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Reply to "Comparison of the Web Tools ARG-ANNOT and ResFinder for Detection of Resistance Genes in Bacteria"

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We read with interest the comment letter of Zankari (1) regarding our previous manuscript on ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance (AR) genes in bacterial genomes (2). Here are responses to those comments. We agree that the *tetR* gene is a regulatory gene and not an AR gene. There are several regulatory genes involved in AR, and though they are not AR genes, they are indirectly involved in AR. One recent example of this is the discovery of insertional inactivation of the *mgrB* regulatory gene in *Klebsiella pneumoniae* as a cause of resistance to colistin in this genus (3). Thus, these regulatory genes cannot be omitted, and we are currently collecting all the information regarding these regulatory genes; an upgraded database including these genes will be available soon. Moreover, we are pleased to learn that the ResFinder tool (4) has been updated since November 2013 in order to detect genes in multiple copies and to change default settings down to 30% identity and 20% length coverage. This current version of ResFinder was not available when our manuscript was published (ahead of print version, 21 October 2013). We believe that this point is critical for genome analysis to detect AR genes for at least two reasons. First, when we built our ARG-ANNOT database, one of our objectives was to be able to analyze any bacterial whole-genome sequence (WGS) in two steps. In a first step, the tool is used with high stringency for high-specificity detection of AR genes, whereas in a second step, a very low specificity (percent identity and percent length of coverage) can be used to detect more-distant AR gene sequences, i.e., putative new AR genes. This was the case in our paper for the detection of a putative new *rmt* gene sequence (absent from our database at the time of manuscript submission), for which we found only short hits (sequences of 17 to 40 bp) with other members of the *rmt* gene family (*rmtD*, *rmtD2*, and *rmtA*), representing only 35% coverage; this sequence was in fact a true new AR gene, *rmtF* (5). Moreover, a lower stringency is warranted since the majority of WGSs actually released in databases are unclosed genomes with contigs and/or scaffolds, meaning that AR genes may be missed if sequences are split into different sets of sequences. We believe that these two approaches are complementary for WGS analysis and also for analysis of metagenome sequences in order to discover new and/or emerging AR gene sequences. Hence, reduced specificity may be helpful in detecting putative new AR genes, but further experimental validation is warranted when a new putative AR gene is detected.

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