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Application of a novel k-mer primer design algorithm for detecting antibiotic resistance determinants

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Motivation and Aim: Last decades the constant expansion of multi-drug resistant microorganism strains became an important problem [2, 4, 7, 9]. As a result, antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality due to reduced antibiotics effectiveness, including those against microorganisms causing common infectious diseases (such as pneumonia, tuberculosis etc.) [2, 4, 7, 9]. However, modern molecular methods of infectious disease diagnostics make it possible to provide timely and effective profiling of the resistance of an infectious agent, and thereby expand and enhance means of clinical analysis [2, 3, 5, 6, 8].
Methods and Algorithms: In the present work, a novel *k*-mer algorithm was used to generate a panel of degenerate multiplexable primers for NGS-based detection of known genetic determinants of drug resistance to colistin, polymyxin, and *β*-lactam antibiotics. The reference database, used for primer design, was based on available open sources (CARD, NDARO and MEGARes) and contained information on 6772 loci associated with the resistance of bacterial pathogens to these groups of antibiotics. We have designed a primer panel suitable for analysis using Illumina (Miseq) platform as well as Nanopore (ONT MinION) sequencing which allowed to identify any all known to date groups of genes of interest in a single study. We then analyzed 62 DNA samples isolated from antibiotic-resistant bacteria of the genera *Acinetobacter, Escherichia, Enterobacter, Pseudomonas, Serratia, Klebsiella*, found in clinical biological material. The studied isolates were obtained from hospitalized patients with laboratory-confirmed manifestations of infection in various localizations, including CNS infection, bloodstream infection, urinary tract infection, intra-abdominal infection, bone and joint infection, pneumonia, skin and soft tissue infection and primary bacteremia.
Results: Using the newly developed profiling method, in the studied set of biological samples we identified genes belonging to the following groups: *bla*OXA, *bla*TEM, *bla*GES, *bla*ADC, *bla*NDM, *bla*CTX-M, *bla*VIM, *bla*CMY, *bla*PER, *bla*SHV, *bla*KPC, *bla*IMP, *bla*DHA, *bla*CRT, *bla*SST, *bla*ACT, and *mcr*-1. All results were confirmed with available qPCR assays and/or whole genome sequencing.
Conclusion: A method based on multiplex PCR with subsequent next generation sequencing for identifying the genetic determinants of drug resistance was developed. This method is more informative than qPCR analysis, much cheaper and standardizable than whole genome sequencing.

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