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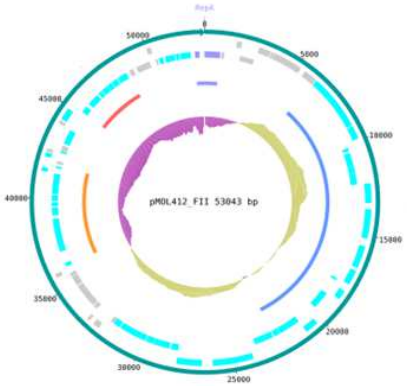

14th EURL-AR Workshop 2020

29th April 2020

NDM-4 carbapenemase gene harboured by a novel IncFII plasmid in *E. coli* of pig origin, Italy

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L 303/26 EN Official Journal of the European Union 14.11.2013

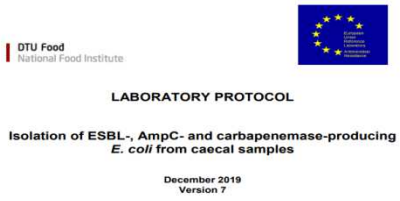
DECISIONS

COMMISSION IMPLEMENTING DECISION
of 12 November 2013
on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria
(notified under document C(2013) 7145)
(Text with EEA relevance)
(2013/652/EU)

• In 2019, one carbapenem-resistant *E. coli* was isolated by the NRL-AR (Italy) from a caecal content of a fattening pig sampled at slaughter, in the frame of the EU harmonized AMR monitoring activities (Decision 2013/652/EU)

•The isolate was detected only by the specific isolation method for carbapenemase-producing *E. coli* (EURL-AR protocols).

The same sample (same epi unit) yielded only ESBL(CTX-M-32)-producing *E. coli* when cultured on CTX-MacConkey agar (ESBL/AmpC/Carba general protocol)


DTU Food
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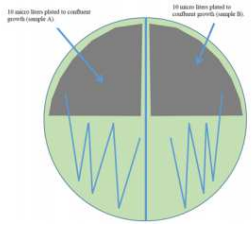
LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples

December 2019
Version 7



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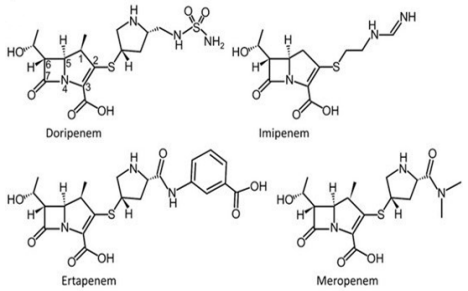


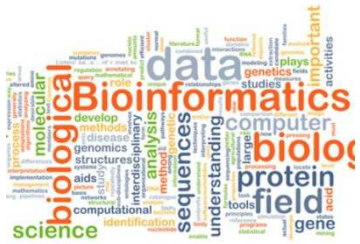
• The *E. coli* isolate was MDR, carbapenem resistant displaying meropenem, imipenem, and ertapenem MIC values of >16, 8, and >2mg/L respectively (according to Decision 2013/652/EU), and tested positive by PCR[1] for the presence of bla_{NDM} genes family

•It was in-depth characterized by Whole Genome Sequencing (WGS) and bioinformatics analysis.

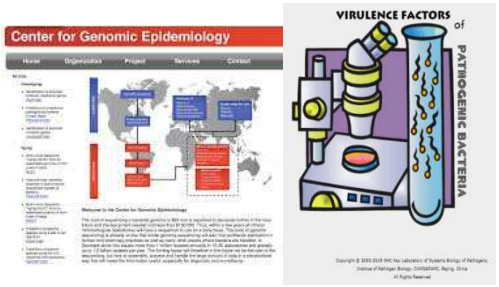
•Combined bioinformatic analysis of Illumina short-reads and Oxford Nanopore long-reads

Illumina Miseq sequencer

MinION nanopore sequencer



[1]Poirel L, Walsh T, Cuvillier V, et al. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn micr infec dis* 2011; 70.1: 119-123



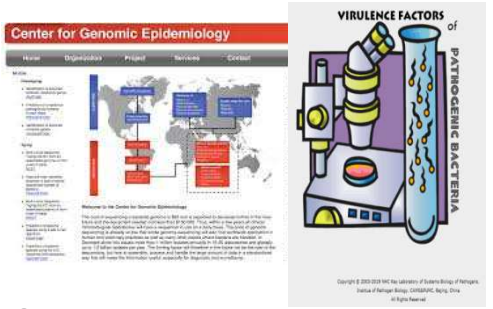
For Illumina sequencing: M&M

✓DNA extraction, library preparation, trimming and de novo assembly of raw reads were performed according to a pipeline we implemented at the NRL-AR[1]

✓The assembly obtained was annotated using The RAST Server and manually curated

✓Molecular characterization was performed using different CGE tools to assign STs, for the genetic basis of AMR, for the detection of plasmid replicons, in silico serotyping and virulence genes detection (using also theVF dataBase)

[1]Alba P, Leekitcharoenphon P, Carfora V, et al. Molecular epidemiology of *Salmonella* Infantis in Europe: insights into the success of the bacterial host and its parasitic pESI-like megaplasmid [published online ahead of print, 2020 Apr 9]. *Microb Genom*. 2020;10.1099/mgen.0.000365. doi:10.1099/mgen.0.000365



For Illumina sequencing: Results

ST **Genoserotype**	• 641 • O108:H23
Plasmid replicons **Virulence genes**	• IncFII, IncY, IncR, IncX1[#], pENTAS02[#], Col440I[#] • Several virulence determinants, but no species-specific swine pathotype
AMR genotypic pattern **AMR phenotypic pattern**	• bla_{TEM-1B}, bla_{NDM-4}, *sul1*, *sul3*, *dfrA12*, *aadA2*[#], *mdf*(A)[#] • AMP, FOT, TAZ, MERO, IMI, ETP, SUL, TMP

[#] % of identity lower than 99%

AMP= ampicillin; FOT= cefotaxime; TAZ=ceftazidime; MERO= meropenem; IMI=imipenem; ETP=ertapenem; SUL= sulphonamides; TMP=trimethoprim

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Discussion

❖According to EU harmonized monitoring, the prevalence of carbapenemase-producing *E. coli* and Salmonella among livestock in Europe has so far remained very low (<1%) (only $bla_{\text{OXA-48}}$ and $bla_{\text{VIM-1}}$ types detected)

❖First description of a NDM-producing E. coli and Enterobacteriales in food-producing animals in Europe and the first report of a NDM-4 producing E. coli and Enterobacteriales detected in a pig sample.

❖The ST641 *E. coli* isolate belongs to a ST already detected in animal samples including pig samples, but not previously associated with NDM-mediated carbapenem resistance.

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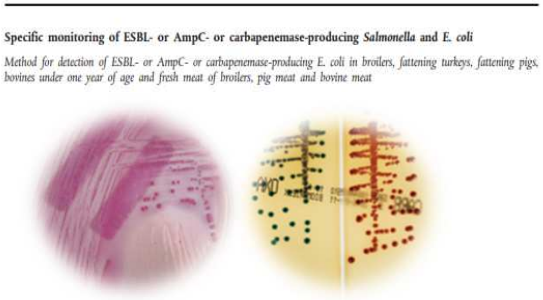
Discussion

The NDM-producing *E. coli* was not detected on the selective agar MC+CTX, on which it may have been overgrown by other ESBL/AmpC-producing *E. coli*. Indeed by using this latter method we only cultured an ESBL-producing E. coli (CTX-M-32 type) from the same sample

Importance of continuous and specific monitoring of carbapenem-producing Enterobacteriales in food-producing animals and along the food chain in the EU



Specific monitoring of ESBL- or AmpC- or carbapenemase-producing *Salmonella* and *E. coli*

Method for detection of ESBL- or AmpC- or carbapenemase-producing E. coli in broilers, fattening turkeys, fattening pigs, bovines under one year of age and fresh meat of broilers, pig meat and bovine meat

Third- and fourth-generation cephalosporins and even the continuous oral usage of aminopenicillins in animals can select for most carbapenemases!!!

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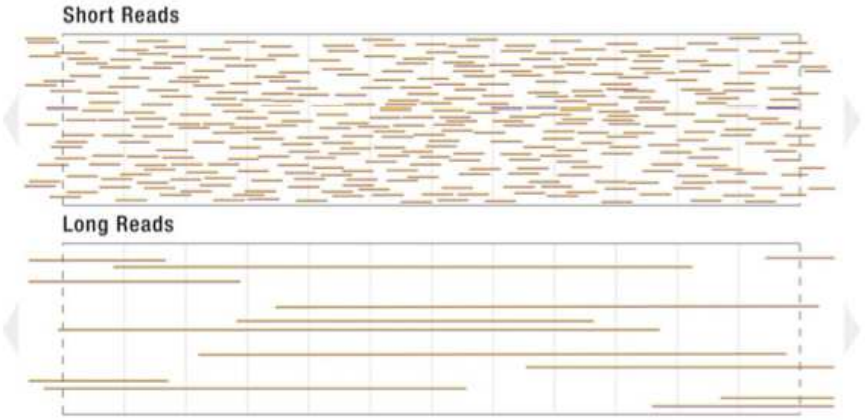
Short reads VS Long reads
Benefiting from both

In order to close the gaps and verify the correct assembly of repetitive regions of the *bla*$_{\text{NDM-4}}$-harbouring plasmid, our isolate was sequenced using the nanopore-based MinION device (Oxford Nanopore Technologies) with the rapid barcoding kit (SQK-RBK004).



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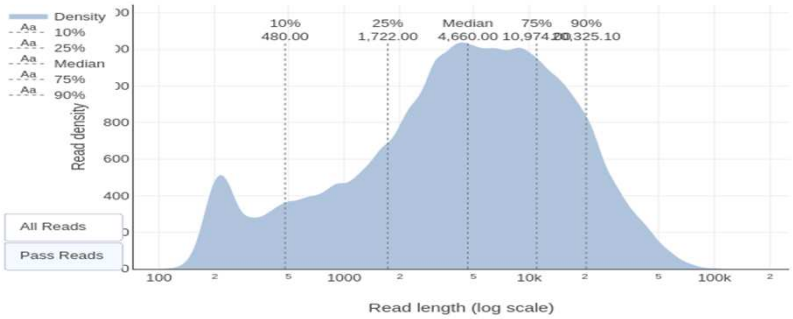
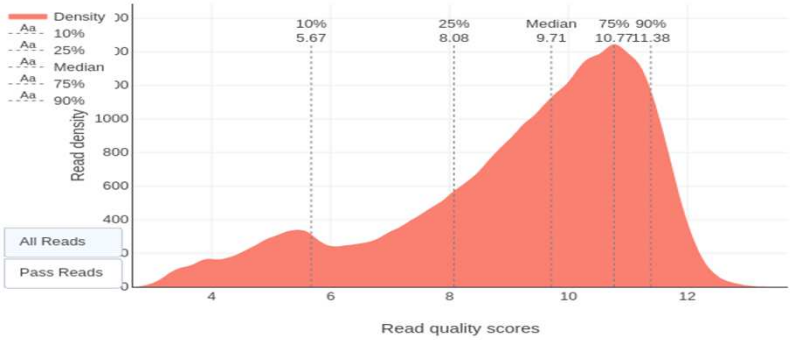
Quality control of ONT raw reads

Distribution of read length

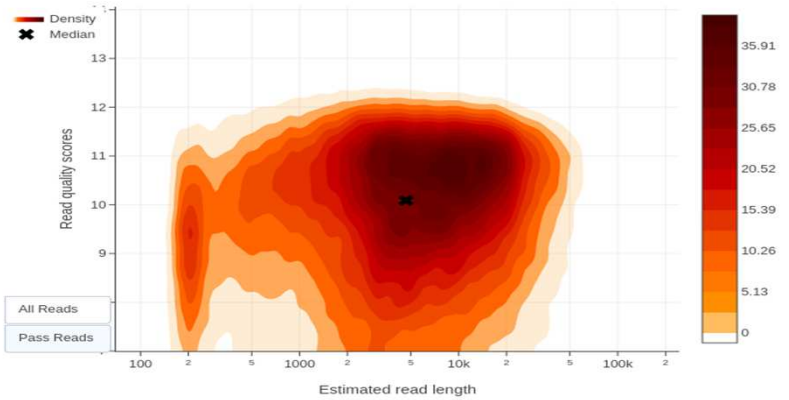


Number of raw reads: 365002
N50 Length: 7744
Median Read Quality: 9.80

Distribution of read quality



Mean read quality per sequence length



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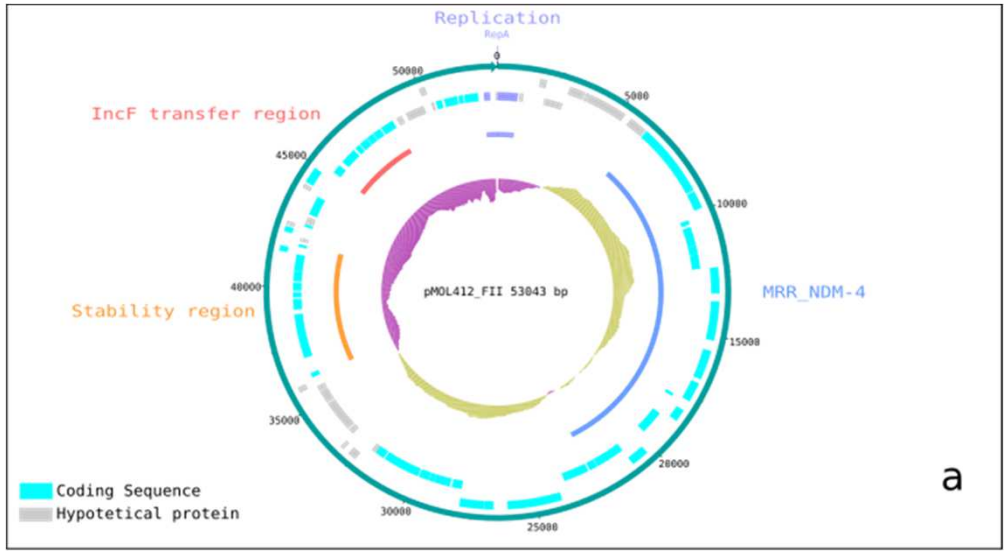
Unicycler

Results from hybrid assembly (Illumina - Oxford Nanopore)

- We have identified and resolved the complete sequence of **a novel IncFII plasmid (pMOL412_FII)** of around 53kb harbouring the carbapenemase gene ***bla*$_{\text{NDM-4}}$**.

- Annotation of plasmid sequence identified four main genetic regions. The plasmid backbone included a replication region containing the *repA* gene encoding a replication initiation protein of IncFII family, a stability region and the IncF transfer region.



Circular representation of pMOL412_FII

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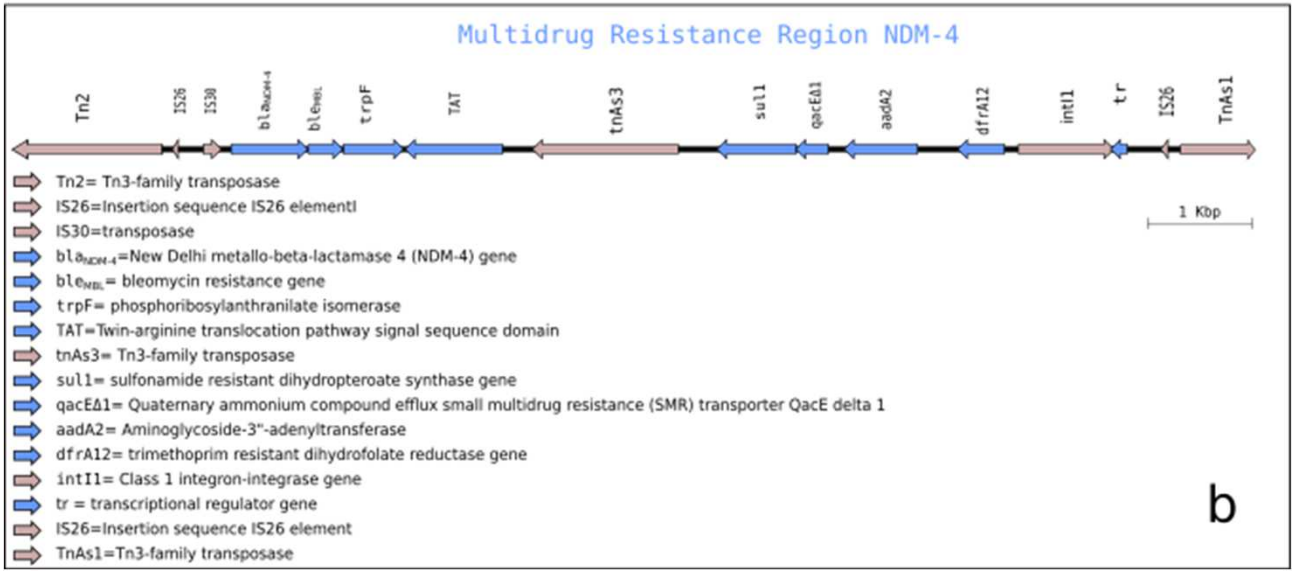
Results from hybrid assembly (Illumina - Oxford Nanopore)

- The variable region was a multidrug resistance region (MRR) of 16 kb harbouring $bla_{\text{NDM-4}}$ (named MRR-NDM-4) and bracketed by two copies of IS26 and transposons.

- MRR-NDM-4 harboured several AMR determinants as *dfrA12, sul1, qacEΔ1* (quaternary ammonium compounds resistance gene), and *aadA2* (streptomycin resistance gene), that were located on a gene cassette array inserted into a class 1 integron



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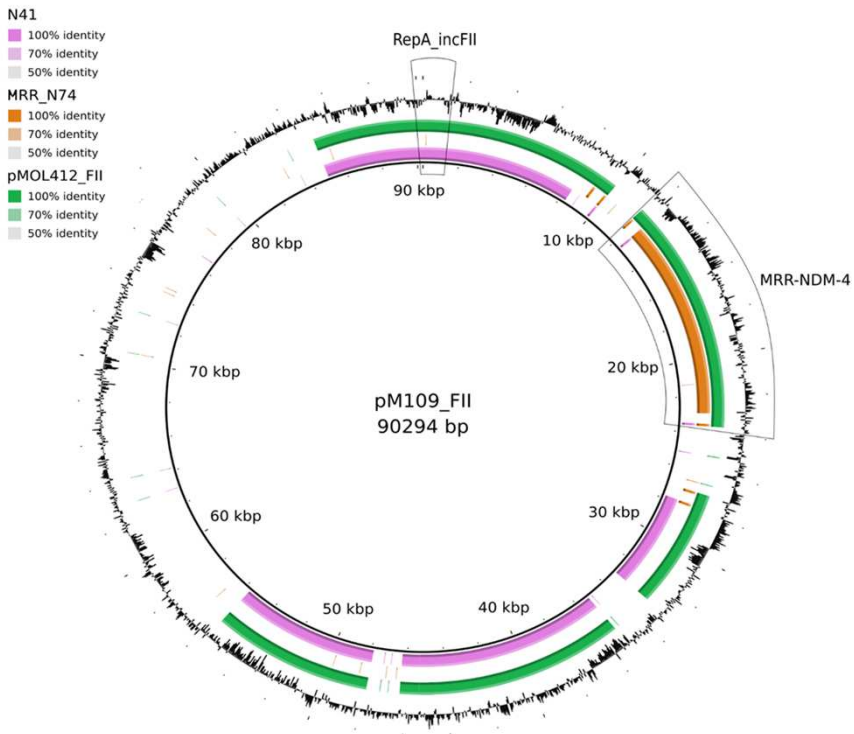
Comparative analysis

- Results of comparison with public databases revealed that the **pMOL412_FII** plasmid was most closely related (57% coverage and 99.82 % identity) to a ~90.3 kb plasmid (pM109_FII)-harbouring *bla*$_{\text{NDM-4}}$ of a carbapenem-resistant *E. coli,* previously isolated from a human patient in Myanmar.

- Additionally, the **MRR-NDM-4** resulted identical to the *bla*$_{\text{NDM-4}}$ region (12388:22899) of the above mentioned carbapenem-resistant *E. coli* (accession number: AP018139).



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Conclusions

Importantly, our results proved how the combined bioinformatics analysis of ONT long-reads with Illumina short-reads, has been decisive to precisely locate class 1 integron containing *bla*$_{\text{NDM-4}}$ in a high resistance region (MRR-NDM-4), and to resolve the IncFII plasmid (pMOL412_FII).

Novel IncFII plasmid harbouring *bla*$_{\text{NDM-4}}$ in a carbapenem-resistant *Escherichia coli* of pig origin, Italy

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UNDER REVIEW

Acknowledgements

All staff of UOC General Diagnostics Department is involved in the EU harmonized AMR monitoring activities:

Head of UOC General Diagnostics Department: Antonio Battisti

Head of Laboratory of Diagnostics of bacterial, mycotic and parasitic diseases: Andrea Caprioli

Samples reception and culturing:
- Carmela Buccella
- Serena Lorenzetti ♥
- Roberta Onorati
- Luigi Sorbara
- Tamara Cerci

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- Paola Di Matteo
- Roberta Amoruso

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- Patricia Alba
- Elena Lavinia Diaconu
- Manuela Iurescia

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Thanks for your attention!