

Nanopore Sequencing- a step forward in pathogen identification and antibiotic resistance gene profiling of urine samples

Katarzyna Schmidt 20-10-2017 ICCMg, Geneva



Conflict of interest

- I received free MinION flow cells and library preparation reagents as member of the ONT MinION Access Programme.
- I received one-off bursary to cover expenses at the International Conference on Clinical Metagenomics conference from ONT.



- Rationale for rapid diagnostics in urosepsis patients
- Workflow for urinary tract infections
- Results
- Problems and solutions
- Conclusions



Rationale for rapid diagnostics for cUTIs

- In the UK, emergency hospital admissions for complicated UTIs among the elderly ('over-65s') doubled from 2002-12.
- cUTIs are a source of many septic episodes
 - > *E. coli* is now the commonest agent of bacteraemia in the UK.
 - Estimated mortality rate 18.2%.
- Global spread of multi-resistant uropathogenic *E. coli* ST131 may lead to failure treatment.



Current Diagnostics for cUTI





Turnaround Time: 24-72 h



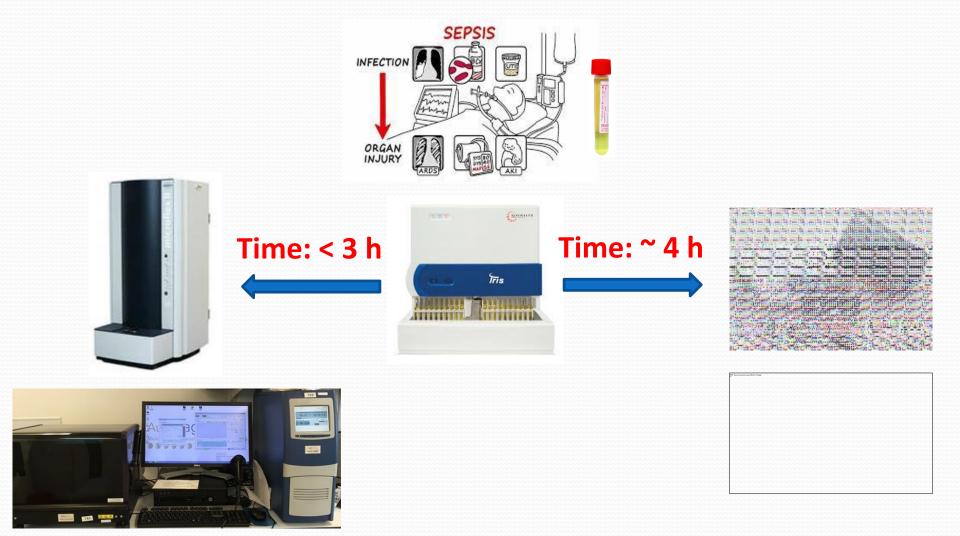








Rapid Diagnostics for cUTI

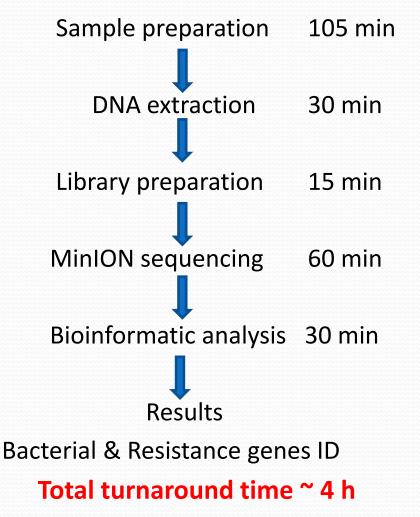




- Clinical urines with bacteria >10⁷ cfu/mL & human cells >10⁵/mL.
- Urine spiked with multi-drug resistant E. coli.
- Host cell and human DNA depletion performed.

Urine sample		





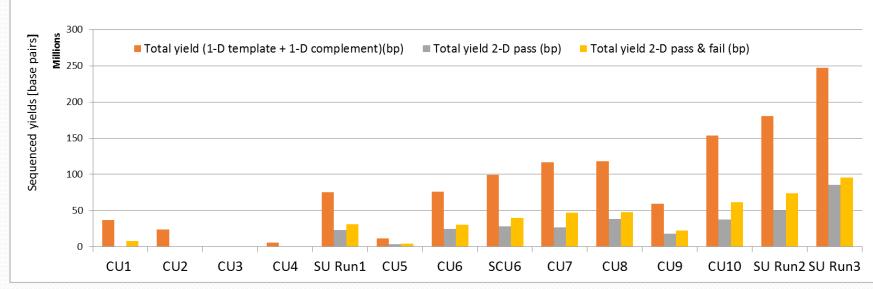




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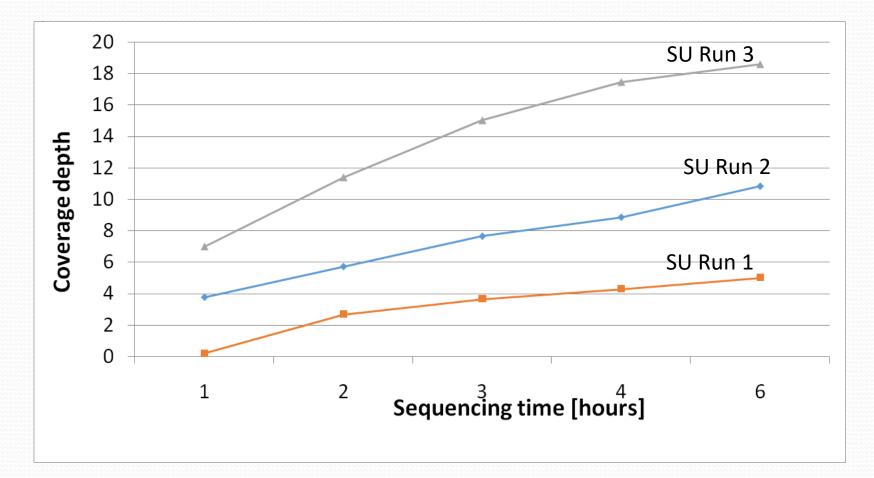
Improvement of MinION sequencing performance and yields over 6 h of sequencing (09.2014-10.2015)



CU: clinical urine; SU: spiked urine



Improvement of MinION coverage depth (SQK-MAP4, SQK-MAP5, SQK-MAP6)





Pathogen Identification

0

Escherichia coli 0139:H28 str. E24377A Escherichia coli APEC 078 Escherichia coli APEC 01 Escherichia coli ATCC 8739 Escherichia coli 0104:H4 Escherichia coli 0104:H4 Escherichia coli 0104:H4 Escherichia coli 0104:H4 Escherichia coli 0104:H4 Escherichia coli 026:H11 str. 11368 Escherichia scoli 026:H11 str. 11368 Escherichia coli 026:H11 str. 11368 Escherichia scoli 026:H11 str. 11368 Escherichia sco		0				
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* Arc angle is proportional to its read count						

E Taxonomic lineage (NCBI) superkingdom - Bacteria class -- Garmaproteobacteria class -- Garmaproteobacteria order --- Enterobacteriales family --- Enterobacteriaceae genus ---- Escherichia species ---- Escherichia coli Species identification in all clinical urines
(3 x E. coli, 2 x K. pneumoniae, E. cloacae).

Depth of coverage:
2.71x (CU5)- 22.84x (CU8).



Resistance gene profiles for Spiked Urines

Genes	Illumina	MinION run 3 ARMA (run time= 1 h)	MinION run 4 BLAST/CARD (run time= 1 h)				
β-Lactamase genes							
bla _{тем}	1	1, mv	1, mv				
bla _{стх-м}	group-1 (15)	mv not including <i>bla</i> _{CTX-M-15}	mv not including <i>bla</i> _{CTX-M-15}				
bla _{oxa}	1, 181	1, 181, mv	181, mv not including <i>bla</i> _{OXA-1}				
bla _{NDM}	4	1	mv				
bla _{сму}	2	mv not including <i>bla</i> _{CMY-2}	mv not including <i>bla</i> _{CMY-2}				
others	-	-	bla _{LAT-1}				
Aminoglycoside resistance genes							
aacC	aacC2	aacC2	аасС2, <mark>аасС8</mark>				
aadA2, aadA3, aadA5	aadA2, aadA3, aadA5	aadA2, aadA3, aadA5, mv	mv not including aadA2,A3, A5				
rmtB	rmtB	rmtB	rmtA				
aac(6')-Ib-cr	aac(6')-Ib-cr	aac(6′)-Ib-cr	aac(6′)-Ib				
strA/B	strA/B	strA/B	strA				
	Quinolone resistance genes						
qnr	qnrS1	qnrS1	qnrS				
aac(6')-Ib-cr	aac(6')-Ib-cr	aac(6')-Ib-cr	aac(6')-Ib				
	Trimethoprim resistance genes						
dfrA	dfrA-12, dfrA-17	dfrA-12, dfrA-17	dfrA7 (A17) , A12, <mark>A21, A22</mark>				
	Others						
cat	not detected	catB3	catB3/B6				
sul	sul1	sul1, <mark>sul2</mark>	sul1, sul2				
tet	tetA, tetR	tetA, tetR	tetA, tetR				



- Acquired resistance genes were readily detectable with 92% sensitivity
 - > mostly agreed with Illumina and phenotypic profile.
- MinION often flagged multiple gene variants (e.g. of bla_{TEM}, bla_{AmpC}, bla_{NDM}, bla_{CTX-M}) while Illumina found specific alleles.
- Chromosomal gyrA and parC mutations were not detected.
- MinION didn't detect mutations causing *ampC* up-regulation & couldn't discriminate chromosomal and plasmid *ampC*.



Future directions

Issues

- Needed ~1 μg DNA (>10⁷ cfu)
- Depletion of human cells
- Turnaround time and laborious library preparation
- Costs and throughput
- Low coverage, yields and high error rate
- Manual bioinformatics pipeline
- CARD database

Solutions

- Low-Input Kit: <10 ng DNA (~10⁵ cfu)
- Commercial or in-house methods
- Rapid Sequencing kit, automated sample processor VolTRAX
- PCR-free rapid barcoding kit, single sample flow-cell (Flongle), GridION X5
- R9.4 or R9.5 flow cells
- WIMP/ARMA software
- Curated clinical databases required



- MinION sequencing can rapidly identify pathogens and acquired antibiotic resistance genes from urine samples.
- Remaining challenges include:
- Choice of patients;
- Catheter urines;
- Improving the bioinformatics;
- Genotype/phenotype;
- ✓ Software output.



Acknowledgement



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Thank you very much!