Homologous Recombination in \textit{Clostridioides difficile} Mediates Diversification of Cell Surface Features and Transport Systems

Hannah D. Steinberg,a* Evan S. Snitkinb

aUniversity of Michigan School of Public Health, Ann Arbor, Michigan, USA
bDepartment of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA

ABSTRACT Illness caused by the pathogen \textit{Clostridioides difficile} is widespread and can range in severity from mild diarrhea to sepsis and death. Strains of \textit{C. difficile} isolated from human infections exhibit great genetic diversity, leading to the hypothesis that the genetic background of the infecting strain at least partially determines a patient’s clinical course. However, although certain strains of \textit{C. difficile} have been suggested to be associated with increased severity, strain typing alone has proved insufficient to explain infection severity. The limited explanatory power of strain typing has been hypothesized to be due to genetic variation within strain types, as well as genetic elements shared between strain types. Homologous recombination is an evolutionary mechanism that can result in large genetic differences between two otherwise clonal isolates, and also lead to convergent genotypes in distantly related strains. More than 400 \textit{C. difficile} genomes were analyzed here to assess the effect of homologous recombination within and between \textit{C. difficile} clades. Almost three-quarters of single nucleotide variants in the \textit{C. difficile} phylogeny are predicted to be due to homologous recombination events. Furthermore, recombination events were enriched in genes previously reported to be important to virulence and host-pathogen interactions, such as flagella, cell wall proteins, and sugar transport and metabolism. Thus, by exploring the landscape of homologous recombination in \textit{C. difficile}, we identified genetic loci whose elevated rates of recombination mediated diversification, making them strong candidates for being mediators of host-pathogen interaction in diverse strains of \textit{C. difficile}.

IMPORTANCE Infections with \textit{C. difficile} result in up to half a million illnesses and tens of thousands of deaths annually in the United States. The severity of \textit{C. difficile} illness is dependent on both host and bacterial factors. Studying the evolutionary history of \textit{C. difficile} pathogens is important for understanding the variation in pathogenicity of these bacteria. This study examines the extent and targets of homologous recombination, a mechanism by which distant strains of bacteria can share genetic material, in hundreds of \textit{C. difficile} strains and identifies hot spots of realized recombination events. The results of this analysis reveal the importance of homologous recombination in the diversification of genetic loci in \textit{C. difficile} that are significant in its pathogenicity and host interactions, such as flagellar construction, cell wall proteins, and sugar transport and metabolism.

KEYWORDS \textit{Clostridioides difficile}, S layer, flagella, homologous recombination, phosphotransferase system

\textit{Clostridium difficile} is a significant cause of nosocomial infections and can result in clinical presentations ranging from mild diarrhea to severe colitis, sepsis, and death. \textit{C. difficile} causes disease when it can colonize and grow in the gastrointestinal tract, most often after antibiotic treatment depletes a patient’s normal gut microbiota (1, 2). Two major toxins, toxin A and toxin B, are associated with the virulence of \textit{C.
*difficile*, and their secretion results in the destruction of intestinal epithelial cells and recruitment of inflammatory mediators and neutrophils (2–5). However, other factors, such as adhesins, cell surface capsule proteins, hydrolytic enzymes, and flagella, have also been suggested to play an important role in *C. difficile* colonization, pathogenesis, and virulence (2, 6).

Five major phylogenetic clades of *C. difficile* have been described (7), and upward of 100 PCR-ribotypes circulate and cause disease in U.S. hospitals (8). Some studies have suggested that epidemic ribotypes 027 (clade 2) and 078 (clade 5) cause more severe disease than other *C. difficile* strains (9, 10), while others fail to find an association between ribotype and disease severity (11). Thus, more research is needed to explore pathogen factors important in disease manifestation and heterogeneity in severity.

Bacteria can acquire genetic diversity through vertically inherited mutations, recombination via transformation of free DNA, transduction by phage, and conjugation of plasmids (12). Recombination can either be homologous, in which a genomic region is exchanged for an allelic variant, or nonhomologous, in which accessory genes can be transferred from one organism to another (12). Studying bacterial recombination is important in genomics studies since it can both weaken phylogenetic signals and reveal important selective pressures (13). Genomic hot spots for recombination have been hypothesized to be due to both mechanistic and evolutionary reasons. For example, lower rates of recombination have been observed at the terminus compared to the origin of replication, and conserved core genes often flank highly recombinant genes in *Escherichia coli* (14). However, recombination events that are disadvantageous to the organism will be lost via negative selection, and the events that remain to be observed are likely to be advantageous or neutral in effect (13).

Homologous recombination has been shown to result in high rates of genetic diversity in adhesion, invasion, and colonization factors, as well as cell membrane/surface structures in various pathogenic bacteria (13, 15–17). These factors each play a role in the pathogenesis and/or host interactions of these pathogens. Elevated levels of genetic diversity in these features could lead to adaptations in virulence, transmission, or evasion of the host immune system. In *C. difficile*, the S-layer cassette that encodes surface proteins important for its antigenicity has been shown to undergo frequent recombination (13, 18). This study utilizes more than 400 *C. difficile* whole-genome sequences to unveil the contribution of homologous recombination within and between clades of this pathogen, and to elucidate specific functions associated with higher rates of realized recombination events.

**RESULTS AND DISCUSSION**

**Strain selection.** A total of 412 strains were selected that cluster into five distinct clades (Fig. 1a), as described previously (7). Of the 412 strains, 306 isolates are in clade 1, 76 in clade 2, 15 in clade 3, 10 in clade 2, and 5 in clade 5. Clade 5 is the most distantly related from the rest of the phylogeny, with only 97% sequence identity to reference strain 630 (calculated as number of identical nucleotides over the length of the aligned sequence). Since the majority of isolates are in clades 1 and 2 and since excluding clades 3, 4, and 5 increased the size of the core genome (from 1.66 to 1.75 Mbp) and allowed more genes to be studied, gene and pathway analyses were performed on just the 382 isolates in clades 1 and 2. Moreover, besides ribotype 078, which is in clade 5 and is relatively distantly related to clades 1 and 2, the majority of *C. difficile* isolates that cause human disease in the United States are in clade 1 (includes ribotype 014/020) and clade 2 (includes ribotype 027) (8), so the results of these analyses can be applicable to much of pathogenic *C. difficile*.

**Homologous recombination in five *C. difficile* clades.** There is a great deal of heterogeneity in the contribution of recombination to the evolution across the *C. difficile* phylogeny, as measured by the proportion of single-nucleotide variants (SNVs) inside compared to outside predicted recombination events (r/m) (Fig. 1b). Most branches have more single nucleotide substitution events than recombination events (see Fig. S1 in the supplemental material); however, each recombination event can lead to
the transfer of multiple SNVs. Throughout the phylogeny, 72% of SNVs fall within predicted recombination events, even though there are about 15 times as many inferred nucleotide substitution events as there are recombination events. In addition to variance on branches of the tree, there is also a significant amount of heterogeneity in
and F1 operons are responsible for early-stage and late-stage recombination, with there being more F3 genotypes than F1 genotypes present in these isolates. The F3 operon between F1 and F3, although these operational units are so diverse that they do not show up in the core genome for clades 1 and 2, and thus homologous recombination in the F2 operon was not analyzed in this study. Supporting the existence of selective pressure for diversification of F2 are previous reports of

The median length of recombination events in all C. difficile clades is $-6.7$ kb, and the maximum length of a predicted recombination event was $>100$ kb in clade 1. Overall, most recombination events are $<10$ kb, with an exponential decay in events with increasing length (Fig. 1c). This distribution is observed in all C. difficile clades, though there are more recombination events observed overall in clades 1 and 2 than in clades 3, 4, and 5, likely because the genome sample size is greatest in clades 1 and 2.

**Areas of enriched homologous recombination.** Twelve percent (486/3,980) of C. difficile genes analyzed were significantly enriched for homologous recombination ($P < 0.05$) according to a permutation test (see Table S2 in the supplemental material), and 40 of these genes were enriched for recombination with a fold change of $\geq 3$ (Table 1). The majority of these highly enriched (3-fold) genes are involved in flagellar activity. Overall, the set of 486 significant genes were significantly overrepresented in four KEGG pathways, and one COG term involved in flagella, sugar transport and metabolism, and the cell envelope and outer membrane (Table 2). The S-layer cassette, which has previously been shown to be highly recombinant in C. difficile (13, 18), also appears in a high-recombination zone (Fig. 2), with many of the genes involved in S-layer structure significantly enriched for recombination (see Table S2 and Fig. S2 in the supplemental material). There is no KEGG pathway specific to S-layer proteins, but the COG ontology “cell envelope biogenesis, outer membrane,” whose genes are enriched for homologous recombination (Table 2), does include some S-layer proteins.

The genomic region from approximately 2.6 to 0.7 Mb appears to be enriched for recombination compared to the other half of the genome (Fig. 2). This could suggest the mechanistic feasibility of recombination in this area and/or grouping of genes where genetic diversity is more evolutionarily advantageous. Low recombination at the terminus of the genome is consistent with findings in *Escherichia coli* (14).

**Flagella.** There have been reports of large-scale structural variations of flagella genes in C. difficile, but the contribution of homologous recombination is not as well described. It is clear that unique flagellar variant patterns are present in distantly related isolates (Fig. 3). In particular, within each clade there appears to be a set of distinct flagellar genotypes distributed sporadically throughout the phylogeny, suggesting that large regions of the flagella operons may have been swapped in homologous recombination events. Further, there appears to be a distinct recombination pattern in the two flagellar regulatory units present in the clades 1 and 2 core genome, with there being more F3 genotypes than F1 genotypes present in these isolates. The F3 and F1 operons are responsible for early-stage and late-stage flagellar genes, respectively (19). F1 genes, such as *flIC* and *flID*, code for the flagellin filament and protein cap (19) that may be involved in protein-protein interactions and recombinant FlIC proteins have been shown to be involved in C. difficile immunogenicity (20). F3 genes are associated with other structural components of the flagella, such as the basal body, hook, and motor proteins (19), and the F3 gene *flIA* (*sigD*) has been suggested to be important not only in regulating late-stage flagellar genes but also in the production of C. difficile toxins (21).

Genomic structural diversity of C. difficile flagella has been described before. For instance, C. difficile ribotype 078 (clade 5) strains lack the F3 regulon completely, rendering that ribotype immotile (19). Furthermore, many strains of C. difficile also have an F2 operon between F1 and F3, although these operational units are so diverse that they do not show up in the core genome for clades 1 and 2, and thus homologous recombination in the F2 operon was not analyzed in this study. Supporting the existence of selective pressure for diversification of F2 are previous reports of
nonhomologous recombination in this region. *C. difficile* 630, for example, has four genes in its F2 region, including genes involved in glycosylation, while ribotype 027 isolate CD196 has six seemingly different genes in this area (19, 22).

These results suggest that homologous recombination, in addition to nonhomologous recombination, may be an important mechanism of flagellar diversity in *C. difficile*. It has been reported that flagella may have a role in motility, colonization, adherence, virulence, and immunogenicity in mucosal pathogens, including *C. difficile* (23), and in vivo studies show that mutants of late-stage flagellar genes *fliC* and *fliD* in *C. difficile* 630 exhibited greater virulence than did the wild type (24). The enrichment in

**TABLE 1** *C. difficile* genes with the greatest increase in recombination events in the clade 1 and 2 core genome

| Gene   | Annotation                                      | PR events | OR events | Fold change | P *\|* |
|--------|-------------------------------------------------|-----------|-----------|-------------|-------|
| fliG   | Flagellar motor switch protein FliG             | 11        | 58        | 5.4         | <0.001|
| fliH   | Flagellar assembly protein FliH                 | 11        | 58        | 5.3         | <0.001|
| fliI   | Flagellar protein FliI                          | 10        | 52        | 5.0         | <0.001|
| fliP   | Flagellar protein MS-ring protein               | 12        | 56        | 4.8         | <0.001|
| fliE   | Flagellar hook basal body protein FliE          | 11        | 50        | 4.7         | <0.001|
| flgD   | Basal-body rod modification protein FlgD        | 10        | 49        | 4.7         | <0.001|
| fliK   | Flagellar hook-length control protein FliK      | 10        | 48        | 4.6         | <0.001|
| flgE   | Flagellar hook protein FlgE                     | 11        | 51        | 4.6         | <0.001|
| flgB   | Flagellar basal body rod protein FlgB           | 11        | 48        | 4.5         | <0.001|
| flgC   | Flagellar basal body rod protein FlgC           | 11        | 47        | 4.3         | <0.001|
| CD630-27960 | Cell surface protein                               | 12      | 52        | 4.2         | <0.001|
| flbD   | Flagellar protein FlbD                          | 11        | 44        | 4.2         | <0.001|
| fls1   | Flagellar protein Fls1                          | 11        | 43        | 4.0         | <0.001|
| motB   | Flagellar motor rotation protein MotB           | 11        | 44        | 3.9         | <0.001|
| motA   | Flagellar motor rotation protein MotA           | 11        | 44        | 3.9         | <0.001|
| csrA   | Carbon storage regulator CsrA                   | 10        | 41        | 3.9         | <0.001|
| fliL   | Flagellar basal body associated protein FliL    | 11        | 41        | 3.9         | <0.001|
| fliZ   | Flagellar protein FliZ                          | 10        | 40        | 3.8         | <0.001|
| fls2   | Flagellar protein Fls2                          | 11        | 41        | 3.8         | <0.001|
| fliQ   | Flagellar biosynthetic protein FliQ              | 10        | 40        | 3.8         | <0.001|
| flhB   | Bifunctional flagellar biosynthesis protein FliR/FihB | 12        | 45        | 3.8         | <0.001|
| flp   | Flagellar biosynthesis protein FlpF             | 11        | 41        | 3.7         | <0.001|
| CD630-27970 | Calcium binding adhesion protein                     | 17      | 62        | 3.7         | <0.001|
| CD630-36440 | Hypothetical protein                                      | 11      | 40        | 3.6         | <0.001|
| flhA   | Flagellar biosynthesis protein FlhA              | 12        | 42        | 3.5         | <0.001|
| flc   | Flagellin C                                     | 11        | 39        | 3.4         | <0.001|
| CD630-30830 | PTS operon transcription antiterminator                  | 11      | 38        | 3.4         | <0.001|
| flid   | flagellar hook-associated protein FliD           | 12        | 40        | 3.3         | <0.001|
| CD630-02380 | hypothetical protein                                      | 11      | 35        | 3.3         | <0.001|
| fliW   | Flagellar assembly factor FliW                   | 11        | 34        | 3.2         | <0.001|
| flhF   | Flagellar biosynthesis regulator FlhF            | 11        | 36        | 3.1         | <0.001|
| CD630-02410 | Phosphoserine phosphatase                        | 10        | 32        | 3.1         | <0.001|
| CD630-02420 | Hypothetical protein                                      | 10      | 32        | 3.1         | <0.001|
| CD630-02430 | Hypothetical protein                                      | 10      | 32        | 3.1         | <0.001|
| CD630-02440 | CDP glycerol poly(glycerophosphate) glycerophosphotransferase | 10        | 32        | 3.1         | <0.001|
| CD630-02420 | Hypothetical protein                                      | 11        | 32        | 3.0         | <0.001|
| flgL   | Flagellar hook-associated protein FlgL            | 11        | 34        | 3.0         | <0.001|
| CD630-022430 | Membrane protein                                         | 11      | 32        | 3.0         | <0.001|
| fliA   | Flagellar operon RNA polymerase a28 factor         | 11        | 33        | 3.0         | <0.001|

*PR events, the predicted number of overlapping recombination events with each gene, calculated as the mean number of recombination events in each of 10,000 permutations randomly placing the identified recombination events.

*OR events, the observed number of overlapping recombination events with each gene, as identified by Gubbins.

*Ratio (fold change) of observed recombination events compared to predicted recombination events.

*Probability (P) that the number of observed recombination events is observed by chance based on 10,000 permutations randomly placing the identified recombination events throughout the clades 1 and 2 core genome.

*Gene in F3 regulon.

*Gene in F2 regulon.

*Gene in F1 regulon.

*PTS gene.

*Membrane protein gene.
flagellar diversity in closely related strains observed here suggests an evolutionary advantage, such as antigenic diversification or a unique role in virulence or pathogenicity. Of note, five genes involved in type IV pilus biosynthesis were also shown to be significantly enriched for homologous recombination (see Table S2), which suggests a

![Figure 2](image-url)

**Figure 2** Distribution of recombination events throughout the *C. difficile* genome. From innermost circle outward: positions in the core genome of clades 1 and 2 (purple) used as input for recombination detection; histogram of recombination events overlapping with each position in the *C. difficile* genome (dark gray represents more recombination than average, light gray represents less recombination than average); flagellar genes (turquoise); S-layer cassette genes (teal); phosphotransferase system (PTS) genes (dark red); genes involved in amino sugar and nucleotide sugar metabolism (salmon); genes involved in fructose and mannose metabolism (orange); and genes annotated as membrane proteins (navy). Areas highlighted in light blue represent genes that have more recombination than expected with a *P* value of <0.05 as determined by a permutation test. Numbers around the circular genome plot mark the nucleotide position in the reference genome *C. difficile* 630.
further role of homologous recombination in the diversification of cell surface structures that act as colonization factors in C. difficile (25).

**PTS and sugar metabolism.** The phosphotransferase system (PTS) is responsible for the active import of certain sugars into bacteria (26). It involves many transmembrane proteins that interact with the extracellular environment, and could be important in host interactions and immunogenicity. The combination of enriched recombination in both PTS and the metabolism of certain sugars (fructose, mannose, and pentose; Table 2) also suggests that recombination may play a role in the ability of C. difficile strains to occupy unique niches via diverse metabolic capabilities. Interestingly, a pleiotropic effect of flIC on genes involved in a number of mechanisms, including PTS and carbon metabolism has been observed (6). Variation in the interaction of flagella, PTS, and metabolism proteins itself may be being selected for via homologous recombination.

**Cell membrane and the S layer.** Genes that code for components on the outer layers of bacteria that interact with the outside world and host environment have previously been shown to undergo a relatively large amount of homologous recombination (13). That appears to be the case here as well, since flagella are environment-exposed appendages, and the PTS system involves a number of transmembrane proteins that regulate what sugars can come into the cell. The COG ontology that includes genes involved in cell envelope biogenesis and the outer membrane, including S-layer proteins, is significantly enriched for recombination (Table 2). These data are in line with previous studies that show high recombination in the S-layer surface proteins of C. difficile (18). The majority of genes involved in the S layer (27) were significantly enriched for homologous recombination in this study (Table 1; see also Table S2 and Fig. S2 in the supplemental material).

**Conclusions.** Homologous recombination is an important contributor to the evolution of C. difficile, accounting for an estimated 72% of SNVs. Furthermore, there are
Further in vitro and in vivo assays should be conducted to confirm these roles.

**MATERIALS AND METHODS**

**Strain selection.** *C. difficile* isolates with whole-genome sequence data were selected from a set of 917 genomes previously analyzed in our lab and 4,443 previously published genomes (30) to maximize representation of clinically important strains. To reduce redundancy of nearly identical genomes, isolates that were differentiated from another isolate in the sample by less than six coding mutations were excluded. All genomes used in this analysis were sequenced previously and are publicly available (see Table S1).

**Genome alignments.** Whole-genome alignments were created for each clade, as well as the combination of clades 1 and 2 and the entire set of genomes. Whole-genome alignments were produced as previously described (31). Briefly, each assembly was first concatenated into a pseudochromosome by using mauve contig mover to order and orient large contigs relative to a reference (32). The finished chromosome of the *C. difficile* 630 strain (included in clade 1) was used as a reference genome where possible, and the most complete whole-genome sequence in each alignment (determined by minimum number of contigs) was chosen as the reference genome otherwise. Each pseudochromosome was aligned to the reference genome using nucmer, and variants were identified relative to the reference genome using the show-snps command (33). Single-nucleotide variants (SNVs) were filtered to remove those SNVs that were likely to be due to alignment or sequencing errors. SNVs were filtered out if (i) they resided in genes annotated as phage, transposase, or integrase; (ii) they resided within 20 bp of the start or end of a contig; (iii) they resided in tandem repeats of total length >20 bp, as determined by the exact-tandem program associated with MUMmer; or (iv) they resided in large inexact repeats as determined by nucmer. Core genomes were calculated based on positions in the reference genome that were aligned in every genome using nucmer.

**Recombination identification.** Gubbins v1.3.4 (Genealogies Unbiased By recom-Binations In Nucleotide Sequences (34)) was used to identify areas likely introduced under homologous recombination in *C. difficile*. Gubbins was run on core genome alignments of each *C. difficile* clade, as well as clades 1 and 2 collectively, and on all 412 representative *C. difficile* genomes used in this study. Genomic regions identified by Gubbins as having densities of SNVs statistically different from background SNV density were inferred to be homologous recombination events, although it is possible that other molecular mechanisms could also have played a role in some of these regions. Maximum-likelihood recombination-filtered phylogenetic trees for the entire set of genomes, as well as just clades 1 and 2, were produced using RAxML v8.2.9 (35) assuming a general time reversible model of
nucleotide variations (-m GTRCAT). The bootstrap convergence test and the autoMRE convergence criteria (-N autoMRE) were used to determine the number of bootstrap replicates. The best-scoring maximum-likelihood tree was identified with rapid bootstrap analysis (-f a).

Identification of genes and pathways with enriched recombination. A permutation test was implemented and run in R v3.4.0 (36) to randomly place each observed recombination event in the C. difficile clades 1 and 2 core genome. Only clades 1 and 2 were used in this analysis since that is where the majority of recombination events were found and, since these two clades are closely related, it allowed for an expanded core genome compared to the entire set. Furthermore, epidemic ribotypes 014 and 027 fall within clades 1 and 2, respectively. The permutation test was run 10,000 times. Information about all deposited C. difficile genes in the reference genome C. difficile 630 were downloaded from the National Center for Biotechnology Information (NCBI) gene database. A recombination event was considered overlapping with a gene if they overlapped in at least one nucleotide position. One-sided P-values were calculated for each gene as the number of permutations that predicted a number greater than or equal to the observed value of

Data visualization. An unrooted phylogenetic tree (Fig. 1a) of all 412 isolates was plotted in R v3.4.0 with the ape package v4.1. Box plots and histograms (Fig. 1b and c; see also Fig. S1 in the supplemental material) were made in R v3.4.0. Genome visualization and a recombination map (Fig. 2) was made in DNAPlotter v1.0 (37). Variant heatmaps (Fig. 3; see also Fig. S2) were made with the ggtree package v1.8.1 (38) in R v3.4.0.

Data availability. All of the genomes discussed here, as well as their accession numbers, are listed in Table S1 in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, PDF file, 0.3 MB.
FIG S2, PDF file, 2.2 MB.
TABLE S1, CSV file, 0.04 MB.
TABLE S2, XLSX file, 0.1 MB.

ACKNOWLEDGMENTS

This study was supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (grant U01AI124255).

The study was conceptualized by H.D.S. and E.S.S. Data analysis, original draft preparation, and visualizations were performed by H.D.S. All authors performed editing and review, and E.S.S. supervised the project.

We have no conflicts of interest to report.

REFERENCES

1. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JL, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. 2015. Burden of Clostridium difficile infection in the United States. N Engl J Med 372:825–834. https://doi.org/10.1056/NEJMoa1408913.
2. Borriello SP. 1998. Pathogenesis of Clostridium difficile infection. J Antimicrob Chemother 41:13–19. https://doi.org/10.1093/jac/41.suppl_3.13.
3. Borriello SP, Ketley JM, Mitchell TJ, Barclay FE, Welch AR, Price AB, Stephen J. 1987. Clostridium difficile: a spectrum of virulence and analysis of putative virulence determinants in the hamster model of antibiotic-associated colitis. J Med Microbiol 24:53–64. https://doi.org/10.1099/00222615-24-1-53.
4. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cokayne A, Minton NP. 2010. The role of toxin A and toxin B in Clostridium difficile infection. Nature 467:711–713. https://doi.org/10.1038/nature09397.
5. Kelly OP, Becker S, Lievevsky JK, Joshi MA, O’Keane JC, Dickey BF, LaMont JT, Pothoulakis C. 1994. Neutrophil recruitment in Clostridium difficile toxin A entersitis in the rabbit. J Clin Invest 93:1257–1265. https://doi.org/10.1172/JCI117080.
6. Barketi-Klai A, Monot M, Hoys S, Lambert-Bordes S, Kuehne SA, Minton N, Collignon A, Dupuy B, Kansau I. 2014. The flagellin Flc of Clostridium difficile is responsible for pleiotropic gene regulation during in vivo infection. PLoS One 9:e96876. https://doi.org/10.1371/journal.pone.0096876.
7. Elliott B, Androga GO, Knight DR, Riley TV. 2017. Clostridium difficile infection: evolution, phylogeny, and molecular epidemiology. Infect Genet Evol 49:1–11. https://doi.org/10.1016/j.meegid.2016.12.018.
8. Wasiawski S, Lo ES, Ewing SA, Young VB, Aronoff DM, Sharp SE, Novak-Weeksley SM, Crist AE, Dunne WM, Hoppe-Bauer J, Johnson M, Brecher SM, Newton DW, Walk ST. 2013. Clostridium difficile ribotype diversity at six health care institutions in the United States. J Clin Microbiol 51:1938–1941. https://doi.org/10.1128/JCM.00556-13.
9. Rao K, Micic D, Natarajan M, Winters S, Kiel MJ, Walk ST, Santhosh K, Mogle JA, Galecki AT, Lebar W, Higgin PS, Young VB, Aronoff DM. 2015. Clostridium difficile ribotype 027: relationship to age, detectability of toxins A or B in stool with rapid testing, severe infection, and mortality. Clin Infect Dis 61:233–241. https://doi.org/10.1093/cid/civ254.
10. Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW, Bergwerff AA, Dekker FW, Kuiper EJ. 2008. Emergence of Clostridium difficile infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis 47:1162–1170. https://doi.org/10.1086/592257.
11. Walk ST, Micic D, Jain R, Lo ES, Trivedi I, Liu EW, Almassah LM, Ewing SA, Ring C, Galecki AT, Rogers MAM, Washler L, Newton DW, Malani PN, Young VB, Aronoff DM. 2012. Clostridium difficile ribotype does not predict severe infection. Clin Infect Dis 55:1661–1668. https://doi.org/10.1093/cid/cis786.
12. Vos M. 2009. Why do bacteria engage in homologous recombination? Evol Microbiol 17:226–232. https://doi.org/10.1091/evim.2009.03.001.
13. Yahara K, Didelot X, Jolley KA, Kobayashi I, Maiden MCJ, Sheppard SK, Falush D. 2016. The landscape of realized homologous recombination in pathogenic bacteria. Mol Biol Evol 33:456–471. https://doi.org/10.1093/molbev/msv237.
14. Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S, Bidet P, Bingen E,
Bonacorsi S, Boucher C, Bouvet O, Calteau A, Chiapello H, Clermont O, Cruveiller S, Danchin A, Diard M, Dossat C, Karoui ME, Frapy E, Garry L, Ghigo JM, Gilles AM, Johnson J, Le Bouguénec C, Lescat M, Mangenot S, Martinez-Jéanne V, Matic I, Nassif X, Oztas S, Petit MA, Pichon C, Rouy Z, Ruf CS, Schneider D, Tourret J, Vacherie B, Vallenet D, Médiécu G, Rocha EPC, Denamur E. 2009. Organized genome dynamics in the Escherichia coli species results in highly diverse adaptive paths. PLoS Genet 5:e1000344. https://doi.org/10.1371/journal.pgen.1000344.

Didelot X, Guillaume M, Falush D, Darling AE. 2012. Impact of homologous and non-homologous recombination in the genomic evolution of Escherichia coli. BMC Genomics 13:256. https://doi.org/10.1186/1471-2164-13-256.

Joseph SJ, Didelot X, Rothschild J, De Vries HJC, Morre SA, Read TD, Dean D. 2012. Population genomics of Chlamydia trachomatis: insights on drift, selection, recombination, and population structure. Mol Biol Evol 29:3933–3946. https://doi.org/10.1093/molbev/mss198.

Lüneberg E, Glenn-Calvo E, Hartmann M, Bär W, Frosch M. 1998. The central, surface-exposed region of the flagellar hook protein FlgE of Campylobacter jejuni shows hypervariability among strains. J Bacteriol 180:3711–3714. https://doi.org/10.1128/JB.180.14.3711-3714.1998.

Dingle KE, Didelot X, Ansari MA, Eyre DW, Vaughan A, Grif D, Ip CLC, Batty EM, Golubchik T, Bowden R, Jolley KA, Hood DW, Fawley WN, Walker AS, Petro TE, Wilcox MH, Crook DW. 2013. Recombinational switching of the Clostridium difficile S-layer and a novel glycosylation gene cluster revealed by large-scale whole-genome sequencing. J Infect Dis 207:675–686. https://doi.org/10.1093/infdis/jis734.

Stevenson E, Minton NP, Kuehne SA, Cd CD. 2015. The role of flagella in Clostridium difficile pathogenicity. Trends Microbiol 23:275–282. https://doi.org/10.1016/j.tim.2015.01.004.

Ghose C, Eugenis I, Sun X, Edwards AN, Mcbride SM, Pride DT, Kelly CP, Ho DD. 2016. Immunogenicity and protective efficacy of recombinant Clostridium difficile flagellar protein FlIC. Emerg Microbes Infect 5:e1–10. https://doi.org/10.1016/j.emi.2016.18.

El Meouche I, Peltier J, Monot M, Soutourina O, Pestel-Caron M, Dupuy B, Pons JL. 2013. Characterization of the SigD regulon of Clostridium difficile and its positive control of toxin production through the regulation of tcdC. PLoS One 8:e83748. https://doi.org/10.1371/journal.pone.0083748.

Stabler RA, He M, Dawson L, Martin M, Valente E, Corton C, Lawdy TD, Sebaiahia M, Quail MA, Rose G, Gerding DN, Gibert M, Popoff MR, Parkhill J, Dougan G, Wren BW. 2009. Comparative genome and phenotypic analysis of Clostridium difficile strain 630C1 and other associated data. Methods Ecol Evol 8:28–36. https://doi.org/10.1111/2041-210X.12628.