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REVIEW PAPER

TRANSFERABLE DRUG RESISTANCE IN BACTERIA  
(Are Fecal Coliforms Becoming a Hazardous Waste)

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## ABSTRACT

Fecal coliforms are generally considered to be harmless indicators of fecal contamination and thus the possibility that pathogens are also present. In many countries there is extensive, and sometimes indiscriminate, non-prescription use of antibiotics; they are also used world-wide as feed additives for livestock. This use has resulted in many pathogens, and fecal coliforms, becoming resistant to antibiotic therapy; often shortly after their development and marketing. No antibiotics have yet been discovered or developed to which bacteria can not develop resistance. This rise in resistance to antibiotics, sometimes to 10 or more antibiotics simultaneously in one organism, has decreased the therapeutic value of antibiotics and caused considerable problems in both human and veterinary medicine. This situation is not necessarily inevitable but is a result of the uncontrolled access to the drugs and the inadequate treatment of wastes containing drugs and viable bacteria. The problem and the solution are more economic and political than technical.

Drug resistance in bacteria is readily transferred from one organism to another, even across specific, generic and familial boundaries. This readily transferable resistance is not carried on chromosomal genes but is in extrachromosomal plasmids which replicate autonomously and are transferred from generation to generation in the cytoplasm and by conjugative processes to other organisms in the same generation. This transfer may occur in the gut, in sewage treatment plants and in the environment. Drug presence affords a selection pressure which results in a higher proportion of resistant organisms in successive generations. Even if drug therapy is successful in eliminating all the pathogens in a patient, harmless fecal coliforms may become resistant and subsequently pass on this resistance to future pathogens. The patient infected by these resistant pathogens, who undergoes drug therapy with one of the drugs to which the pathogen is resistant, continues to get sicker. If the pathogen is a

dangerous one like that causing typhoid fever this can become a serious problem before a new treatment can be found. The recent typhoid fever epidemic in Mexico, where antibiotic use is not regulated, is a good example of the public health problems which may ensue.

Serious thought needs to be given to devising new and innovative waste-treatment processes, in concert with restricted drug availability and solution of the economic and political problems, if we are to avoid finding ourselves back in pre-antibiotic conditions in our health care system. Source segregation and sterilization of wastes containing drugs and viable organisms may be necessary. Development of drugs to which bacteria can not develop resistance, or at least can not transmit this resistance, is another potential solution.

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## INTRODUCTION

A number of good review papers are available on the subject of infectious or transferable drug resistance (R-Factor) in bacteria and the resulting consequences. Examples include Anderson - 1968(1), Richmond - 1972(3), Grabour et al - 1974(4), Watenabe - 1971(2), - 1963(6), Mitsuhashi - 1967(61) and Davies and Round - 1972(62).

"The magnitude of man's achievements is frequently equalled only by that of his stupidity in their use." Anderson - 1968(1).

This quote just about sums up the problems with antibiotics; their abuse and the negligence in disposal of resistant bacteria resulting from this abuse. No drugs have yet been developed to which bacteria can not develop resistance. The increase in multiple drug resistance among pathogenic bacteria has been rising and the problem is now of a scale which is causing a serious medical problem (2,3). Drugs are used for legitimate therapy and also for various other purposes such as preventatives and feed additives, which encourages the shift from susceptible to resistant bacteria in the environment. Coliform bacteria, generally regarded as harmless indicators, can transfer drug resistance to pathogens(4). The nearly universal use of antibiotics in animal feeds means that most livestock harbour drug resistant bacterial floras. When this resistance is transferred to a pathogen, veterinarians may have difficulty finding a drug to which the pathogens are not already resistant. Every use of a drug runs the risk of adding one more drug to the resistance spectrum(2).

Two steps are involved in the spread of infectious drug resistance in the population. Drug therapy selects for the resistant organism within a patient, whether pathogen or coliform bacteria. Transmission occurs when waste treatment processes allow viable resistant bacteria to reach the environment and transfer this resistance. Many organisms survive conventional sewage treatment processes in large numbers and some multiply in the system(11).

Sewage which is not adequately treated is the major source of drug resistant coliforms in rivers, reservoirs and swimming beaches; such polluted water is instrumental in the spread of resistant organisms. Sewage should not be discharged without sufficient treatment to eliminate such organisms(4,11,16,17).

The bacterial genes responsible for transmissible drug resistance are not chromosomal but are in plasmids which are comprised of DNA. They replicate autonomously and more often than the chromosomes. These plasmids, which contain genes for drug resistance (R-factors), may also contain a gene which determines the transferability of this resistance to other bacteria by exchange of plasmids or portions of the plasmid DNA. Such transfer appears to occur readily within the enterobacteriaceae and also among diverse groups of bacteria(30). These transfers take place by cellular contact in such diverse habitats as the gastrointestinal tract, sewers, sewage treatment plants, sludge and compost heaps and in the water and sediments of lakes and rivers where waste and effluent are discharged. Resistance to many drugs is transferred under these conditions and some bacteria have been found with resistance to as many as 12 antibiotics simultaneously(26), and the ability to transfer up to 6(26), 7(25), or 8(31) of these resistances all at once. In Nigeria antibiotics and drugs are available without prescription to the general public in pharmacies as well as in market stalls(48). This practice is widespread in many other countries of the world including Mexico and the abuse by ignorant or unconcerned people is widespread, leading to widespread resistance in the bacteria of local mammalian and bird populations.

The constant pressure on pathogens of antibiotics in widespread use, as in animal feeds which selects for large populations of resistant strains, is a serious threat to antibiotic therapy, and medicine may soon find itself back in the pre-antibiotic days when it comes to treating infectious diseases unless steps are taken to limit this spread(30).

## RESERVOIRS AND SOURCES OF BACTERIA WITH TRANSFERABLE DRUG RESISTANCE

Food/Animals

In Ireland there is no legislation regulating the use of antibiotics in livestock by farmers. Escherichia coli isolated from sausages, yearling pigs, sows and the hands of butchers and their staff, were resistant to ampicillin, streptomycin, tetracycline, chloramphenicol and neomycin. Even the inside of cooked sausages showed viable E. coli resistant to ampicillin, streptomycin and tetracycline. Many of the resistant strains of E. coli were capable of transferring this resistance (7). Pork and beef carcasses in Britain were tested for coliform bacteria both internally and surficially. Beef samples were 52% positive and pork samples 83% positive for coliforms and of the positive samples, 38% of the beef, and 80% of the pork, were resistant to one or more antibiotics. Most resistant strains were capable of transferring this resistance. Resistance to streptomycin, tetracycline, ampicillin and chloramphenicol was tested(8).

In Nigeria antibiotics are freely available even in market stalls(48), and poultry is routinely fed antibiotics. All 2190 strains of Escherichia coli isolated from the poultry were resistant to tetracyclines, streptomycin and sulfonamide(49). In Hong Kong animals, including man, pigs, cows, dogs, geese and ducks, which were not fed antibiotic containing food, still showed positive for drug resistance and transferability to chloramphenicol, penicillin, tetracycline and streptomycin. Cows did not transmit tetracycline resistant bacteria though they did harbour them in their gut. Only cows, pigs and man transmitted chloramphenicol resistant bacteria. Chickens receive a diet containing chlortetracycline, 3,5-dinitrobenzamide, acetyl (paranitrophenyl) sulfanilimide, 3-nitro-4-hydroxyphenylarsonic acid and bacracin. They harbour bacteria with transferable drug resistance to these drugs(21).

Fish culture in Japan uses antibiotics to maintain stock health, as in other parts of the world. This has resulted in antibiotic resultant strains of Aeromonas liquifaciens in Trionyx sinensis (soft-shelled turtle), Anguilla japonica (eels), Cyprinus carpio (carp), Plecoglossus altivelas (ayu) and Carassius auratus (goldfish). The Aeromonas liquifaciens appears to have a 'natural resistance' to aminobenzyl penicillin since most cultures were resistant, even though this antibiotic is not used in fish culture. This resistance was not transferable to E. coli in culture and may have been chromosomal rather than plasmid mediated resistance(28).

Antibiotic resistant E. coli on commercially available chicken carcasses were eventually found to be present in the gut of people who handled, cooked and ate the chickens. Extensive serotyping and plasmid characterization, before and after handling the chickens, showed that the strains were the same(46). In Britain cows(1) and in Sweden pigs(2) are the principal reservoirs. Cross transfer from these reservoirs, maintained at high resistance levels by continuous drug addition in their feed, are responsible for transmissible drug resistance in other species. The ubiquitous E. coli serves as a bacterial reservoir and often is an intermediary in transfer of drug resistance from one pathogen to another and from animals to humans(30). In farm animals the incidence of multiple resistance to antibiotics by E. coli was 85% in animals given continuous exposure to drugs in their feed as opposed to 16% in other animals. The animals given a higher drug exposure also had bacteria with a higher rate of drug transfer ability(50).

Freshwater mussels, Hydridella menziesii, from 3 lakes in New Zealand contained total and fecal coliforms and E. coli resistant to ampicillin, rifampin, trimethoprim, sulfafurazole, tetracycline, cephalothin, nalidixic acid, kanamycin and streptomycin. Many had multiple resistance but more were resistant to chloromycetin, gentamicin or paromomycin. No isolates resistant to streptomycin, tetracycline or kanamycin were able to transfer this resistance to E. coli, strain K-12(59). Prior to installation of a marine outfall in New Zealand no fecal coliforms were found in shellfish or

the seawater. Subsequently the mean fecal coliform level in shellfish rose to 60/100 gm. (5-160) in the first year and 225/100 gm in the second year. In these shellfish 89% of the total coliforms and 73% of the fecal coliforms were resistant to one or more of ampicillin, sulfafurozole, cephalothin, tetracycline, chloromycetin, streptomycin, nalidixic acid, kanamycin, trimethoprim, rifampin and paromomycin. Multiple resistance was found in 62% of the total coliforms and 65% of the fecal coliforms. Selected isolates susceptible to nalidixic acid and resistant to streptomycin, tetracycline, chloromycetin or kanamycin were tested for transmission of resistance. Transfers occurred except for kanamycin resistance in fecal and total coliforms(60).

People

Rectal swabs were made from 771 British school children, 709 contained a strain of E. coli of which 102 were resistant to one or more of sulfosomidine, tetracycline, streptomycin, ampicillin, chloramphenicol, nitrofurantoin, nalidixic acid and trimethoprim(43). Bacteroides fragilis is an anaerobe that normally resides in the human gut where it is a major portion of the fecal flora and an opportunistic pathogen. It is usually found to be resistant to penicillin G, ampicillin, cephalothin and the aminoglycoside antibiotics. It may also be resistant to clindamycin, tetracycline, chloramphenicol and erythromycin. Tetracycline resistance is increasing. Resistance can be transferred to E. coli in vitro and may also occur in vivo(54).

The resistance of 224 strains of Salmonella, isolated from people in a California hospital, was tested against 12 antibiotics: streptomycin, tetracycline, ampicillin, neomycin, sulfisoxazole, kanamycin, cephalothin, chloramphenicol, nalidixic acid, colistin, nitrofurantoin and gentamicin. No strains were resistant to colistin or gentamicin; Salmonella enteritidis was not resistant to any of these antibiotics. The other strains of Salmonella had variable resistance to the other 10 drugs ranging from 0 to 43% of the samples of each serotype. Of the isolates, 32% were resistant to 1 or more drugs and 70% of those resistant demonstrated the capability to transfer this resistance. Thus 17% of the original isolates were capable of transferring multiple resistance. For some serotypes this percentage was in the 90-100% range of the multiple resistant strains(57).

Bacteroides ochraceus is a major constituent of the gastrointestinal tract of mammals. Some strains are resistant to penicillin, ampicillin, tetracycline, chloramphenicol, erythromycin, clindamycin, rifampin and the aminoglycosides. They can transfer resistance to tetracycline, kanamycin, chloramphenicol and streptomycin to E. coli. This resistance is in plasmids but non-transferable resistance to ampicillin and penicillin is presumably chromosomal(37).

In a study of adults and children in rural and urban settings, 67% of the children but only 46% of the adults carried resistant fecal coliforms regardless of where they lived. Rural people more often carried resistant bacteria; adults working with farm animals had 67% as opposed to only 29% of those in other occupations. Of the resistant strains 61% were transmissible for various patterns of single or multiple resistance to ampicillin, neomycin, tetracycline, sulfonamide, chloramphenicol and streptomycin(41).

## Sewage

sewage contamination of coastal bathing waters is widespread in Britain. E. coli isolated from 15 different bathing beaches showed variable amounts of resistance to chloramphenicol, tetracycline, neomycin, streptomycin and ampicillin and variable ability to transfer this resistance. Many were resistant to more than 1 drug and could transfer their resistance to Salmonella typhi and other Escherichia coli strains. Bathing water contaminated to the extent found in this survey helps disseminate E. coli with transfer capability among people and also helps to spread pathogens. Since the life span of these organisms in sea water is not long, the high concentrations found in this study were a result of constant ongoing pollution(33).

Little difference was found in the number of organisms with transferrable drug resistance between hospital and city sewage but there was a broader spectrum of resistance patterns in hospital sewage organisms. E. coli with transferable resistance is present in such effluent(11). Large numbers of resistant bacteria occur in wastewater, particularly that from hospitals(18). If hospitals receive special stringent wastewater disinfection procedures their relative load of infectious organisms may be lower than that of general city sewage, but if not specially treated their load will be higher. Hospital sewage receiving only primary treatment should not be discharged into streams used for recreation, irrigation or animal drinking. High Pseudomonas aeruginosa levels are found in hospital sewage since it is a common hospital contaminant and is quite resistant to disinfection(19).

Although hospital sewage contains higher proportions of bacteria with drug resistance and transmissible drug resistance than does domestic urban sewage, due to the greater numbers of people contributing to the urban sewage and the widespread level of resistant bacteria in the general population, over 90% of the resistant bacteria will be found in the urban

sewage rather than the hospital sewage. The proportion of resistant to non resistant bacteria was similar in hospital and residential sewage but the incidence of multiple resistance higher in hospital sewage. The maintenance of this reservoir of resistant bacteria in the population may have several causes: food the people eat may be contaminated by drug resistant organisms, poor sanitary practices spread and maintain existing bacterial flora and treatment with antibiotics produces a resistant flora which may continue to be excreted for up to 3 months after treatment(42).

Open sewer bacteria in Hong Kong showed transmissible drug resistance to chloramphenicol, streptomycin, tetracycline and penicillin(21). There is a significant level of infectious drug resistance in fecal coliforms found in urban sewage; many show multiple resistance to streptomycin, tetracycline and ampicillin(22,23). In Manitoba, Saskatchewan and the North West Territories 9% of the total coliforms and 11% of the fecal coliforms sampled carried resistance to up to 7 antibiotics simultaneously. Final discharges to the environment of fecal coliforms containing drug resistance reached  $6 \times 10^2/100$  ml; for total coliforms the number was  $3 \times 10^4/100$  ml. Such bacteria, with transferable drug resistance, should be removed from sewage before discharge to the environment(25).

## Environment

Prior to installation of a marine outfall in New Zealand no fecal coliforms were found in shellfish or seawater. Subsequently, 72% of the total coliforms and 69% of the fecal coliforms found in the seawater were resistant to one or more of ampicillin, sulfafurazole, cephalothin, tetracycline, chloromycetin, streptomycin, nalidixic acid, kanamycin, trimethoprim, rifampin and paromomycin. Of these, 50% of the total coliforms and 42% of the fecal coliforms had multiple resistance(60).

Wild animals, primarily impala, wildebeast, Kudu and warthog, as well as Kalahari bushmen were tested for antibiotic resistance of their fecal coliforms. The bushmen selected had not had any contact with other people for 10 years. Of the 582 specimens tested, 10% of the animals and men tested carried antibiotic resistant bacteria, but none of the resistance was transferable to donor bacteria. Resistant bacteria included E. coli, Proteus vulgaris, Proteus morgani, (Aerobacter) Enterobacter aerogenes and Alcaligen faecalis. The antibiotics to which they showed resistance included streptomycin, ampicillin, chloramphenicol and tetracycline(32).

Surface drainage water (stormwater runoff) appears to have a high level of intrinsically ampicillin resistant bacteria(42). The proportion of bacteria that are resistant appears to be rising. This is not unexpected and was demonstrated in Japan with Shigella in the 1945 - 1952 period(61). In 1970 a study showed 27% of the organisms in some rivers were ampicillin resistant; while in 1974 73% of the enterobacteria in the Stour River, Kent, England were resistant. Of the 71 strains, resistance or multiple resistance, was found for ampicillin -50, streptomycin -40, tetracycline -38, chloramphenicol -16, cephaloridine -32, kanamycin -8 and nalidixic acid -13. An overnight transfer to E. coli, K-12, of all of their resistance, was demonstrated for 21 of the 70 strains tested(36).

Water and sediments in coastal canal areas of the southern United States heavily used for recreation, were sampled for drug resistance and

transferability to ten antibiotics in Salmonella, fecal coliforms and total coliforms. Many bacteria showed multiple resistance and the ability to transfer this multiple resistance. More drug resistant bacteria were found in the sediments than in the overlying water. Some coliforms were resistant to 9 drugs and some Salmonella to 7 drugs simultaneously(10). Multiple drug resistant E. coli isolated from an estuary were about 8% of the total E. coli population. They transferred resistance to 6 antibiotics with a frequency of 60%, to laboratory strains of E. coli. However, the overall transfer frequency for all strains was low,  $10^{-4}$ , so risk of infection and transfer of drug resistance in recreational waters is low(15).

Salmonella and total and fecal coliforms isolated from the Red River had transferable drug resistance. Some of the fecal coliforms were resistant to all 12 antibiotics tested and 18% of the Salmonella were resistant to 1 or more drugs. Cross transfer between E. coli and Salmonella occurred. Populations of 1400/100 ml. of fecal coliforms with transferable drug resistance were present(26). Bacterial isolates from rivers and the bay at Tillamook, Oregon, were tested for antibiotic resistance patterns with chloramphenicol, streptomycin, ampicillin, tetracycline, chlortetracycline, oxytetracycline, neomycin, nitrofurazone, nalidixic acid, kanamycin and penicillin. The resistance patterns were well correlated, even across generic boundaries, indicating a common pool in which drug resistance transfers took place(27).

Coliform and fecal coliform bacteria from potable water supplies, streams, seawater and effluents were examined for the incidence of antibiotic resistance to ampicillin, sulfafurazole, cephalothin, tetracycline, chloromycetin, rifampin, streptomycin, nalidixic acid, kanamycin, trimethoprim and paromomycin. In drinking water 40% of the isolates were resistant to one or more drugs, in streams and seawater 60% and in effluents 70%. Of those isolates resistant to streptomycin or tetracycline, 40% could transfer this resistance to E. coli(45).

### Mechanisms of Drug Resistance

While the exact mechanism by which all bacteria are able to survive the attacks by drugs is not known, there are some known mechanisms for some drugs and some bacteria. Since most of this resistance is carried in the plasmid genes and is readily transferable, most species of enterobacteria share the same mechanism of resistance to any given antibiotic.

**Tetracyclines.** Resistance is due to an inhibition of drug uptake into the cell.

**Chloramphenicol.** Resistance is due to an acetyltransferase which acetylates the 1- and 3-hydroxyl groups on the sidechain of the ring.

**Penicillins.** Resistance is due to the production of a penicillinase (a beta-lactamase) which hydrolyses the beta-lactam ring of penicillins between the nitrogen and the carbonyl group. Beta-lactamases are known which will inactivate all penicillin and cephalosporin derivatives in this manner.

**Aminoglycosides.** Resistance is due to a series of enzymes which inactivate one or more of the drugs in this group. The 3-hydroxyl group on the L-glucosamine ring of streptomycin may be phosphorylated by one enzyme or adenylated by another enzyme. An acetyltransferase inactivates kanamycin and a phosphorylase attacks a hydroxyl group of kanamycin and neomycin. A different hydroxyl group of kanamycin, gentamycin and tobramycin are inactivated by an adenylating enzyme. Some strains of Pseudomonas aeruginosa can also inactivate gentamycin by acetylation of the deoxystreptamine group.

This, and more detailed, information on deactivation mechanisms may be found in a review paper by Davies and Rownd(62).

### Factors Affecting the Transfer of Drug Resistance

The transfer of resistance to chloramphenicol, tetracycline, streptomycin and sulfonamide from Salmonella typhi, was low at 37°C but higher at 28° or 22°C. However, Shigella dysenteriae can transfer readily to E. coli - K12 at 37°C. It is likely that most transfers occur in sewage or in feces after voiding when temperatures are lower than in the gut(35). The frequency of drug resistance transfer by plasmids is controlled by regulatory chromosomal genes(38). Under normal clinical and gut conditions the transfer of resistance to ampicillin, kanamycin, nalidixic acid, rifamycin, tetracycline and vancomycin, from E. coli to Bacteroides fragiles, Bacteroides spp., Fissobacterium spp. and other obligate anaerobes, does not occur. However, if the recipient cells were heated to 50°C for 20 minutes prior to attempting transfer than the transfer was successful(47).

A human volunteer was given oral doses of E. coli strains originating in pigs, oxen, chickens and other people. These strains were selected for antibiotic resistance to various drugs. Except at high dose rates, over 10<sup>9</sup>/day, such donor strains did not colonize the gut or transfer resistance to gut bacteria. Very low transfer rates were found for ampicillin, streptomycin, sulfonamide, neomycin, tetracycline and chloramphenicol. The donor strains and the resistance factors in resident strains all disappeared from the gut within a month(34).

The Effects on Bacterial Survival of Carrying a Gene for Drug Resistance Transfer

Bacterial cells with drug resistance genes do not have survival disadvantages in non-drug conditions but do, of course, survive better in habitats containing drugs(16,18,24,25,27). Escherichia coli, bearing plasmids for rifampicin or nalidixic acid transferable drug resistance were fed to people along with strains without such plasmid mediated transferable resistance. Under non drug conditions in the gut the cells with the drug resistance did not survive as well, or as long, as the chromosomally marked tracers without the plasmid resistance(52). E. coli with transferable drug resistance plasmids survives just as well in the sea as E. coli without such R-factors(24).

## Treatments for the Removal of Bacteria Carrying Transferable Drug Resistance

Resistance is stable and wide spectrum bacteria survive best. Removal of such bacteria is best in processes involving rapid passage over stony surfaces such as biofilters and sand filtration. The number of resistant bacteria may increase in sedimentation units and maturation ponds(18,20). The resistance of 260 strains of fecal coliforms isolated from raw domestic sewage and aerobic lagoon effluent was determined for the antibiotics ampicillin, aureomycin, chloromycetin, gentamicin, streptomycin, sulfodiazine and tetracycline. The aerobic lagoon reduced the fecal coliform number by a factor of 20, but the proportion of singly or multiply resistant bacteria in the aerobic lagoon was the same as that in the raw domestic sewage(44). Sewage maturation ponds used in tertiary treatments decrease coliform counts by 90 - 99%, but drug resistant bacteria, especially those with transferable drug resistance (60% E. coli), have increased survival rates and are found to be a greater proportion of the coliforms leaving the ponds than of those entering the ponds. Self purification by rivers and lakes can not be relied upon to eliminate transferable drug resistant pathogens; sterilization methods are required(51).

Water quality standards should either not allow viable coliforms to be present or else specify that any present should not carry transferable resistance to therapeutically valuable drugs. Such coliforms are not harmless indicators but sources of resistance genes which can be used by pathogens to our detriment. This affects the discharge of such coliforms in water used for irrigation, recreation, shellfish growing or wildlife and livestock use(18).

### Consequences of Pathogens with Transferable Drug Resistance

Twenty-five years ago if you contracted a serious bacterial infection such as dysentery, pneumonia, typhoid fever or systemic infections of staphylococcus or streptococcus, your doctor would prescribe the appropriate antibiotic and within 10 days you would be well; now you could be in some difficulty. The reason is simple and well known; the solution is not clear and will likely be expensive, but could have been avoided, or at least delayed, for a very long time. Many pathogenic bacteria are resistant to a whole group of antibiotics and the problem gets worse as we try to control it with new antibiotics. While the doctor and the laboratory are screening to find an antibiotic which will control the infection, and not have adverse side effects, the patient gets sicker. The problem will be worse next time as yet one more antibiotic will likely not be useful.

Ampicillin, streptomycin and tetracycline are the most commonly used drugs for people, and are thus the drugs to which bacteria are most often resistant(10). Chloramphenicol is the drug of choice for typhoid fever and generally Salmonella typhi remains susceptible, but several epidemics of typhoid fever have occurred, in Mexico and Belgium, in which chloramphenicol resistant S. typhi were implicated. In the 1972 Mexican epidemic the bacteria were also resistant to streptomycin, tetracycline and the sulfonamides; some were also resistant to ampicillin(12).

"The emergence of strains of bacteria resistant to common antibacterial agents has become one of the most important problems in clinical medicine. These strains...are commonly resistant to several drugs simultaneously"(61).

Klebsiella, Proteus and E. coli resistant to chloramphenicol, streptomycin and tetracycline have transferred this resistance to Salmonella typhi. In countries like India and Africa where typhoid fever is endemic and chloramphenicol is the drug of choice for its control, resistant S. typhi is a serious threat to health care. Ampicillin is still able to

control typhoid fever but this may not last too long as resistance will likely develop and spread(39).

In Japan sulfonamides were first effective against bacillary dysentery due to Shigella, and by 1945 large amounts were being used. However, the effectiveness only lasted about 5 years and by 1952 Shigella resistant strains reached a peak. By then streptomycin, tetracycline and chloramphenicol were beginning to be used and were, initially, quite effective. Only a few resistant strains were reported through the 1950's, but as the use of these antibiotics continued and increased, the rate of resistant Shigella strains also rose to reach 80% by 1966. Multiple resistance to all the drugs was common and transfer of this resistance to gut E. coli began to be noticed. The rate was low in normal healthy people, 1%, but in patients undergoing drug therapy the rate was 60% for chloramphenicol patients and 21% for streptomycin patients with TB. Surveys in Japanese hospitals showed E. coli, Enterobacter (Aerobacter), Klebsiella and Proteus strains resistant to 1 or more of the four drugs at rates from 30% in Proteus to 82% in E. coli. Multiple resistance to all 4 drugs simultaneously was found in over 50% of the resistant strains for each genus(61).

Diseases caused by antibiotic resistant bacteria are increasing and are the most frequent hospital infection. Reports from United States sources indicate an annual frequency of 1/100 hospital patients and a fatality rate of 30-50%, or 100 000 fatal infections per year in the United States(5). The increased occurrence of infections by drug resistant bacteria has serious consequences for the growing number of cancer and transplant patients who receive immunosuppressive drugs, and AIDS patients. If their own immune systems can not deal with an infection, and the pathogens are resistant to antibiotics, the patients are in serious, often fatal, difficulty, even though they may have survived the surgery quite successfully.

Coliforms possessing transferable drug resistance are not harmless indicators of water quality. Once in the gut they may colonize the gut, or transfer their resistance to pathogens or gut bacteria(10,18). Their presence has a bearing on the treatment of the infected person and should affect how that person's waste is treated. When drugs are used routinely in feed the proportion of bacteria resistant to a given drug is higher than for farms where such drug feeding does not occur. In one farm the swine herd had 100% multiple resistance to drugs in the gut bacteria; other farms ranged from 89 to 95%. In the case of a disease outbreak finding a useful antibiotic would be difficult since these multiple resistance bacteria also had high transfer rates of plasmids with resistance to other bacteria(50).

In 1969-1970 there was an epidemic of Shigella dysenteriae, type 1, in Central America and many of the cases were imported into the United States. The extent of the epidemic, and its severity were unusual. S. dysenteriae had been a major cause of bacillary dysentery prior to 1915 but had virtually disappeared by 1950 except for isolated populations in some developing countries. Most of the strains isolated during the epidemic were resistant to sulfonamides, streptomycin, tetracycline and chloramphenicol and all could transfer such resistance to E. coli(53).

High levels of fecal coliforms with transferable drug resistance, in shellfish and water near marine sewage outfall are not harmless indicators of pollution. Since shellfish are often eaten raw they are a health hazard because they may result in gut microflora becoming resistant to many drugs, even if no disease symptoms occur from eating the shellfish. During a subsequent illness the pathogen may become resistant by transfer from existing gut bacteria. Multiple resistance transfer occurs regularly as one plasmid accumulates resistance factors to many different drugs(60).

Documentation of Transferable Drug Resistance

Pseudomonas aeruginosa was able to transfer resistance to streptomycin and sulfonamides, to E. coli, and resistance to tetracycline to another strain of P. aeruginosa(55). Klebsiella, Proteus and E. coli with resistance to chloramphenicol, streptomycin and tetracycline can transfer this resistance to Salmonella typhi(39). Documentation of transferable drug resistance is given in Tables 1 and 2. Table 1 is arranged by drug group and Table 2 by organism; both give lists of references to the documentation. These are the numbers of the references in the bibliography. Table 1 lists drug groups, rather than individual drugs, since drugs are often in families of related or derived drugs, the mechanism of resistance is often common for a whole group and cross-resistance is common. Table 3 lists the drug groups and the individual drugs in each group. Only transferable drug resistance is documented in the tables; if drug resistance were the only criterion the tables would be much more extensive.

TABLE 1

Documented Transferable Drug Resistance - By Drug Group

drug group	species/genus/group*	references
Aminoglycosides - dihydrostreptomycin - gentamicin - kanamycin - neomycin - paromomycin - streptomycin	<u>Acinetobacter</u> spp. <u>Aeromonas</u> spp. <u>Aeromonas hydrophila</u> <u>Aeromonas liquifaciens</u> <u>Bacteroides ochraceus</u> <u>Citrobacter</u> spp. <u>Enterobacter</u> spp. <u>Escherichia coli</u>  fecal coliforms  <u>Flavobacter</u> spp. <u>Klebsiella</u> spp. <u>Moroxella</u> spp. <u>Proteus</u> spp. <u>Pseudomonas</u> spp. <u>Pseudomonas aeruginosa</u> <u>Salmonella</u> spp.  <u>Salmonella paratyphi</u> <u>Salmonella typhi</u> <u>Salmonella typhimurium</u> <u>Shigella</u> spp. <u>Shigella dysenteriae</u> <u>Shigella flexneri</u> total coliforms	27 13,25,26,28 13 28 37 25, 25,26 7,8,11,12,15,23, 25,26,27,34,50, 51,52 7,8,10,11,12,15, 21,23,25,26,27, 34,39,40,41,45, 50,51,52 27 25,26,39 27 39 27,55 55 10,12,14,29,35, 39,40,57 40 12,14,29,35,39 40 12,29,35,53 12,29,35,53 12 7,8,10,11,12,15, 20,21,23,25,26 27,34,39,40,41, 45,50,51,52
cephalosporins - cephaloridine - cephalothin	<u>Aeromonas</u> spp. <u>Bacteroides fragilis</u> <u>Citrobacter</u> spp. <u>Enterobacter</u> <u>Escherichia coli</u> fecal coliforms <u>Flavobacter</u> spp. <u>Klebsiella</u> spp. <u>Salmonella</u> spp. total coliforms	25,26 54 25 25,26 23,25,26,50 10,23,25,26,50, 26 25,26 10,57 10,11,23,25,26, 50

Continued

drug group	species/genus/group*	references
Chloramphenicol - chloromycetin	<u>Acinetobacter</u> spp. <u>Aeromonas</u> spp. <u>Aeromonas hydrophila</u> <u>Aeromonas liquifaciens</u> <u>Bacteroides</u> <u>Bacteroides fragilis</u> <u>Bacteroides ochraceus</u> <u>Enterobacter</u> spp. <u>Escherichia coli</u>  fecal coliforms  <u>Flavobacter</u> spp. <u>Klebsiella</u> spp. <u>Moraxella</u> spp. <u>Proteus</u> spp. <u>Pseudomonas</u> spp. <u>Salmonella</u> spp.  <u>Salmonella paratyphi</u> <u>Salmonella typhi</u>  <u>Salmonella typhimurium</u> <u>Shigella</u> spp. <u>Shigella dysenteriae</u> <u>Shigella flexneri</u> <u>Streptococcus pneumoniae</u> total coliforms	27 13,26,28 13 28 37,54 54 37 25 7,8,11,12,15,23, 25,26,34,39,51, 52 7,8,10,11,12,15, 21,23,25,26,34, 39,41,51,52 27 39 27 39 27 9,12,14,29,35, 39,40,57 40 9,12,14,29,35, 39 40 12,29,35 12,29,35,53 12 58 7,8,10,11,12,15, 20,21,23,25,26, 34,39,41,51,52
furazolidone - furoxone	fecal coliforms <u>Salmonella</u> spp. total coliforms	10 10 10
nalidixic acid	<u>Acinetobacter</u> spp. <u>Escherichia coli</u> fecal coliforms <u>Flavobacter</u> spp. <u>Moraxella</u> spp. <u>Pseudomonas</u> spp. <u>Salmonella</u> spp. total coliforms	27 23,27 10,23,27 27 27 27 10 10,23,27

Continued

drug group	species/genus/group*	references
nitrofurantoin - nitrofurant	<u>Aeromonas liquifaciens</u>	28
	<u>Escherichia coli</u>	25
	fecal coliforms	25
	<u>Klebsiella spp.</u>	25
	total coliforms	25
nitrofurazone - nitrofuraz	<u>Acinetobacter spp.</u>	27
	<u>Escherichia coli</u>	27
	fecal coliforms	27
	<u>Flavobacter spp.</u>	27
	<u>Moraxella spp.</u>	27
	<u>Pseudomonas spp.</u>	27
total coliforms	27	
Penicillins - aminobenzyl penicillin - amoxicillin - ampicillin - carbenicillin - penicillin	<u>Acinetobacter spp.</u>	27
	<u>Aeromonas spp.</u>	13,25,26,28
	<u>Aeromonas hydrophila</u>	13
	<u>Aeromonas liquifaciens</u>	28
	<u>Alcaligenes faecalis</u>	32
	<u>Bacteroides fragilis</u>	54
	<u>Citrobacter spp.</u>	25,26,27
	<u>Enterobacter spp.</u>	26,32,43
	<u>Enterobacter aerogenes</u>	32
	<u>Escherichia coli</u>	7,8,11,15,23,24, 25,26,27,32,34, 50,51
	fecal coliforms	7,8,10,11,15,21, 23,24,25,26,27, 32,34,41,50,51
	<u>Flavobacter spp.</u>	26,27
	<u>Klebsiella spp.</u>	25,26
	<u>Moraxella spp.</u>	27
	<u>Pseudomonas spp.</u>	27,56
	<u>Pseudomonas aeruginosa</u>	56
	<u>Salmonella spp.</u>	10,12,14,40,57
	<u>Salmonella paratyphi</u>	40
	<u>Salmonella typhi</u>	12,14
<u>Salmonella typhimurium</u>	40	
total coliforms	7,8,10,11,15,20, 21,23,24,25,26, 27,32,34,41,50, 51	

Continued

drug group	species/genus/group*	references
Polymixins - colistin - colistin sulfate - polymyxin B - polymyxin E	<u>Aeromonas</u> spp. <u>Aeromonas hydrophila</u> <u>Escherichia coli</u> fecal coliforms total coliforms	13 13 25,26 10,25,26 10,25,26
Sulfonamides - sulfachlorpyridazine - sulfadiazine - sulfadimidine - sulfafurazole  - sulfamethazine  - sulf amethoxypridazine  - sulfanilamide - sulfasomadine - sulfisoxazole	<u>Aeromonas</u> spp. <u>Aeromonas liquifaciens</u> <u>Citrobacter</u> spp. <u>Enterobacter</u> spp. <u>Escherichia coli</u>  fecal coliforms  <u>Pseudomonas aeruginosa</u>  <u>Salmonella</u> spp.  <u>Salmonella paratyphi</u> <u>Salmonella typhi</u> <u>Salmonella typhimurium</u> <u>Shigella</u> spp. <u>Shigella dysenteriae</u> <u>Shigella flexneri</u> total coliforms	25,28 28 25 25,26 11,12,15,23,25, 34,50,52 11,12,15,23,25, 26,34,41,50,52, 55  12,14,29,35,40, 57 40 12,14,29,35 40 12,29,35 12,29,35,53 12 11,12,15,23,25, 26,34,41,50,52
tetracyclines - chlortetracycline - minocycline - oxytetracycline - tetracycline	<u>Acinetobacter</u> spp. <u>Aeromonas</u> spp. <u>Aeromonas hydrophila</u> <u>Aeromonas liquifaciens</u> <u>Bacteroides</u> spp. <u>Bacteroides fragilis</u> <u>Bacteroides ochraceus</u> <u>Citrobacter</u> spp. <u>Enterobacter</u> spp. <u>Escherichia coli</u>  fecal coliforms  <u>Flavobacter</u> spp. <u>Klebsiella</u> spp. <u>Moraxella</u> spp. <u>Proteus</u> spp. <u>Pseudomonas</u> spp. <u>Pseudomonas aeruginosa</u>	27 13,28 13 28 37,54 54 37 25 25,26 7,8,11,12,15,23, 25,26,27,34,50, 51,52 7,8,10,11,12,15, 23,25,26,27,34, 39,41,45,50,51, 52 27 25,39 27 39 27,55 55

Continued

drug group	species/genus/group*	references
	<u>Salmonella</u> spp. <u>Salmonella typhi</u> <u>Salmonella typhimurium</u> <u>Shigella</u> spp. <u>Shigella dysenteriae</u> <u>Shigella flexneri</u> total coliforms	10,12,14,27,29, 35,39,40,57 12,14,29,35,39 40 12,29,35,53 12,29,35,53 12 7,8,10,11,12,15, 20,23,25,26,27, 34,39,41,45,50 51,52

\* Aerobacter is found under Enterobacter; Cytophaga is found under Flavobacter

TABLE 2

Documented Transferable Drug Resistance-By Organism

species/genus/group*	drug group	references
<u>Acinetobacter</u> spp.	aminoglycosides chloramphenicol nalidixic acid penicillins nitrofurazone tetracyclines	27 27 27 27 27 27
<u>Aeromonas</u> spp.	aminoglycosides cephalosporins chloramphenicol nitrofurantoin penicillins polymixins sulfonamides tetracyclines	13,25,26,28 25,26 13,26,28 28 13,25,26,28 13 25,28 13,28
<u>Aeromonas hydrophila</u>	aminoglycosides chloramphenicol penicillins polymixins tetracyclines	13 13 13 13 13
<u>Aeromonas liquifaciens</u>	aminoglycosides chloramphenicol nitrofurantoin penicillins sulfonamides tetracyclines	28 28 28 28 28 28
<u>Alcaligenes faecalis</u>	penicillins	32
<u>Bacteroides</u> spp.	aminoglycosides cephalosporins chloramphenicol penicillins tetracyclines	37 54 37,54 54 37,54

Continued

species/genus/group*	drug group	references
<u>Bacteroides fragilis</u>	cephalosporins	54
	chloramphenicol	54
	penicillins	54
	tetracyclines	54
<u>Bacteroides ochraceus</u>	aminoglycosides	37
	chloramphenicol	37
	tetracyclines	37
<u>Citrobacter spp.</u>	aminoglycosides	25
	cephalosporins	25
	penicillins	25
	sulfonamides	25
	tetracyclines	25
<u>Enterobacter spp.</u>	aminoglycosides	25,26
	cephalosporins	25,26
	chloramphenicol	25
	penicillins	25,26,32
	sulfonamides	25,26
	tetracyclines	25,26
<u>Enterobacter aerogenes</u>	penicillins	32
<u>Escherichia coli</u>	aminoglycosides	7,8,11,12,15,23 25,26,27,34,50, 51,52
	cephalosporins	23,25,26,50
	chloramphenicol	7,8,11,12,15,23, 25,26,34,39,51, 52
	nalidixic acid	23,27
	nitrofurantoin	25
	nitrofurazone	27
	penicillins	7,8,11,15,23,24 25,26,27,32,34, 50,51
	polymixins	25,26
	sulfonamide	11,12,15,23,25, 34,50,52
	tetracyclines	7,8,11,12,15,23, 25,26,27,34,50, 51,52

Continued

species/genus/group*	drug group	references
fecal coliforms	aminoglycosides cephalosporins chloramphenicol furazolidone nalidixic acid nitrofurantoin nitrofurazone penicillins polymixins sulfonamides tetracyclines	7,8,10,11,12,15, 21,23,25,26,27, 34,39,40,41,45 50,51,52 10,23,25,26,50 7,8,10,11,12,15, 21,23,25,26,34, 39,41,51,52 10 10,23,27 25 27 7,8,10,11,15,21, 23,24,25,26,27, 32,34,41,50,51 10,25,26 11,12,15,23,25, 26,34,41,50,52 7,8,10,11,12,15, 23,25,26,27,34, 39,41,45,50,51, 52
<u>Flavobacter</u> spp.	aminoglycosides cephalosporins chloramphenicol nalidixic acid nitrofurazone penicillins tetracyclines	27 26 27 27 27 26,27 27
<u>Klebsiella</u> spp.	aminoglycosides cephalosporins chloramphenicol nitrofurantoin penicillins tetracyclines	25,26,39 25,26 39 25 25,26 25,39
<u>Moraxella</u> spp.	aminoglycosides chloramphenicol nalidixic acid nitrofurazone penicillins tetracyclines	27 27 27 27 27 27

Continued

species/genus/group*	drug group	references
<u>Proteus</u> spp.	cephalosporins	39
	chloramphenicol	39
	tetracyclines	39
<u>Pseudomonas</u> spp.	aminoglycosides	27,55
	chloramphenicol	27
	nalidixic acid	27
	nitrofurazone	27
	penicillins	27,56
	sulfonamides	55
	tetracyclines	27,55
<u>Pseudomonas aeruginosa</u>	aminoglycosides	55
	penicillins	56
	sulfonamides	55
	tetracyclines	55
<u>Salmonella</u> spp.	aminoglycosides	10,12,14,29,35, 39,40,57
	cephalosporins	10,57
	chloramphenicol	9,12,14,29,35,39 40,57
	furazolidone	10
	nalidixic acid	10
	penicillins	10,12,14,40,57
	polymixins	10
	sulfonamides	12,14,29,35,40, 57
	tetracyclines	29
	<u>Salmonella paratyphi</u>	aminoglycosides
chloramphenicol		40
penicillins		40
sulfonamides		40
<u>Salmonella typhi</u>	aminoglycosides	12,14,29,35,39
	chloramphenicol	9,12,14,29,35, 39
	penicillins	12,14
	sulfonamides	12,14,29,35
	tetracyclines	12,14,29,35,39

Continued

species/genus/group*	drug group	references
<u>Salmonella typhimurium</u>	aminoglycosides chloramphenicol penicillins sulfonamides tetracyclines	40 40 40 40 40
<u>Shigella spp.</u>	aminoglycosides chloramphenicol sulfonamides tetracyclines	12,29,35 12,29,35 12,29,35 12,29,35
<u>Shigella dysenteriae</u>	aminoglycosides chloramphenicol sulfonamides tetracyclines	12,29,35,53 12,29,35,53 12,29,35,53 12,29,35,53
<u>Shigella flexneri</u>	aminoglycosides chloramphenicol sulfonamides tetracyclines	12 12 12 12
<u>Streptococcus pneumoniae</u>	chloramphenicol	58
total coliforms	aminoglycosides  cephalosporins  chloramphenicol  furazolidone nalidixic acid nitrofurantoin nitrofurazone	7,8,10,11,12,15, 20,21,23,25,26, 27,34,39,40,41, 45,50,51,52 10,11,23,25,26, 50 7,8,10,11,12,15, 20,21,23,25,26, 34,39,41,51,52 10 10,23,27 25 27

continued

species/genus/group*	drug group	references
	penicillins	7,8,10,11,15,20, 21,23,24,25,26, 27,32,34,41,50, 51
	polymixins	10,25,26
	sulfonamides	11,12,15,23,25, 26,34,41,50,52
	tetracyclines	7,8,10,11,12,15, 20,23,25,26,27, 34,39,41,45,50, 51,52

\* Aerobacter is found under Enterobacter; Cytophaga is found under Flavobacter.

TABLE 3

## Groups of Related Drugs\*

<b>Aminoglycosides</b> dihydrostreptomycin gentamicin kanamycin neomycin paromomycin streptomycin	<b>Penicillins</b> aminobenzyl penicillin amoxicillin ampicillin carbenicillin
<b>Cephalosporins</b> cephaloridine cephalothin	<b>Polymixins</b> colistin colistin sulfate polymixin B polymixin E
<b>Chloramphenicol</b> chloromycetin	<b>Sulfonamides</b> sulfachlorpyridazine sulfadiazine sulfadimidine sulfafurazole sulfamethazine sulfamethoxypyridazine sulfanilamide sulfasomazine sulfisoxazole
<b>Furazolidone</b> furoxone	
<b>Nalidixic acid</b>	
<b>Nitrofurantoin</b> nitrofuran nitrofurant	
<b>Nitrofurazone</b> nitrofural	<b>Tetracyclines</b> chlortetracycline minocycline oxytetracycline

\* includes some synonyms for identical drugs

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