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# Letter to the Editor

## Clonal Origin, Virulence Factors, and Virulence

The recent report from Picard et al. regarding extraintestinal *Escherichia coli* infections provides new insights into the relationships between clonal origin, virulence factor repertoire, and virulence (1). That clonal origin was only secondarily associated with virulence in this study was suggested by the finding that although mouse lethality was more frequent overall among strains of phylogenetic group B2 than among other strains, lethality was proportional to the number of virulence traits present (other than *aer* and *afa*) and was similar among B2 and non-B2 strains after stratification according to virulence factor numbers (Table 1).

Stepwise logistic regression analysis of the data in Table 1 of Picard et al. (1) showed that, when competing with the individual virulence factors, phylogenetic group B2 status was never the strongest predictor of lethality for any of the three lethality endpoints analyzed (data not shown). When the presence or absence of two or more non-*aer* non-*afa* virulence factors was entered as a single dichotomous variable into logistic regression analysis along with B2 status, the presence of multiple virulence factors accounted for almost all the observed lethality, with B2 status exhibiting a weak and marginally significant residual association with lethality for only one of the three lethality endpoints (Table 2).

These observations suggest that the group B2 genomic background is not required for virulence, whereas specific virulence properties are. They further suggest that the association of phylogenetic group B2 with extraintestinal virulence is attributable to the abundance of virulence factors in this lineage rather than to some undefined quality of the B2 genomic background per se. This concept is consistent with the traditional "virulent clone" hypothesis (4). What remains unclear is why extraintestinal virulence factors are so selectively concentrated within group B2. The major competing hypotheses to explain this phenomenon include (i) the existence of a special compatibility between the B2 genome and virulence genes and (ii) chance and timing.

As proposed by Picard et al. (1), preexisting features of the B2 genome may have made this lineage preferentially receptive to the initial acquisition of exogenous virulence genes and/or may have promoted retention of such genes once acquired. Or, compensatory (adaptive) mutations which now help maintain virulence genes in group B2 may have appeared subsequent to the entry of these genes into the lineage, which initially may not have been particularly receptive to the foreign genes.

Alternatively, there may be no special affinity between group B2 and virulence genes. Virulence genes may have been acquired by chance by a group B2 ancestor soon enough after the group's emergence as to be inherited vertically by most members of the group as the group underwent clonal expansion. This could account for the broad prevalence of virulence genes within group B2. Furthermore, because of the comparative "youth" of group B2, the initial virulence gene acquisition event(s) may have occurred so recently that there simply has not been sufficient evolutionary time for these newcomer sequences to diffuse outward from group B2 through the rest of the species despite their comparatively high rates of horizontal transfer relative to other components of the genome. Future studies designed to select between these competing hypotheses will refine our understanding of the origins of virulent extraintestinal *E. coli* and perhaps suggest new preventive strategies.

It should be noted that knockout studies are required to confirm that the suspected virulence traits themselves (rather than associated bacterial properties) are actually responsible for enhanced virulence (2). Furthermore, knockout studies can identify bacterial traits which, although widely prevalent among commensal as well as pathogenic *E. coli* (e.g., *guaA* and *argC*), are necessary (albeit not sufficient) for virulence and hence may constitute potential targets for preventive or therapeutic interventions despite having no epidemiological associations with disease (3).

TABLE 1. Lethality for mice according to B2 phylogenetic group status and number of virulence factors[a]

| No. of virulence factors[c] | Proportion of strains lethal to mice[b] | | |
|---|---|---|---|
| | non-B2[d] ($n$ = 45) | B2[d] ($n$ = 37) | Total ($n$ = 82) |
| 0 | 0/30 | 0/3 | 0/33 |
| 1 | 1/7 | 1/2 | 2/9 |
| 2 | 6/8 | 6/8 | 12/16 |
| >2 | (none) | 24/24 | 24/24 |

[a] Data from reference 1.
[b] Lethal to one or more mice.
[c] Factors including hemolysin, MRHA, K1 antigen, *sfa*/*foc*, *pap*, *hly*, and *ibe*10 (not *afa* and *aer*).
[d] $P > 0.10$ for proportion lethal to mice for phylogenetic group B2 versus non-B2 (i.e., A, B1, and D) strains within each virulence factor stratum (no, one, or two virulence factors).

TABLE 2. Logistic regression analysis of multiple virulence factors and B2 status as predictors of mouse lethality[a]

| Lethality criterion | Multiple virulence factors[b] | | B2 phylogenetic group | |
|---|---|---|---|---|
| | RR[c] | $P$ | RR | $P$ |
| ≥1 mouse (model 1) | 82 | <0.001 | 6 | 0.04 |
| All 10 mice (model 2) | 31 | 0.003 | 2 | 0.33 |
| Accelerated lethality[d] (model 3) | 37 | 0.001 | 3 | 0.11 |

[a] Results are based on data from reference 1.
[b] Two or more factors including hemolysin, MRHA, K1 antigen, *sfa*/*foc*, *pap*, *hly*, and *ibe*10 (not *afa* and *aer*).
[c] RR, relative risk.
[d] Accelerated lethality as indicated by + in the column labeled "Lethality in mice" of Table 1 of reference 1.

### REFERENCES

1. **Picard, B., J. Sevali Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.
2. **Russo, T. A., J. J. Brown, S. T. Jodush, and J. R. Johnson.** 1996. The O4 specific antigen moiety of lipopolysaccharide but not the K54 group 2 capsule is important for urovirulence in an extraintestinal isolate of *Escherichia coli*. Infect. Immun. **64:**2343–2348.
3. **Russo, T. A., J. J. Brown, S. T. Jodush, and J. R. Johnson.** 1996. Identification of two previously unrecognized genes (*guaA*, *argC*) important for uropatho-

genesis. Mol. Microbiol. **22:**217–229.
4. **Vaisanen-Rhen, V., J. Elo, E. Vaisanen, A. Siitonen, I. Orskov, F. Orskov, S. B. Svenson, P. H. Makela, and T. Korhonen.** 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun. **43:**149–155.

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## Author's Reply

We agree with the analysis that Johnson and Kuskowski have made of our recently published data (6). Actually, lethality is directly proportional to the number of virulence factors regardless of the phylogenetic group. This probably should have been clearly stated in our report. However, our major focus was to highlight the selective concentration of virulence factors in phylogenetic group B2, thus establishing a link between virulence and phylogeny. In that respect, Johnson and Kuskowski argue that two competing hypotheses can be made: not only (i) the existence of a special compatibility between the B2 genome and virulence genes (i.e., the hypothesis we defend) but also (ii) chance and timing. Although we accept that virulence genes may have been acquired by chance, we disagree with the timing scenario. Indeed, based on the sequence data of several genes among strains of the *Escherichia coli* reference (ECOR) collection, we have reconstructed the *E. coli* phylogeny and shown that group B2 is the most basal group of the species (4). Group D then emerges as the sister group of the rest, and finally groups A and B1 are sister groups (4). Assuming a molecular clock (a rather controversial concept [7]), the time of divergence between monophyletic group B2 and the rest of the species is 25.8 to 30.8 million years. Thus, group B2 cannot be considered "comparative youth," as stated by Johnson and Kuskowski, but instead is the most primitive taxon within *E. coli* in terms of branching pattern. The most parsimonious scenario accounting for the presence of virulence genes in most members of this group, compared to the other phylogenetic groups, is their acquisition once in a common B2 ancestor followed by vertical transmission. Phylogenetic reconstructions from the virulence gene sequences yield trees which are not congruent with the *E. coli* phylogeny, indicating that these genes are transferred horizontally between members of the species (2, 3). However, within the same divergence time, plasmid genes or genes submitted to selective pressure (such as the *hsd* locus or the *gnd* locus close to the O antigen gene complex, which is subject to hitchhiking) show a considerably higher rate of horizontal transfer (1, 4). In this context, and assuming that selection favors virulent strains (5), it seems that the simplest explanation for the rarity of horizontal transfer of virulence genes toward non-B2 strains resides in a "fine tuning" between the virulence genes and the B2 genetic background.

### REFERENCES

1. **Barcus, V. A., A. J. B. Titheradge, and N. E. Murray.** 1995. The diversity of alleles at the *hsd* locus in natural populations of *Escherichia coli*. Genetics **140:**1187–1197.
2. **Bingen, E., B. Picard, N. Brahimi, S. Mathy, P. Desjardins, J. Elion, and E. Denamur.** 1998. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J. Infect. Dis. **177:**642–650.
3. **Boyd, E. F., and D. L. Hartl.** 1998. Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. J. Bacteriol. **180:**1159–1165.
4. **Lecointre, G., L. Rachdi, P. Darlu, and E. Denamur.** 1998. *Escherichia coli* molecular phylogeny using the incongruence length difference test. Mol. Biol. Evol. **15:**1685–1695.
5. **Lipsitch, M., and E. R. Moxon.** 1997. Virulence and transmissibility of pathogens: what is the relationship? Trends Microbiol. **5:**31–37.
6. **Picard, B., J. Sevali Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.
7. **Strauss, E.** 1999. Can mitochondrial clocks keep time? Science **283:**1435–1438.

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