

## High Frequency of Mutator Strains among Human Uropathogenic *Escherichia coli* Isolates

Erick Denamur,<sup>1</sup> Stéphane Bonacorsi,<sup>2</sup> Antoine Giraud,<sup>3</sup> Patrick Duriez,<sup>1,3</sup> Farida Hilali,<sup>4</sup> Christine Amarin,<sup>1</sup> Edouard Bingen,<sup>2</sup> Antoine Andremont,<sup>4</sup> Bertrand Picard,<sup>5</sup> François Taddei,<sup>3</sup> and Ivan Matic<sup>3\*</sup>

INSERM U 458<sup>1</sup> and Laboratoire d'Étude de Génétique Bactérienne dans les Infections de l'Enfant (EA3105),<sup>2</sup> Hôpital Robert Debré, INSERM EPI 9916, Faculté de Médecine Necker-Enfants Malades, Université René Descartes—Paris V,<sup>3</sup> and INSERM EPI 9933, Hôpital Bichat-Claude Bernard, AP-HP,<sup>4</sup> Paris, and Laboratoire de Microbiologie, Hôpital Morvan, Brest,<sup>5</sup> France

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**By using a panel of 603 commensal and pathogenic *Escherichia coli* and *Shigella* isolates, we showed that mutation rates of strains vary considerably among different ecotypes. Uropathogenic strains had the highest frequency of mutators, while strains from patients with bacteremia had the lowest mutation rates. No correlation between the mutation rates and antibiotic resistance was observed among the studied strains.**

Bacterial populations with a high level of genetic variability have a higher probability of survival in constantly changing environments (18). Since genetic variability is generated mostly by mutagenesis, bacterial strains with high mutation rates are expected to have higher capacities for adaptation. Such strains are favored by selection when the advantage of beneficial mutations is greater than the cost of being a mutator due to the overproduction of lethal and deleterious mutations (6, 17, 19). Mutator strains, having a defective mismatch repair system, have indeed been observed in natural populations of *Escherichia coli*, *Salmonella enterica*, *Neisseria meningitidis*, and *Pseudomonas aeruginosa* (5, 7, 10, 12). Because most of these isolates are pathogens, it has been hypothesized that mutator and hyperrecombination phenotypes may accelerate the evolution of pathogenic strains by, e.g., increasing the variation of surface antigens, as well as by facilitating the acquisition of pathogenic determinants and antibiotic resistance. Indeed, it has been observed that the levels of resistance to antibiotics were significantly higher in mutator than in nonmutator pathogenic *P. aeruginosa* isolates (10) and that mismatch repair-deficient *N. meningitidis* strains displayed high phase variation rates (12).

However, from the available data, it is not clear whether high mutation rates are particularly important for the evolution of pathogens in general or for the evolution of only some pathogenic groups (5, 7, 10, 11). Furthermore, a mutator phenotype may not be specific to pathogens, since mutators have also been observed in commensal populations (7). In order to examine the link between a particular bacterial lifestyle, mutation rate, and antibiotic resistance, we used a collection of 603 human *E. coli* (including *Shigella*) isolates, either commensal isolates or ones involved in various pathologies, such as enteroinvasive and enterohemorrhagic diseases, urinary tract

infection (UTI), bacteremia, pus production from miscellaneous infections, and newborn meningitis (NBM). A detailed list of strains is given in Table 1.

**Variations of mutation rates.** The mutation rates of the studied strains were estimated by monitoring the strains' capacities to generate mutations conferring resistance to rifampin in at least six independent cultures for each strain (Fig. 1 and 2), as described previously (16). Between  $10^2$  and  $10^3$  cells from an overnight culture were inoculated onto nitrocellulose filters (NC45; Schleicher and Schuell) laid on plates containing fresh 869 medium (NaCl, 5 g/liter; Bacto Tryptone, 10 g/liter; yeast extract, 5 g/liter; agar, 15 g/liter). The plates were incubated at 37°C for 24 h. The cells were resuspended in 1 ml of 869 medium and incubated for 1 h at 37°C to allow for rifampin resistance expression. Appropriate dilutions were then spread on 869 medium plates with rifampin (100 µg/ml; Sigma) or without. The rifampin-resistant mutants were counted after 24 h at 37°C.

We found mutators among commensals and pathogens, but the frequencies of mutators in these two groups of strains were not significantly different (Fig. 1 and 2). However, when pathogenic strains were analyzed as members of different ecotypes, mutator strains were found to occur significantly more frequently among UTI strains than they did among commensals and also more frequently among UTI strains than among all other pathogenic strain groups. It is interesting to note that bacteremia strains, which include urosepsis isolates, had the smallest fraction of mutator strains but also, significantly (according to the *t* test), the lowest mutation rates of all strains (Fig. 1 and 2). Strains isolated from pus also have significantly lower mutation rates than all other strains (except bacteremia strains) (Fig. 1 and 2).

We confirmed that strains generating rifampin-resistant mutants at a high rate correspond to generalized mutators by measuring the frequencies of the mutations that confer resistance to the following four additional antibiotics (at indicated concentrations) in at least six independent cultures for each strain: nalidixic acid (40 µg/ml), phosphomycin (30 µg/ml),

\* Corresponding author. Mailing address: INSERM EPI 9916, Faculté de Médecine Necker-Enfants Malades, Université René Descartes—Paris V, 156 Rue de Vaugirard, 75730 Paris Cedex 15, France. Phone: (33) 1 40 61 53 25. Fax: (33) 1 40 61 53 22. E-mail: matic@necker.fr.

TABLE 1. Strains studied

Strain ecotype or pathology	Total no. of strains	Origin(s) <sup>a</sup> (no. of strains)	Reference(s) or source
Bacteremia	93	Paris (three university hospitals) (88), Avignon (one general hospital) (5)	4a, 11a; unpublished data
Commensal organisms	217	Croatia (61), Mali (57), France (70), ECOR collection (Sweden and United States) (29)	2a, 4a, 9a
Enteroinvasive disease	86	Mainly France: <i>Shigella</i> (73), EIEC (13)	13a
Enterohemorrhagic disease	26	France	Unpublished data
NBM	60	Mainly France	1
Pus from miscellaneous infections	30	Paris (two university hospitals) (24), Avignon (one general hospital) (5), Paris (outpatient) (1)	11a; unpublished data
UTI	91	Paris (one university hospital) (49), Avignon (one general hospital) (21), Paris (outpatient) (10), ECOR collection (Sweden) (11)	9a, 11a; unpublished data

<sup>a</sup> ECOR, *E. coli* reference; ECEI, enteroinvasive *E. coli*.

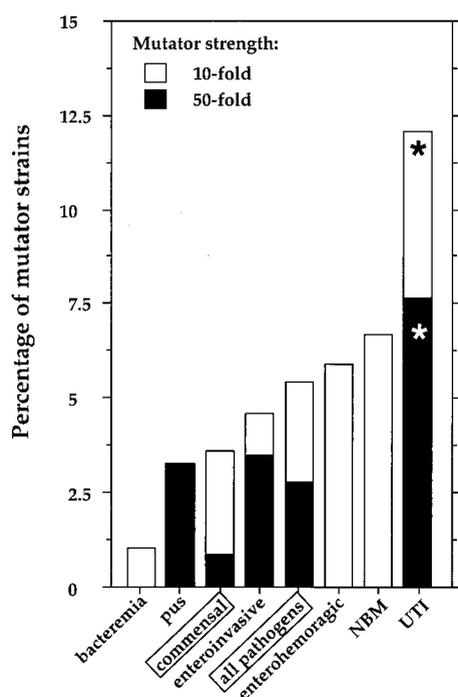


FIG. 1. Mutator strains belonging to different human *E. coli* and *Shigella* commensal and pathogenic groups. Bacterial strains are grouped according to their origins and the different pathologies in which they have been involved, including bacteremia strains ( $n = 93$ ), strains isolated from pus ( $n = 30$ ), commensal strains ( $n = 217$ ), enteroinvasive strains ( $n = 86$ ), enterohemorrhagic strains ( $n = 26$ ), NBM strains ( $n = 60$ ), and UTI strains ( $n = 91$ ). In addition, all pathogenic strains are presented as one group in order to facilitate a comparison of that group with commensal strains. Strains were considered mutators when they exhibited frequencies of mutations conferring resistance to rifampin ( $100 \mu\text{g/ml}$ ) that were 10-fold higher than the median value of mutagenesis ( $5.04 \times 10^{-9}$ ) observed for all studied strains ( $n = 603$ ) (10-fold mutators). Strains that displayed a  $>50$ -fold increase in mutagenesis were considered strong mutators (50-fold mutators). Percentages of mutator strains were calculated for every group of studied strains. UTI strains had significantly higher (according to the  $\chi^2$  test) fractions (\*) of 10- and 50-fold mutators than commensal strains ( $P = 0.005$  and  $P = 0.001$  for 10- and 50-fold mutators, respectively), as well as other pathogens ( $P = 0.001$  and  $P = 0.001$  for 10- and 50-fold mutators, respectively).

spectinomycin ( $100 \mu\text{g/ml}$ ), and streptomycin ( $100 \mu\text{g/ml}$ ) (all from Sigma) (Fig. 3).

**Why do UTI strains have the highest frequency of mutator strains?** One possible explanation for the high frequency of mutators in UTI strains is that mutator strains belong to one

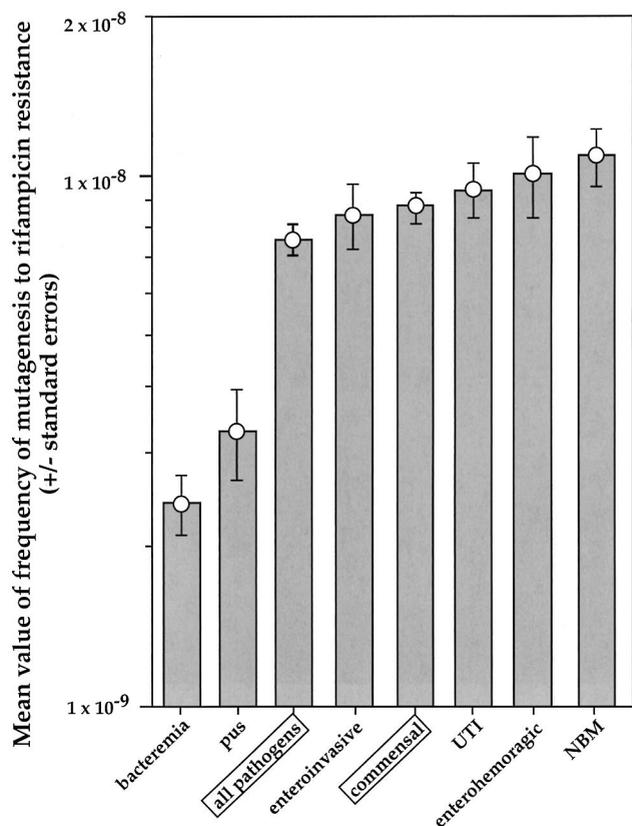


FIG. 2. Variability of mutation rates of human *E. coli* and *Shigella* isolates after elimination of mutators. The mean value ( $\pm$  standard error) of mutation frequency, after removal of those for 10- and 50-fold mutators (see legend to Fig. 1), is shown for each group.

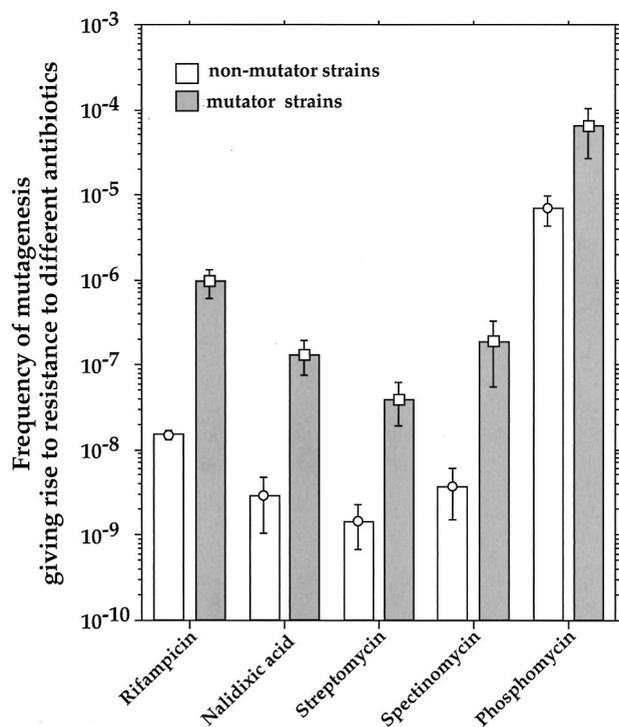


FIG. 3. Capacities of mutator strains to generate mutations conferring resistance to different antibiotics. The results are presented as mean values ( $\pm$  standard errors) for mutator ( $>10$ -fold increase in mutagenesis;  $n = 21$ ) and nonmutator ( $n = 47$ ) strains.

clone which has increased in frequency in populations of UTI strains due to the action of positive selection. However, we found that strong UTI mutator strains belong to different *E. coli* phylogenetic groups: A, B2, and D. Furthermore, by sequencing metabolic genes (*trpA*, *trpB*, *putP*, and *papB*), we have also confirmed that the group B2 UTI mutator strains (most abundant among UTI strains) did not belong to the same clone (data not shown). Therefore, our data suggest that there is no correlation between mutation rate and phylogenetic group.

The possibility that UTI mutators are better adapted to growth in urine, due to the acquisition of adaptive mutations or to a pleiotropic effect linked to a modified DNA repair ability, seems to be marginal at best, since both mutator and nonmutator UTI strains grow easily in fresh urine (with no significant difference between them), reaching concentrations of about  $10^8$  CFU/ml (data not shown).

Another possibility is that mutators are less frequently counterselected in the urinary tract than in other body compartments. It has been demonstrated that mutators can suffer a reduction of fitness due to the accumulation of deleterious mutations (3, 4). One of the measurable phenotypes of fitness reduction is loss of the capacity to grow on minimal synthetic medium. This handicap might be less important, at least in the short run, in urine, as suggested by a higher incidence of auxotrophs (25%) among UTI strains than among strains from fecal samples (5.8%) (13). However, we did not observe more auxotrophs among UTI mutator strains than among UTI nonmutators (data not shown).

Finally, it is possible that UTI mutators are selected because they generate mutations that increase adaptation to the urinary tract at a higher rate than that generated by nonmutators. For example, it has been shown that point mutations in *fimH* genes increase binding of the adhesin to monomannose residues, structures that are abundant in the urothelial glycoproteins, conferring increased virulence in a UTI mouse model (15) as well as an increased capacity for biofilm formation (14). However, this hypothesis must be confirmed by in vivo and in vitro reconstruction experiments.

**Antibiotic resistance.** It is possible that antibiotic treatments contribute to selection of the mutators, as has been demonstrated in in vitro experiments (6). Mutators can be favored under such conditions because they generate antibiotic resistance-conferring mutations at a higher rate than that generated by nonmutators (Fig. 3). In addition, they also generate more mutations that compensate for the fitness reduction associated with antibiotic resistance (1a).

Since most of the antibiotics we used to demonstrate the mutator phenotype of the strains were not of medical relevance, we performed standard antibiogram testing of the activities of amikacin, amoxicillin, amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, and phosphomycin (8), as well as a determination of the MICs of ceftazidime, amikacin, and ciprofloxacin (9) for 26 mutator and 42 nonmutator strains. The majority of mutator strains yielded colonies inside the growth inhibition zone (squatter colonies), while no nonmutator strains exhibited that phenotype (Fig. 4). The presence of squatter colonies reflects the high frequency of mutations conferring resistance to antibiotics. The squatter colonies were not observed only when ceftazidime or ciprofloxacin was used. However, mutator strains are not more resistant than nonmutators are, and no mutator strain was resistant to multiple antibiotics (data not shown).

Furthermore, additional UTI strains that are resistant to quinolones ( $n = 9$ ) or that have an overexpressed cephalosporinase ( $n = 7$ ) (both resistance mechanisms resulting from point mutations) did not show a higher mutation rate than nonmutator strains (data not shown). Therefore, it can be concluded that antibiotics are probably not the major selective pressure that favors mutator strains in natural *E. coli* populations.

**Conclusions.** Although UTI strains have the highest frequency of mutators, the link between high mutation rates and pathogenicity cannot be generalized. Other pathogenic groups do not have more mutators than commensal organisms do. Furthermore, bacteremia and pus isolates have very low mutation rates (Fig. 1 and 2). The reason for the observed high frequency of mutators in populations of UTI strains remains to be determined.

Our finding that mutators are present in almost all studied groups of *E. coli* ecotypes supports recently published observations which suggest that the majority of *E. coli* strains repeatedly pass through periods of high mutation rates during their evolutionary history, regardless of whether they are commensal or pathogenic or to which phylogenetic group they belong (2).

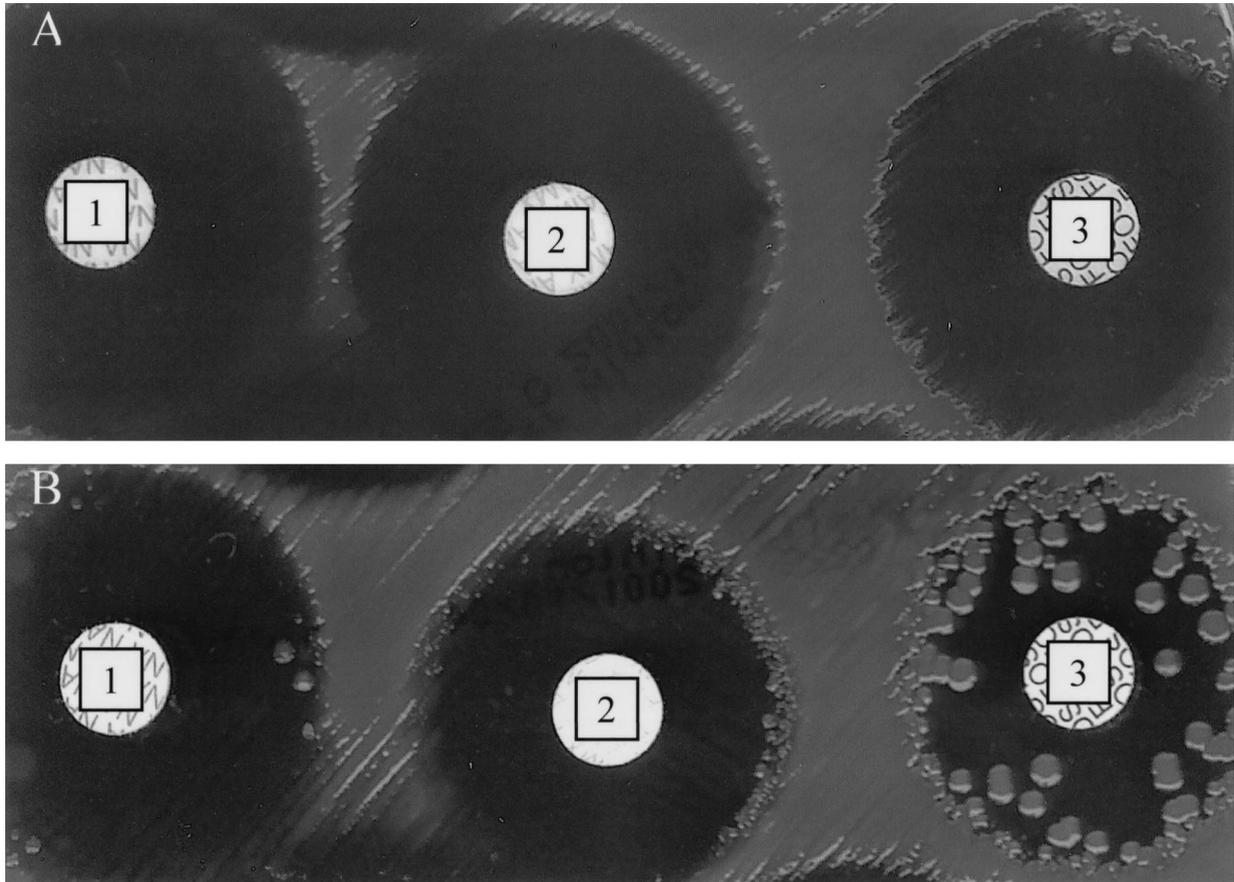


FIG. 4. Squatter colonies inside growth inhibition zone. Growth inhibitory zones for nalidixic acid (disk 1), amoxicillin (disk 2), and phosphomycin (disk 3) are presented for nonmutator (A) and mutator (B) strains. Note the presence of squatter colonies for the mutator strain only.

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