The Emergence of Bacterial "Hopeful Monsters"

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ABSTRACT The global spread of antibiotic-resistant bacteria has largely been driven by the dissemination of successful lineages. A particularly important example is sequence type (ST) 258 of *Klebsiella pneumoniae*, a common cause of health care-associated infections. Representatives of this lineage carry a variable array of plasmid-borne resistance genes, typically including a carbapenemase effective against the full range of clinically important β-lactams. In their recent *mBio* article, Chen et al. [*mBio* 5(3): e01355-14] described how ST258 emerged through "hybridization" between two other strains, with a second recombination resulting in the diversification of a key antigen. This commentary describes the findings in the context of other examples where saltational evolution has resulted in the sudden emergence of important pathogenic bacteria.

Growing concern about the future effectiveness of antibiotics was recently highlighted by the publication of the World Health Organization's first global report on the distribution of antimicrobial resistance (1). At the heart of this crisis are *Klebsiella pneumoniae* strains harboring carbapenemases, which have spread worldwide and were found to constitute the majority of *K. pneumoniae* clinical isolates in some regions (1). As infection with carbapenem-resistant *K. pneumoniae* is associated with high rates of mortality, above those from disease caused by carbapenem-susceptible strains, the dissemination of these bacteria is a clear threat to public health (2).

Although multiple carbapenem-resistant *K. pneumoniae* strains have been identified, the most commonly sampled sequence type (ST) worldwide is ST258 (2). Recent genomic analysis of 85 *K. pneumoniae* ST258 isolates from the United States found them not to have descended from a single resistant common ancestor, but instead to represent two clades, each having apparently acquired resistance on multiple occasions independently of one another (3). Clade 1 was generally associated with the carbapenemase allele KPC-2, whereas clade 2 tended to carry KPC-3, both of which were carried on Tn*4401*-type transposons. These transposons were in turn inserted into plasmids, which also carried a diverse set of further antibiotic resistance genes. Isolates in the two separate clades were also distinguished by at least 310 single nucleotide polymorphisms (SNPs), which were concentrated within a 215-kb "region of divergence" that included the capsule polysaccharide synthesis (*cps*) genomic island.

One key finding of a fascinating *mBio* paper by Chen and colleagues (4) was how this substantial difference between the two clades appears to have arisen through a large recombination. This was established through sequencing an ST258 clade 1 strain and a representative of *K. pneumoniae* ST42, which had been suggested to have similar *cps* loci by previous genotyping (3). Alignment of these genomes identified a region of near identity between them, containing just two SNPs, which spanned the *cps* locus. This exchange of sequence introduced almost 90% of the SNPs in the "region of divergence" that differentiated the two clades of ST258. Hence, at some point in ST258's history, one isolate appears to have acquired a novel *cps* island as part of a ~52-kb recombination, likely from an ST42 donor. The recombinant generated by this event then gave rise to clade 1, which consequently produced a different capsule polysaccharide than the existing ST258 isolates of clade 2.

Over 70 different *K. pneumoniae* capsule polysaccharide, or K antigen, types are known (3), and recombinations affecting the *cps* locus may be an important mechanism by which lineages diversify their antigenic profile in response to immune-mediated selection pressures. Similarly, in the Gram-positive commensal and pathogen *Streptococcus pneumoniae*, over 90 different capsule polysaccharide types have been characterized (5). Recombinations at the pneumococcal *cps* locus (6), typically of a similar size as those observed in *K. pneumoniae* (7), have driven capsule "switching" that has resulted in some lineages evading the polysaccharide conjugate vaccine (8). The emergence of lineages having undergone such switching has not been observed so frequently following the introduction of similar vaccines against *Neisseria meningitidis*, but nevertheless cases of disease have been caused by a "hyperinvasive" lineage having apparently evaded the vaccine through capsule switching (9). Similarly, in *Streptococcus agalactiae*, a "hypervirulent" strain was found to have switched capsule through a 35.5-kb recombination spanning the *cps* locus, raising concerns about such replacements complicating future vaccine development targeting this antigen (10). Recombination has also been observed to be important in altering other similar surface structures that might be under immune selection: pneumococcal antigenic proteins are frequently affected by homologous recombination events (7), horizontal exchange of sequence has made an important contribution to the distribution of the *Vibrio cholerae* O-antigen lipopolysaccharide component across the species (11), and recombinations spanning the ~10-kb "cell wall protein" locus of *Clostridium difficile* have altered the type of paracrystalline S-protein layer produced by these cells (12).

A second important observation in the paper by Chen and colleagues (4) was the identification of an even larger recombination event originally giving rise to the *K. pneumoniae* ST258 strain. This required comparisons with two other sequence types: *K. pneumoniae* ST11, which shares six of seven multilocus sequence typing loci (all except *tonB*) with ST258, and ST442, which

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differs from ST258 at the *tonB* sequence typing locus by only a single SNP. The ST258 genome was found to be closely related to ST11 across ~80% of its length; however, the remaining ~1.1 Mb (including the *cps* locus) was highly similar to the ST442 representative. Hence, *K. pneumoniae* ST258 is likely a "hybrid" generated through an ST11-like strain acquiring a single large segment of sequence from an ST442-like donor. However, ST258's acquisition of carbapenemase appears to have been an independent, subsequent event distinct from this exchange.

"Hybridization" between two parental strains has been observed to drive the emergence of other important pathogen genotypes. The methicillin-resistant *Staphylococcus aureus* ST239 lineage represents a similar case, in which approximately 20% of the sequence appears to represent the transfer of a single large genomic fragment from a donor distantly related to the likely parental strain, an event that preceded ST239's acquisition of β-lactam resistance (13). Similar proportions of the *Salmonella enterica* serovar Typhi and *S. enterica* serovar Paratyphi A genomes are likely to have been exchanged between these serovars at some point in their recent evolutionary history (14). This latter horizontal transfer event appears to have involved multiple fragments, which is also characteristic of large-scale recombination in other species. For instance, the exchange of numerous large segments of sequence is observed in clinical isolates of *S. agalactiae*, frequently affecting the *cps* locus; this has been suggested to occur through conjugation, found to be an efficient mechanism of sequence transfer between members of this species *in vitro* (15). A similar mechanism, or homologous recombination, was suggested as an explanation for the large-scale exchange of sequence observed in the diversification of the *C. difficile* 017 ribotype (16). In *S. pneumoniae*, transformation is the most likely mechanism by which large-scale transfer of sequence is observed. Again, the recombinations affecting the *cps* locus are temporally associated with the cotransfer of other noncontiguous loci (17, 18). In instances where it has been possible to identify the likely source of the sequence, such as in the emergence of some multidrug-resistant lineages, it appears that these sudden changes again might be considered a "hybridization" event in which DNA from a single donor is integrated at multiple sites around the chromosome (19).

As KPC-encoding genes are found on mobile elements, their association with particular lineages of *K. pneumoniae* suggests that the genetic background in which they are found may contribute significantly to the fitness of the resistant phenotype. While the antibiotic resistance profile of *K. pneumoniae* ST258 seems to have been relatively plastic, owing to the variable complement of plasmids, the chromosome appears to have recently diversified primarily through two discrete recombination events affecting the *cps* locus. The first, larger event was substantial enough for the ST258 recombinant progeny to be considered a lineage distinct from the parental strains, while the second smaller exchange distinguishes the two clades within the lineage. It may be that in both cases selection acting at the *cps* locus is the most important factor, and the variation in the quantity of cotransferred sequence simply reflects the intrinsic heterogeneity in the mechanism of horizontal sequence transfer (18). Alternatively, it may be that multiple beneficial loci were exchanged in the larger recombination, akin to a haploid version of "hybrid vigor," accounting for the subsequent success of ST258. While it seems likely that many of the progeny of such "hybridization" events are unsuccessful and never observed, at least some seem to emerge as "hopeful monsters" (20). This saltational form of evolution means there is considerable potential for public health threats to be generated suddenly in a manner that is hard to predict, necessitating effective surveillance measures to monitor the emergence of bacteria resistant to key antibiotics or able to evade vaccines designed to target specific bacterial proteins or carbohydrates.

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