ANTIBIOTIC SENSITIVITY TESTING: CORRELATIONS BETWEEN IN VITRO TESTS AND IN VIVO SITUATIONS

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Résumé

L'ANTIBIOGRAMME: L'APPLICATION DES TESTS IN VITRO AUX SITUATIONS CLINIQUES.

Les critères de sensibilité basés sur les relations entre les taux sanguins et les concentrations minimales inhibitrices employées dans l'interprétation de l'antibiogramme, ne sont pas toujours applicables. La présente revue traite des situations cliniques dans lesquelles ces critères font défaut. Des exemples importants se situent dans les traitements locaux, nombreux en médecine vétérinaire et les traitements d'infections situées dans les parties du corps où les antibiotiques sont concentrés. Dans une deuxième partie l'effet clinique des résistances acquises par des bactéries appartenant à des espèces normalement sensibles est examiné. L'exposé conclut par une description du problème de l'interprétation de la sensibilité des souches intestinales envers les facteurs de croissance possédant une activité antibactérienne.

From a medical point of view a bacterium is sensitive to an antibacterial agent when the agent has a curing effect on natural infections in which the organism in question is of primary or secondary importance. In vivo sensitivity or resistance of specific organisms in infections is difficult to prove adequately. The main difficulties lie in the complexity and diversity and often also in the sporadic or erratic nature of infection caused by organisms belonging to the same bacterial species. Host-linked factors may contribute more to the success or failure of a treatment than the actual sensitivity of the bacteria involved. Usually the in vivo sensitivity or resistance of bacterial strains belonging to a given bacterial species has been well demonstrated in controlled clinical trials in one type of infection in one host species (usually the human) in which this organism plays a preponderant and uncontested role. From this it is postulated that other bacteria of the same species and sometimes also of related species, having a similar in vitro sensitivity, will respond similarly in other infections, eventually in other animal species. In antibiogram testing they will be reported as sensitive.

Ericsson et al. (1960), Pullen (1960) and their many successors compiled data on serum and tissue levels obtainable with ordinary doses of antibiotics, which provide a useful alternative sensitivity criterion. They showed that a bacterium can be considered sensitive to an antibiotic when the minimal inhibitory concentration (MIC, expressed in μg/ml) of the agent is lower than commonly achievable blood or serum levels (also expressed in μg/ml). The interpretations of most agar-diffusion antibiogram tests used in routine bacteriology are indirectly based on this criterion (Bauer et al., 1966; Ericsson and Sherris, 1971).
In the present critical review an attempt is made to apply these sensitivity criteria to in vivo situations. More specifically, blood level-MIC relationships are studied, the clinical significance of acquired resistance is discussed and the problem of the interpretation of sensitivities of intestinal bacteria to growth promoters is described.

Blood level-MIC relationships and in vivo antibacterial activity.

Blood level-MIC relations are only one, admittedly an important facet of a complex in vivo situation. Attempts to develop precise and generally applicable mathematical predictive relationships between blood levels and MIC are not justified (Sherris, 1977). The following paragraphs may well serve to illustrate this.

Minimal inhibitory concentrations measure growth inhibition, only one of the many ways by which antibacterial agents affect bacteria. In one well documented condition, bacterial endocarditis, minimal bactericidal concentrations (MBC) offer a much better picture of in vivo conditions because at this site the normal host defense mechanisms which kill growth-inhibited bacteria are absent. Lower than growth inhibitory concentrations measured by MIC determinations may affect metabolism and structure of bacteria. These have been called minimal antibiotic concentrations (MAC). Their significance in vivo in conjunction with body defense factors is unclear (Symposium, 1979). To date, the efficacy of MAC in natural diseases has been proved only in synergic combinations of antibiotics.

The in vitro cultural conditions to which bacteria are subjected in MIC testing differ greatly from the in vivo situations and in certain cases this has dramatic implications. Polymyxins appear to be in vitro active (MIC lower than blood levels) against Pseudomonas and many Enterobacteriaceae, but these antibiotics are virtually inactive against P. aeruginosa at physiological calcium concentrations (Davis et al., 1971). Calcium does not inhibit effects of polymyxins on E. coli. These observations parallel and explain clinical experiences. MIC against major mastitis pathogens determined in milk are 2 to 6 times higher than corresponding values in broth (Ziv, 1969). This is most probably of importance in the systemic and local treatment of mastitis. Quinoxaline-di-N oxydes such as carbadox are more active in anaerobic conditions than aerobically (Henessey and Edwards, 1972) whereas the activity of aminoglycosides is markedly impaired by anaerobiosis (Bondi et al., 1946; Verklin and Mandell, 1977). Antibiotic sensitivity testing of facultative aerobes in air may not reflect the action of these antibacterials in the large intestine, in abscesses and in certain other types of inflammation.

Serum levels only incompletely reflect the active concentrations of antibiotics in the body. It is not clear whether peak, valley or mean levels are most significant. Distribution of antibiotics may differ in different body compartments. This also occurs at the microscopic level: antibiotic concentrations inside cells may be lower than in extracellular fluids and their effect on intracellularly located organisms is still a controversial topic (Easmon, 1979). Binding of antimicrobials to serum proteins and tissue affects their distribution, elimination and in vitro antibiotic activity but the literature does not contain well controlled studies that clearly demonstrate an inhibitory effect of protein binding on in vivo antibacterial activity (Craig and Suh, 1978). Small or important differences may occur in drug distribution, excretion or metabolism in different animal species including pharmacological extremes such as birds or cold blooded animals.

Most of these observations help to explain why there is truth in the dictum that antibiotic results indicating resistance are more trustworthy than those indicating sensitivity. Only the possible effects of MAC do not fit into this rule. There are, however, two other much more important exceptions to this: local treatments and drug concentrating mechanisms at certain body sites.

Many localized infections receive local treatments. Antibiotic concentrations at these sites are not correlated with blood levels and the blood level-MIC criterion is not applicable under these circumstances. The importance of this should not be underestimated, especially in veterinary medicine where local (intramammary, enteral, uterine, skin) treatments are perhaps of even greater importance than in human medicine. It is very well conceivable that locally attainable levels surpass the MIC of antibacterials against bacteria with acquired resistance which are reported in vitro resistant. A similar situation exists in cases where the drugs are concentrated in certain sites after systemic dosage. Clinical success in the cure of infections caused by in vitro resistant
bacteria have been well documented in human and canine urinary tract infections (Ling, 1979). Also in the large intestine certain antibacterials are concentrated and may reach high levels (Jeffries et al., 1977), but the effects of this are largely unknown.

**Acquired resistance**

When collections of strains belonging to the same bacterial species are tested one or more strains may be found with MICs definitely higher than those of a more sensitive group. The collection shows a bimodal distribution of strain sensitivities. This is illustrated with Staphylococcus aureus and tetracycline in Table 1. Different resistance mechanisms in the same bacterial species may cause different resistance levels and as a result trimodal or even quadrimodal MIC distributions with two or three resistant groups clearly differing from the sensitive one are seen. All strains which are in the less sensitive group(s) are considered to have acquired resistance. Genetic studies eventually may show that these strains possess chromosomal or extrachromosomal resistance genes. In other much rarer cases, the distribution of sensitivities of strains belonging to the same bacterial species may be extended without being clearly separated in two or more populations. The sensitivity of E. coli to nitrofurans is an important example in farm animal medicine (table 1). These differences in sensitivity between strains of a given species are objective and uncontestable criteria of in vitro sensitivity. However they are only a bacteriologist's tools. They can be relevant to the clinical situation but are not intended as such. Only when the bacteriological categories have been clearly related to treatment failure or success can clinically useful predictive value be attributed to them.

In the example of furazolidone (table 1) the strains with MIC of 2 to 32 μg/ml are to be considered as resistant from a bacteriological point of view but it is difficult to imagine how these strains will behave in the gut when furazolidone is given. Strains with gradually decreasing sensitivities may respond to higher than usual doses of an antibiotic to which they are, bacteriologically speaking, resistant. This has been most abundantly documented in the treatment of gonococcal infections with penicillins.

When the sensitivity levels of the strains of a species are neatly bimodally distributed, the responses of sensitive and resistant strains can be expected to show more clearcut differences.

Surprisingly few reports relate clinical results of treatments in animals with antibacterials to which the infecting organisms show acquired resistance in vitro. In the local treatment of staphylococcal mastitis, a condition with a low response to treatment, some investigators (Pearson and Mackie, 1979; Postle et al., 1979) found that in vitro test results did not correspond with clinical responses whereas others (Weight and Bleckman, 1977) came to opposite conclusions. The latter authors did not distinguish between staphylococcal and streptococcal infections and it cannot be excluded that their good correlation was due to this.

Information on in vivo response of strains with acquired resistance are also scanty in experimental infections. Dey et al. (1977) reported the failure of oral tetracycline in the

**Table 1.** Sensitivity levels of two collections of bacterial strains.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antibacterial agent</th>
<th>Number of strains with MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25  0.5 1  2  4  8  16 32  64 128 &gt;128</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>Tetracycline HCl</td>
<td>59  4  3  9  1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Furazolidone</td>
<td>1  21**  9  10  19  6  16  3</td>
</tr>
</tbody>
</table>

**Notes:**

(1) Unpublished results with 76 strains from poultry.

(2) From Devries (1975).

(3) Including reference strains.
treatment of experimental colibacillosis in piglets caused by an *in vitro* tetracycline-resistant and chloramphenicol-sensitive *E. coli* strain. Unfortunately this experiment was only compared with antibiotic-free control and chloramphenicol treated groups.

More abundant results can be found in the human medical literature. Tuberculosis provides an outstanding example in which relations of *in vitro* sensitivity to treatment results have been most thoroughly studied (Mitchison, 1970). A useful review on the significance of *in vitro* resistance in the care of patients infected with other organisms has been compiled by Sanders and Sanders (1977). Sugarman and Pesanti (1980) reviewed reports on the treatment failures attributed to emergence of resistance during therapy with aminoglycosides, penicillins, chloramphenicol, macrolides, lincomycins, sulphonamides and tetracyclins. The effect of penicillinase-labile penicillins on infections caused by penicillinase-producing staphylococci has long been controversial. Burn et al. (1957) did not find any differences in responses of superficial infections caused by penicillinase-negative and penicillinase-producing staphylococci. Following later recommendations (Ericsson and Sherris, 1971), penicillinase-producing staphylococci are nowadays always reported as being resistant to penicillin G or V and ampicillin. The clinical significance of resistance against penicillinase-stable penicillins in staphylococci remains unclear (Lacey, 1974). This resistance type is, however, rare in veterinary medicine.

**Sensitivity and resistance to growth promoters**

The interpretation of test results with growth promoters on intestinal bacteria poses a special problem. In this important field of farm animal science and veterinary medicine, bacterial sensitivity cannot be measured in terms of clinical effects or in blood level-MIC relationships. Many growth promoting antibacterial agents are not absorbed or very poorly absorbed from the gut and the relationships of changes in composition of intestinal flora with growth enhancing properties are still unknown (Visek, 1978). Gut level-MIC relationships could offer an alternative sensitivity criterion but information on concentrations of these products in the different compartments of the gut is not available.

Intestinal bacteria with MIC definitely higher than those of other strains of the same species can be said to have acquired resistance. In these interpretations, however, a purely bacteriological criterion of sensitivity is handled. The findings may be of importance in the case of antibiotics which are also used in therapy but the significance of resistances to compounds used only for growth promotion remains unknown.

*In vivo* suppressive effects in the intestines on *Clostridium perfringens* have been recorded in trials with low (growth promoting) doses of virginiamycin, tylosin, bacitracin and nitrovin (Smith, 1972), lincomycin (Truscott and Al-Sheikhly, 1977) and avoparcin (Prescott, 1979), on unspecified lactobacilli with virginiamycin (Decuyper et al., 1973) and on *Streptococcus faecalis* subsp. *liquifaciens* with bacitracin (Barnes et al., 1978). The penicillins and tetracyclines, no longer in use in Western Europe for growth promotion, have been extensively studied with respect to their activity on broad bacterial families or genera rather than single species, with *C. perfringens* as a notable exception. Detailed results have been described with therapeutically dosed antibiotics in humans (Seeliger and Schroter, 1969). These effects are site-dependent (Sieburth et al., 1954) and dose-dependent (Cardon et al., 1971). They may also differ according to host species and interactions of other intestinal inhabitants may influence the behaviour of individual bacterial strains or species (Ducluzeau et al., 1971).

*In vivo* bacteriological observations of this type can also be taken as evidences of sensitivity. Species suppressed *in vivo* by common dosages of growth promoter can be said to be naturally or intrinsically sensitive to the agent. *In vitro* sensitivity levels, similar to those observed in strains or species with which *in vivo* suppression has been demonstrated, can be interpreted as indicating sensitivity to the growth promoter under study. These notions of *in vivo* sensitivity again remain without firmly established etiological connections with beneficial effects to the host.

**Concluding remarks**

Although it was necessary to borrow heavily from human medical experience, the examples of good correlations between *in vitro* sensitivity results and clinical responses given in the
section on acquired resistance demonstrate
the predictive value of antibiotic sensitivity
testing.

Antibiogram results with bacterial species
whose in vivo response to antibiotics is well
known and predictable give useful informa-
tion. They will show whether the strains have
the usual sensitivity characteristics of the spe-
cies or have acquired resistance. Strains with
resistance of this type are unlikely to respond
to treatment except in local medication or in
the treatment of sites where the antibacterial is
concentrated. When they belong to the infre-
cquent category of strains whose resistance level
is not clearly distinct from the usual sen-
sitivity of the species, they may respond to
higher doses. Strains with normal sensitivity
levels will behave as can be expected for the
species.

These conclusions illustrate the fact that all
valuable antibiogram interpretations are
based, or are to be based on sound clinical
experience with infections of well defined
types caused by organisms with known in vitro
sensitivity. The interpretation of antibiotic-susceptibility to growth promoters
not used in therapy remains an unresolved
problem. The in vitro, results cannot be inter-
preted as long as the significance of changes
of the intestinal bacterial flora after feeding
these compounds is unknown.

It should be kept in mind that many discre-
pancies between in vitro reports and clinical
results still originate from bad test techniques,
particularly in tests with penicillins, oligoglyco-
sides, sulphonamides and sulphonamide-
potentiators (George, 1974) and, not less
important, in bad diagnostics. An antibiogram
of an organism which has nothing to do with
infection to be treated may give frankly mislead-
ing information.

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Summary

This review discusses the correlations between in vitro antibiotic sensitivity test results and in vivo clinical situations. It is concluded that the relations between blood levels and minimal inhibi-
tory concentrations of antibacterial drugs cannot always be applied as criteria of sensitivity. Important exceptions are local treatments and treatment of infections in body sites where anti-
biotics are concentrated. Examples of agreement and lack of agreement between in vitro test
results and treatment results in infections caused by bacteria with acquired resistance are given.
Finally, the special problem of the interpretation of results obtained in tests with intestinal bacte-
ria and antibacterials used for growth promotion is discussed.

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