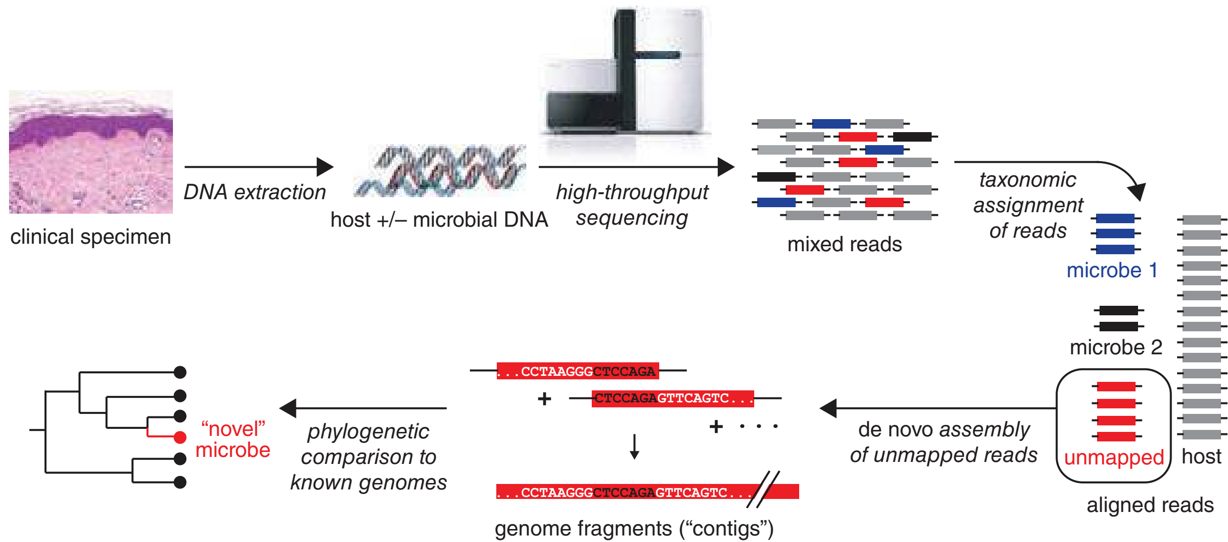

FIGURE 146-3 Workflow of metagenomic assembly for pathogen discovery. DNA is isolated from a specimen of interest (e.g., tissue, body fluid) containing a mixture of host DNA and nucleic acids from coexisting microbes, either commensal or pathogenic. All DNA (and RNA if a reverse transcription step is added) is then sequenced, yielding a mixture of DNA sequence fragments ("reads") from organisms present. These reads are then aligned to existing reference genomes for the host or any known microbes, leaving reads that do not align ("map") to any known sequence. These unmapped reads are then computationally assembled de novo into the largest contiguous stretches of DNA possible ("contigs"), representing fragments of previously unsequenced genomes. These genome fragments (contigs) are then mapped onto a phylogenetic tree based on their sequence. Some may represent known but as-yet-unsequenced organisms, while others will represent novel species. *(Figure prepared with valuable input from Dr. Ami S. Bhatt, personal communication.)*

from viruses to bacteria to parasites. As sequencing-based discovery expands, microbes may be found to be associated with conditions not classically thought of as infectious. Studies of bowel flora in laboratory animals and even humans are already beginning to suggest correlations between microbe composition and various aspects of metabolic and cardiovascular health. Improved methods for pathogen detection will continue to uncover unexpected correlations between microbes and disease states, but the mere presence of a microbe does not establish causality. Fortunately, once the relatively laborious and computationally intensive metagenomic sequencing and assembly efforts have identified a pathogen, further detection can easily be undertaken with targeted methods such as PCR or hybridization, which are much more straightforward and scalable. This capacity should facilitate the additional careful investigation that will be required to progress beyond correlation and to draw causal inference.

ANTIBIOTIC RESISTANCE

At present, antibiotic resistance in bacteria and fungi is determined by isolating a single colony from a cultured clinical specimen and testing its growth in the presence of a drug. The requirement for multiple growth steps in these conventional assays has several consequences. First, only culturable pathogens can be readily processed. Second, this process requires considerable infrastructure to support the sterile environment required for culture-based testing of diverse organisms. Finally, and perhaps most significantly, even the fastest-growing organisms require 1–2 days of processing for identification and 2–3 days for determination of susceptibilities. Slower-growing organisms take even longer: for instance, weeks must pass before drug-resistant *M. tuberculosis* can be identified by growth phenotype. Given the clinical imperative in serious illness to begin effective therapy early, this inherent delay in susceptibility determination has obvious implications for empirical antibiotic use: broad-spectrum antibiotics often must be chosen up front in situations where it is later shown that preferred narrower-spectrum drugs would have been effective or even that no antibiotics were appropriate (i.e., in viral infections). With this strategy, the empirical choice can be incorrect, often with devastating consequences. Real-time identification of the infecting organism and information on its susceptibility profile would guide initial therapy and support judicious antibiotic use, ideally improving patient outcomes while aiding in the ever-escalating struggle with antibiotic resistance by reserving the use of broad-spectrum agents for cases in which they are truly needed.

Molecular diagnostics and sequencing offer a way to accelerate detection of a pathogen's antibiotic susceptibility profile. If a genotype that confers resistance can be identified, this genotype can be targeted for molecular detection. In infectious disease, this approach has most convincingly come to fruition for HIV (Fig. 146-2*A*). (In a conceptually parallel application of genomic analysis, molecular detection of certain resistance determinants in cancers is beginning to inform chemotherapeutic selection.) Extensive sequencing of HIV strains and correlations drawn between viral genotypes and phenotypic resistance have delineated the majority of mutations in key HIV genes, such as reverse transcriptase, protease, and integrase, that confer resistance to the antiretroviral agents that target these proteins. For instance, the single-amino-acid substitution K103N in the HIV reverse transcriptase gene predicts resistance to the first-line nonnucleoside reverse transcriptase inhibitor efavirenz, and its detection thus informs a clinician to choose a different agent. The effects of these common mutations on HIV susceptibility to various drugs—as well as on viral fitness—are curated in publically available databases. Thus, genotypes are now routinely used to predict drug resistance in HIV, as phenotypic resistance assays are far more cumbersome than targeted sequencing. Indeed, current recommendations in the United States are to sequence virus from a patient's blood before initiating antiretroviral therapy, which is then tailored to the predicted resistance phenotype. As new targeted therapies are introduced, this targeted sequencing–based approach to drug resistance will likely prove important in other viral infections (e.g., hepatitis C).

For several reasons, the challenge of predicting antibiotic susceptibility from genotype has not yet been met in bacteria to the same degree as in HIV. In general, bacteria have evolved diverse resistance mechanisms to most antibiotics; thus, the task cannot be reduced to probing for a single genetic lesion, target, or mechanism. For instance, at least five distinct modes of resistance to fluoroquinolones are known: reduced import, increased efflux, mutated target sites, drug modification, and shielding of the target sites by expression of another protein. Further, we lack a comprehensive compendium of genetic elements conferring resistance, and new mechanisms and genes emerge regularly in the face of antibiotic deployment. As bacteria have far more complex genomes than viruses, with thousands of genes on their chromosomes and the capacity for acquiring many more through