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Letters to the Editor

Use of Mutator Strains for Characterization of Novel Antimicrobial Agents

A recent minireview by Martinez and Baquero (8) provides a useful discussion on various aspects of mutational resistance to antibiotics in bacteria. As noted by these authors, bacteria displaying strong mutator (hypermutator) phenotypes exhibit significantly increased rates of mutation conferring antibiotic resistance (up to 10,000-fold that of the wild type). We would like to further highlight the value of such strains for basic antimicrobial drug discovery research, an aspect that was only partially addressed in the minireview.

Novel antimicrobial drug candidates are invariably evaluated with respect to the frequency with which resistant bacterial genotypes arise in vitro (3, 4, 7, 14). This provides an indication of whether resistance to the agent is likely to arise rapidly, either during therapy or within the environment. In addition, mutants recovered during such determinations may be important for elucidation of the drug's mode of action (4, 7, 12, 13) and for predicting the mechanism of resistance that may arise in the clinical setting.

We would like to stress the point touched upon in the minireview that hypermutators, e.g., *Escherichia coli* and *Salmonella enterica* with defects in the mismatch repair pathway (5, 9), should be used alongside wild-type isolates to examine the frequency with which drug resistance to a particular agent arises. This will yield mutation frequencies that represent worst-case scenarios. In turn this allows expression of the frequency of mutations conferring resistance as a range, not as a single value.

The rationale is that populations of pathogenic bacteria do not exhibit homogeneous mutation rates. For example, >1% of natural pathogenic *E. coli* and *S. enterica* populations exhibit a strong mutator phenotype (5). In addition, 0.0001 to 0.001% of some, and possibly all, bacterial populations are hypermutators (6), and a single selection event (e.g., antibiotic selection) can enrich the mutator population to 0.5% of the total (6). As Martinez and Baquero (8) point out, it is therefore erroneous to assume that a bacterial population exhibits uniform mutation rates. This could be particularly relevant during infection when in vivo mutation rates may be elevated (1).

Hypermutator strains may also be used to enhance the recovery of rare resistance mutations, e.g., for elucidation of modified drug targets within the cell. We have established that a fully grown 2YT or TB (11) culture of *E. coli* reaches cell densities of about 10^{10} CFU/ml (unpublished data). Resuspension of this culture in 1/10 the volume and incorporation of 1-ml aliquots in 10 agar pour plates allow mutants arising at frequencies approaching 10^{-12} to be detected. Using *E. coli* hypermutators such as *mutS* or *uvrD* mutants, which exhibit 1,000-fold increases in mutation rate under certain conditions, allows detection of drug-resistant mutants that effectively occur at frequencies as low as 10^{-15}. Indeed, we have used this approach to detect rare *ampC* promoter mutations in *E. coli* that confer increased ampicillin resistance (unpublished data).

There is little doubt that new antimicrobial agents are needed to combat the growing problem of antibiotic-resistant bacteria (2, 10). We suggest that hypermutator strains have an important role in the evaluation of such new agents.

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Authors' Reply

O'Neill and Chopra discuss the possibility of using hypermutator bacterial strains for enhancing the recovery of rare antibiotic-resistant bacterial mutants. Hypermutation in bacterial populations may occur in either a transient or a permanent way. In the first case, error-prone DNA polymerases may be involved (5); in the second, mutations of the mismatch repair system are thought to be implicated (3, 4). Both types of adaptive strategies may be triggered during the infective process or during antibiotic therapy (1, 4; M. C. Negri, M. R. Baquero, J. Blázquez, and F. Baquero, Abstr. 40th Intersci.

Conf. Antimicrob. Agents Chemother., abstr. 1918, p. 116, 2000). As we pointed our in our minireview, the frequency of mutation is highly dependent on the environment. Thus, conventional mutation rate determinations carried out on culture tubes may totally fail in predicting a probability of emergence of mutational resistance under in vivo circumstances. In other words, very rare mutants or even double mutants may emerge in vivo if the bacterial population has the adaptive benefits of hypermutation. Consequently, only the use of hypermutators in the in vitro testing could predict the emergence of resistance in some cases. As discussed by O'Neill and Chopra, mutants that arise at frequencies as low as 10^{-15} might be easily selected by using hypermutator strains. In looking for rare mutants, hypermutator strains will therefore be very useful, not only for the analysis of antibiotic resistance but also for the generation of interesting bacterial mutants showing novel metabolic capabilities with biotechnological relevance. In fact, biotechnology companies are well aware of this situation, and Stratagene (La Jolla, Calif.) offers in its catalogue Epicurean Coli AE XL1-Red Competent Cells, which are "useful to generate random mutations because they have a mutation rate 5,000-fold higher than the wild-type parents."

We therefore agree with the suggestion of O'Neill and Chopra. Testing of the mutants that are selectable from hypermutator strains is needed for a correct prognosis of the probability of emergence of antibiotic resistance. This approach enabled us to detect mutations in the structural gene of the *Acidaminococcus* beta-lactamase ACI-1, leading to resistance associated with beta-lactams and beta-lactam inhibitors, when conventional methods with normal mutators failed (J. C. Galán, M. R. Baquero, M. Reig, F. Baquero, and J. Blázquez, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1919, p. 116). Another approach that we have analyzed is the possibility that a given concentration of an antimicrobial agent will select the hypermutable population in a mixed population. In the case of *Streptococcus pneumoniae*, a *hexA*-negative hypermutator strain was selected over the normal mutator in the presence of cefotaxime. Selection was due to the hitchhiking effect of the cefotaxime-resistant mutation Thr550-Ala that emerged in the hypermutable population (Negri et al., 40th ICAAC). Note that this selected variant also has more possibilities to become resistant to other antibiotics. In summary, we support the recommendation of O'Neill and Chopra to use hypermutator strains for predicting mechanisms of action and mechanisms of resistance to new drugs. We would like to add that these experiments should include selection at different antibiotic concentrations (2) and, eventually, prolonged incubation times (1).

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