University of East Anglia

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

Katarzyna Schmidt

20-10-2017

ICCMg, Geneva

University of East Anglia

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.

University of East Anglia

Overview

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

University of East Anglia

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.

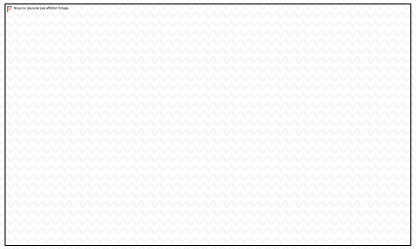
Current Diagnostics for cUTI

Turnaround Time:
24-72 h

Rapid Diagnostics for cUTI

Time: < 3 h

Time: ~ 4 h



Workflow for urine sample

- Clinical urines with bacteria >10^7 cfu/mL & human cells >10^5/mL.
- Urine spiked with multi-drug resistant *E. coli.*
- Host cell and human DNA depletion performed.

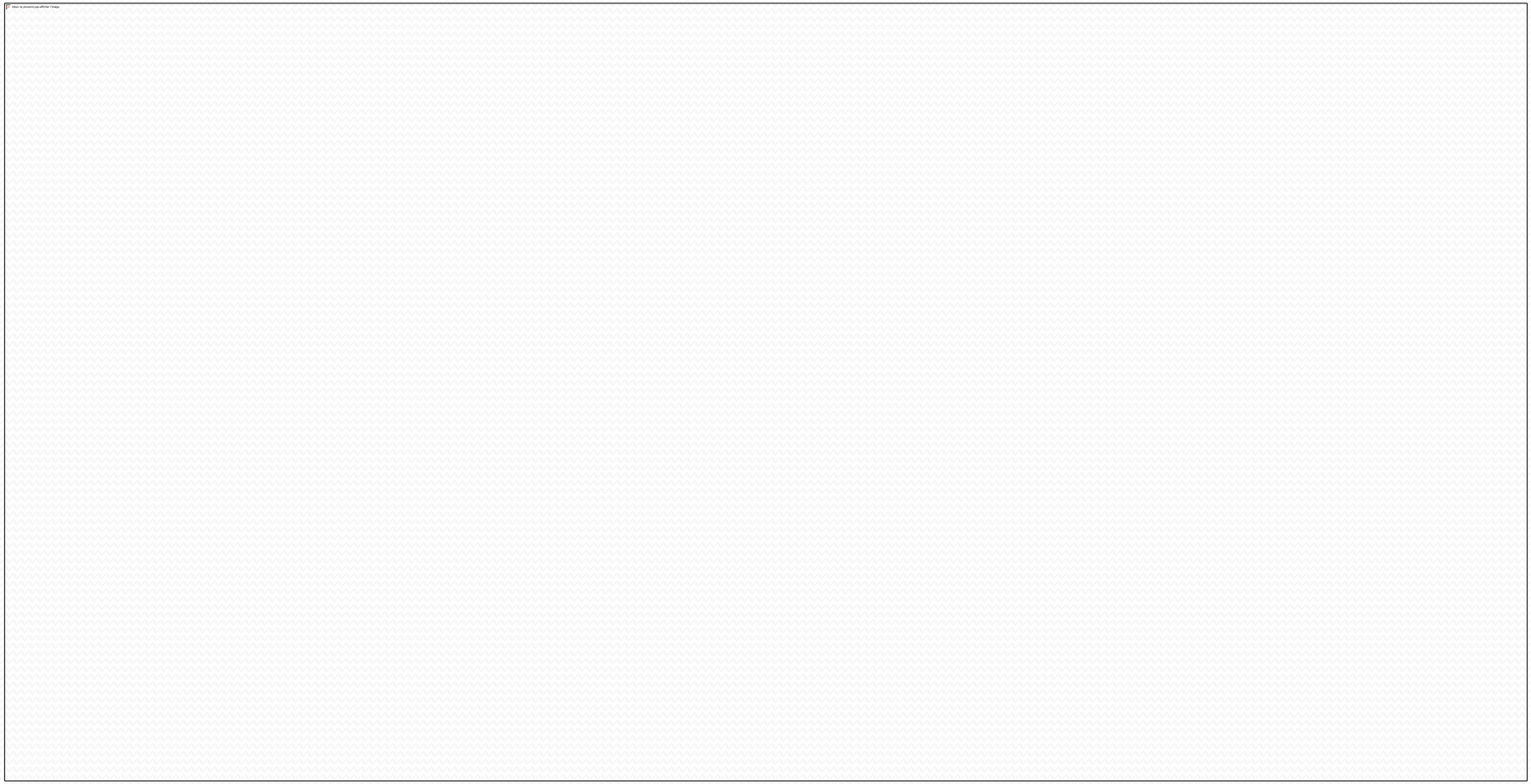
Timeframe of MinION sequencing

Sample preparation	105 min
↓	
DNA extraction	30 min
↓	
Library preparation	15 min
↓	
MinION sequencing	60 min
↓	
Bioinformatic analysis	30 min
↓	
Results	

Bacterial & Resistance genes ID

Total turnaround time ~ 4 h

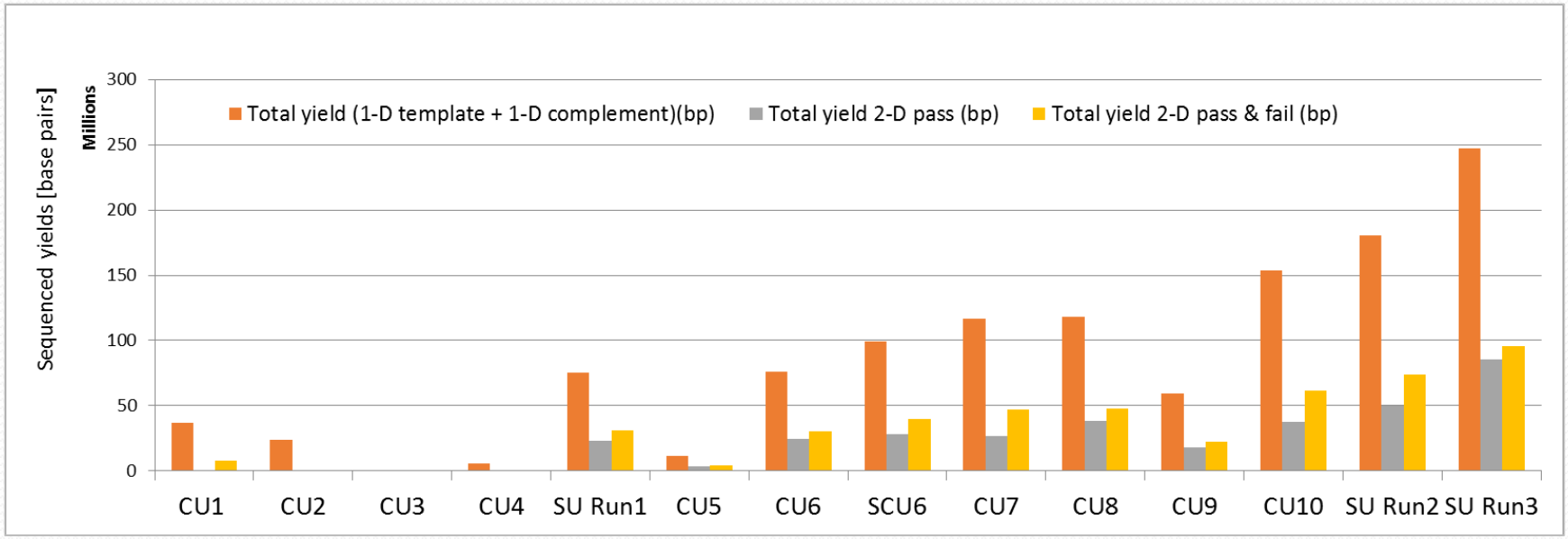
University of East Anglia



University of East Anglia

MinION performance

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

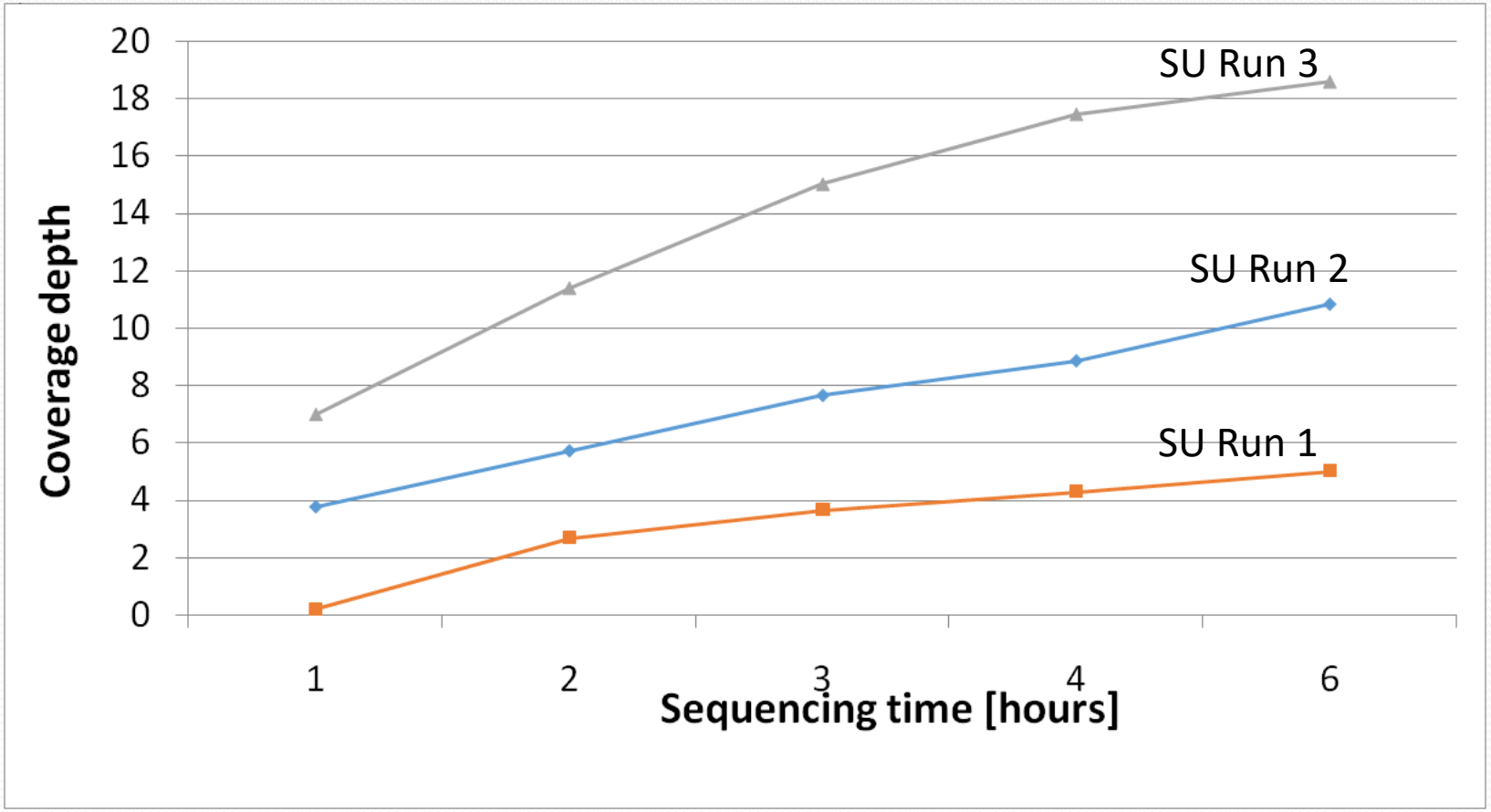


CU: clinical urine; SU: spiked urine

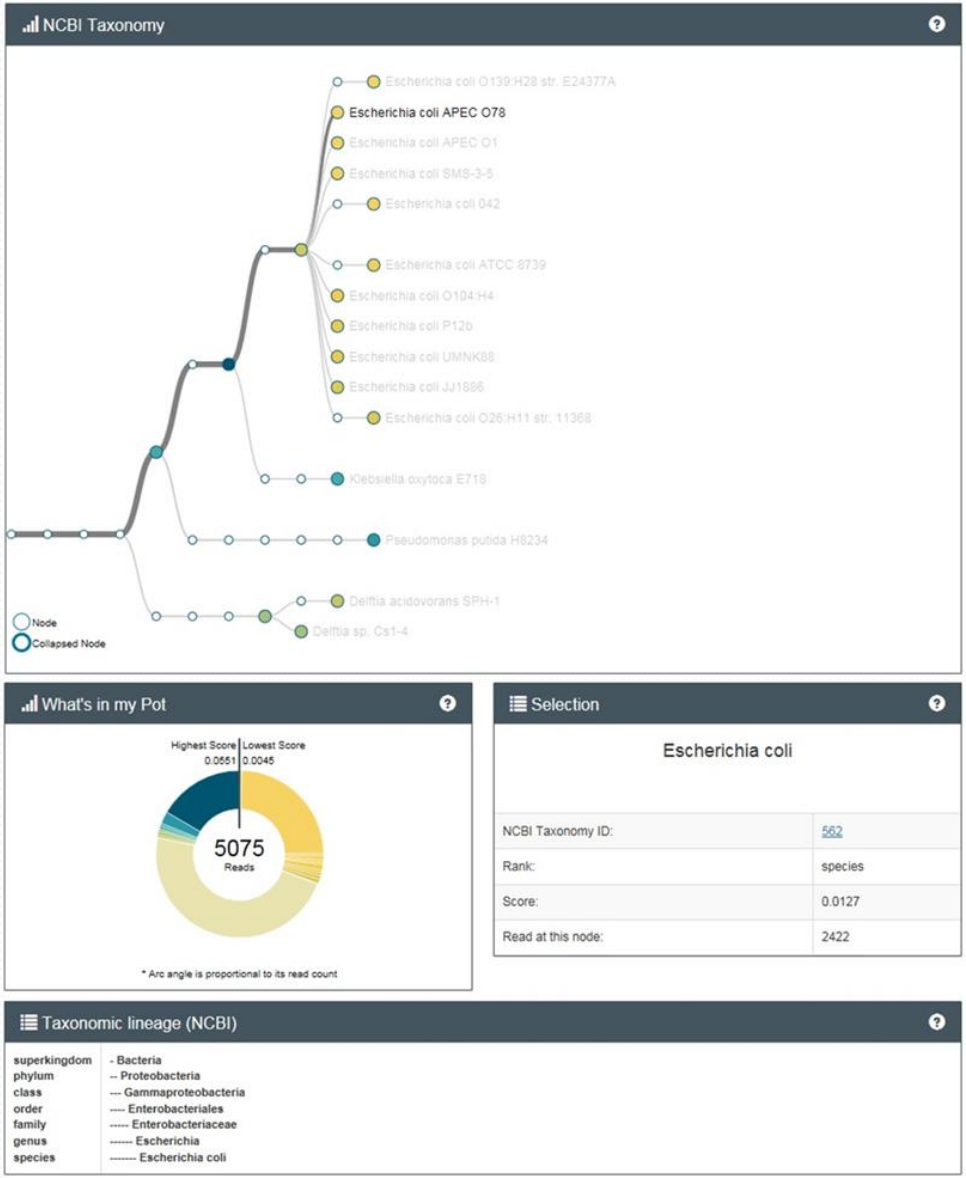
University of East Anglia

Coverage depth

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



Pathogen Identification



- 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).

University of East Anglia

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*$_{TEM}$**	1	1, mv	1, mv
***bla*$_{CTX-M}$**	group-1 (15)	mv not including *bla*$_{CTX-M-15}$	mv not including *bla*$_{CTX-M-15}$
***bla*$_{OXA}$**	1, 181	1, 181, mv	181, mv not including *bla*$_{OXA-1}$
***bla*$_{NDM}$**	4	1	mv
***bla*$_{CMY}$**	2	mv not including *bla*$_{CMY-2}$	mv not including *bla*$_{CMY-2}$
others	-	-	***bla*$_{LAT-1}$**
Aminoglycoside resistance genes			
aacC	*aacC2*	*aacC2*	*aacC2*, ***aacC8***
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,* ***A21, A22***
Others			
cat	not detected	***catB3***	***catB3/B6***
sul	*sul1*	*sul1,* ***sul2***	*sul1,* ***sul2***
tet	*tetA, tetR*	*tetA, tetR*	*tetA, tetR*

Resistance gene profiles for Clinical Urines

- 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}, $bla_{CTX\text{-}M}$) 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 (>10^7 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 (~10^5 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

University of East Anglia

Conclusions

- 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

- Prof. David Livermore
- Prof. John Wain
- Dr Justin O'Grady
- Solomon Mwaigwisya
- Dr Lisa Crossman

- Microbiology Department of NNUH

- Dr Michael Doumith
- Prof. Neil Woodford

- Dr Carlos Pires
- Dr Arshad Khan
- Prof. Nigel Saunders

Thank you very much!