DOI: 10.1534/genetics.105.045526

Note

Intermediate Mutation Frequencies Favor Evolution of Multidrug Resistance in *Escherichia coli*

Erick Denamur,* Olivier Tenaillon,* Catherine Deschamps,*,† David Skurnik,† Esthel Ronco,‡ Jean Louis Gaillard,‡ Bertrand Picard,† Catherine Branger*,† and Ivan Matic§,1

**INSERM U722 and Université Paris 7, Paris, France,* †*Laboratoires de Microbiologie, GHU Nord, AP-HP, Colombes, Paris, Bobigny, France,* ‡*Laboratoire de Microbiologie, GHU Ouest, AP-HP, Garches, France and* §*INSERM U571 and Université Paris 5, Paris, France*

Manuscript received May 11, 2005
Accepted for publication May 31, 2005

ABSTRACT

In studying the interplay between mutation frequencies and antibiotic resistance among *Escherichia coli* natural isolates, we observed that modest modifications of mutation frequency may significantly influence the evolution of antibiotic resistance. The strains having intermediate mutation frequencies have significantly more antibiotic resistances than strains having low and high mutation frequencies.

THE comprehension of how cells having high mutation frequencies arise and proliferate is important for the understanding of the evolution of antibiotic resistance. *In vitro* and *in vivo* studies show that high mutation frequencies can significantly contribute to the appearance of multiresistant bacteria (Mao *et al.* 1997; Giraud *et al.* 2002). As strong mutators were found in natural populations of many pathogenic species (LeClerc *et al.* 1996; Matic *et al.* 1997; Oliver *et al.* 2000; Denamur *et al.* 2002; Richardson *et al.* 2002; Morosini *et al.* 2003; Prunier *et al.* 2003; Watson *et al.* 2004), it was hypothesized that a positive correlation between antibiotic resistance and high mutation frequencies should be frequent in natural bacterial populations (Blazquez 2003).

To study the relationship between mutation frequencies and antibiotic resistance, we examined three different collections of *Escherichia coli* natural isolates, composed of 312 human commensal and extra-intestinal pathogenic strains having very different frequencies of antibiotic resistances. We estimated mutation frequencies by measuring the capacity of strains to generate resistance to rifampicin (Denamur *et al.* 2002), whereas the susceptibility to antibiotics was tested by the disk diffusion technique according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (http://www.sfm.asso.fr). Because an identical pattern of relationship between mutation frequencies and antibiotic resistance was obtained for all three collections of *E. coli*, we present the data obtained with only one collection.

Surprisingly, we did not observe a positive correlation between increase of mutation frequencies and increase in antibiotic resistance. This was due to the fact that strains having low and high mutation frequencies have significantly less antibiotic resistance than strains having intermediate mutation frequencies (Figure 1, A and B). The range of mutation frequencies that correlates with the maximal antibiotic resistance is between 2.9×10^{-9} and 9.3×10^{-9}, *i.e.*, starting just above the median value of mutation frequencies for this collection (2.47×10^{-9}). These data suggest that even modest modifications of mutation rate can significantly influence the evolution of antibiotic resistance. Furthermore, this phenomenon does not depend on one antibiotic or on one family of antibiotics (Figure 1C), suggesting that mutations can participate in various modes of evolution of antibiotic resistance. Indeed, whenever mutations can confer or increase resistance to the antibiotics or reduce the biological cost of resistance on bacterial fitness, it is more likely that those mutations will appear in the populations of cells having higher mutation frequencies. Furthermore, when resistance is associated with an acquisition of several mutations, the advantage of being a mutator increases significantly (Tenaillon *et al.* 1999).

Why are strains that have many antibiotic resistances underrepresented among isolates with high mutation frequencies? Mutator alleles are enriched in bacterial populations via selection for the adaptive mutations that they generate (Taddei *et al.* 1997). Once adaptation is achieved, the counterselection of mutators will start as the result of the accumulation of deleterious mutations

[1]*Corresponding author:* INSERM U571 Faculté de Medecine-Necker, 156 rue de Vaugirard, 75730 Paris, Cedex 15, France.
E-mail: matic@necker.fr

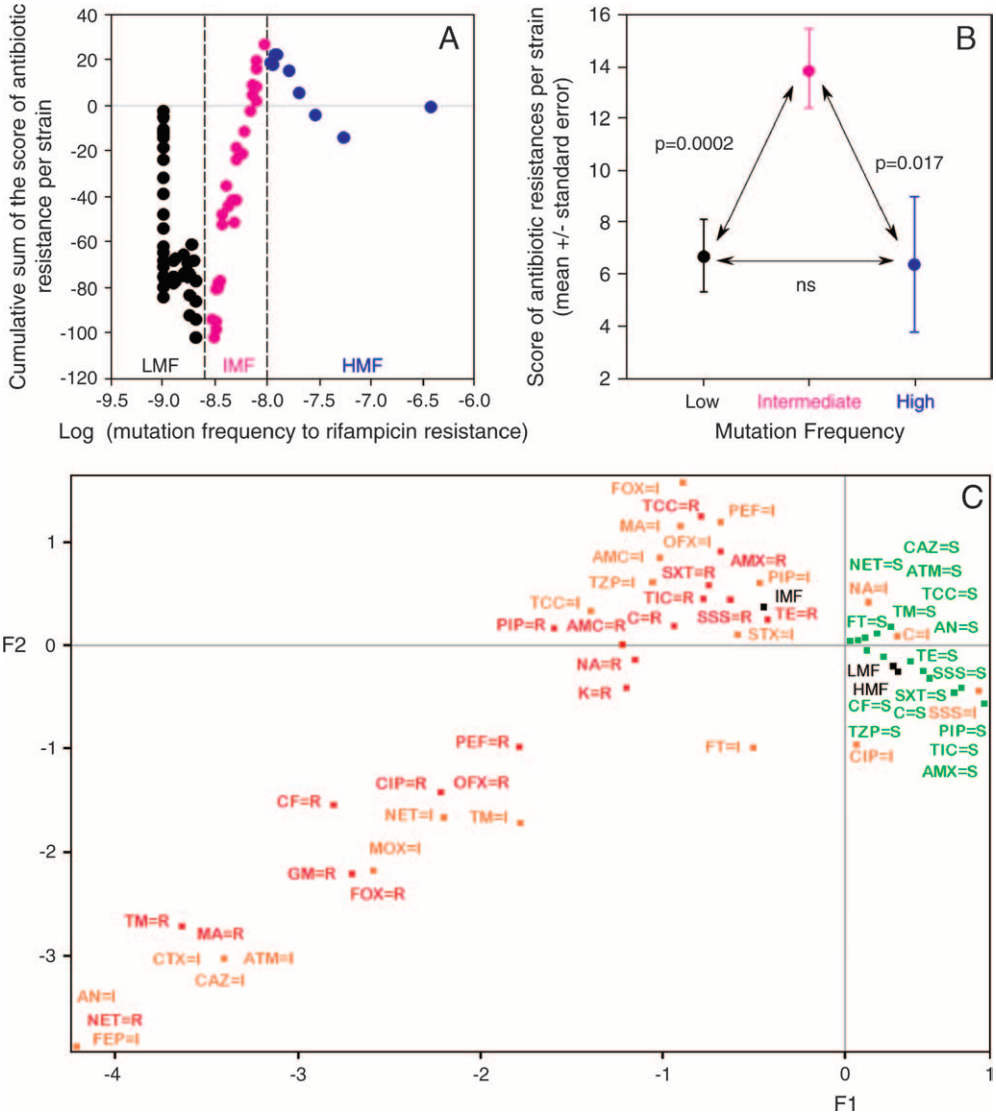

FIGURE 1.—Relationship between antibiotic resistance and mutation frequencies. These data were obtained using a collection of *E. coli* natural isolates composed of 76 strains isolated from the urine of patients with spinal cord injuries either as asymptomatic carriage ($n = 32$) or as urinary tract infections ($n = 44$). The following antibiotics were used: amikacin, AN; amoxicillin, AMX; amoxicillin + clavulanic acid, AMC; aztreonam, ATM; cefalotin, CF; cefamandol, MA; cefepime, FEP; cefotaxime, CTX; cefoxitin, FOX; ceftazidime, CAZ; chloramphenicol, C; ciprofloxacin, CIP; fosfomycin, FOS; gentamicin, GM; imipenem, IPM; kanamycin, K; moxalactam, MOX; nalidixic acid, NA; netilmicin, NET; nitrofurantoin, FT; ofloxacin, OFX; pefloxacin, PEF; piperacillin, PIP; piperacillin + tazobactam, TZP; sulfamethoxazole, SSS; sulfamethoxazole + trimethoprim, SXT; tetracycline, TE; ticarcillin, TIC; ticarcillin + clavulanic acid, TCC; tobramycin, TM; trimethoprim, TMP. Sensitive, intermediate, and resistant phenotypes were scored as 0, 1, and 2, respectively. (A) The curve was obtained by plotting the cumulative sum of the score of antibiotic resistance per strain as the function of the increase of mutation frequencies. The plotted values correspond to $f[M(x_i)] = \sum_{[M(x_j) \le M(x_i)]} [R(x_j) - R]$. $M(x_j)$, $R(x_j)$, and R being the mutation frequency, the score of antibiotic resistance of the strain, and the average score of antibiotic resistance in the collection, respectively. A decrease/increase in the plot reveals a succession of strains with a lower- or higher-than-average level of resistance. LMF, low mutation frequency; IMF, intermediate mutation frequency; HMF, high mutation frequency. (B) Three groups of strains having low, intermediate, and high mutation frequencies, with significantly (Mann-Whitney test) different levels of the score of antibiotic resistance per strain were identified using breaking points of the cumulative sum analysis curve in A (dotted lines). (C) Factorial analysis of correspondence. LMF, IMF, and HMF phenotypes were considered as illustrative variables. The levels of sensitivity (sensitive, S; intermediate, I; resistant, R) to each antibiotic were considered as active variables. This plane clearly distinguishes the LMF and HMF strains, grouped with the S phenotype on the positive values of the F1 axis, from the IMF strains with I and R phenotypes on the negative values. This collection has 52.6% of strains resistant to amoxicillin, a well-known indicator of the frequency of antibiotic resistance in bacterial populations. Identical patterns of the relationship between antibiotic resistance and mutation frequencies were obtained with two other collections encompassing both commensal and extra-intestinal pathogenic strains: a collection of 117 highly resistant strains producing extended spectrum β-lactamases (100% of strains resistant to amoxicillin) (BRANGER *et al.* 2005) and a collection of 119 strains isolated in the 1980s with a low level of antibiotic resistance (11.8% of strains resistant to amoxicillin) (data not shown).

(FUNCHAIN *et al.* 2000; GIRAUD *et al.* 2001). The rise and decline of mutator alleles is modulated by the strength of the mutator allele. Computer simulations predict that intermediate mutators can be selected and that, once selected, they have a much longer persistence time than strong mutators (TADDEI *et al.* 1997). Therefore, in the long run, intermediate mutators might have more chance to accumulate multiple antibiotic resistances than the strong mutator, a prediction in agreement with the higher prevalence of multiple resistances among intermediate mutators.

Selection acting on mutator alleles is also modulated by variation in environment and the opportunity for competition between strains. Mutator clones can hyperspecialize to their local conditions, whereas bacteria that experience many different environments are very sensitive to the overspecialization (through mutation accumulation or antagonistic pleiotropy) (COOPER and

Lenski 2000; Giraud *et al.* 2001). Hence, we predict that the large fraction of intermediate mutator strains will be associated with the appearance and maintenance of multiresistance among most free-living bacteria experiencing periods of life in diverse environments. Indeed, the correlation between high frequency of resistance to antibiotics with high mutation frequency was reported only for bacteria isolated from the lungs of cystic fibrosis (CF) patients (Oliver *et al.* 2000; Prunier *et al.* 2003; Roman *et al.* 2004). This is probably the consequence, in association with multiple antibiotic cures, of the strong compartmentalization and low migration rates within and between the lungs of CF patients that limit competition between strains and therefore increase the persistence of strong mutator alleles.

Knowledge of selective forces governing the evolution of mutation rates is extremely important for designing therapeutic strategies aiming to control appearance and dissemination of multidrug-resistant bacteria, but also of other pathologies. For example, the intermediate mutation rates, albeit at a much higher level than bacterial ones, also maximize human immunodeficiency virus (HIV) fitness. It was reported that lowering the HIV mutagenesis rate delays the appearance of drug resistance and is associated with the lower viral burden *in vivo* (Wainberg *et al.* 1996), while increasing the rate of HIV mutagenesis reduces viral infectivity due to accumulation of deleterious mutations (Smith *et al.* 2005). Similarly, while, on one hand, mutator phenotypes can accelerate tumor progression by, for example, facilitating the evasion of host immune defenses (Branch *et al.* 1995), on the other hand, the mutator phenotype reduces tumor fitness in the longer run and consequently renders it more responsive to chemotherapy than nonmutator tumors (Bignami *et al.* 2003).

We thank Guy Perriere for help with statistical analysis and Christine Amorin for technical assistance. This work was partially supported by a grant from the Fondation pour la Recherche Médicale.

LITERATURE CITED

Bignami, M., I. Casorelli and P. Karran, 2003 Mismatch repair and response to DNA-damaging antitumour therapies. Eur. J. Cancer **39:** 2142–2149.

Blazquez, J., 2003 Hypermutation as a factor contributing to the acquisition of antimicrobial resistance. Clin. Infect. Dis. **37:** 1201–1209.

Branch, P., D. C. Bicknell, A. Rowan, W. F. Bodmer and P. Karran, 1995 Immune surveillance in colorectal carcinoma. Nat. Genet. **9:** 231–232.

Branger, C., O. Zamfir, S. Geoffroy, G. Laurans, G. Arlet *et al.*, 2005 Genetic background of *Escherichia coli* and extended-spectrum beta-lactamase type. Emerg. Infect. Dis. **11:** 54–61.

Cooper, V. S., and R. E. Lenski, 2000 The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature **407:** 736–739.

Denamur, E., S. Bonacorsi, A. Giraud, P. Duriez, F. Hilali *et al.*, 2002 High frequency of mutator strains among human uropathogenic *Escherichia coli* isolates. J. Bacteriol. **184:** 605–609.

Funchain, P., A. Yeung, J. L. Stewart, R. Lin, M. M. Slupska *et al.*, 2000 The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. Genetics **154:** 959–970.

Giraud, A., I. Matic, O. Tenaillon, A. Clara, M. Radman *et al.*, 2001 Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. Science **291:** 2606–2608.

Giraud, A., I. Matic, M. Radman, M. Fons and F. Taddei, 2002 Mutator bacteria as a risk factor in treatment of infectious diseases. Antimicrob. Agents Chemother. **46:** 863–865.

LeClerc, J. E., B. Li, W. L. Payne and T. A. Cebula, 1996 High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science **274:** 1208–1211.

Mao, E. F., L. Lane, J. Lee and J. H. Miller, 1997 Proliferation of mutators in a cell population. J. Bacteriol. **179:** 417–422.

Matic, I., M. Radman, F. Taddei, B. Picard, C. Doit *et al.*, 1997 Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. Science **277:** 1833–1834.

Morosini, M. I., M. R. Baquero, J. M. Sanchez-Romero, M. C. Negri, J. C. Galan *et al.*, 2003 Frequency of mutation to rifampin resistance in *Streptococcus pneumoniae* clinical strains: *hexA* and *hexB* polymorphisms do not account for hypermutation. Antimicrob. Agents Chemother. **47:** 1464–1467.

Oliver, A., R. Canton, P. Campo, F. Baquero and J. Blazquez, 2000 High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science **288:** 1251–1254.

Prunier, A. L., B. Malbruny, M. Laurans, J. Brouard, J. F. Duhamel *et al.*, 2003 High rate of macrolide resistance in *Staphylococcus aureus* strains from patients with cystic fibrosis reveals high proportions of hypermutable strains. J. Infect. Dis. **187:** 1709–1716.

Richardson, A. R., Z. Yu, T. Popovic and I. Stojiljkovic, 2002 Mutator clones of *Neisseria meningitidis* in epidemic serogroup A disease. Proc. Natl. Acad. Sci. USA **99:** 6103–6107.

Roman, F., R. Canton, M. Perez-Vazquez, F. Baquero and J. Campos, 2004 Dynamics of long-term colonization of respiratory tract by *Haemophilus influenzae* in cystic fibrosis patients shows a marked increase in hypermutable strains. J. Clin. Microbiol. **42:** 1450–1459.

Smith, R. A., L. A. Loeb and B. D. Preston, 2005 Lethal mutagenesis of HIV. Virus Res. **107:** 215–228.

Taddei, F., M. Radman, J. Maynard-Smith, B. Toupance, P. H. Gouyon *et al.*, 1997 Role of mutators in adaptive evolution. Nature **387:** 700–702.

Tenaillon, O., B. Toupance, H. Le Nagard, F. Taddei and B. Godelle, 1999 Mutators, population size, adaptive landscape and the adaptation of asexual populations of bacteria. Genetics **152:** 485–493.

Wainberg, M. A., W. C. Drosopoulos, H. Salomon, M. Hsu, G. Borkow *et al.*, 1996 Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science **271:** 1282–1285.

Watson, M. E. J., J. L. Burns and A. L. Smith, 2004 Hypermutable *Haemophilus influenzae* with mutations in *mutS* are found in cystic fibrosis sputum. Microbiology **150:** 2947–2958.

Communicating editor: T. Stearns