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The resistome of a multi-resistant outbreak Providencia stuartii strain

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Background: *Providencia stuartii* has emerged as an important nosocomial pathogen. *P. stuartii* displays intrinsic resistance to many common antimicrobials, limiting our treatment options and therefore resulting in a significant impact on patient morbidity, mortality, treatment, and management costs. Recently, we describe an outbreak of pan-drug-resistant *P. stuartii* infections in a Greek intensive care unit, involving a virulent strain harbouring a unique complement of resistance determinants. The aim of this study was to use next generation sequencing in order to gain better insight into the resistome of this outbreak strain.

Material/methods: Genomic DNA of the outbreak strain PS71 was subjected to whole-genome sequencing (WGS) using 2x250bp paired-end reads on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA). Generated reads were trimmed using Trimmomatic algorithm (v0.36) and their quality was assessed by *in-house* scripts combined with SAMtools (v1.3.1) (2), BedTools (v2.25.0) and BWA mem (v2) algorithms. High-quality filtered reads were subsequently assembled *de novo* using SPAdes algorithm (v3.7.1), while the *ab initio* gene finder algorithm Prokka (version 1.11) was used for annotation. ResFinder (version 2.1) and PlasmidFinder (version 1.3) were used to determine the presence of resistance genes and plasmid replicon types, respectively.

Results: WGS generated a total of 843,412 reads with an average length of 475 bp, which were subsequently assembled into 83 scaffolds with a N50 of 455,952 bp. The sequence coverage of the de novo assemblies was approximately 83 reads per assembled base. The draft genome sequence of

PS71 revealed a 4,400,597 bp genome size with 41.75 % average G+C content. Provisional annotation shows a total of 4026 coding sequences (CDSs), including at least 76 tRNAs and 8 rRNAs. PlasmidFinder identified the presence of three replicon types, namely ColE, IncA/C and IncR, which we have proven earlier are located on a multi-replicon plasmid (IncA/C-R). ResFinder revealed a unique complement of 24 genes mostly encoded on this IncA/C-R plasmid, conferring resistance to aminoglycosides (*aadA1*, *aadA2*, *aadB*, *aac(6')-Ia*, *aph(3')-Ia*, *strA*, *strB* and *rmtB*), β-lactams (bla_{TEM-1b}, bla_{OXA-10}, bla_{SHV-5}, bla_{VEB-1} and bla_{VIM-1}), macrolides, lincosamides and streptogramin B (*mph(A)*), phenicols (*cmlA1* and *catA3*), rifampicin (*arr-2*), sulphonamides (*sul1* and *sul2*), tetracyclines (tet(A), *tet(B)* and *tet(G)*) and trimethoprim (*dfrA1* and *dfrA12*).

Conclusions: To our knowledge this is the first whole genome sequence of an emerging multi-resistant *Providencia stuartii* strain associated with outbreak episodes in Greece. Our data indicate that the acquisition of a multi-resistant plasmid, in combination to the species-inherent resistance to polymixins resulted in the emergence of this extremely resistant strain. The increasing number of reports of multi-resistant strains of *Providencia* spp in the Mediterranean region warrants enhanced surveillance so as to limit their dissemination and impact on human health.