Laboratory Testing of Cephalosporins

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Summary

Cephalosporin drugs are stable, soluble at high concentrations and possess a characteristic ultraviolet absorption spectrum, which allows easy quantification. They are therefore relatively easy to work with under laboratory conditions. This chapter provides an overview of the laboratory tests available for assessing antimicrobial activity in research and clinical practice, and highlights the usefulness and drawbacks of such tests in the prediction of clinical efficacy with particular reference to newer cephalosporins, which are for oral administration.

Laboratory testing of antimicrobials is used to predict their potential efficacy. In the research environment, during the development of a drug, microbial susceptibility is a relative determinant that helps in the decision-making process. In the hospital microbiology laboratory it is a standard measurement, which aids interpretation of the clinical usefulness of a drug against particular infections. In this latter setting, susceptibility testing has to serve 2 purposes: (i) to assist in the choice of a suitable antimicrobial for an infected patient and (ii) to obtain data about the developing patterns of resistance for epidemiological purposes. The latter function is the most important, as in most instances the antimicrobial has to be administered before microbiological testing is possible. Thus, statistics of resistance patterns for specific hospital wards facilitate the choice of therapy. However, until now there has been little agreement between scientists about the most important factors influencing the outcome of therapy. An indication of this uncertainty is the differing breakpoints for susceptibility in various countries, which occur despite homogeneity in the characteristics of bacteria and patients, and use of the same drugs.

1. Interaction of Cephalosporins with Penicillin-Binding Proteins

At present, our understanding of the mechanism of action of β-lactam antibiotics, including cephalosporins, remains incomplete. However, we do know that this action involves binding to and specific inactivation of penicillin-binding proteins (PBPs) located on the inner aspect of the bacterial membrane. PBPs are enzymes important in the synthesis of the peptidoglycan component of the cell wall. Multiple PBPs (up to 8) have been detected in aerobic and anaerobic bacteria. However, most work has been conducted using a small number of species, mainly Escherichia coli. Some PBPs (PBP 1a, 1b, PBP 2, and PBP 3) are of critical overall importance and their inactivation leads to cell death. Actual lysis of a bacterium by a β-lactam appears to involve inhibition of protein synthesis and the loss from the cell of an inhibitor of an enzyme of autolysis. Decreased PBP binding affinity is another mechanism of bacterial resistance to cephalosporins, and is the reason that these drugs are ineffective against methicillin-resistant Staphylococcus aureus (MRSA).

It has been suggested that lysis of Gram-nega-
tive bacteria occurs because of inhibition of PBP 1b, 1a plus 1b, 3 followed by 1a, 1a plus 2, 2 plus 3.\textsuperscript{[9]} Binding to PBP 1a/1b causes rapid lysis of the bacteria, whereas binding to PBP 2 causes formation of osmotically stable large round cells (spheroplasts) that lyse slowly. Binding to PBP 3 inhibits the synthesis of cross-walls, leading to filamentation and slow death. At low concentrations, cephalosporins in general have high affinity for PBPs 1 and 3 of Gram-negative bacteria, but at high concentrations there is usually also binding to PBP 2.\textsuperscript{[10]} Preferential binding of PBP 3 at low concentrations favours filament formation, decreased viability and short postantibiotic effects (PAEs). At high concentrations, spheroplasts may be formed.

The function of PBPs in Gram-positive bacteria is less clear than in Gram-negative species. Morphological responses of Gram-positive isolates to antimicrobial agents are generally limited to spheroplast formation and rapid lysis.\textsuperscript{[11]} Identifiable PBPs essential for viability are PBPs 1 and 3 in \textit{S. aureus}, mainly PBPs 1 and 3 in streptococci and pneumococci and PBPs 3 and 4 in \textit{Clostridium perfringens}. In general, cephalosporins bind to these PBPs and cause a variety of morphological changes.\textsuperscript{[11]}

Among the cephalosporins, cefdinir generally has high affinity for PBPs, particularly in comparison with other drugs of this class. Indeed, cefdinir has shown higher affinity for PBPs 1, 2, and/or 3 of \textit{S. aureus} than cefaclor,\textsuperscript{[12,13]} cefalexin,\textsuperscript{[12]} and for all PBPs (except PBP 2b) of \textit{Streptococcus pneumoniae} than cefaclor.\textsuperscript{[13]} Cefdinir also possesses higher affinity for PBPs 1bs, 2, 3 and 4 of \textit{E. coli} than cefaclor.\textsuperscript{[12]} Against this background, electron microscopy has revealed that the \textit{in vivo} activity of cefdinir against methicillin-susceptible strains of \textit{S. aureus} and \textit{S. epidermidis} reflects alterations in cell wall composition mostly due to interactions with PBPs.\textsuperscript{[14]} Similarly, \textit{in vitro} changes in the morphology of \textit{S. aureus} and \textit{S. pyogenes} were caused by binding of cefdinir to all PBPs.\textsuperscript{[15]}

2. Measurement of Susceptibility

There are several variables that may affect the clinical efficacy of an antimicrobial agent \textit{in vivo} and that should be considered when interpreting the results of \textit{in vitro} susceptibility tests (table I). From the technical point of view, the information sought is the minimum inhibitory antimicrobial concentration (MIC) against clinical isolates of pathogens. This and the achievable serum drug concentration are the cornerstones on which the selection and dosage regimens of antimicrobial therapy have been constructed. Nevertheless, logistical constraints almost always prevent the precise measurement of MICs.

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<th>Principal factor</th>
<th>Detailed information required</th>
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| Antimicrobial concentration at the site of the infection | Site of infection  
|                                                      | Degree of protein binding  
|                                                      | Tissue antimicrobial concentrations  
|                                                      | Intracellular antimicrobial concentrations  
|                                                      | pO\textsubscript{2}  
|                                                      | pH  
|                                                      | pCO\textsubscript{2}  
|                                                      | Ionic strength  
|                                                      | Presence of various ions  |
| Physical conditions at the site of the infection     |                                                   |
| Inoculum size                                         |                                                   |
| Temperature                                           |                                                   |
| State of body defences                                | Surfactant  
|                                                      | Natural; complement activation  
|                                                      | Acquired; cellular and humoral immunity  |
| Nature of normal flora locally                        |                                                   |