EDITORIAL ANNOUNCEMENT

Over the last few years the *Journal of Medical Microbiology* has undergone several changes with the development of its position as an international primary journal publishing the results of research in all aspects of microbiology relevant to human or veterinary medicine and dentistry. The *Journal* expanded during the 1980s to become a monthly publication, adopted modern approaches to scientific publication such as the 'Vancouver-style', and has recently moved to an A4 format.

In a further development to widen its purpose of providing medical microbiologists with information about developments in the subject, the *Journal* begins publishing editorials. It is intended to publish one or two editorials in each issue; subject matter will reflect the general balance of the papers currently published in the *Journal* and will include basic bacteriology, virology, diagnostic microbiology, pathogenicity, antimicrobial chemotherapy and specific infections. The editorials will be signed and will reflect the personal opinion of the individual author rather than the editors and publishers of the *Journal*. Whenever possible, topics will reflect areas of current interest or controversy. In this issue, an editorial on the classification of trimethoprim resistance by Amyes *et al.* is published. Future issues will contain editorials on the use of antibiotics in veterinary practice, the epidemiology of pertussis in adults, human herpes virus 6 and giardiasis.

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EDITORIAL

Classification of plasmid-encoded dihydrofolate reductases conferring trimethoprim resistance

The classification of bacterial resistance genes and the enzymes that they may encode is vital to our understanding of the epidemiology of resistance and the factors which may stimulate its emergence. Such classification is often contentious and can lead to considerable confusion. Often, it is not based on good scientific nomenclature and may even include personal considerations, such as a name or initials. A classification scheme with a limited number of different names may be acceptable; however, if the number of enzymes which express a specific type of resistance is high, then such a scheme becomes very difficult to operate. This became particularly evident with the plasmid-encoded β -lactamases in gram-negative bacteria; there are about 30 such enzymes and, although some were classified by their ability to hydrolyse oxacillin (OXA enzymes), most were just given three initials plus a suffix (TLE-1, HMS-1, etc).

Plasmid-mediated trimethoprim resistance is the result of production of an additional plasmid-encoded, trimethoprim-resistant dihydrofolate reductase (DHFR).¹ At least 14 such enzymes have been reported (table I),^{2,3} and we are aware of further enzymes whose properties are being elucidated. Initially it was shown that these plasmid-encoded enzymes had a

larger M_r than their chromosomal counterparts.¹ Pattishall *et al.*⁴ distinguished two different types of plasmid-encoded enzyme—types I and II—entirely on biochemical properties. The type I enzyme was

Table I. Classification of plasmid-mediated dihydrofolate reductases by biochemical properties

Group 1: K_i for Trimethoprim = 10–100 mM High trimethoprim MIC	Type Ia Type Ib Type V Type VII Type S1
Group 2: High K_i for trimethoprim (> 100 mM) High trimethoprim MIC	Type IIa Type IIb Type IIc Type VI
Group 3: Low K_i for trimethoprim (< 1 mM) Low trimethoprim MIC Not inducible	Type IIIa Type IIIb Type IIIc
Group 4: Low K_i for trimethoprim (< 1 mM) Low trimethoprim MIC Inducible	Type IV
Group 5: ID_{50} * for trimethoprim = 10–100 mM Low trimethoprim MIC	Type IX

* A K_i value has not yet been published for this enzyme.

produced in greater quantity than the chromosomal-encoded enzyme and was about 10 000-fold more resistant to trimethoprim. Subsequently, another type I-like enzyme was identified and termed type Ib,⁵ with the original type I enzyme renamed type Ia. The type II DHFR was produced in smaller quantity than the type Ia enzyme, but was very much more resistant to trimethoprim. DNA sequence analysis of type II DHFR genes from different sources revealed that they were not an homogenous group; three closely-related genes, all encoding enzymes of identical biochemical properties (types IIa, IIb and IIc) were identified. Both the type I and II DHFR groups confer very high levels of trimethoprim resistance (MIC > 1000 mg/L) and this fact was used to infer the presence of plasmid-encoded genes. The emergence of a type III DHFR, first in New Zealand and, later, in the UK^{6,7} added a new dimension as this enzyme had completely different biochemical properties and conferred a much lower level of resistance (MIC 64 mg/L). Even following the first report of an inducible plasmid-encoded enzyme (type IV), which also conferred low-level resistance,⁸ all DHFR types were still distinguishable clearly on enzymic properties alone. DNA sequence analysis has now revealed that there are considerable differences between the genes coding for the various DHFR types and, in particular, between the type II enzymes and the rest.

However, the subsequent recognition of the DHFR types IIIb, IIIc, V, VI, VII and IX has now caused confusion in the scheme (table I). Types IIIb and IIIc were found in *Shigella* spp. in the USA;⁹ both had biochemical properties very similar to the original type III, now renamed type IIIa, and were, therefore, placed in the same group, but DNA sequence analysis suggests that the type IIIb DHFR is not closely related to type IIIa. Type IIIc has yet to be sequenced. DHFR types V and VII have biochemical properties very similar to the type Ia enzyme and are as closely related to it as the type Ib enzyme. However they have been designated separate numerals, so to the unfamiliar researcher they may appear to be as distinct from type Ia as types II or III. Type VI DHFR has biochemical properties similar to the type II enzymes but the DNA sequence of its gene is more related to that of the type Ia.¹⁰ The type IX enzyme has inhibition properties similar to type Ia but does not confer the same degree of resistance.³

We are now faced with a dilemma: should we abandon a system that has been used for nearly 15 years? For the most part, the allocation of an enzyme to a specific type has reflected its biochemical properties and to some extent its emergence in clinical practice; for example, those enzymes that confer high trimethoprim MICs have been found predominantly in urinary bacteria, which have been challenged with high drug concentrations, whereas those that confer lower MICs have been found predominantly in bacteria isolated from sites with lower drug concentrations. On the other hand, as more and more resistance

Table II. Classification of plasmid-mediated dihydrofolate reductase genes (or their products) by sequence homology

Group A: Related to <i>Escherichia coli</i> chromosomal DHFR	Type IIIa Type IV
Group B: Related to type Ia plasmid-encoded DHFR	Type Ia Type Ib Type V Type VI Type VII
Group C: Related to type IIa plasmid-encoded DHFR	Type IIa Type IIb Type IIc
Group D: Related to <i>Staphylococcus aureus</i> chromosomal DHFR	Type S1
Group E: No substantial relationship with other DHFR types	Type IIIb Type IX

(A sequence is not yet available for the type IIIc gene).

genes are sequenced, DHFR types could be re-allocated according to sequence homology, although this is no guarantee that the gene products will have similar biochemical properties (table II). In defence of the *status quo*, the criteria of clinical microbiologists or epidemiologists are different from those of molecular biologists. The latter would see a 50% similarity between genes as highly significant in the long-term evolutionary process whereas, in immediate clinical terms, such similarities are not important because there is no possibility that genes with this degree of relationship could have evolved from each other in the short time scale of the antibiotic era. This contrasts with genes that differ from one another by only a few base pairs and which clearly have evolved recently from each other; this has been demonstrated with plasmid genes encoding broad-spectrum β -lactamases and here classification by sequence may be of particular value. The plasmid-encoded DHFR types do not belong to this latter category, and it seems foolhardy to change radically a system that has been used for so long. Furthermore, it is extremely difficult to alter classifications that are already widespread in the scientific literature. It seems logical, therefore, that a system should be used that takes account of what has gone before.

We propose that types I–VII, IX and the type S1 (the only staphylococcal plasmid-encoded enzyme) should remain, including their sub-types. However, in future, no sub-types should be used and each newly discovered enzyme, *when it has been shown to be completely different from all others by both biochemical properties and sequence analysis*, should be allocated a new type.

There remains a problem: which is the next available type number? We have established a register of all plasmid-encoded DHFR types, and suggest that if potential new enzymes (or their genes) are discovered the investigator should contact the registry so that the next available type number can be allocated. This overcomes the confusing situation in which the same

number is inadvertently used by different investigators for different enzymes or genes. Gene probes for each of the existing recognised DHFR types can be supplied so that investigators can ensure that they are not duplicating previous work. We would also request that when each investigator is ready to confirm the identification of a new gene, a copy of the new gene, or the appropriate probe, should be donated for future distribution. By this approach, although DHFR type numbers will gradually less reflect biochemical prop-

erties, we can ensure that duplication will not occur and DHFR types will be correctly placed in the classification scheme.

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