Infection Control Hospital Epidemiology

 MS #38707

Identification of four patients with colistin resistant Escherichia coli containing the mcr-1 (mobile colistin resistance) gene from a single health system in Michigan, USA

Supplementary Data

DNA preparation, library construction and whole genome sequencing

DNA was extracted using the automated nucleic acid purification Maxwell® 16 MDx Research (Promega, Madison, WI) instrument according to the manufacturer’s recommendations. High quality input gDNA from the samples, indicated by an absorbance ratio of 1.8-2.0, was fragmented by Covaris® (Woburn, Massachusetts) ultrasonic fragmentation. The gDNA sample libraries were then prepared using the NuGen® Ovation Ultralow DR Multiplex System 1-96 kit (San Carlos, CA). Lastly, libraries were multiplexed and sequenced with the Illumina® Miseq (San Diego, CA) yielding 250 bp paired-end reads. Reads were filtered for PhiX contamination using the BBDuk program <https://sourceforge.net/projects/bbmap/>). Adapter sequences and low quality bases were trimmed using Trimmomatic (sliding window size of 20 with a quality threshold of 30).6 The SPAdes assembler was used for assembly, and contigs less than 300bp were removed.7

Molecular characterization

Using assemblies as input, in silico MLST was performed using the freely available software (<https://github.com/tseemann/mlst>), according to the PubMLST scheme. ResFinder was used to determine antibiotic resistance genes (including *mcr* variant) and PlasmidFinder was employed to identify plasmid replicon (Inc) genes. 8,9,10

Additionally, ST-types were determined by PCR and sequencing using the Pasteur scheme11(http://bigsdb.pasteur.fr/ecoli/ecoli.html). PCR was performed in order to identify the phylogenetic group12, as well as for screening for virulence factors (*papA/papC*, *sfa/focDE*, *afa/draBC*, *iutA*, *kpsMTII*), beta-lactamase genes (*bla*CTX-M, *bla*OXA-48, *bla*NDM, *bla*KPC, *bla*IMP, and *bla*VIM) and mcr genes (*mcr-1*, *mcr-2*, *mcr-3*).

 Plasmid identification

Conjugation assays using clinical strains as donors, as well as plasmid DNA transformation into *E. coli* DH10B cells was performed followed by selection on Polymyxin B (2 ug/ml) LB agar plates. PCR-based plasmid typing was performed on clinical strains and recipient *E.coli* DH10B using the Diatheva PCR-based Replicon Typing Kit 2.0 (Fano, Italy).

RESULTS

Conjugation assays were performed twice, however transconjugants could not be obtained in any of the cases, suggesting *mcr-1* plasmids might not be transferable. In order to characterize the *mcr-1* containing plasmids, plasmid DNA from each clinical strain was transformed into *E.coli* DH10B. Plasmid typing revealed that the isolate from patient #2 carried the *mcr-1* on an IncX4 plasmid while the isolates from the other three patients carried the *mcr-1* on IncI2 plasmids.