical plasmids occurred in the fecal flora of the personnel, healthy human beings which were not in contact with agriculture. They occurred also in E. coli isolated from infections of the urinary tract and last not least in outbreaks of shigellosis. Table 13 summarizes the data concerning the streptothricin resistance plasmids pIE636, pIE638 and pIE663. The genetic and molecular identity of the corresponding plasmid isolates from the different sources was confirmed by DNA fingerprintings. Additionally, the complex typing of the different host bacteria by biotyping, serotyping and plasmid profiles showed clearly, that independent of the distribution of the bacteria some sort of clonal plasmid spread really took place independently of the distribution of the bacteria and that particulary this kind of plasmid circulation contributes to the overall streptothricin resistance development. Such plasmid spread is very likely to occur in sewage and surface waters as described in chapter 6.

8. Assessment of human health risks

Sewage and surface waters can be regarded as a monitoring system for incidence and spread of antibiotic resistant bacteria and their plasmids in a distinct country or area. Such a surveillance programme reflects the overall antibiotica resistance development but can not include conclusions about the health risks posed especially by water. Generally, the human intestinal tract shows a high load with antibiotic resistant bacteria and the food chain is considered to be the main source for resistant gut flora of healthy human beings (9) (table 13). The daily uptake of few antibiotic resistant bacteria by drinking water is rather unlikely to have any hazard to human health. A similar conclusion for bathing in R^+ bacteria contaminated surface waters was documented (12).

However, there is at least one exception with regard to patients with a damaged intestinal flora, because the attachment and colonization of antibiotic resistant bacteria is facilitated by the mucosa exhibiting such conditions.

The question which conditions drug resistant bacteria might "flow back" to human beings remains a matter of speculation and might be restricted to the problem of waste water and sludge application in agriculture. Therefore, a "flow back" of antibiotica resistance from sludge, sewage and contaminated surface waters via the plants and animals has to be taken into account to an increasing extent. It is suggested that a "flow back" of antibiotica resistance might occur and

if detailed studies were performed to underline this suggestion the human health risk can be better assessed as it is possible today.

9. Conclusions and recommendations

1. Human faeces represent a large reservoir of multiple resistant E. coli strains. About 75 % of healthy individuals are carriers of tetracycline resistant coliforms in the range from 1.000 to 10.000.000.000 per g feaces. 50 % of the fecal samples of healthy individuals contained $\geq$ 1.000 chloramphenicol and kanamycin resistant coliforms per g faeces, respectively.

2. In municipal raw sewage at average 100 - 10.000 antibiotic resistant coliforms/ml were found to be resistant to the older agents tetracycline, chloramphenicol and kanamycin. In hospital sewage concentration of about on log higher values can be assumed. The prevalence of antibiotic resistant coliforms in hospital sewage reflects the therapeutic regime of the clinics.

3. The primary treatment of sewage is ineffective in the reduction of R^+ bacteria. In conventional biological sewage treatment plants approximately a one log reduction of R^+ bacteria can be found. Stable elimination rates of more than 95 % can be reproduced in low rate oxydation pond systems. Similar results were found for pathogenic bacteria (Salmonella, Campylobacter).

4. Sewage and surface waters are loaded with R^+ bacteria via faeces and semisolid animal wastes (slurry). They represent a pool of all plasmid types circulating.

5. Sewage investigations offer a reliable additional possibility to be used for drug surveillance programmes. Sewage investigations should be involved in nationwide surveillance programmes to control the efficiency of policies, laws and regulations pertaining antibiotics. But regular testing of water systems should not be performed. Sewage investigations should be restricted to gain basic information for an assessment of the resistance situation in a certain area. Additional testing of hospital sewages should be focussed on the emergence and dissemination of genes encoding resistance to new generations of antibiotics introduced. It may be useful to study the development of cross resistance among members of the various groups of antimicrobial agents and to analyse the possibility of the appearance of novel resistance mechanisms.

6. Studies of appropriate sewage samples are recommended for countries without any drug surveillance programme to anti-

biotic resistance in order to obtain basic information on the level of the dissemination of antibiotic resistance determinants to both, older antimicrobial agents and newer ones.

7. Sewage represents a natural medium in which R plasmid transfer can be observed under certain physical, chemical and biological conditions. However, the transfer rates are low and the recipients will fail to reach a detectable population size in natural waters without selective pressure. Nevertheless, collecting ponds or poorly operating sewage treatment plants enhance the intensity of the spread of infectious drug resistance in the environment. On the other hand, well operating sewage treatment plants reduce the load of plasmid-containing bacteria in surface waters.

8. R plasmids represent important vehicles of foreign DNA in genetic engineering experiments. From this point of view several R plasmids demonstrate to some extent the fate of genetically modified organisms in the environment. Therefore, in further research R plasmids and their easy identification offer a qualified model for studying the gene exchange under natural conditions.

9. R^+ bacteria may contaminate food, drinking water, fodder, milk etc. The origins therefore may be sewage, slurry or surface waters. The circulation of hygienically relevant bacteria can be traced by the help of plasmid profiles and definite plasmid-encoded markers in the environment. These modern molecular tools enable the hygienist to give a clear-cut evidence of the routes of bacterial pollutions in the environment.

10. The intake of a few R^+ bacteria via drinking water or bathing water does not imply health hazard. The suggested route of R^+ bacteria or their plasmids from the sewage and surface water via agricultural application to plants, animals and back to human population via food chain has to be proved by modern technologies of bacteria labelling and has to be elucidated in the near future.

11. Nevertheless, the spread of R^+ bacteria in the environment should be limited by reducing their transmission via sewage polluted water. Therefore, the complete treatment of sewage, including primary, secondary and tertiary treatment steps, must be enforced. Low rate oxydation pond systems are recommended for sewage treatment in rural areas and as a possibility of a further treatment step of biological sewage treatment plant effluents. The complete sewage treatment technology improves the protection of the population against this hazard, but does not completely prevent the dissemination of resistant bacterial strains

in the environment. Particularly the agricultural application of water polluted with sewage represents a main source of the contamination of forage, vegetables, and fruits, which has to be restricted more and more. Sludge disposal must always be accompanied by certain hygienic precautions.

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TABLE 1

The prevalence (%) of antibiotic resistant coliforms in the fecal samples of healthy individuals

percentage (%) of resistant coliforms on total coliforms in fecal samples	Resistant to tetracycline	chloramphe-nicol	kanamycin
10 %	14.3 (11)[1)]	3.9 (3)	2.6 (2)
1.1 - 10	20.8 (16)	3.9 (3)	3.9 (3)
0.1 - 1.0	18.2 (14)	11.7 (3)	10.4 (8)
0.01 - 0.1	15.6 (12)	11.7 (9)	14.3 (11)
0.01	6.5 (5)	9.5 (15)	13.0 (10)
percentage (%) of fecal samples containing antibiotic resistant coliforms	75.4 (58)	50.7 (39)	44.2 (34)

1) number of fecal samples

TABLE 2

The most frequent resistance patterns of coliforms[1] isolated from fecal samples of healthy individuals

No.	number	resistance pattern[2]	%
1	217	Tc	23.7
2	123	TcSmCmSuApKm	13.4
3	106	TcSmCmSu Km	11.6
4	77	TcSmCmSu	8.4
5	63	Tc Su	6.9
6	58	TcSm Su Km	6.3
7	52	TcSm Su	5.7
8	37	SmCmSuApKmTp	4.0
9	28	TcSm	3.1
10	24	TcSmCm ApKm	2.6

[1] Coliforms were typed: 98.0 % E. coli, 1.0 % Klebsiella pneumoniae, 0.9 % Citrobacter freundii and 0.1 % Enterobacter agglomerans.

[2] Abbreviations used for antibiotics in this paper:
Tc, tetracycline
Sm, streptomycin
Cm, chloramphenicol
Su, sulfonamide
Ap, ampicillin
Km, kanamycin
Tp, trimethoprim
Gm, gentamicin
Ak, amikacin
Si, sisomycin
Tb, tobramycin
Nm, neomycin
Sp, spectomycin
St, streptothricin

TABLE 3

The occurrence of antibiotica resistant fecal E. coli in fecal samples of healthy human populations in areas with various pressure of antibiotics.

population	percentage (%) of fecal samples positive with resistant E. coli	resistance quotient (%)
workers in antibiotica manufacturing	100	100
workers applying antibiotics	80	0.1 - 10
family members	80	0.1
human beings without contacts	50	0.0001 - 1
hospitalized childrens	100	10 - 100
hospitalized adults	80	0.1 - 1
children 6 weeks after hospitalization	60	0.001 - 1

TABLE 4

Co-selection[1)] among coliforms by using different drugs

number of strains tested	selective drug	co-selected resistance in % Tp	Km	Tc	Cm	Gm	Ap
100	Tp	100	35	63	43	1	71
100	Km	11	100	68	47	1	51
100	Tc	6	14	100	15	2	38
100	Cm	24	32	83	100	2	89
100	Gm	32	53	85	95	100	87
100	Ap	1	11	47	22	-	100

1) The sewage samples were plated on endoagar supplemented with the respective antibiotics. Pay attention to the high incidence of co-selection on Gm-plates.

TABLE 5

Resistance quotients (%) among coliforms from hospital sewage in comparison to drug consumption

Source	resistance quotients in % to the respective antibiotics						mainly therapeutical used antibiotics
	Cm	Tc	Km	Tp	Ap	Gm	
hospital A	1.2	32.4	0.3	3.6	6.2	n.d.[1)]	Tc, Ap, Doxycyclin, Pn
hospital B	9.9	12.9	2.6	7.9	27.2	0.65	CmTp, Ap, Gm
hospital C	14.9	20.8	0.1	1.9	34.2	n.d.	Cm, Tc, Tp Ap

[1)] n.d. = non detectable

TABLE 6

Occurrence of the most frequent resistance patterns (%) of coliforms isolated from sewage samples of the investigated sewage plant

resistance types isolated from endoagar supplemented with tetracycline in %		resistance types isolated from endoagar supplemented with chloramphenicol in %		resistance types isolated from endoagar supplemented with kanamycin in %		resistance types isolated from endoagar supplemented with gentamicin in %	
Tc	29.3	TcSmCmSuApKm	28.4	TcSmCmSuApKm	30.9	TcSmSuApGm[1)]	30.1
TcSmSu	10.8	TcSmCmSuAp	18.9	TcSmSuKm	13.0	SmCmSuApTpGm[2)]	15.6
TcSu	9.6	TcSmCmSu	8.1	TcSmCmSuKm	12.4	TcSmCmSuApKmGm	6.7
TcSm	8.3	TcSm	5.4	TcSmCmSuApKmTp	7.4	TcSmCmSuApGm	5.8
TcSmCmSuApKm	7.0	TcSmCmSuApKmTp	5.4	TcSmSuApKm	6.2	TcSmCmSuApKmTpGm	4.2
TcSmSuAp	5.1	TcCmSuAp	3.4	SmCmSuApKm	5.6	TcSmSuApTpGm	4.0
TcSmAp	3.2	SmCmSuAp	3.4	SmSuApKm	4.9	SmSuApGm	3.0
TcSmCmSuAp	3.2	TcSmCmSuKm	3.4	SmCmSuApKmTp	2.5	SmCmSuApKmGm	2.8
TcSmCmSuApKmTp	3.2	CmSuApTp	2.7	TcKm	1.8	TcSmSuApKmGm	2.8
TcSmSuKm	2.6	SmCmSuApKm	2.7	SmKm	1.2	ApGm[3)]	2.6
number of strains tested	157		148		162		429
E. coli	93.0 %		83.1 %		81.5 %		31.0 %
Klebsiella	4.4 %		6.8 %		11.8 %		13.1 %
Enterobacter	2.6 %		4.8 %		2.5 %		43.3 %
Citrobacter	-		5.3 %		4.2 %		12.6 %

[1)] among 120 strains tested 1 E. coli strain was detected only

[2)] 64 (95.5 %) of the strains of this resistance type, were typed as E. coli

[3)] this resistance type was exclusively detected in Citrobacter freundii

TABLE 7

The prevalence (%) of antibiotica resistant coliforms in river waters

river system	prevalence % of coliforms resistant to tetracycline	chloramphenicol	kanamycin	gentamicin
Elster[1]	0.5-5.1	8.0-2.1	1.2-2.7	0.15-0.3
Elbe[1)]	1.6-7.3	0.4-1.1	0.3-1.8	0.01-0.3
Saale[2)]	0.9	0.9	6.2	0.04
Schwarza[2)]	1.2	1.1	0.6	0.06
Goeltzsch[2)]	0.5	0.8	0.4	0.01
Warnow[2)]	2.2	0.8	1.1	0.25

[1)] arithmetic mean values of different sampling sites
[2)] arithmetic mean values of one sampling site

TABLE 8

Prevalence of drug resistance plasmids in E. coli isolated from a municipal sewage plant surveyed between 1978 and 1988

prevalence in %	drug resistance patterns	plasmid group	size in Md	incidence in %
12.3	CmSmSuTcAp(TEM)	FII	65	73.7
		FI	70	15.8
		B	0	5.3
		K	55	2.6
		M	55	2.6
11.7	Tc	J1	70	88.2
		FII	65	8.8
		FI	60	3.0
11.0	CmSmSuTcApKm(APH-3)	FI	70	50.0
		FII	65	40.0
		H1	120	10.0
4.4	CmSmSuTcAp(TEM)KmTp	FI	75	44.5
		FII	65	33.3
		H1	110	7.4
		J1	70	7.4
		M	60	3.7
		C	110	3.7
3.4	TcSmSuAp(SH-1)	J1	70	51.7
		Z	65	41.4
		N	35	6.9
3.4	CmSmSuTcKm(APH-3)	FI	70	35.8
		HI	120	32.1
		H3	130	32.1
2.9	TcAp	J1	70	80.0
		FII	65	20.0
2.8	TcSmSuApTp	J1	70	67.6
		F1	70	19.4
		Z	60	6.5
		M	60	6.5
2.7	CmSmSuTcApKmGm(AAC-3)	M	60	100.0
2.6	TcCmAp	K	60	40.0
		M	55	30.0
		U	30	30.0
2.1	CmSmSuTcTpAp	FI	65	46.9

		FII	60	43.8
		M	60	6.2
		H1	110	3.1
2.0	CmSmSuTpApGm(ANT-2)	OF	55	40.0
		D	55	30.0
		C	105	30.0
1.8	CmSmSuTcApKmTpGm(AAC-3)	H2	150	30.0
		H3	130	30.0
		M	60	20.0
		C	110	20.0
1.7	TpSmSuAp	J1	70	80.0
		FI	65	20.0
1.6	CmSmSuTcApGm(ANT)	H3	20	50.0
		FII	65	50.0

TABLE 9

Gentamicin resistance plasmids originated in enteric bacteria from surface waters

No.	year	origin, species and towns (GDR)	size (Md)	plasmid group	drug resistance patterns
pIE459	1979	E. coli, Berlin	65	M	GmSiCmSmSuTcApKm (AAC-3/I)
pIE507	1980	C. freundii, Berlin	105	C	GmSiKmTbSmSuAp (ANT-2")
pIE613	1980	C. freundii, Berlin	55	OF	GmSiTcCmSmSuApTp (ACC-3/II)
pIE567	1980	K. pneumoniae, Magdeburg	30	W	GmSiKmTbSu(ANT-2")
pIE706	1980	E. coli, Wernigerode	35	N	GmSiKmTbSmSu (ANT-2")
pIE584	1981	P. mirabilis, Schwerin	55	OF	GmSiTbCmSmSuApTp (AAC-3/II)
pIE583	1982	K. pneumoniae, Magdeburg	110	C	GmSiTbCmSmSuApTp (AAC-3/II)
pIE510	1982	K. pneumoniae, Dresden	110	C	GmSi(Tb)CmSmSuTp (AAC-3/II)
pIE577	1982	E. cloacae,	130	H4	GmSiCmSmSuAp(AAC-3)
pIE579	1982	C. freundii, Gotha	70	I1	GmSiCmSmSuTcApKmNm (AAC-3/I)
pIE705	1982	E. coli, Dresden	65	FII	GmSiKmTbCmSmSuTcAp (ANT-2)"
pIE713	1983	S. marcescens, Dresden	150	H2	GmSiKmTbCmSmSu (ANT-2)
pIE715	1983	E. coli, Wernigerode	65	B	GmSiCmSmSuTc (AAC-3/I)
pIE866	1987	E. coli, Berlin	140	H2	GmSiKmNmTbSmCmApHg Kt(AAC-6)

TABLE 10

Trimethoprim resistance plasmids originated in enteric bacteria from surface waters

No.	year	origin, species and towns (GDR)	size (Md)	plasmid group	drug resistance patterns
pIE415	1977	E. coli, Wernigerode	75	I5	TpSmSpSuKmNm
pIE465	1978	C. freundii, Dresden	35	N	TpSmSpSu
pIE716	1978	E. coli, Berlin	25	W1	TpSmSpSu
pIE421	1978	P. stuartii, Schierke	110	C	TpSmSpSu
pIE491	1978	E. coli, Wernigerode	30	U	TpSmSpSu
pIE422	1979	C. freundii, Wernigerode	65	FII	TpSmSpSu
pIE612	1979	E. coli, Berlin	60	FI	TpSmSpSu
pIE458	1979	E. coli, Halberstadt	60	M	TpSmSp
pIE417	1980	E. coli, Magdeburg	60	K	TpSmSpSuAp
pIE572	1980	E. coli, Schwerin	120	H1	TpSmSpSuCm
pIE574	1980	E. coli, Osterwieck	160	H2	TpSmSpSuCm
pIE614	1982	E. coli, Wernigerode	65	Z	TpSmSpAp
pIE717	1982	E. cloacae, Berlin	70	V	TpSmSpSuAp

TABLE 11

Streptothricin resistance plasmids originated in E. coli from slurry

No.	year	origin (slurry)	size (Md)	plasmid group	drug resistance patterns
pIE638	1981	animal farm Lo.	28	W3	StSmSp
pIE636	1981	animal farm Bu.	50	I2	StSmSp
pIE657	1982	animal farm Th.	140	H2	StSmSpCmTcKmNm TpSu
pIE660	1982	animal farm Be.	35	N	StSmSp
pIE662	1982	animal farm Th.	70	I1	StSmSp
pIE663	1983	animal farm Th.	35	X	StSmSp
pIE667	1983	animal farm Th.	65	FII	StSmSp
pIE	1984	animal farm A.	50	OF	StSmSp
pIE7271	1985	animal farm J.	55	M	StSmSp
pIE873	1986	animal farm Th.	105	H1	StSmSp

TABLE 12

Incidence of the IncOF plasmid pIE584 (CmSmSuTpApGm) among the fecal flora of hospitalized persons and sewage

origin	percentage of total drug resistance
<u>hospital A:</u>	
HAI (ICU)[1)]	53
hospital sewage	30
municipal sewage	0.01
<u>hospital B:</u>	
HAI (UTI ward)[2)]	0.5
hospital sewage	1.0
municipal sewage	0.01

1) hospital-equired infections in an intensive care unit
2) hospital-equired infections in an urinary tract infection ward

TABLE 13

Occurrence of the streptothricin resistance plasmids[1)]

source of E. coli	occurrence of respective streptothricin resistance plasmids				
	1982	1983	1984	1985	1986
animals (pigs)	-	W3,I2	I2,W3,X	I2,W3,X	I2,W3,X
slurry	-	W3,I2	I2,W3,X	I2,W3,X	I2,W3,X
farmers	-		I2,X	X,W3,I2	X,W3,I2
family members	-		I2,X	X,W3	X,W3,I2
sick animals	-		I2,W3	I2,X	I2
urban population	-		-	I2,X	X,W3
patients with UTI[2)]			-	X,I2	X,I2
patients with shigellosis	-		-	-	X

1) pIE636 (IncI2, 55 MD, Tn1825), pIE638 (IncW3, 25 MD, Tn1826) and pIE663 (IncX, 35 MD, Tn1826) in E. coli strains with different clonal relatedness

2) infections of the urinary tract.

TABLE 14

Uptake of R plasmids by the plasmid free recipient strain E. coli K12 Nal^r Rif^r in waste water. The recipient strain E. coli K12 Nal^r Rif^r was added to 2 l of raw sewage to a final concentration of about 10^7 - 10^8 colonies/ml

recipient strains isolated from raw	resistance pattern			R plasmid picked up (Md)		transfer frequency
	first determination	after one week	after two weeks	first determination	after two weeks	
1	TcAp	susceptible	susceptible	110	110 (week)	
2	TcSmSuApKm	Su	Su	60	-	10^{-5}/recipient
3	TcSuAp	Su	Su	60	-	

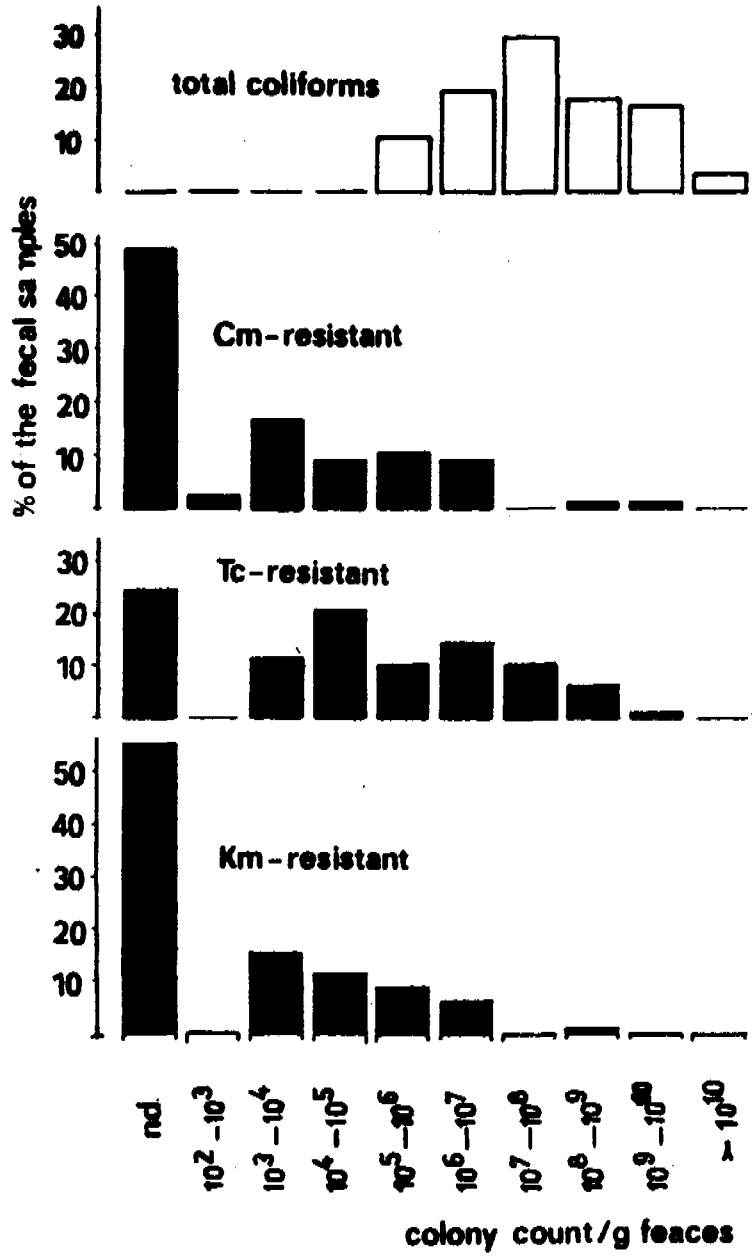
Tc, tetracycline; Ap, ampicillin; Sm, streptomycin; Su, sulfonamide; Km, kanamycin; Nal^r, nalidixicacid resistant; Rif^r, rifampicin resistant

TABLE 15

The occurrence of antibiotic resistant coliforms in forage after a fortnights waiting period subsequent to of the irrigation with primarily treated sewage effluents

resistance type	prevalence (%) of E. coli	Klebsiella	Enterobacter	Citrobacter
Tc	18.1		18.1	
Ap		10.0		
Km		5.0	9.1	
ApKm		55.0		
ApTc		5.0		
CmSm			9.1	
KmTc				50.0
SmTc	9.1			
ApCmSm			9.1	
CmSmTc	9.1	15.0	18.1	
ApSmTc				50.0
KmSmTc	9.1			
ApCmKmGm		5.0		
ApKmSmTc	9.1			
CmKmSmT	18.1			
ApCmKmSm			9.1	
ApCmSmTc		5.0	18.1	
ApCmKmSmTc	9.1			
ApKmCmSmGm			9.1	
ApCmKmSmTcTp	18.1			

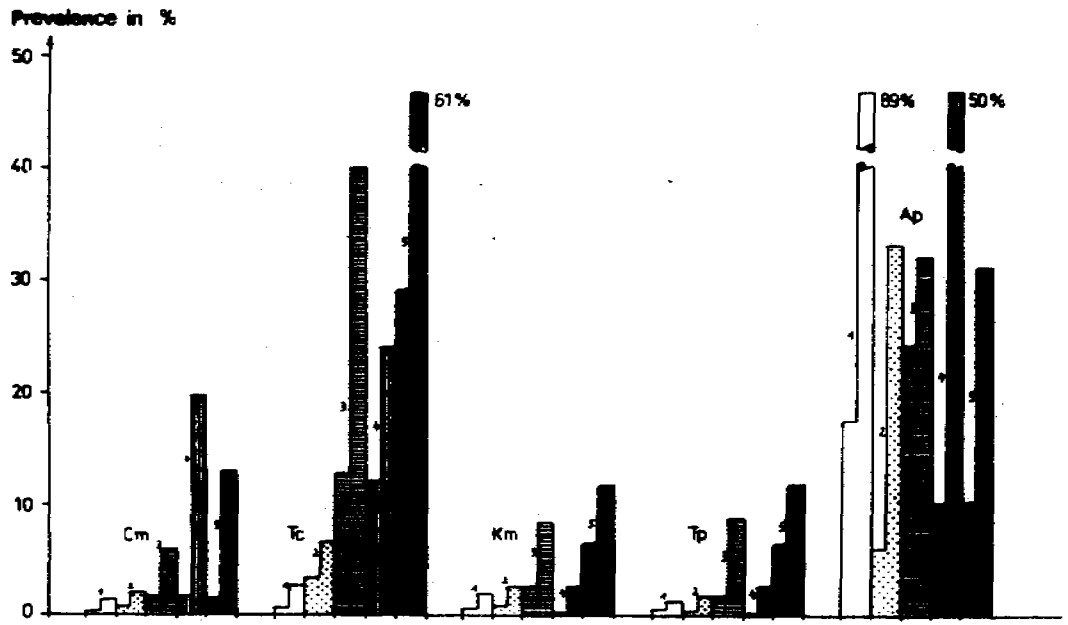
FIG. 1



The occurrence of total coliforms and antibiotic resistant coliforms (Cm, chloramphenicol; Tc, tetracycline; Km, kanamycin resistant coliforms) in fecal samples of healthy individuals.

FIG. 2

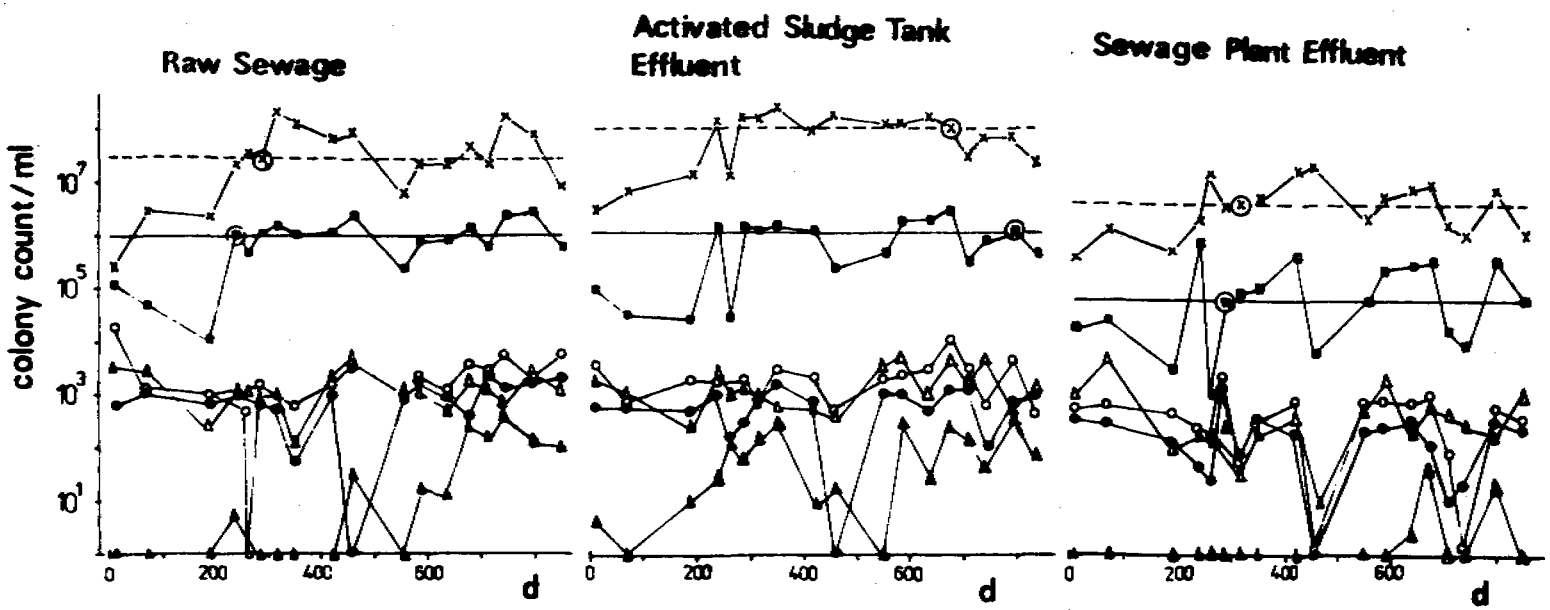
COMPARISON OF MINIMAL AND MAXIMAL RESISTANCE QUOTIENTS (%) AMONG COLIFORMS FROM DIFFERENT ECOLOGICAL SOURCES



1 ▭ , Elbe river
2 ▭ ; municipal sewage treatment plant
3 ▬ , municipal sewage treatment plant with outlets of an antibiotic producing plant
4 ▬ , hospital sewage treatment
5 ▬ , slurry

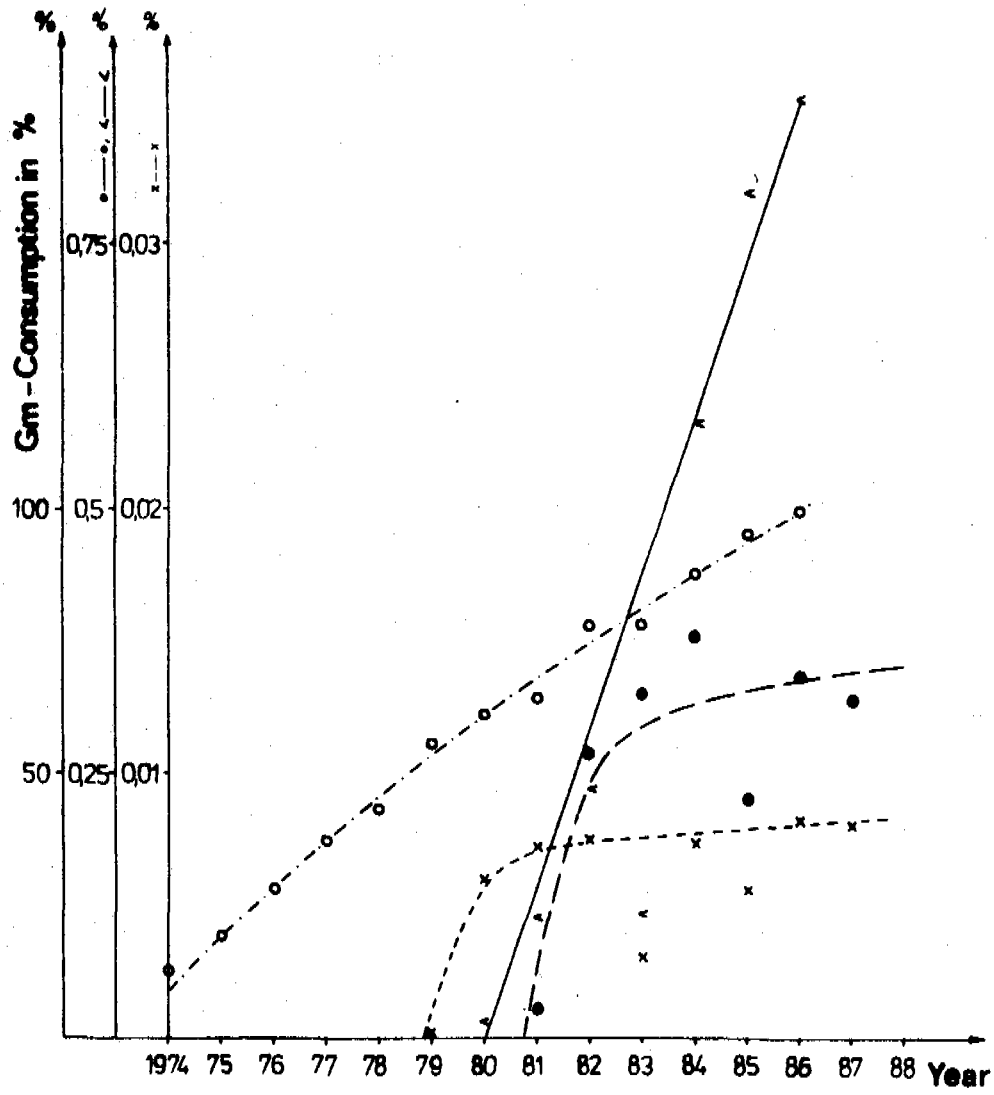
FIG. 3

COLONY COUNTS (20°C) (x——x), TOTAL COLIFORMS (●——●) AND ANTIBIOTICA RESISTANT COLIFORMS IN THE PURIFICATION STEPS OF THE INVESTIGATED SEWAGE PLANT



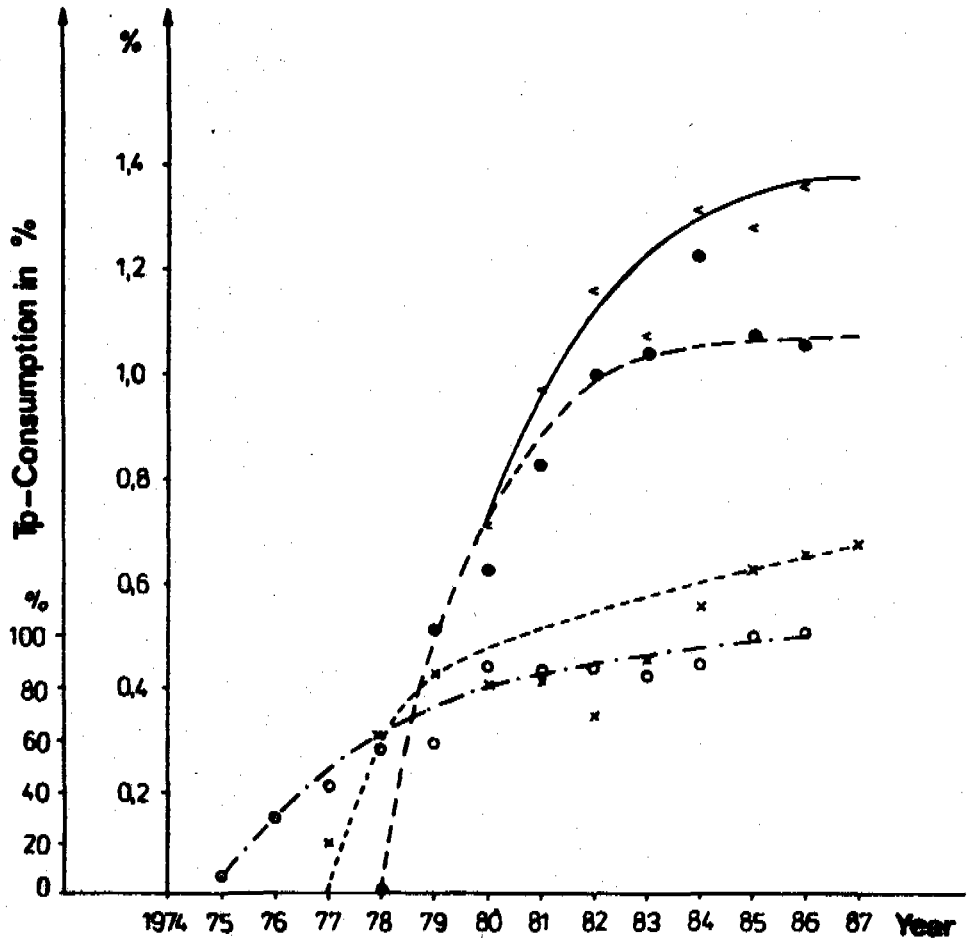
Δ—Δ ; tetracycline resistant coliforms
●—● ; chloramphenicol resistant coliforms
o—o ; kanamycin resistant coliforms
▲—▲ ; gentamicin resistant coliforms
--⊗-- ; median values of colony counts (20°C)
——◉—— ; median values of total coliforms

FIG. 4



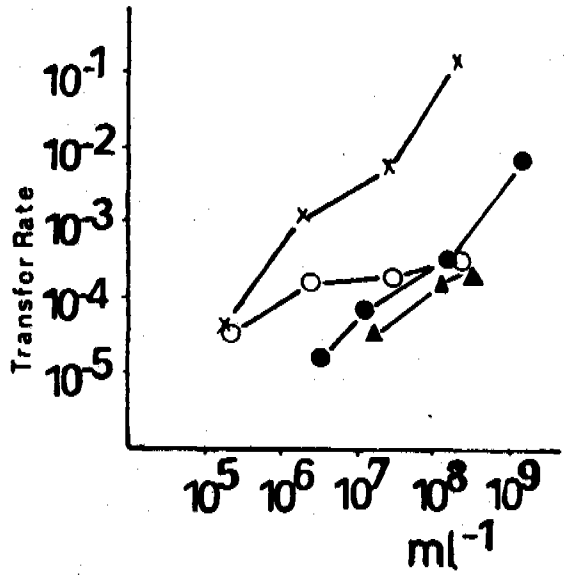
Gentamicin resistance development among coliforms from sewage (x——x); E. coli isolates from urinary tract infections (●– – –●) and E. coli isolates from a hospital (>——>) in comparison with the gentamicin (o–·–o) consumption in the G.D.R. between 1974 and 1986 (gentamicin consumption 1986 = 100 %)

FIG. 5



Trimethoprim resistance development among coliforms from sewage (x- - -x), E. coli isolates from infections of the urinary tract (●- - -●) and E. coli isolates from a hospital (>——— >) in comparison with the trimethoprim (o—·—o) consumption in the G.D.R. between 1974 and 1986 (trimethoprim consumption 1986 = 100 %)

FIG. 6



Relationship between transfer frequency and bacterial density. Different densities of donor and recipient bacteria were mixed in nutrient broth and conjugated at 37°C for 2h.

donor strains

E. coli K12 (R 64), SmTc ●—●

E. coli 14, isolated from water, SmSuTp x—x

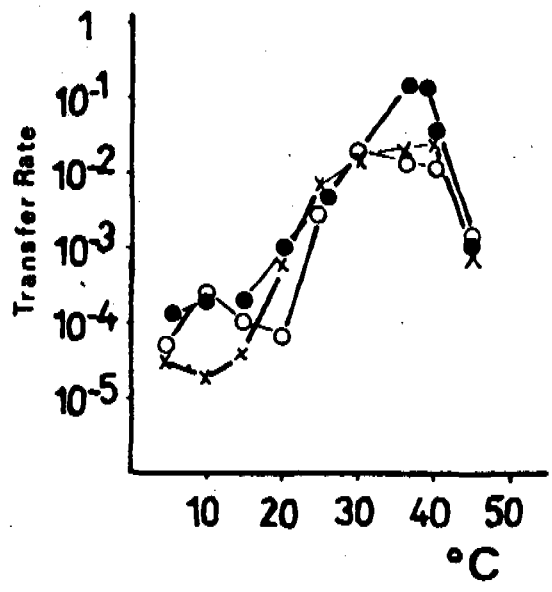
K. oxytoca 16, isolated from water, SmSuCm o—o

K. pneumoniae 9, isolated from water, SmSmSuApKm ▲—▲

recipient strain

E. coli K12, thi$^-$ thr$^-$ leu$^-$ lac$^-$ Rif^r

FIG. 7



Relationship between transfer frequency and temperature. Densities from 10^7 to 10^8/ml of donor and recipient bacteria were mixed in nutrient broth (SIFIN) and conjugated over night at different temperatures.

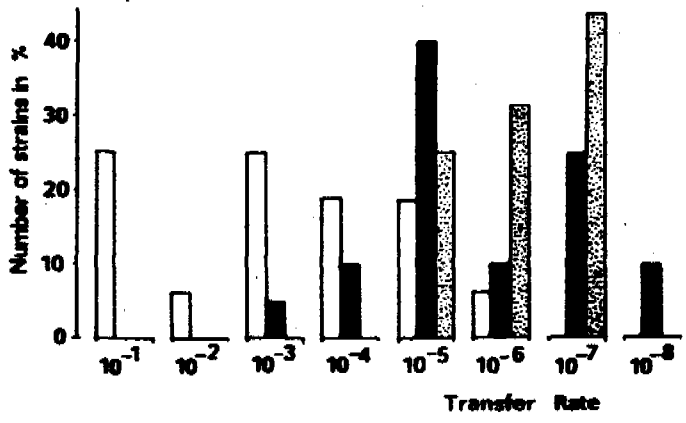
donor strains (10^7 - 10^8 cells/ml):
E. coli K12 (RN3), TcSmSu ●——●
E. coli 14, isolated from water, SmSuTp X——X
K. oxytoca 16, isolated from water, SmSuCm o——o

recipient strain (10^7 - 10^8 cells/ml):

E. coli K12, thi^- thr^- leu^- lac^- Rif^r

FIG. 8

R plasmid transfer in nutrient broth (▭), waste water (▬) and river water (▨)



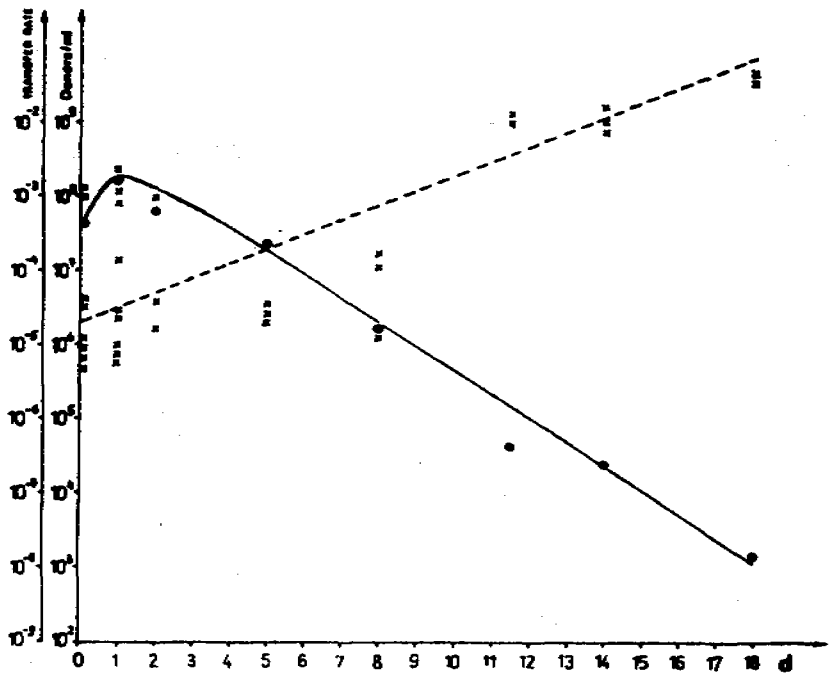
Sterilized dialysis membrane bags were filled with 10 ml of sterilized waste water or river water, respectively. Donor bacteria and recipient bacteria were added to a final concentration of about 10^8 colonies/ml, and the prepared dialysis membrane bags were incubated in 3 l untreated waste water or river water at room temperature for 3 hours.

donor strains:	E. coli and Klebsiella strains isolated from water	
E. coli	53	TcSmSu
	14	SmSuTp
	29	SmCmKmSuApTc
	31	SmCmKmSuApTc
	20/2	TcSmCmSuApKmTp
reference plasmid	RN3	TcSmSu
K. pneumoniae	1	
	2	
	3	TcSmCmSuApKmGm
	4	
	5	
	9	SmCmSuApKm
K. oxytoca	1	TcSuApGm
	2	TcSmCmSuApKmTpGm
	4	TcSuApGm
	16	SmSuCm

recipient strain: E. coli K12, thi^- thr^- leu^- lac^- Rif^-

FIG. 9

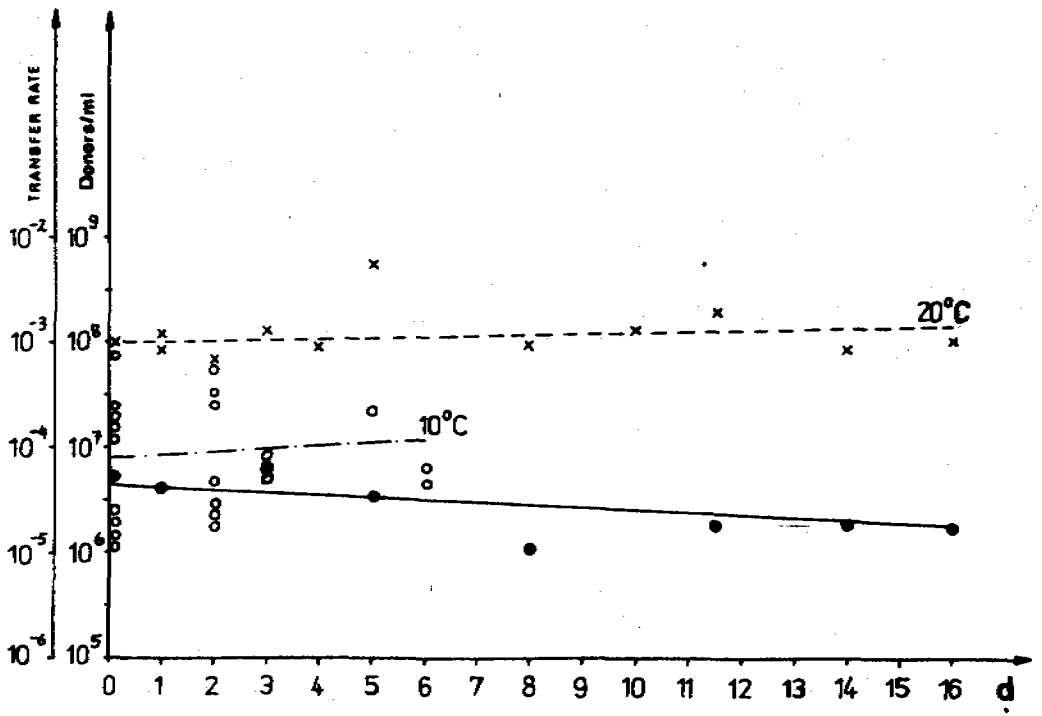
R100-TRANSFER FREQUENCIES IN STERILIZED SEWAGE



Primarily treated sewage (BOD 58-104 mg O_2/l, COD 21-83 mg O_2/l, pH 6.7 - 7.2)was boiled for 20 min. After cooling at room temperature the sewage samples were inoculated with plasmid-carrying donor bacteria (10^7 - 10^8 cells/ml) (●——●) and recipient bacteria E. coli K12, thi^- thr^- leu^- lac^- Rif^r; $8.0 \cdot 10^6$ - $1.2 \cdot 10^8$ cells/ml) and the transfer frequencies (×---×) were estimated at different times from various experiments.

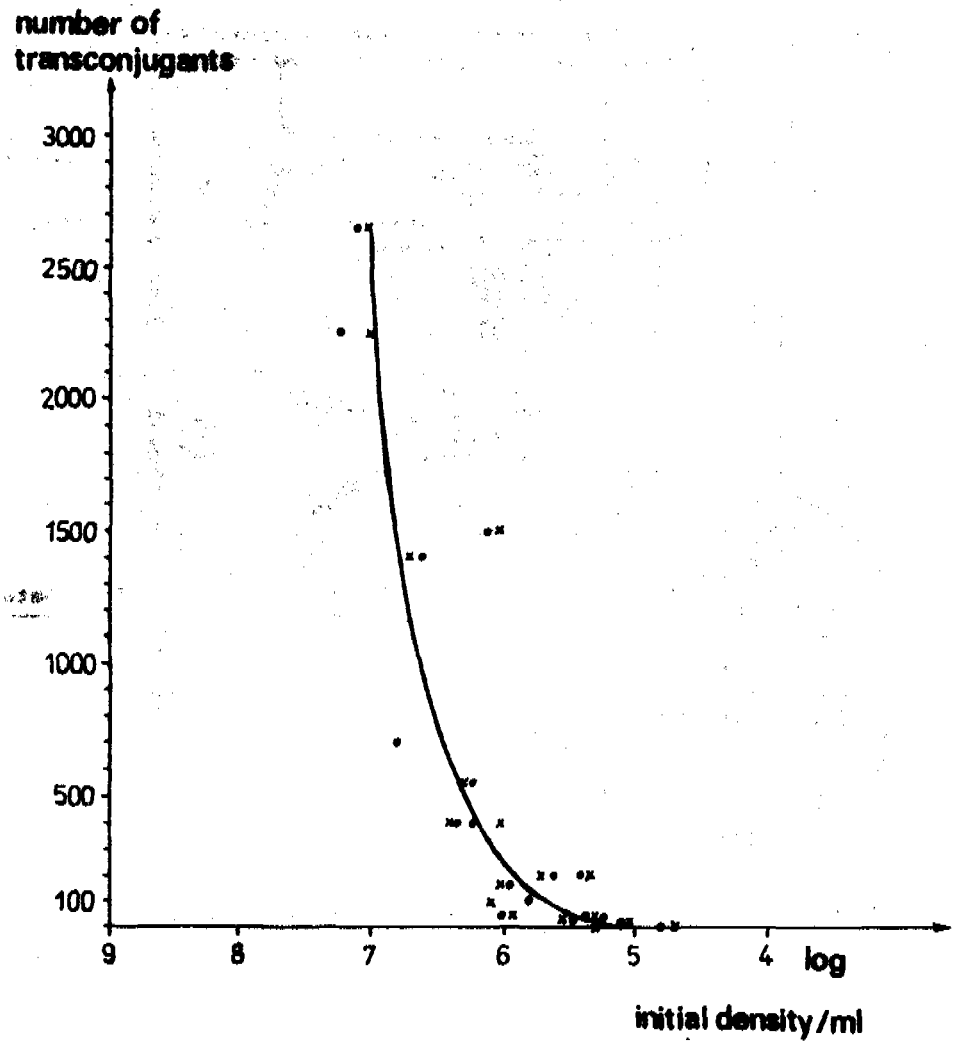
FIG. 10

Rp1-TRANSFER FREQUENCIES IN STERILIZED SEWAGE



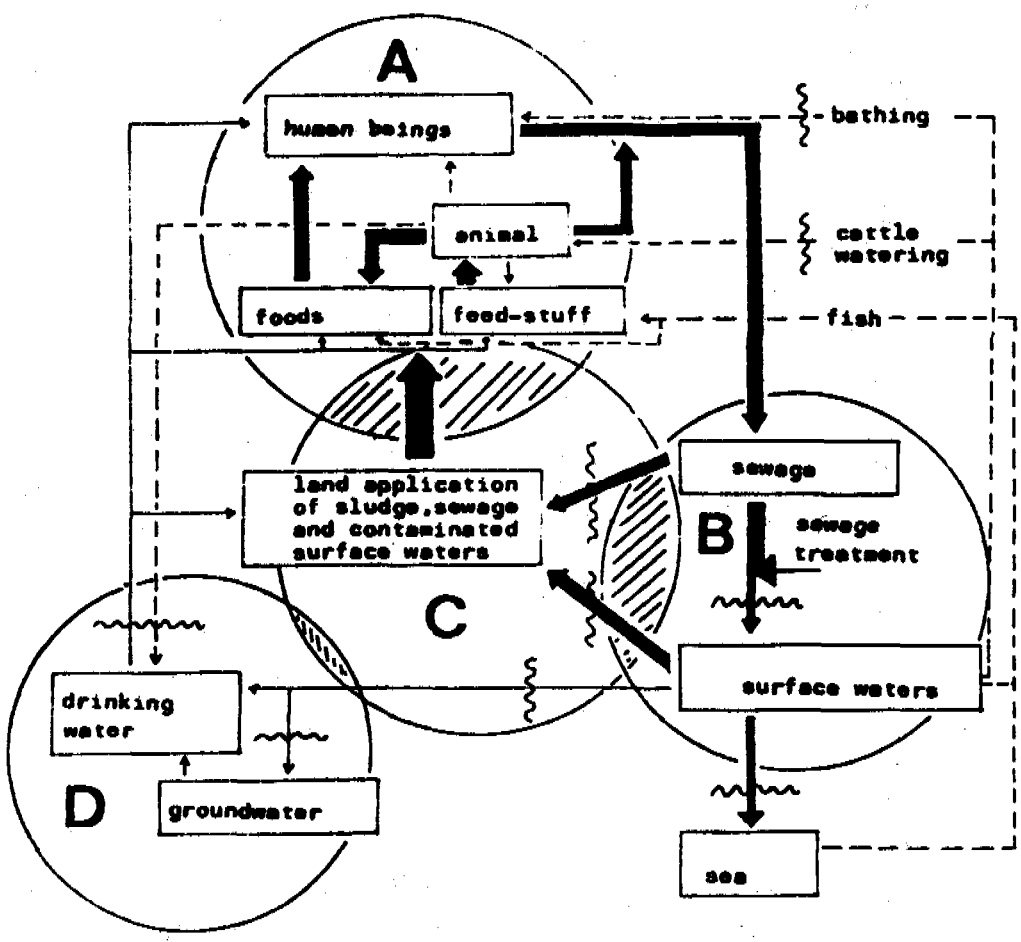
Primarily treated sewage was boiled for 20 min (see above). The sterilized and cooled sewage samples were inoculated with plasmid-carrying donor bacteria (10^7 - 10^8 cells/ml (●——●) and recipient bacteria (see legende figure 9) ($5 \cdot 10^6$ - $6.0 \cdot 10^7$ cells/ml) and the transfer frequencies were estimated at different times and temperatures (x----x 20°C, o——o 10°C) from various experiments.

FIG. 11



Influence of bacterial density on "plate conjugation"
The sterilized sewage samples were inoculated with altered population densities of donor (R 100) and altered population densities of recipient bacteria (E. coli K12, thi thr leu lac Rif^r), respectively. Immediately after mixing (temperature 20°C) the samples were plated on selective agar plates for counting transconjugant colonies (×——×, inoculum size of recipient bacteria about 10^6/plate and variable inoculum sizes of donor bacteria; ●——●, inoculum size of donor bacteria about 10^6/plate and variable inoculum sizes of recipient bacteria).

FIG. 12



The transmission of R^+ bacteria in the environment