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Beyond ordinary molecular diagnostics: next-generation sequencing analytic pipeline for understanding antibiotic resistance mechanisms in clinical diagnostics

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Background: Alarmingly, multidrug resistant (MDR) gram-negative bacteria are rapidly increasing around the globe. Currently several mechanisms of resistance are emerging such as various Carbapenemases or plasmid borne Colistin resistance (mcr-1). For the most frequent genes associated with antibiotic resistance different phenotypic and genotypic diagnostic assay are available. However, in some cases it is difficult to verify rare mechanisms and understand why certain isolates develop resistance under treatment within the patient. In addition, epidemiological surveillance is a key element in further reducing the spread of MDR-pathogens. Therefore, methods are needed to unveil resistant strains, which cannot be elucidated by standard phenotypic and genotypic methods to follow potential new upcoming resistance mechanisms.

Material/methods: We collected several highly antibiotic resistant isolates from clinical routine diagnostics, in which antibiotic resistance mechanism could not be determined using our standard approach - including in-depth phenotypic testing with VITEK2 and Etest (both bioMérieux, Lyon, France) and disks testing (ROSCO, Taastrup, Denmark) following EUCAST interpretation rules. As well as, broad genotypic testing with different Xpert (Cepheid, Sunnyvale, USA) and eazyplex assays (Amplex, Giessen, Germany). DNA was extracted from MDR-isolates and library preparations were carried out using Illumina Nextera XT Kits. Next generation sequencing (NGS) was carried out with an Illumina MiSeq V3 2x300bp chemistry. Bioinformatics analysis was done using CLC Genomics Workbench (Qiagen).

Results: We present here our NGS pipeline based on commercial software and publicly available resources to determine acquisition of resistance genes within bacteria. Our pipeline includes 2154 resistance conferring genes and is based on Zankari et al. 2012 (J Antimicrob Chemother). Here, we demonstrate how NGS can be used to identify resistance genes in an efficient and reliable way. Furthermore, we present several cases for special resistance mechanisms: (i) resistance to colistin in *E. coli* by *mcr-1*. (ii) *Pseudomonas aeruginosa* acquiring resistance to carbapenems by porin loss, which could be proved by tandem-masspectrometry. Additionally, mutations were detected, which cause overexpression of the *ampC* gene and thus lead to resistance to piperacillin and ceftazidime. (iii) Detection of rare β-lactamases in several isolates, such as an *imi-1*, *shv-1* or *imp-18* gene.

Conclusions: NGS data together with antibiotic resistance determination pipeline are powerful tools to identify uncommon resistance mechanisms and allow for understanding resistance causing mutations mechanistically. In addition, it allows monitoring spread of the according genes in a clinical context.