Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria

Report of a WHO Meeting

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WHO MEETING ON SURVEILLANCE FOR THE PREVENTION
AND CONTROL OF HEALTH HAZARDS DUE
TO ANTIBiotic-RESISTANT ENTEROBACTERIA

Geneva, 18-24 October 1977

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SURVEILLANCE FOR THE PREVENTION AND CONTROL OF HEALTH HAZARDS DUE TO ANTIBIOTIC-RESISTANT ENTEROBACTERIA

Report of a WHO Meeting

A WHO Meeting on Surveillance for the Prevention and Control of Health Hazards due to Antibiotic-resistant Enterobacteria was held in Geneva from 18 to 24 October 1977. The Meeting was opened on behalf of the Director-General by Dr A. Zahra, Director, Division of Communicable Diseases.

1. INTRODUCTION

The World Health Organization has taken an active interest in the problem of antibiotic resistance. In 1960 a first attempt was made to standardize the laboratory test for determining susceptibility (1), although it was only in 1976 that the WHO Expert Committee on Biological Standardization drew up requirements for antibiotic susceptibility tests using the disc method (Requirements for Biological Substances No. 26) (2). In the meantime, the public health aspects of antibiotics in foodstuffs and in the environment were examined by three WHO expert committees (3–5) and by two meetings organized by the WHO Regional Office for Europe.1,2 In 1974, a WHO Collaborating Centre for Research and Reference Services for Biological Products, in Particular Antibiotics and Antibiotic Substances, was established in Atlanta, GA, USA, and in 1975 the WHO Collaborating Centre for Reference and Research on Enteric Phage-typing at the Enteric Reference Laboratory, London, England, expanded its terms of reference to include international studies on antibiotic resistance in enterobacteria.


Outbreaks of infection due to drug-resistant organisms are an increasing problem in both the developing and the developed countries. This problem has been brought into prominence by the recent widespread outbreaks of enteric diseases caused by drug-resistant organisms; delayed recognition of drug resistance has, on several occasions, caused unnecessary suffering and loss of life. An equally serious problem is that of nosocomial infections with drug-resistant opportunistic pathogens of intestinal origin which invade the bloodstream, surgical wounds, the urinary tract and other tissues, particularly in enfeebled hosts. The problem is global and is the result of the widespread and indiscriminate use of antimicrobial drugs in man and animals.

There is a need for more rational and coordinated administration of antibiotics and for strict measures against their abuse. Uncontrolled and excessive use of antibiotics in man and animals results in an increase in antibiotic resistance and diminishes the effectiveness of these life-saving drugs. The problem is a man-made one and could be mitigated if agreement were reached on a more rational approach to the use of antibiotics. Unfortunately, current practices and the limited awareness both of health administrations and of the general public make such an approach difficult to adopt.

The Meeting was convened to review the current problems caused by antibiotic resistance among the enterobacteria and related organisms on a worldwide basis, and the factors involved in the spread of resistant organisms. The participants also reviewed methods of surveillance of resistance in enterobacteria and recommended simple methods whose operation would generate internationally comparable data. This could facilitate the appropriate use of antibiotics and permit early recognition of epidemics caused by resistant bacteria, so that control measures could be rapidly applied. The objective was to promote the development of national and international policies for antibiotic use.

This report summarizes the present state of the problem and proposes action which the participants considered might be feasible and effective for reducing the frequency and ill effects of antibiotic resistance in the enterobacteria.

REFERENCES


2. EXPLANATION OF CERTAIN TERMS

The term “resistance” is used to describe several different phenomena, and the following explanations provide more precise descriptions.

2.1 Antibiotic resistance in vitro

A susceptible strain is one that is consistently inhibited by a particular low concentration of a given antibiotic. An organism is resistant when it tolerates a concentration of antibiotic significantly higher than that which inhibits the growth of susceptible strains of the same species in vitro. In vitro predictions generally serve as a useful guide for clinical purposes, though the clinical outcome may sometimes vary for a number of reasons.

2.2 Natural resistance and acquired resistance

Some organisms are naturally resistant to antibiotics—for example, pseudomonads generally possess a barrier to the penetration of ampicillin and are therefore uniformly resistant. They are susceptible to carbenicillin but can acquire genetically the ability to produce an enzyme that inactivates the drug; this is an example of acquired resistance. Many other instances of natural resistance occur among the enterobacteria, such as that of most Proteus species to the tetracyclines and to colistin, of most Klebsiella to ampicillin, and of most Enterobacter to some cephalosporins.

2.3 Biochemical and genetic mechanisms of resistance

The biochemical mechanisms by which bacteria display resistance include the production of enzymes that destroy or modify the antibiotic, possession of a permeability barrier that prevents access to the bacterial cells, or alteration of the target site that is normally attacked by the antibiotic.
Genetic mechanisms by which bacteria acquire resistance include chromosomal mutation and also the acquisition of extrachromosomal elements (resistance plasmids). Plasmids code for a variety of characters and are now by far the major source of acquired antibiotic resistance in enterobacteria. They usually code for a high level of resistance, which may be multiple. The mechanisms of plasmid-mediated resistance are generally different from those of resistance of chromosomal origin; the resistances to nalidixic acid and nitrofurans are of chromosomal origin, while those to other antibiotics are plasmidborne.

2.4 Abbreviations and definitions

For the sake of convenience, the following abbreviations of the names of antimicrobial agents are used in this report when reference is made to multiple resistance:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Abbreviation</th>
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<td>Ampicillin</td>
<td>A</td>
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<tr>
<td>Amikacin</td>
<td>Ak</td>
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<tr>
<td>Benzylpenicillin</td>
<td>P</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>Cb</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Ce</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>C</td>
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<tr>
<td>Clindamycin</td>
<td>Cl</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>G</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>K</td>
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<tr>
<td>Nalidixic acid</td>
<td>Nx</td>
</tr>
<tr>
<td>Neomycin</td>
<td>N</td>
</tr>
<tr>
<td>Nitrofuran</td>
<td>Nu</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>Po</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Ri</td>
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<tr>
<td>Sisomicin</td>
<td>Si</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
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<tr>
<td>Sulfonamides</td>
<td>Su</td>
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<tr>
<td>Tetracyclines</td>
<td>T</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>To</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Tm</td>
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The word "antibiotic", as used in this report, signifies all antibacterial drugs of natural or synthetic origin. The names of groups of antibiotics used in the text are aminoglycosides (gentamicin, kanamycin, streptomycin), β-lactams (penicillins and cephalosporins) and macrolides (erythromycin, spiramycin and related compounds).

The term "enterobacteria" as used herein refers to Gram-negative aerobic rods found in the intestine which are members of the family Enterobacteriaceae.

3. PRESENT STATUS OF ANTIBIOTIC RESISTANCE IN ENTEROBACTERIA

3.1 Occurrence of antibiotic resistance in bacteria infecting man and animals

A search of the world literature, including a MEDLARS survey of literature on antibiotic resistance, highlighted the following points:
(1) There is a preponderance of reports on the occurrence of resistant strains in developed countries, and from investigators with a special interest in antibiotic resistance, and a shortage of information from the developing countries.

(2) In many countries there is a lack of standardization of methodology in the testing and reporting of bacterial resistance. This makes comparisons of information difficult.

(3) The time-span over which studies are made varies considerably—which is important because the situation may show considerable fluctuations with time; rapid changes may occur because of the emergence and spread of resistant strains.

However, allowing for the patchy distribution of the reported results and the variations in defining resistance, a few examples are quoted below to illustrate discernible trends.

3.1.1 Escherichia coli

Antibiotic-resistant strains of E. coli are prevalent in the general population of healthy individuals. For example, in the United Kingdom, about 15% of strains are resistant to ampicillin and 20% to streptomycin and/or the tetracyclines (1). In Japan, in 1974, tetracycline resistance was found in 70%, streptomycin resistance in 75%, and chloramphenicol resistance in 50% of isolates of E. coli from human sources. Multi-resistant strains were also shown to be frequent in some areas: 12% of the isolates in Sweden in 1971 were resistant to ampicillin, chloramphenicol and tetracycline (2). No distinction has been made in this review between strains isolated from the community and those isolated in hospital. Moreover, attention should be directed to the patchy distribution of resistance that occurs within a country, which may be due to local predominance of a single resistant strain of E. coli.

The few studies that have been carried out on animal strains—for example, in the USA—show a very high prevalence of resistance, particularly to the tetracyclines (91% in pigs) and to streptomycin (94% in calves) (3). The low prevalence of resistant strains of E. coli in wild animals is an indication of the role that animal husbandry, especially the use of antibiotics in animal feeds, may play in producing the high isolation rate of resistant strains from livestock.
3.1.2 Shigella

Antibiotic resistance is very frequent in *Shigella*. For example, 90% of strains of *S. sonnei* in the United Kingdom were resistant to ampicillin in 1970 (4); 48–54% of strains of these shigellae were resistant to the tetracyclines and streptomycin in New York City, USA, in 1973 (5). Chloramphenicol resistance has not been a predominant feature in survey data, although epidemics have been caused by chloramphenicol-resistant strains. The prevalence of resistance in shigellae in Algeria (6), in developing countries of the Western Pacific, and in Papua New Guinea (7) is reported to be considerably lower than in developed countries.

3.1.3 Salmonella

Considerable variation is found in the incidence of antibiotic resistance in salmonellae, particularly in multiresistant strains, in reports from different countries and, at times, from different areas of the same country (8–10). It was noted in a survey of animal strains that resistance was more prevalent in *S. typhimurium*, generally the commonest *Salmonella* serotype, than in other salmonellae. Isolated foci of resistance are also reported, such as the high incidence (90%) of streptomycin resistance in *S. dublin* in the Netherlands in 1971 (11). A large outbreak may, however, be caused by a single strain—i.e., a clone of one or more *Salmonella* serotypes—which makes it difficult to compare incidence in different areas or countries.

Information derived from data collected in the FAO/WHO Collaborating Centre for Research and Training in Food Hygiene, Berlin (West), emphasizes the variations in antibiotic resistance seen in salmonellae of animal origin. Over 1000 strains of salmonellae were examined each year from 1971 to 1976. Table 1 shows that during this period there was a rise and fall in resistance in *S. typhimurium*, a fall in resistance in *S. Panama*, and a rise in resistance in *S. dublin*.

Tetracycline resistance in *Salmonella* in general remained at a constantly high level over the 6-year period and the use of tetracyclines as a feed additive was discontinued at the end of 1976. Resistance to chloramphenicol had risen to almost 50% and that to kanamycin to 25% by 1976. The association of these strains with infections in man in the Federal Republic of Germany remains unclear.

In the USA, overall resistance more than doubled in the period 1967–1975, the proportion of resistant strains rising from 22% to 50%.

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1 Unpublished data.
Table 1
Variation in antibiotic resistance in *Salmonella* strains isolated from domestic animals in the Federal Republic of Germany

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Year of isolation</th>
<th>Percentage resistant</th>
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<tbody>
<tr>
<td><em>S. typhimurium</em></td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td><em>S. typhimurium</em> (var. Copenhagen)</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td><em>S. paratyphi</em> a</td>
<td>65</td>
<td>58</td>
</tr>
<tr>
<td><em>S. dublin</em></td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Resistance in *S. typhimurium* increased 1.7 times and a greater increase, 2.8-fold, was seen in resistance of other serotypes. Multiple resistance increased significantly, from 15% to 26%, and the proportion of *Salmonella* strains with resistance to 6 or more antibiotics increased from 0.8% to 9.2%. As has been indicated, this may have been determined by the prevalence of a resistant clone, which could have been recognized by examination for genetic markers.

### 3.2 Outbreaks of human infection due to drug-resistant enterobacteria

Several types of infection may occur with antibiotic-resistant strains of enterobacteria—namely:
- shigellosis;
- typhoid fever;
- *Salmonella* enteritis associated with strains of animal origin;
- nosocomial infections with *Salmonella* species, particularly in neonatal and paediatric units;
- nosocomial infections of extraintestinal sites with other enterobacteria, such as *Klebsiella*, *Proteus*, *Serratia* and *Enterobacter*.

#### 3.2.1 Shigellosis

*Shigella dysenteriae* type 1 (Shiga's bacillus), a highly virulent pathogen, caused an extensive outbreak that was first recognized in Guatemala and spread throughout Central America from 1968 to 1972. Severe
bacillary dysentery affected hundreds of thousands or persons and caused
tens of thousands of deaths (12). Disease caused by this antibiotic-
resistant (CSSuT) strain was initially misdiagnosed as amoebiasis—partly
because it did not respond to the antimicrobial drugs in common use;
this delay in diagnosis contributed greatly to the formidable mortality.
A widespread outbreak due to drug-resistant S. dysenteriae type 1 also
occurred in Bangladesh, which persisted for several years; in 1973, 33%
of the entire population of the island of St Martin in the Bay of Bengal
was affected over a period of 3 months (13).

Infections with multiresistant strains of S. sonnet have been a persistent
problem in many countries. For example, in the USA, 194 students
and 14 staff members were involved in a waterborne outbreak due to a
resistant (ASSuT) strain in a school in 1972 (14). In 1973, 12 cases with
2 deaths occurred at an institution for mentally retarded children; the
responsible strains were resistant to AST (15). A high prevalence of
ampicillin resistance in S. sonnet has been reported from many centres
in the USA and elsewhere since 1967.

3.2.2 Typhoid fever

Although chloramphenicol-resistant Salmonella typhi had been
described as early as 1950, the first recorded epidemic caused by a
resistant strain occurred in Mexico in 1972–1973 (16, 17). This outbreak
was due to a multiresistant (CSSuT) strain with a characteristic phage-
typing pattern; the compatibility group to which the plasmid carrying
chloramphenicol resistance belonged was designated H1. The outbreak
was unique, not only because it was caused by a drug-resistant strain,
but also because of its long duration (over 2 years) and wide distribution
(several states in central Mexico). There is no accurate record of the
number of cases that occurred, but conservative estimates suggest that
there were many thousands. The case-fatality rate early in this epidemic
was similar to that experienced before the antibiotic era, but after it was
determined that the organism was chloramphenicol-resistant, treatment
with ampicillin is reported to have led to a dramatic reduction in the
death rate.

In India, the first documented outbreak due to a resistance (R)-factor-
carrying, multiresistant (CSSuT) strain of S. typhi (Vi phage type D1-N,
later replaced by type C5 with the same resistance pattern) took place
in Calicut (Kerala) in 1972 (18). The type C5 focus has persisted but has
not spread widely. A localized outbreak occurred in 1976 at Ernakulum,
about 20 kilometres south of Calicut, due to phage type A with the same
CSSuT resistance pattern. This focus, too, has shown no tendency to
spread (19). The plasmid responsible belonged in all cases to compatibility group H₁. Since then, outbreaks due to R-factor-carrying salmonellae of other serotypes have been reported from other parts of India. In 1976 single sporadic isolates were received from Trivandrum (Kerala), Madurai (Tamil Nadu) and Bombay (Maharashtra) (19).

In a study carried out by the Southeast Asian Medical Information Center, Tokyo, Japan, strains of *S. typhi* from 3 centres were compared: 87 from Singapore, 198 from Thailand and 390 from Japan. Resistance was not detected in Singapore, and in Japan it was at a very low level, while in Thailand 39% of the strains were resistant, the predominant pattern being CTS with A as an additional resistance in some cases. Antibiotic resistance in *S. paratyphi* A and *S. paratyphi* B was negligible. In Vietnam about 80% of *S. typhi* strains were found to be resistant to many antibiotics (20, 21). Information from Peru indicates that the proportion of strains of *S. typhi* susceptible to chloramphenicol fell from 90% in 1974 to below 30% in 1976; strains remained susceptible to ampicillin. Wherever chloramphenicol-resistant outbreaks of typhoid fever have been detected, the resistance has been mediated by plasmids of compatibility group H₁.

3.2.3 *Salmonella enteritis* associated with strains of animal origin

The drug-resistant strain of *S. typhimurium* that proved most important in early studies in the United Kingdom belonged to phage type 29, which first appeared in epidemic form in 1963–1964. It was found mostly in man and bovines, and its increased prevalence in 1963 coincided with the appearance of transferable resistance to streptomycin and the sulfonamides. Resistance to the tetracyclines became evident early in 1964, followed by that to ampicillin, chloramphenicol, furazolidone and neomycin-kanamycin in succession, while the frequency of the type greatly increased (22).

Studies of cultures from the United Kingdom and a number of other countries revealed that drug-resistant clones of *S. typhimurium* and other salmonellae were causing widespread outbreaks of infection, some on an international scale (23).

Two types of outbreak due to drug-resistant salmonellae (except *S. typhi*) have been identified: those which clearly have an animal origin and those in which no animal source can be identified. The first type is exemplified by the *S. typhimurium* outbreak in the United Kingdom, where the drug resistance is acquired in the animal host and the distribution is related to that of its animal source. The second type is com-
monly seen in paediatric units and is described in the following paragraphs.

3.2.4 Nosocomial infections with Salmonella species

In recent years, multiresistant salmonellae have caused many outbreaks. Morbidity and mortality have been especially great in paediatric units and nurseries.

In these outbreaks no animal source can be identified; they do not coincide with animal epidemics and the strains are not even present in livestock in the affected areas. The outbreaks appear quite suddenly; they are severe, with a morbidity rate of up to 50% and a case-fatality rate of up to 20–30%; septicaemia and meningitis are common. The infecting strain is almost always resistant to a multiplicity of drugs and may carry three or more plasmids.

Documented outbreaks have been associated with *S. typhimurium* (Argentina, Brazil, Chile, Paraguay, USA (Kentucky), and Uruguay), *S. heidelberg* (Puerto Rico), *S. newport* (India (Delhi) and Peru), *S. isangi* (Zaire), *S. virchow* (United Kingdom), *S. alachua* (India (Calcutta)), *S. wien* (Algeria, Belgium, France, Iraq, Italy, United Kingdom, and Yugoslavia). Nosocomial infection has featured prominently in the national spread of multiresistant strains of *S. typhimurium* in Argentina and Uruguay, *S. newport* in Peru, *S. isangi* in Zaire and *S. wien* in Iraq and countries of the Mediterranean region.

3.2.5 Nosocomial infections with other drug-resistant enterobacteria

During the past 20 years Gram-negative bacilli, particularly enterobacteria and pseudomonads, have been the most important agents causing hospital-acquired infections in many countries. Multiple resistance to antibiotics has been the rule in these infections.

Debilitated patients are easily colonized and subsequently infected by opportunistic Gram-negative bacilli, which may gain access to the body by way of intravascular catheters, bladder catheters, inhalation therapy devices, and respirators, or by other invasive techniques.

In 1970–1971 a protracted nationwide outbreak of septicaemia in the USA due to *Enterobacter cloacae* and *E. agglomerans* was traced to contaminated intravenous fluids (24). A similar episode occurred in the United Kingdom in 1973 (25); this was an outbreak due to *Citrobacter freundii* and *E. agglomerans*, also caused by contaminated intravenous fluid.

Most nosocomial outbreaks of infection are due to contact spread. A few examples of a very widespread phenomenon are cited here. One
hundred and thirty cases of infection with *Proteus rettgeri*, with 5 cases of septicaemia and 1 death, were observed in a hospital in Memphis, TN, USA, in 1972. Twenty-eight patients in a hospital in Minneapolis, MN, USA, were infected or colonized with *Klebsiella pneumoniae* resistant to ACbCeCGKSu during 1976. Nosocomial outbreaks involving hundreds of cases of infection with strains of *Serratia marcescens* resistant to many antibiotics, including gentamicin and tobramycin, have been reported from other centres in recent years.

REFERENCES

4. RESISTANCE FACTOR SPREAD FROM ENTEROBACTERIA

During the past few years, it has been found that plasmidborne resistance may spread from enterobacteria to other bacterial species; some significant epidemiological events relating to other Gram-negative bacteria are listed below.

(1) Pseudomonas aeruginosa

In March 1969, Lowbury et al. (1) isolated from patients in a burns unit in Birmingham, England, strains of Pseudomonas that were highly resistant to carbenicillin through the production of β-lactamase. Subsequently, Sykes & Richmond (2) showed resistance transfer from these strains to Escherichia coli K12; the R factor concerned was designated RP1. The resistance was linked to those of ATKCe and the same R factor was also found to be widespread in Proteus spp.

The appearance of gentamicin-resistant Pseudomonas aeruginosa strains reported by Witchit & Chabbert (3) in France was a matter of
considerable clinical concern. These strains had ACSu resistances which were transferred by conjugation to *E. coli* K12. R-factor-carrying strains isolated in the USA (4) were also resistant to the newer aminoglycosides, tobramycin and amikacin.

(2) *Haemophilus influenzae*

In 1974, cases of meningitis were reported that failed to respond to ampicillin because of resistance to that drug. The mechanism of resistance in some of these strains was shown to be due to a β-lactamase similar to the plasmid-mediated β-lactamase \( R_{\text{TEM}} \) (3).

Current prevalence of β-lactamase-producing strains in the United Kingdom remains at a fairly low level. Of 950 strains tested in early 1977, 14 (1.5%) were β-lactamase producing. However, there is some evidence that β-lactamase production is more likely to occur in the encapsulated strains which cause the more serious infections, including meningitis, than in the noncapsulated variants. In the USA ampicillin-resistant strains of *H. influenzae* are responsible for 2–8% of serious infections caused by this organism.

(3) *Neisseria gonorrhoeae*

The acquisition of R factors by *N. gonorrhoeae* created widespread public health concern during 1976. Cases of penicillin-resistant gonorrhoea were observed in visitors or military personnel returning from countries of the Western Pacific area or West Africa to Europe and the USA (6–8). The production of β-lactamase by these strains was shown to be plasmid-mediated. Plasmids of two different sizes have been identified, and there is some evidence that the β-lactamase produced was the same as the β-lactamases of *H. influenzae* and enterobacteria (9).

(4) *Vibrio cholerae*

Resistance is very rarely seen in *V. cholerae*. However, some multi-resistant strains have been described which can transfer their resistance by conjugation, thus confirming the plasmid-mediated nature of their resistance (10). These plasmids are generally unstable in *V. cholerae*, though a stable plasmid has been found in a few strains (11).

The above examples show that resistance plasmids can affect the ecology not only of the enterobacteria but also of other important pathogens. There is some evidence to support the hypothesis that the plasmids concerned are being derived from enterobacteria, which can share a habitat with other pathogens in clinical situations.
Two possible organisms that may be the next to acquire resistance plasmids are *Bacteroides* species and *Neisseria meningitidis*. The latter is normally distributed in the body in a fashion similar to that of *H. influenzae* and the occurrence of penicillin-resistant meningococcal meningitis would create enormous problems in the treatment of cases and the control of epidemics. The acquisition of β-lactamase by the related species *Branhamella catarrhalis* (12) is an indication that such drug resistance could well develop.

REFERENCES


5. FACTORS CONTRIBUTING TO THE EMERGENCE AND SPREAD OF ANTIBIOTIC RESISTANCE

5.1 Antibiotic use in man

The appearance of antibiotic-resistant strains of bacteria is closely linked to antibiotic use for the treatment of human infection (1, 2). Resistance may appear rapidly or slowly, depending on the organism concerned, the volume and type of antibiotic used, and the method of application.

The most clear-cut reports of antibiotic use and resistance have resulted from hospital studies in which outbreaks of nosocomial infections were related to the extensive use of antibiotics. The following are examples: infections with Klebsiella related to the use of ampicillin in a neurosurgical unit (3), infections with pseudomonads resistant to carbenicillin in a burns unit (4), and resistant infections with Serratia associated with the use of gentamicin in an intensive care unit.

In community infections, data from Japan (1) and Poland clearly showed that a rise in tetracycline resistance in pneumococci was closely associated with an increase in tetracycline use. There was a sharp increase in macrolide use in Japan from 1967 onwards, the quantities administered having risen from about 50 000 kilograms a year to almost 200 000 kilograms a year by 1973. Macrolide-resistant strains of group A haemolytic streptococci were first recognized in Japan during the early 1970s and, by 1974, 75% of strains isolated were resistant to erythromycin and lincomycin. Resistance to the tetracyclines and that to chloramphenicol were at a level of 90% and 75% respectively. In a paediatric unit in Tokyo in 1963, all of 250 isolates of Escherichia coli showed CSSuT resistances. Since then, rises in antibiotic resistance have been observed with other agents as they have been introduced. An increase in ampicillin use occurred about 1964 and 27% of E. coli strains were resistant by 1969; an increase in the use of cephalosporin occurred in about 1972, and 31% of strains were resistant by 1975.

Unfortunately, precise data on antibiotic use in many countries are not widely available, but consumption appears to be rising on a worldwide scale. The factors contributing to this rise are complex but include such obvious reasons as the fact that improved medical care has resulted in wider coverage of the population. Recognition of new clinical syndromes

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may require further antibiotic use; there is also an increase in the number of new antibiotics available.

Much of the increasing use of antibacterial drugs is unnecessary (5). On many occasions they are employed without any clinical or laboratory examination. It is not suggested that laboratory support is always feasible or necessary, but there are many cases where rational therapy is possible only if culture data are available. Antibiotics are also used inappropriately in viral infections, in mild bacterial infections, which do not require such treatment, and in the many diarrhoeal diseases in which they are ineffective. In Salmonella gastroenteritis these drugs prolong the period of excretion (6). Authorities in some countries have already advised against the use of antibiotics in the treatment of salmonellosis except in special circumstances (7). There is often prophylactic application without a rational basis, as, for example, in mass chemotherapy against cholera. Other factors, such as pressure exerted by patients and their relatives and promotion by the pharmaceutical industry, also contribute to the rapidly growing use of antibiotics.

5.2 Antibiotic use in animals

The uses of antibiotics in animals are as feed additives and for the prophylaxis or therapy of bacterial infections.

The use of antibiotics as feed additives for growth promotion is widespread. The commonest drugs currently used for this purpose are the tetracyclines—one of the most potent agents for provoking the emergence and selection of resistance plasmids and at the same time a very useful therapeutic agent. Antibiotics have been extensively but unsuccessfully used in animals for the prevention of infectious diarrhoea caused by salmonellae.

It is generally recognized that the use of antibiotics for animal salmonellosis may prolong excretion in animals as it does in man. Salmonella septicemia is, however, accepted as an indication for antimicrobial treatment in animals as well as man, when chloramphenicol is the drug of choice if the strain proves susceptible in laboratory tests.

The rapid emergence and spread of drug-resistant salmonellae resulted from antibiotic use in animals during the 1960s (8). Transmission of these salmonellae to man resulted in many human infections, and the resistances of such strains, plasmidborne and chromosomal, were acquired in the animal host. Resistant enterobacteria colonizing man may not invariably have an animal source.
5.3 The role of food in the spread of antibiotic-resistant bacteria

This subject has been recently reviewed by a WHO Expert Committee on Microbiological Aspects of Food Hygiene (with the participation of FAO) (9) and by a Working Group convened by the WHO Regional Office for Europe. The prevalence of drug-resistant enterobacteria in food has been demonstrated by many workers. The presence of antibiotic-resistant nonpathogenic enterobacteria in food is of public health significance. It is recognized that, although some food containing resistant bacteria is decontaminated during cooking, before being cooked it may contaminate other cooked or uncooked food in the kitchen and so transmit drug-resistant enterobacteria to man. Multiresistant nonpathogenic bacteria may add to the number of drug-resistant bacteria, with their plasmids, in the human intestine, but when the organisms concerned are pathogenic they may also produce foodborne diseases.

5.4 The role of sewage and surface waters

Sewage and surface waters contribute to the distribution and circulation of resistant organisms. They represent a natural medium in which R-plasmid transfer can occur under certain physical, chemical, or biological conditions. Sewage and surface waters contain resistant bacteria from human and animal wastes and can be regarded as a source of all plasmid types, which circulate and are selected under appropriate environmental conditions. These resistant bacteria from sewage and surface waters can be transferred under some circumstances to food and drinking-water, which leads to a recycling to man and animals (10–12).

REFERENCES


6. MEANS OF CONTROLLING THE SPREAD OF ANTIBIOTIC-RESISTANT BACTERIA

To ascertain whether a country has a problem of drug resistance, appropriate clinical specimens from nosocomial and enteric infections must be cultured regularly and the bacteria thus isolated tested for drug resistance on a continuing basis. Systematic collection and analysis of these data could provide an epidemiological basis for clinical and administrative guidance in the prevention and control of spread.

6.1 Rationale of surveillance

Information concerning the drug-resistance patterns of the prevailing pathogenic bacteria and the appearance of new resistance characteristics is of the utmost value for a proper selection of antimicrobial agents for therapeutic purposes. Unawareness of local drug-resistance patterns in pathogens may foster misuse and often overuse of antibiotics, with all their harmful consequences.

A national surveillance programme, in addition to ensuring early detection of the appearance of multiresistant strains, may guide clinicians in choosing the most suitable antimicrobial agent without laboratory examination of the particular isolate (for which facilities may in any case
not be available), and may preclude the blind use of multiple antibiotics. It may also provide a scientific basis for the development of national and international policies concerning the manufacture, importation and use of antibiotics.

6.2 Health and economic benefits

Surveillance data, if properly used by health administrators and others responsible for the formulation of health policies governing the use of antibacterial drugs, could bestow both health and economic benefits. The economic benefits could follow from prevention of wastage and overuse of these agents. The costs of surveillance represent only a small fraction of the expenditure on improper use of antibiotics.

6.3 Surveillance and control of antibiotic-resistant bacteria in man

In the developed countries laboratory investigations are carried out to determine the organisms causing infections in man, and susceptibility tests are performed for clinical purposes as a guide to treatment. Such investigations are also undertaken in developing countries, although they are often limited to large cities. In many countries, such clinical data have also been used to provide epidemiological material for determining the incidence of resistant bacteria in human infections both in the hospital and in the community.

The main requirements for providing useful information in man are therefore to ensure: (a) that the medical microbiological services of a country are sufficiently well developed to study the problem of drug resistance; (b) that susceptibility tests are performed in a reliable manner to identify those antibiotics that need to be excluded because of drug resistance and those that might be clinically useful; and (c) that efficient means are available for collecting, processing and disseminating data as quickly as possible. The means for fulfilling these requirements are discussed in sections 8 and 9. Collection of information of this type should be given a high priority by health authorities.

Control of antibiotic use in man is very difficult, but every effort should be made to ensure the rational use of these drugs. This may be achieved by monitoring the emergence of antibiotic-resistant enterobacteria, formation of national antibiotic utilization committees, nationwide distribution of information concerning antibiotic-susceptibility patterns with profiles of antibiotic use, and establishment by WHO of an effective system for the wide dissemination of appropriate information on these topics.
The problems and control of antibiotic use in man can be dealt with only by educational methods. Steps should be taken to improve undergraduate and postgraduate education in the problem of drug resistance and the importance of rational chemotherapy of bacterial infection. Antibiotic substances should be made available only on prescription. The freedom of physicians to prescribe whatever antibiotics they may deem fit is difficult to limit, but some regulation of the use of a particular antibiotic in special circumstances may be required.

6.4 Surveillance and control of antibiotic-resistant bacteria in animals

The monitoring of enterobacterial resistance in domestic animals, which constitute a reservoir of resistance plasmids and a source of human infection, should be a function of veterinary and public health laboratories. The methodology of sampling and testing should be similar to that used in man.

There are several uses of antibiotics in food and in animals that require attention. The use of antibiotics as food preservatives should be discontinued, as other effective means of prevention of spoilage are available.

Therapeutic antibiotics cannot be used in the United Kingdom as feed additives (I) and the same is true for an increasing number of countries, such as Czechoslovakia, the Federal Republic of Germany and the Scandinavian countries. Restriction of the use of tetracyclines and penicillin in animal feeds appears to be imminent in the USA.

Animal growth promotion is achievable without the use of therapeutic antibiotics as feed additives.

The prophylactic use of antibiotics against infections in domestic animals should be critically reviewed. It should not be a substitute for high standards of animal husbandry. At present, it is not feasible to treat infections with different antibiotics from those used in man. However, such treatment must be on veterinary prescription. The educational needs of veterinarians in the appropriate use of antibiotics are similar to those of physicians.

6.5 Surveillance and control of antibiotic-resistant bacteria in the environment

The environment is continually being polluted by drug-resistant bacteria, but the public health importance of this contamination has not
been fully ascertained. Investigation of the following factors, which will probably vary from place to place, may be important:

1. Transmission of resistant bacteria by food likely to be eaten raw or undercooked.
2. Presence of resistant bacteria in waste waters, sewage and animal wastes.
3. Monitoring and disposal of refuse, particularly from hospitals, abattoirs and buildings used for livestock rearing.
5. Carriage of resistant bacteria by pets and wildlife, including birds, insects and rodents.

Proper legislation to govern food handling and effective implementation of the regulations adopted are of basic importance.

6.6 Control of outbreaks of disease in man

Like all disease outbreaks, those caused by resistant bacteria should be brought under control, but they need energetic epidemiological investigation with a high standard of laboratory support to define the resistance pattern and distribution of the causative agent.

In the absence of trained epidemiologists, microbiologists with suitable experience serve well as epidemiologists. When an outbreak occurs, an appraisal must be made by grouping cases by date of onset, location and special features to enable the source to be traced. Case-control studies or cohort analysis may be necessary. As soon as the evidence of the source and vehicle is available, the relevant laboratory investigation should be instituted. Epidemiological findings should indicate the need for and determine the scope of laboratory investigations. The sampling procedures used will depend on the nature of the outbreak and the prevailing circumstances.

Community-acquired outbreaks of enterobacterial infection are usually foodborne or waterborne. If the responsible vehicle can be identified it must be withdrawn from circulation. If it is not possible to determine the vehicle of transmission, the public—especially in developing countries—should be urged to take hygienic measures (for example, boiling drinking-water and thoroughly cooking meat, shellfish, etc.). Facilities for treating ill persons should be available, as this encourages the reporting of cases, and prompt and effective treatment of cases also
reduces transmission to other persons and to the environment. Restrictions on the movement of people and goods create hardships and economic losses, inhibit the reporting of cases and generate public alarm, thereby hampering efforts to deal rationally with the problem.

Although foodborne and waterborne illness may occur in hospitals, most resistant enterobacteria involved in nosocomial outbreaks are transmitted either by interpersonal contact or via contaminated fomites, although their relative importance varies by site and by pathogen. Control measures should be based on findings in epidemiological investigations. They include the enforcement of handwashing and the use of sterile techniques, the grouping of infected patients and carriers, appropriate isolation of infected patients, the elimination of contaminated fomites, and spatial dispersal of certain patients to minimize cross-infection. Antibiotic therapy is no substitute for recognized measures for the control of infectious diseases.

REFERENCE


7. LABORATORY METHODS FOR THE STUDY OF RESISTANCE IN ENTEROBACTERIA

7.1 Methods for determining the in vitro susceptibility of bacteria

Antibiotic resistance is usually tested with antibiotic-impregnated paper discs which allow diffusion of antibiotic into agar. This is a simple procedure if performed by a standard method. However, obtaining accurate, reliable and reproducible results from susceptibility tests is not simple, as several factors influence the results of testing. These factors can usually be controlled by performing the test simultaneously with reference strains.

Determination of the minimum inhibitory concentrations (MICs) of antibiotics for bacteria is performed on solid or liquid media containing graded concentrations of antibiotics. This gives a numerical answer which may be correlated with attainable blood or other body-fluid levels to
assess whether infections with the organism are likely to respond to therapy.

Routine disc tests for antibiotic resistance are assessed by the diameter of the zone of inhibition, which varies with the diffusibility of the antibiotic and with the growth phase and rate of the organism (1). The problems inherent in this quick and simple method include variations in the composition of the medium recommended for growing the organism, in the standardization of the inoculum, in the method of applying it to the plate, and in the content and stability of the antibiotic used in the disc. The presence of any of these problems should be detected when the control plates are read.

There are two aspects of susceptibility testing that require consideration: standardization of methods and quality control of the results.

Several attempts have been made to produce an internationally acceptable disc method for antibiotic susceptibility testing. These include the modified Kirby-Bauer technique (1975) (2, 3), which is widely used in the USA and elsewhere, the ICS¹ method (1971) (4) and the Stokes technique with internal standards (1972) (5). The objective of a standard method is to provide a defined technique that can be applied to almost all antibiotics and organisms, that will yield results indicating resistance or susceptibility in the majority of tests, and that is comparable with the methods used elsewhere.

It is unlikely that any one method will become the standard method of testing. It is therefore important to ensure that, whatever technique is employed, it has been fully evaluated and standardized before being used for reporting and that the technical details are carefully and precisely followed to avoid variations that will give rise to misleading results.

The main method for checking the correlation of agar diffusion test results with estimation of MICs is the construction of regression lines relating the MIC to the size of the inhibition zone produced by the antibiotic-containing disc. In drawing a regression line the zone diameters are plotted against the log₂ MIC for each organism and a line is drawn to fit the points. The construction of these regression lines for each antibiotic to be tested is essential for the evaluation of each antibiotic/organism combination.

If the modified Kirby-Bauer method is performed according to directions (6), the interpretative tables given for that method for the rapidly growing Enterobacteriaceae may be used, and the contraction

¹ International Collaborative Study, sponsored by WHO and developed by the Karolinska Institute, Stockholm, Sweden.
of regression lines is not required. However, if another established method is employed, then the regression lines specifically developed for that method must be used.

Validation of the results of testing

There are several ways of validating the results. This is necessary to ensure that the in vitro results obtained are of value in prescribing suitable antibiotics for individual patients and also to permit the comparison of results obtained from different centres. Unless validation is carried out, results cannot be compared with any confidence. To ensure the reliability of the disc test all the following controls should be applied:

1. **Reference culture used for internal quality control**

   Reference strains are necessary to control the performance of the tests. With some methods a reference organism is included on the plate so that each test is controlled. Other methods require the use of a separate plate with reference strains for control. The number of such reference strains for control varies considerably between laboratories. The cultures that are usually used are susceptible strains of *Escherichia coli* (ATCC 25922; NCTC 10418), *Staphylococcus aureus* (ATCC 29213; NCTC 6571) and *Pseudomonas aeruginosa* (ATCC 27853; NCTC 10662), which indicate deficiencies in the medium, inoculum and disc content. The appropriate control strain (or strains) should be used with each batch of routine daily tests. Sets of sequential values of zone diameter for these cultures are used for the evaluation of precision, accuracy and variability.

2. **Control strains for resistance**

   In order to test the performance of a method, some laboratories use a number of strains of *E. coli* with different antibiotic susceptibility patterns. These can also be used selectively in a control test on a batch of clinical isolates. The 12 strains listed in Annex 1 are satisfactory for checking the performance of methods devised for determining susceptibility in rapidly growing Enterobacteriaceae.

   The twenty-eighth report of the WHO Expert Committee on Biological Standardization (6) gives details for the performance of the modified

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1. These strains can be obtained from the American Type Culture Collection, 12301 Parkland Drive, Rockville, MD 20852, USA, or the National Collection of Type Cultures of Micro-organisms, Central Public Health Laboratory, Colindale Avenue, London, NW9, England.

2. These strains, along with details of methods for preserving the plasmids, are available from the Department of Medical Microbiology, The London Hospital Medical College, London E1 2AD, England.
Kirby-Bauer technique. The recommendations for disc antibiotic content are based on those required for that technique.

In some countries where there is variation in the composition of the media and disc content, a method using controls on each plate—e.g., the Stokes method—may provide a more satisfactory procedure for clinical purposes than techniques which use a separate plate for examining the reference strains. Some technical details with regard to disc testing are summarized in Annex 1.

7.2 The nature and identification of resistance plasmids

A bacterium possesses a circular chromosome determining its genetic characters. The chromosome consists of DNA which is about 1000 \( \mu \)m in length and is regarded as constituting a single molecule.

Like the bacterial chromosome, resistance plasmids consist of circular DNA molecules the size of which is about 1–5% of that of the chromosomal DNA. Transferable resistance plasmids (R factors) consist of two different parts: one codes for drug resistance and the other for transfer. These parts may be linked so as to form a single molecule which transfers intact to the recipient bacterium. However, in some resistance-transfer systems, the molecule coding for resistance is independent in the host cell of that responsible for transfer. It is natural to postulate that in the second class of resistance factors, the molecule coding for resistance is associated with that coding for transfer while transfer is actually occurring.

Transfer in Gram-negative bacilli takes place by direct contact between donor and recipient cells—that is, by conjugation. This contact requires the presence of specific bacterial appendages known as pili or fimbriae, the synthesis of which is governed by the transfer factor.

The antibacterial drugs against which plasmid-mediated resistance has been detected in the enterobacteria are as follows: penicillin and related antibiotics; chloramphenicol; tetracyclines; aminoglycosides; and sulfonamides and trimethoprim.1 Resistance to heavy metals such as mercury may also be plasmid-mediated. Other characters that may be determined by these agents are synthesis of enterotoxins, haemolysins and certain surface antigens, which may play an important part in bacterial pathogenicity and resistance.

1 Resistance to furazolidone, nalidixic acid and rifampicin is of chromosomal origin. Selection by the respective drugs of resistant mutants that carry resistance plasmids will also select for the plasmids concerned.
There are numerous different transfer plasmids in the enterobacteria. They can be classified according to their ability to coexist stably in the bacterial cell. It has been established that those that cannot coexist usually have a high degree of DNA homology, while those that can coexist do not show such homology.

In the resistance transfer systems in which the transfer plasmid is physically linked to the resistance determinant, the compatibility is that of the transfer plasmid. By contrast, when the resistance plasmid is separate from the transfer plasmid it has its own properties of compatibility with related plasmids which do not transfer themselves.

A single bacterial strain may carry several plasmids. Genetic and physical studies are important in the detection, isolation and identification of these plasmids, and in tracing the distribution and transmission of drug resistance in the enterobacteria. The relevant laboratory techniques are described in Annex 2.

Resistance plasmids have been encountered in all species of enterobacteria: *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Proteus*, *Providencia* and *Yersinia*. They have also been identified in *Pseudomonas*, *Aeromonas*, *Vibrio* and *Haemophilus*.

It is apparent that resistance transfer takes place in vivo in the intestine, but such transfer must also occur outside the human or animal host—probably in sewage or other highly contaminated surface waters.

The result of pressure by antibiotics is the selection of organisms carrying either complete resistance-transfer systems or resistance plasmids that can be mobilized by transfer plasmids. The maintenance of the selection pressure by antibacterial drugs expands the latitude of resistance and the density of the resistant bacterial population, which now forms an important part of the biosphere.

### 7.3 Sampling and examination of sewage and surface waters

The increasing frequency of antibiotic-resistant bacteria in surface waters may reflect ecological exchange between man and his environment. The bacteria involved are mostly enterobacteria and *Aeromonas* species. In urban waste waters, the concentrations of enterobacteria and *Aeromonas* are similar. The percentage of resistant bacteria varies greatly depending on a multiplicity of factors, from as little as 0.1% to as much as 100%.

Resistance to ampicillin seems to be most frequent, especially in *Aeromonas* species, which are also often streptomycin-resistant. Resistance to the tetracyclines is more frequent in faecal *E. coli* than in other
enterobacteria. Many faecal strains of *E. coli* are chloramphenicol-resistant. In surface waters, the frequency of occurrence of resistant strains varies from 31% to 73% for at least one antibiotic, being highest for streptomycin. Over 80% of strains isolated from drinking-water after concentration are resistant to antibiotics. Multiple resistance is more frequently seen in strains from drinking-water, except in the case of *E. coli*, than in those from other types of water.¹

Methods for sampling and testing the environment for resistant bacteria are described in Annex 3.

REFERENCES


8. COLLECTION AND PROCESSING OF DATA

As discussed earlier, it is necessary to utilize for epidemiological purposes data which have already been collected for clinical purposes. It is therefore important in setting up surveillance programmes to gather information in a form adaptable to epidemiological purposes without involving a great deal of time in the transcription of results. The data should thus be set out in a manner suitable for computer handling because a large volume of information will require processing. In peripheral hospitals transcription would presumably be manual.

The minimum of information required for surveillance of antibiotic-resistant enterobacteria is the following: patient identification, name of

hospital or community source (whether urban or rural), relationship to any prevailing epidemic situation, date and source of isolation of the strain, name of reporting laboratory, and identification of the organism and its resistance pattern.

The antibiotics that are often tested for clinical purposes include ampicillin, chloramphenicol, carbenicillin, the cephalosporins, gentamicin, kanamycin, neomycin, streptomycin, the sulfonamides, the tetracyclines and trimethoprim.

A centralized computer system, when available, is very useful for the analysis of raw data, and help should be sought from computer specialists to determine the type of programme that would be suitable for the available hardware and the health information system in use. Several systems of data analysis exist on a local, national or international level, and a pooling of expertise and facilities would permit much needed and accelerated advances in this field.

For the central collection and analysis of data to be successful and for a rapid response and offers of help to be forthcoming when epidemic situations arise, it will be necessary to ensure the rapid feedback of results to the participating institutions and to government agencies.

In multicentre studies of any type, problems of free exchange of information arise, particularly when infectious agents and antibiotic resistance are being dealt with. Disclosure of information may be impeded by a number of factors and the importance of exchange of information may not be sufficiently appreciated. If health workers are not fully aware of the significance of the information, specimens and data are collected in an irregular manner. This results in a marked variation in the amount and range of material received from different areas.

Communications between central reference laboratories and peripheral laboratories are often inadequate. The peripheral laboratories may on occasion be reluctant to refer strains and the relevant information to the central laboratory. Peripheral laboratories are often left in relative isolation with lack of feedback information, technical support, advice, quality control and proficiency testing. National reference laboratories have responsibilities in raising standards in peripheral laboratories and in ensuring continuous collaboration.

There is often no local mechanism for the exchange of laboratory information on current patterns of bacterial resistance to antibiotics in hospitals, communities, livestock and the environment. This information is required not only by the laboratories involved but also by practitioners and health administrators to ensure that appropriate action is taken.

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9. ORGANIZATION OF A SURVEILLANCE PROGRAMME

9.1 Problems in surveillance of antibiotic resistance

There are several difficulties to be overcome before satisfactory surveillance programmes can be implemented. The problems which arise are common to all countries but have a different order of magnitude in developing countries.

There is often a lack of appreciation of the value of such information by practitioners and health authorities and consequently a lack of support for the activity in many countries. Only a small proportion of the total infections are monitored bacteriologically; laboratory support is usually reserved for severe infections and treatment failures. In many instances laboratory facilities are either inaccessible or inadequate and the techniques are slow to deal with the clinical requirements. The lack of adequate facilities and of uniformity in techniques for susceptibility testing in different laboratories makes standardization difficult.

There is frequently a shortage of suitably qualified medical and technical staff to cope with the microbiological work. This shortage becomes more acute in countries trying to accelerate the development of their health services. In some countries efficiency may be improved by consolidating existing separate laboratory services for hospital, public health, university work, etc.

The establishment of an agreed simple system of surveillance may help to solve some of the obstacles outlined above. It could also bring about improvements in the general standards of laboratory work, make medical and veterinary personnel and administrators more aware of the problems, and improve communication not only between peripheral and central reference laboratories but also between veterinary and public health laboratories.

9.2 Use of information derived from a surveillance programme

The possible uses of information on resistant enterobacteria are as follows:

— to guide clinicians and veterinary workers on the appropriate use of antibiotics in their practices;
— to form a basis for policies on the use of antibiotics in hospitals, in the community and in animal husbandry;
— to provide information early on the drug-resistant pathogens in an outbreak of infection;
— to indicate where special surveys are required in medical and veterinary fields;
— to assist in revisions of hospital and national drug formularies;
— to assist health administrators in decisions on the provision of antibiotics;
— to ensure more reliable susceptibility testing for clinical purposes.

9.3 Requirements for the organization of surveillance of drug resistance

The prerequisites for organizing drug-resistance surveillance are listed below. The existing facilities and personnel should be used as far as possible.

(1) Appropriate government health officials must be convinced that worthwhile benefits will be derived and that the initial cost will not be great if existing laboratories and the professional and technical personnel can be persuaded that the programme of surveillance is feasible and important to the health of the country. However, in time, the programme, if it is to continue and be practicable, may require some additional funding. It is conceivable that this will be a small fraction of the amount derived from savings on antibiotic expenditure and disease treatment costs.

(2) The services of trained peripheral health workers, epidemiologists, public-health-oriented physicians and veterinarians, bacteriologists and technicians should be sought.

(3) A network of peripheral health and veterinary laboratories should be created.

(4) A central reference laboratory at national level (at regional level for several countries if they are small, or at area level if the country is big) would be required.

(5) A central epidemiological or disease intelligence unit to collect, interpret and disseminate the information should be set up.

Peripheral laboratories. The term "peripheral" is used in an administrative sense and is not intended to indicate that such laboratories are less efficient or have less capability than "central" or "national" laboratories. Peripheral laboratories may be hospital or university labora-
tories or local public health laboratories and should therefore have adequate facilities for basic bacteriological work. Many such laboratories will already be performing a number of the following necessary functions for the area in which they are situated: (a) ensuring the proper collection of specimens; (b) isolating and identifying strains; and (c) determining the drug-susceptibility pattern according to methods acceptable to the national reference laboratory. The results obtained should be sent to the clinicians and epidemiologists concerned, as well as to the national reference laboratory along with an agreed number of random strains when these are needed to validate the reporting.

National (central) reference laboratories. Ideally there should be a national reference laboratory for antibiotic resistance in each country. Failing this, a national laboratory for enteric bacteria could be expanded to cope with the responsibility if the capabilities exist. At national level it is desirable that human and veterinary reference laboratories should be closely associated.

The functions of a reference laboratory, whether at the national, regional or area level, should be:

— to develop standard methodology for sampling, for susceptibility testing and for preserving the strains and materials;
— to collect, collate and interpret data from the peripheral laboratories and feed back to them information regarding technical performance;
— to ensure that peripheral laboratories use proper techniques for identifying the strains, in collaboration with the national laboratory for enteric bacteria, and for susceptibility testing by quality control and by supplying appropriate reference strains and materials, etc.;
— to provide training for bacteriologists and technicians in techniques, and for epidemiologists and peripheral health workers in the collection of specimens, etc.;
— to evaluate the in vitro effectiveness of new antibiotics against the prevalent organisms in the country by well-controlled qualitative and quantitative tests;
— when appropriate, to undertake genetic studies on the resistant strains to determine the mechanism of acquisition of resistance, or arrange for such studies to be undertaken with the assistance of WHO collaborating centres;
— to maintain liaison with WHO collaborating centres for the exchange of information, the supply of reference strains and reagents, etc.;
— to collaborate with the national epidemiology unit or disease intelligence centre in the training of health personnel, investigation of epidemics and dissemination of information to physicians and epidemiologists;
— to provide information to the health authority for the development of appropriate policies concerning the importation, stockpiling and manufacture—as the case may be—of the country's supply of antibiotics;
— to make recommendations to clinicians, veterinarians and public health authorities regarding appropriate action to be taken with regard to antibiotic use in man and animals.

**WHO collaborating centres.** The international or regional collaborating centres could provide valuable technical guidance and support at their respective levels to national laboratories. In addition, they might include training programmes, continuing education programmes, the administration of a quality control programme and the dissemination of periodic reports to interested individuals and institutions among their responsibilities.

10. SUMMARY

The Meeting was convened to review serious public health problems caused by infections with antibiotic-resistant enterobacteria and recommend methods for their surveillance and action to control their spread. Antibiotic resistance was defined and the mechanisms of resistance and the role of plasmids in the spread of resistance were reviewed.

Information on antibiotic resistance in the developing countries is in short supply and often there is no correlation of test results between countries. Most reports, however, show that antibiotic-resistant strains of enterobacteria, including *Salmonella*, *Shigella*, and *Escherichia coli*, are very common.

Several serious outbreaks of infection with resistant bacteria have occurred in recent years:
— antibiotic-resistant shigellosis in Central America, with many thousands of deaths, and in Bangladesh, with very high attack and case-fatality rates;
— chloramphenicol-resistant typhoid fever in several parts of the world—for example, in Mexico in 1972, with many thousands of cases and a death rate similar to that of the pre-antibiotic era;
— antibiotic-resistant salmonellosis associated with strains of animal origin;
— resistant *Salmonella* infections in children's hospitals, with many deaths;
— numerous hospital infections of many types caused by antibiotic-resistant strains of *Klebsiella, Serratia, Proteus, Pseudomonas* and *E. coli*.

In addition, resistance has now spread to involve other bacteria such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*. There is a fear that further epidemics may result from this spread of resistance to other bacterial species.

The emergence of antibiotic-resistant bacteria is closely linked to antibiotic use in man, which is growing in all parts of the world. In some cases, the increase in antibiotic use is explicable because more people are receiving improved medical care. It is also associated, however, with the unnecessary administration of antibiotics in many cases. Control of antibiotic use in man is difficult but every effort should be made to ensure rational use—mainly by educational means.

The increase in antibiotic resistance is also connected with the use of these drugs in animals. The addition of antibiotics to feedstuffs as a means of growth promotion is still widespread, though this practice is declining as a result of the recognition of the role such feed additives play in the emergence of antibiotic resistance.

Many components of the environment—surface waters, waste waters, and sewage, for example—are becoming polluted with antibiotic-resistant bacteria, a development which requires careful monitoring.

Reliable laboratory methods for performing and reporting susceptibility tests need to be employed; in this context the problems which arise in many countries over the supply of materials and equipment and the shortage of experienced personnel in microbiology laboratories must be borne in mind.

Other methods, which have a role in the elucidation of epidemiological problems, include those for determining the presence of resistance plasmids and for the study of drug-resistant enterobacteria in surface waters, waste waters, and sewage.

The training of personnel in adequate testing methods, in the use of antibiotics in treatment, and in the carrying out of epidemiological investigations is an essential part of the programme. The need is apparent for an effective system for data collection and analysis and for the dissemination of information, which would necessitate uniform
reporting and the use of computers for data collection, processing and analysis.

Surveillance programmes for antibiotic-resistant enterobacteria should, as far as possible, use existing clinical and public health laboratory facilities. Such programmes would provide continuous assessment of the problems with antibiotic-resistant organisms and could give early warning of impending outbreaks of infection with the bacteria concerned. They would also furnish physicians with information enabling them to apply a more rational antibiotic therapy, and permit health authorities to make decisions on drug-related national policies, such as those concerned with the importation and production of antibiotics and their use in man and animals.

It is important to achieve close collaboration between epidemiologists, clinicians, veterinarians, health administrators and public health laboratories if surveillance programmes are to be effective. Surveillance would provide more appropriate antibiotic use and better health care. These benefits would more than offset the initial costs of establishing an effective system of surveillance.

11. RECOMMENDATIONS

(1) National programmes for surveillance should be established to monitor antibiotic resistance in enterobacteria.

(2) Monitoring of enterobacterial strains of animal origin and strains recovered from the environment should be included in a surveillance programme with the cooperation of veterinary and environmental scientists.

(3) Continuing education of medical practitioners, veterinarians and epidemiologists in the use of antibiotics in therapy and in feeds is required. Training programmes for laboratory workers, and a suitable training manual, are needed to ensure accurate and reproducible testing of antibiotic susceptibility.

(4) Laboratories should be encouraged to perform susceptibility testing of enteric bacteria by a reliable method that will generate comparable data (such as the susceptibility disc technique for which requirements have been established by WHO) and to submit their data for analysis. For this purpose, laboratories should report test results by zone diameter of inhibition, in addition to the notations “susceptible”, “resistant”, “intermediate” (or “reduced susceptibility” or “partial resistance”).
(5) Clinical, microbiological and epidemiological data collected at the local, national and regional levels should be compiled and analysed and made available to clinicians and health authorities for the formulation of national policies on the importation, manufacture and use of antibiotics.

(6) An information system for the collection of data from clinical and other sources should be established so that all the essential data are available on a form suitable for data processing.

(7) It is important to reach agreement on a computer system that would be the most appropriate for electronic data processing of information on antibiotic resistance; for this purpose an international working group is considered essential.

(8) There is an urgent need to promote a programme of international surveillance of antibiotic-resistant enterobacteria and to establish international collaborating centres.

(9) Further research is needed on:

(a) simple methods for the standardization and evaluation of the means used to test bacteria for antibiotic resistance;
(b) development of more rapid methods of susceptibility testing;
(c) methods for the selection and preservation of reference strains for testing for antibiotic resistance;
(d) methods of analysis of the results of susceptibility testing;
(e) development of sampling methods to allow comparison of antibiotic resistance in hospital and community patients;
(f) the clinical and epidemiological relevance of resistant bacteria in the environment;
(g) development of effective methods for the control of resistant microorganisms of human and animal origin;
(h) the factors influencing the emergence and disappearance of resistant bacterial strains and resistance plasmids;
(i) the effect of susceptibility testing on physicians' attitudes and practices in prescribing antibiotics;
(j) the effects of limiting or discontinuing the use of certain antibiotics in man or animals.
ACKNOWLEDGEMENTS

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Annex 1

NOTES ON THE PERFORMANCE OF DISC TESTING

Media for performance of the test

Bacteriological media vary considerably in their ability to support the growth of microorganisms. The size of the inhibition zone produced around a disc of antibiotic-impregnated blotting-paper will vary with the rate of growth of the bacteria being tested and the rate of diffusion of the antibiotic from the disc into the agar. Therefore, when deciding on the break-points of zone sizes between susceptible and resistant strains, it is necessary to correlate the zone size with the MIC of the antibiotic. To do this the zone sizes are measured and plotted graphically against the MIC. If an adequate number of strains are tested a regression line can be drawn for that antibiotic and the relationship of the zone size to sensitivity can be determined. Once a reliable relationship is drawn for a given drug/organism combination, that line can be used while that particular formulation of medium and a specified procedure are being used.

It is important to distinguish between agars for susceptibility testing and agars for antibiotic assay. The latter are not suitable for susceptibility testing because the constituents and pH vary in accordance with the requirements of the particular antibiotic assay for which they were designed.

Content of antibiotic on the disc

The content of antibiotic used on the disc has been defined for laboratories using an established method of testing, and the relevant information may be found in the bibliographical references in section 7.

If other techniques are being followed it should be noted that the correct disc content required to discriminate between susceptible and resistant strains will vary with the medium used and can only be determined by adequate testing in the user laboratories.

Inoculum size and method of inoculation

The size of the zone produced around a disc will vary with the inoculum size and the method used for inoculation of the plate. If a
recommended method is not used it is important to employ a technique for obtaining an inoculum that gives a reproducible amount of growth resulting in a lawn evenly distributed over the plate. Most methods require a semiconfluent lawn but the Kirby-Bauer technique requires a confluent growth. Several methods of inoculation are used—e.g., flooding the plate or spreading the inoculum with a glass spreader or a swab. Flooding the plate is hazardous when highly pathogenic enteric organisms are being dealt with and is not recommended. Wire loops are unsuitable for adequate spreading of the inoculum but the other spreading methods are satisfactory provided that the optimum inoculum size is known. The optimum inoculum size varies with the method of application, but the correct inoculum can easily be obtained by suitable dilution of an overnight broth culture. However, routine susceptibility testing is usually performed on organisms isolated after primary cultures. For epidemiological purposes it is desirable that the susceptibility test should be performed on these isolates and not from the direct plating of specimens. To produce a semiconfluent growth the following method may be used. A wire loop is used to touch 5 colonies of the organism to be tested and the collected growth is emulsified in 3 ml of peptone water (or normal saline). A suitable growth is obtained by using a swab to spread a 3-mm loopful of the emulsion over the surface of the medium in a 9-cm Petri dish. The emulsion needs to be tenfold less concentrated if glass spreaders are used to inoculate the plate. The necessary judgement for preparing the amount of inoculum required to produce the correct amount of growth can only be acquired by experience.

In the modified Kirby-Bauer technique the inoculum suspension is judged with a barium sulfate standard to ensure a constant inoculum density.

Training in methods of susceptibility testing

In training programmes it is essential to aim for an understanding of the principles underlying the test and methods used to validate the results. Materials are likely to be of a different standard and no universal standard method can be applied. Therefore, workers performing the test will require good control methods to obtain reliable results.

The various methods of quality control were discussed earlier. The provision of reference strains of known sensitivity and resistance is one simple method of enabling peripheral laboratories to control their own tests. Annex Table 1 shows the MIC of 12 strains when these were tested by a technique employing the incorporation of graded concentrations of
the antibiotics into solid agar and employing an inoculum of $10^4$-$10^5$ organisms. Annex Table 2 shows the susceptibility of these strains when tested by a variety of disc methods on different media and represents consensus findings of several laboratories. Zone sizes are not given, as they will vary with the technique employed. It is possible that the reporting of zone sizes for these reference strains could provide some degree of validation of a disc test.

All the strains listed are nonpathogenic *Escherichia coli* and may contain plasmids. Like all plasmid-bearing strains, they are not completely stable and need to be kept lyophilized or on a defined agar containing antibiotic; in addition, they may have to be renewed periodically if the plasmids are lost. Other reference strains may need to be added as new antibiotics are introduced and resistances develop.
### Annex Table 1

Minimum inhibitory concentration (MIC) (μg/ml) for reference (WL) strains

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Strain number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WL 1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
</tr>
<tr>
<td>Cefacloridone</td>
<td>2</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>4</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Note: V = Variable MIC and zone size.*
<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>W1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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<tr>
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<td>S</td>
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<td>R</td>
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<tr>
<td>Cefadroxil</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Cefuroxime</td>
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<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>Nalidixic acid</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<tr>
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<td>R</td>
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<td>R</td>
</tr>
<tr>
<td>Colistin</td>
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<td>S</td>
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<td>R</td>
<td>S</td>
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<td>R</td>
</tr>
</tbody>
</table>

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*Note: S = susceptible, R = resistant. Numbers refer to strain number.*
*The table above represents the spectrum of susceptibility for various antimicrobial agents.*

### Reference strains for the determination of antibiotic susceptibility

*Strains are provided by the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH).*
Many of the techniques for the study of enterobacterial plasmids have been described by Anderson & Threlfall (1).

Tests for transferability of drug resistances

Broth cultures of donor and recipient strains are grown with shaking at 37 °C to exponential phase and contain approximately $2 \times 10^6$ organisms/ml.

(1) Short crosses. The cultures are mixed in a ratio of 1 : 10, usually 0.2 ml and 1.8 ml, and the mating is interrupted by the use of a high-speed blender. The duration of these crosses is usually 30 minutes, 1 hour or 2 hours.

(2) Overnight crosses (usually 18 hours). Donor and recipient cultures are mixed in equal volumes, usually 1 ml each, and incubated overnight. Crosses are performed at both 28 °C and 37 °C, because a few resistance plasmids are transferred with much higher frequency at 28 °C than at 37 °C. After each cross, decimal dilutions of the mixture are prepared in phosphate buffer, and either 0.01-ml or 0.1-ml quantities are plated in duplicate. The 0.01-ml quantities are streaked in linear fashion with a standard wire loop: 0.1-ml amounts are measured by pipette and plated with glass spreaders. Crosses are plated on minimal, MacConkey, nutrient or L agar, with and without suitable concentrations of the appropriate antibiotics. The plates are incubated overnight at 37 °C, and suitable plates are scored with a colony counter. The frequency of transfer in interrupted crosses is expressed as the proportion of resistant progeny per donor cell, while the transfer frequency in overnight crosses is calculated as the proportion per recipient cell.

Concentration of drugs in selective media

Drugs are used at the following concentrations to select for transfer of resistance:

- benzylpenicillin . . . . . 100 µg/ml
- chloramphenicol . . . . . 20 µg/ml
gentamicin 20 µg/ml
kanamycin 20 µg/ml
streptomycin 20 or 40 µg/ml
sulfathiazole 100 µg/ml
tetracyclines 5, 10 or 20 µg/ml

Counterselection against the donor strain

If the donor strain is sensitive to nalidixic acid, rifampicin or streptomycin, a strain chromosomally resistant to one of these agents is used as recipient. The drugs are used at the following concentrations for counterselection: nalidixic acid, 40 µg/ml; rifampicin, 100 µg/ml; streptomycin, 500 µg/ml.

In some experiments, colicin E2 is used to eliminate *Escherichia coli* K12 strains by spreading 0.3 ml of the colicin preparation on to each plate. Counterselection against *Salmonella typhimurium* strains is effected with the *Salmonella* 01 phage of Felix & Callow (2).

Most of these techniques for the detection of resistance transfer were described by Anderson & Lewis (3).

Mobilization of non-autotransferring plasmids

When there is no direct transfer of drug resistance, strains are examined for determinant mobilization in a triparental cross (4, 5). Equal quantities (usually 1 ml) of a late exponential growth broth culture of the donor strain carrying a transfer factor, and of the intermediate strain with the non-autotransferring resistance, are incubated together for at least 2 hours. One millilitre of a broth culture of the plasmid-free final recipient is then added. The mixture is incubated overnight. The cross is plated on a medium which selects for the resistance carried by the intermediate strain, but counterselects against both donor and intermediate strains. A control mixture of the intermediate and final recipient is tested to establish that no transfer takes place in the absence of the transfer factor from the donor strain.

Plasmid-specific phage propagation experiments

Resistance plasmids are examined for their ability to stimulate their host strains to synthesize F- or l-type sex fimbriae. These are detected by testing for multiplication of the F-specific phages µ2 and fd or the l-specific phage If1 on the strains concerned (6). The strains are grown
in nutrient broth to about $5 \times 10^8$ bacteria/ml, and 1 ml of each culture is diluted into 8-ml volumes of fresh broth. One millilitre of phage (titre $5 \times 10^8$ plaque-forming units/ml) is added to each diluted culture, so that the phage : bacterium ratio is about 1 : 1000. The mixture, and a control using a plasmid-free strain, are incubated overnight without shaking. After centrifugation, each supernatant is titrated in surface spot tests, using $K12F^+$ as indicator for phages $\mu 2$ and $f_6$, and $K12$ carrying a derepressed mutant of the $l$-like $R$ factor $T\Delta$ as indicator for phage $f1$. The plates are incubated for at least 5 hours, after which the plaques are counted and the titre of phage is calculated for each test and control experiment.

**Surface exclusion and compatibility tests**

Exclusion of one resistance plasmid by another is measured by comparing the transfer frequency of one of the plasmids into an *E. coli* $K12$ plasmid-free recipient with the frequency into the same *E. coli* $K12$ strain carrying the second $R$ factor. When exclusion is present, the frequency of transfer into the recipient carrying the second $R$ factor is lower than that into the plasmid-free recipient.

Compatibility between two plasmids is determined by introducing one plasmid into a strain carrying the other, and examining recipient lines for persistence of this second plasmid. When both $R$ factors are present in the progeny, segregation is monitored by growing the clones for 6 hours or longer in broth and plating suitable dilutions on nutrient agar. The following day, the master plates are replicated on to plates containing drugs corresponding to the resistances carried by each $R$ factor. In general, three hybrid clones are plated and more than 100 colonies of each are replicated. Compatible $R$ plasmids show a rate of segregation no higher than the rate of spontaneous loss of either parent factor. When the two plasmids are incompatible, however, less than 50% of the colonies usually retain both $R$ factors. Compatibility experiments are generally performed on strains of *E. coli* $K12$. Plasmids are assigned to compatibility groups by their ability to coexist with $R$ factors of known compatibility groups (7).

**Tests for the fertility inhibition ($\beta$) character**

1. *E. coli* $K12 F^+$ lines carrying the test plasmid are investigated for inhibition of visible lysis by the F-specific phage $\mu 2$. These tests are performed by spreading a loopful (0.01 ml) of a broth culture over an area of about 1.5-cm diameter on a nutrient agar plate. About 0.01 ml
of the phage is spotted in the centre of the inoculated area with a loop or pipette. The plates are incubated at 37 °C for about 5 hours (8).

(2) The resistance plasmids are transferred into \textit{E. coli} K12 HfrH and recipient lines carrying the plasmid are tested for visible lysis by phage μ2, and for the frequency of transfer of chromosomal markers to \textit{E. coli} K12 F<sup>−</sup> in the 1-hour crosses. Cultures of donor and recipient strains in L broth are mixed in a ratio of 1 : 10, and recombinants are selected on minimal medium supplemented with appropriate amino acids, with 0.15% glycerol as the carbon source. Streptomycin (500 μg/ml) is used to counterselect against the \textit{E. coli} K12 HfrH donor strain. Fertility inhibition caused by the plasmid in HfrH is detected by loss of visible lysis by F-specific phages, and by a reduction in frequency of transfer of chromosomal markers compared with that from the HfrH strain itself.

K12 F<sup>+</sup> and Hfr strains are both used in fertility inhibition tests, because certain plasmids displace the F factor from K12 F<sup>+</sup> lines (9). This displacement of F does not occur in Hfr strains where the chromosomal integration of the F factor gives it security of tenure.

\textit{Phage restriction by plasmids}

Each resistance plasmid is transferred into the standard \textit{E. coli} and \textit{Salmonella} strains and the resistant progeny are tested for sensitivity to phages (1).

(1) \textit{E. coli} K12 : Lines are examined for visible lysis by the “female-specific” phage q2 (10) in surface spot tests.

(2) \textit{S. typhimurium} phage type 36 : tested with routine typing phages (11).

(3) \textit{S. paratyphi B} phage type 1 var. 2 : tested with routine typing phages (2).

(4) \textit{S. typhi} phage type A : tested with routine typing phages (12, 13).

\textit{Investigations of plasmid DNA preparations}

(1) Isolation of plasmid DNA and preparation of radioactively labelled plasmid DNA.

(2) Determination of plasmid molecular weights by electron microscopy.

(3) Analysis of plasmid DNA by agarose gel electrophoresis.
(4) Studies on the cleavage of plasmid DNA with restriction endonucleases.

(5) Radioactively labelled plasmid preparations are required for DNA-DNA reassociation experiments, in combination with total unlabelled DNA from plasmid-carrying strains, prepared by the method of Marmur (14).

REFERENCES

Annex 3

METHOD FOR SAMPLING AND TESTING THE ENVIRONMENT FOR RESISTANT ORGANISMS

Sampling

The detection and counting of antibiotic-resistant bacteria can be done in waste waters, surface waters (rivers, lakes, ponds and bathing places), and drinking-water. With waste water and surface water the untreated sample must be homogenized. Ultrasound treatment in a suitable apparatus has always proved satisfactory (30 seconds at 45 kHz).

After homogenization the samples are diluted serially tenfold down to $10^{-2}$ in 25% Ringer's solution and then seeded on suitable media.

With drinking-water, concentration of the microorganisms, theoretically few in number, is obtained by filtration of 100 ml through membranes. These are placed on Chapman's medium with triphenyl tetrazolium chloride (TTC) (I) and incubated at either 44°C or 37°C. Suspect colonies are then identified and an antibiotic susceptibility test is performed.

Bacterial species

The Escherichia coli group, including the faecal coli (FC), are sensitive and specific indicators of organic contamination, whether human or animal. They are indicators of faecal contamination, and the frequency with which they prove resistant to antibiotics will indicate how frequently they are resistant in the human flora. These bacteria are often abundant in the aquatic environment; they survive relatively well and theoretically do not multiply.

The "total coli" (TC) can refer to the FC and to other enterobacteria that are lactose and ubiquitous. A parallel search can be made for these two groups of microorganisms by simple, rapid and relatively inexpensive techniques. The concentration of antibiotic-resistant FC in water will be an indicator of the utilization of antibiotics in hospitals, as additives in animal feeds, etc. The TC count will be more difficult to interpret in view of the heterogeneous origins of these microorganisms.

The Aeromonas group are aquatic bacteria. Their abundance and the frequency of resistant strains represent ecological changes.
Technical protocol

Organism counts are performed by spreading 0.1 ml of raw or diluted suspension on appropriate selective media:

— Lactose agar or bromocresol purple is suitable for counting FC.
— The reading of the dishes is done after 48 hours' incubation at 44 °C.
— Deoxycholate citrate lactose medium is used for studying TC after 48 hours' incubation at 30 °C.
— Ven Graevenitz's medium contains deoxyribonucleic acid, toluidine blue and ampicillin, and can be used to demonstrate Aeromonas. Suspect colonies are counted after 72 hours' incubation at 25 °C.

For each sample of water, pure or diluted, and for the three types of microorganism under study, platings are made on solid media without antibiotics, which serve as controls (AT⁺), and on media in which antibiotics have been incorporated (AT⁻) at the following final concentrations: ampicillin (20 μg/ml), streptomycin (12.5 μg/ml), kanamycin (12.5 μg/ml), chloramphenicol (12.5 μg/ml) and tetracycline (12.5 μg/ml). The solutions of antibiotics are made in sterile distilled water and stored at 4 °C. Colonies may be picked from the plates for identification and for investigation by the susceptibility test performed with standard methods.

This procedure permits determination of the concentrations of FC, TC and Aeromonas present in the samples analysed, and calculation of the percentages of resistant bacteria for each antibiotic studied in relation to the total bacteria counts. Although precise and reproducible, this technique is relatively difficult to apply in routine practice, for it requires the seeding of 90 dishes of agar medium for the analysis of a single sample of water. However, it would seem to be the only practicable method. Any simplification in its application would compromise the information obtained.

REFERENCE