Resistance phenotype prediction from whole genome data in ESBL or plasmid-AmpC producing *E. coli* or *K. pneumoniae*: results from bloodstream isolates collected during an international randomised trial (the "MERINO" Trial)

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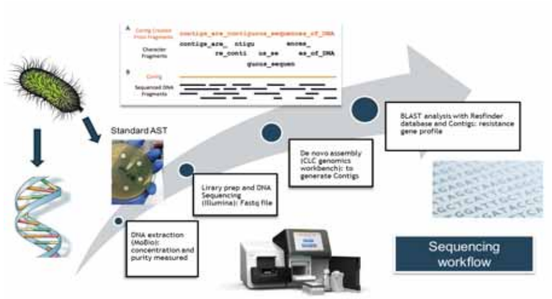
Introduction

Real-time detection of antimicrobial resistance determinants using next-generation sequencing (NGS) may be possible within the near future for clinical use.[1,2] However, we require additional data to ascertain how reliably detection of resistance genes by NGS can predict the observed phenotype and provide actionable information to safely direct therapy. We aimed to correlate resistance genes identified using NGS with the observed antimicrobial resistance profile in a collection of isolates cultured from patients from Australia, New Zealand and Singapore enrolled in a randomized trial comparing treatment options for bloodstream infections (BSI) caused by 3rd-generation cephalosporin (3GC)-resistant *E. coli* or *K. pneumoniae* (the "MERINO" Trial)[3] – i.e. likely extended-spectrum β-lactamase (ESBL) or plasmid-AmpC (p-AmpC) producers.

Methods



Whole genome DNA was extracted using MoBio Ultrapure kits. Paired-end libraries were prepared using Illumina Nextera and sequenced using Illumina MiSeq or HiSeq platforms. *De novo* assembly was performed using CLC Genomics Workbench v8.0 to generate contigs to be used in a BLAST analysis against a curated database of known resistance genes (ResFinder)[4], >with 80% identity and >80% match length filter parameters. BLAST results were curated to provide a resistance gene profile for each strain. Algorithms were developed to predict phenotypic resistance profiles according to detected resistance genes. All isolates were tested against a panel of antibiotics using disc diffusion, and minimum inhibitory concentrations (MICs) determined by Etest for ceftriaxone (CTX), piperacillin-tazobactam (PTZ) and meropenem (MER), according to EUCAST standards. Categorical agreement between the NGS predicted phenotype and the observed phenotype was then determined and visualized using discrimination summary plots[5] (Plotly Inc.). ESBL and p-AmpC types were geocoded according to city of recruitment and mapped using Tableau (version 9.2 Professional Edition).

Results

A total of 107 isolates (86.9% *E coli*, 13.1% *K. pneumoniae*) from 87 patients were included. The majority (60.2%) of *E. coli* were ST-131, mostly with CTX-M-type ESBLs (Figs. 1, 2 & 3).

Of the 100 isolates with phenotypic resistance to ceftriaxone or ceftazidime, 33% harboured $bla_{\text{CTX-M-15}}$, 23% $bla_{\text{CTX-M-27}}$ and 9% $bla_{\text{CTX-M-14}}$ (all of which belong to the CTX-M-9 lineage). Plasmid-mediated AmpC genes were seen in 11% (10 $bla_{\text{CMY-2}}$ and 1 $bla_{\text{DHA-1}}$). 17% of isolates had more than one ESBL or *ampC* gene (Figs. 1 and 2). Other ESBLs (such as $bla_{\text{SHV-28}}$ or $bla_{\text{SHV-9}}$) were present only in combination with $bla_{\text{CTX-M}}$.

Detection of ESBL or *ampC* was highly predictive of resistance to ceftriaxone (predicting resistance correctly in 97%, 95% CI 94-100%) (Fig. 4), but less reliable for ceftazidime (predicting resistance in 74%, 95% CI 66-83%). For other beta-lactams (AMC, FEP, ATM) resistance prediction was excellent (range 95-99%), although less reliable for cefoxitin (75%).

Prediction of susceptibility to AMC or ATM was unreliable (45% and 33% respectively) (Fig. 4, 5a). As the trial selected patients infected with strains susceptible to PTZ and MER, no categorical resistance prediction could be reliably determined for these agents. Falsely predicting susceptibility (a very major error), was less common but occurred most frequently for fluoroquinolones (predicting resistance in only 39%, 95% CI: 28-50%), reflecting deficiencies in the ResFinder database for chromosomal resistance mutations (Fig. 5b). Less than optimal prediction of resistance was also seen for gentamicin (85%, 95% CI: 73-96%) (Fig. 4).

Fig. 1: ESBL and plasmid-AmpC types in *E. coli* or *K. pneumoniae* causing bloodstream infections – MERINO trial

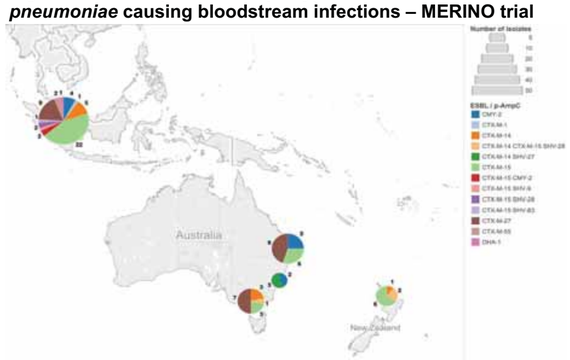


Fig. 2: Number of isolates by beta-lactamase type

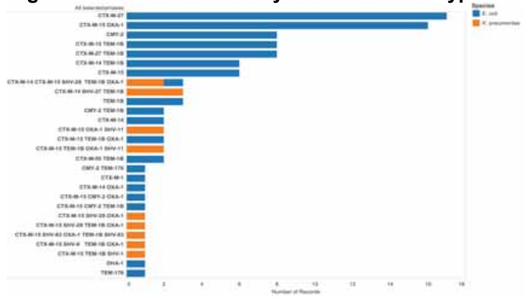


Fig. 3: *in silico* Multi-locus sequence type (MLST)

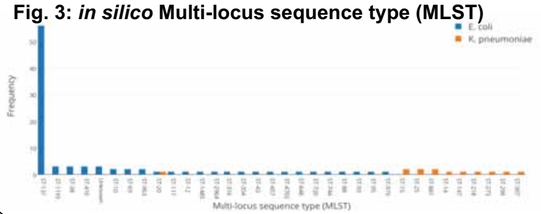
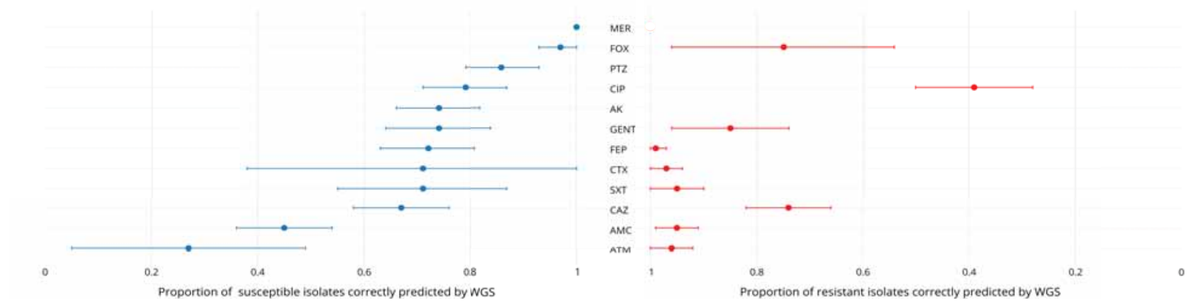


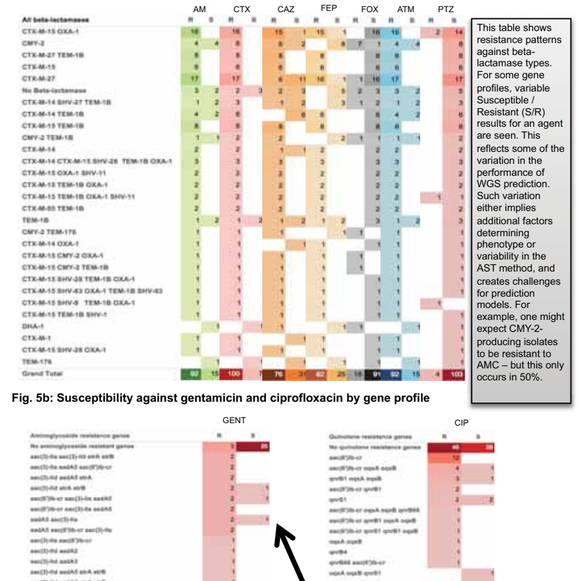
Fig. 4: Discrimination summary plots for phenotype prediction by whole genome sequencing



MER=meropenem, FOX=cefoxitin, PTZ=piperacillin-tazobactam, CIP=ciprofloxacin, AK=amikacin, GENT=gentamicin, FEP=cefepime, CTX=ceftriaxone, SXT=co-trimoxazole, AMC=amoxicillin-clavulanate, ATM=aztreonam

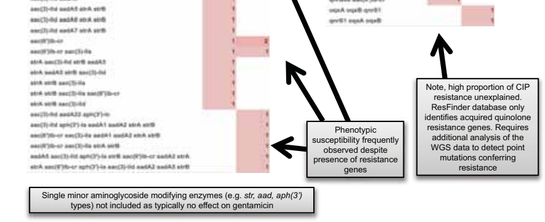
Note: No resistance predictions provided for meropenem, amikacin or piperacillin-tazobactam as too few resistant isolates

Fig. 5a: Susceptibility against beta-lactam agents by gene profile



This table shows resistance patterns against beta-lactamase types. For some gene profiles, variable Susceptible / Resistant (S/R) results for an agent are seen. This reflects some of the variation in the performance of WGS prediction. Such variation either implies additional factors determining phenotype or variability in the AST method, and creates challenges for prediction models. For example, one might expect CMY-2-producing isolates to be resistant to AMC – but this only occurs in 50%.

Fig. 5b: Susceptibility against gentamicin and ciprofloxacin by gene profile



Phenotypic susceptibility frequently observed despite presence of resistance genes

Note, high proportion of CIP resistance unexplained. ResFinder database only identifies acquired quinolone resistance genes. Requires additional analysis of the WGS data to detect point mutations conferring resistance

Single minor aminoglycoside modifying enzymes (e.g. *str, aad, aph(3')* types) not included as typically no effect on gentamicin

Conclusions

Resistance phenotype prediction from NGS data is a promising approach for the rapid characterisation of bacteria causing serious infections. Limitations currently exist, but are likely to be solvable by improvements in databases and bioinformatic pipelines. Although gene detection alone can reliably predict the resistance phenotype for several antibiotics, optimal predictive models are required that detect and characterise additional resistance determinants, such as chromosomal resistance mutations, regulators of gene expression, efflux pumps and porin changes, which are often not well addressed in currently available databases.

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