Emergence of carbapenemase producing *Enterobacteriaceae*, Malawi

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Since ceftriaxone was introduced into the Malawian national formulary in 2005, there have been rapid increases in the incidence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E), which are often untreatable due to a lack of locally available alternative treatment options.[1] Carbapenem antibiotics, the treatment of choice for invasive ESBL-E, were introduced to the Malawian essential medicine list in 2015, but remain sporadically available even in tertiary level facilities, frequently curtailing empiric therapeutic regimens prior to clinical improvement or completion of a course. Surveillance of bloodstream infection via automated blood culture (Biomerieux, France) has yet to detect any carbapenemase-producing organisms in Malawi[2], however , we report the detection of an NDM-5 producing *E. coli,* despite the low availability of carbapenems.

On 19 March 2018, a 67-year-old man attended Queen Elizabeth Central Hospital (QECH), Blantyre, with fever, headache and cough of a week's duration. He was HIV-infected and stable on antiretroviral therapy, but was not taking co-trimoxazole preventative therapy. He had received no antibiotics in the previous month and had no history of foreign travel. He had not been admitted to hospital in the previous 6 months. Malaria rapid diagnostic test was negative for *P. falciparum* and aerobic culture of blood and cerebrospinal fluid yielded no pathogens. He was treated with seven days of intravenous ceftriaxone, made an uneventful recovery and was discharged after seven days of admission.

As part of an observational study investigating acquisition of gut mucosal carriage of ESBL-E during admission to QECH (approved by ethics committees of the Malawi College of Medicine [P.11/16/2063] and Liverpool School of Tropical Medicine [16-

062]), the patient's stool was selectively cultured for ESBL-E on CHROMagar ESBL media (CHROMagar, Paris, France) on admission and on day seven of hospital admission. Morphologically distinct bacterial colonies growing on CHROMagar were confirmed to be ESBL producers using combination disc testing, speciation was carried out using the API system (Biomerieux, France) and antimicrobial sensitivities were determined using disc diffusion testing as per British Society of Antimicrobial Chemotherapy (BSAC) guidelines. All analyses were undertaken in the Malawi-Liverpool Wellcome Trust clinical laboratory, which subscribes to the UK National External Quality Assessment Service (NEQAS).

An ESBL-producing *Escherichia coli* was isolated from stool collected on day 7 of hospital admission, resistant to ciprofloxacin, co-trimoxazole, gentamicin, ceftriaxone and meropenem, with sensitivity to amikacin and chloramphenicol. Minimum inhibitory concentration to meropenem was 4mg/L by E-test (bioMerieux, France), and an ESBL/carbapenemase high resolution melt (HRM) PCR assay confirmed the presence of a New-Delhi metallo-beta-lactamase gene (NDM).[3]

In view of this resistance pattern, genomic DNA was extracted using the Qiagen DNA mini kit (Hilden, Germany) as per the manufacturer's instructions, and paired-end short-read whole genome sequencing was undertaken at the Wellcome Trust Sanger Institute using Illumina HiSeq-X10. De novo assembly was performed using SPAdes V3.11.0 followed by annotation with Prokka v1.5; assemblies were deposited in GenBank (accession number ERS2493547). Multi-locus sequence typing (MLST) using ARIBA V2.12.1 showed that this bacterium belonged to *E. coli* Sequence Type 2083, and a search for known antimicrobial resistance genes against the Comprehensive Antibiotic Resistance Database again using ARIBA V2.12.1 confirmed the presence of $bla_{\text{NDM-5}}$ as well as other genes encoding products conferring resistance to aminoglycosides (*aac(3)-IIa*, *aac(6')-IIb*, *aph(3')-Ib*, *aph(6)-Id*, *aadA5*), tetracyclines (*tet(R)*, *tet(A)*, *tet(D)*), trimethorprim (*dfrA17*) and suphonamides (*sul1*, *sul2*) as well as a CMY-42 *ampC* and $bla_{\text{TEM-95}}$ narrow-spectrum beta lactamase, but no plasmid-mediated quinolone resistance.

Plasmid replicons were identified using ARIBA v2.1.2.1 and the PlasmidFinder database[4]. The bla_{NDM-5} gene was carried on a partially assembled Inc-X3 plasmid,

which had 99% identity with a previously sequenced plasmid, pNDM-MGR194, a 46.2 kbp *bla*$_{NDM-5}$ containing Inc-X3 plasmid found in India between 2011-13.[5] We therefore fully assembled the plasmid from our isolate by mapping reads to this reference using Burrows-Wheeler alignment and found it to be extremely similar, with only 13 single nucleotide polymorphisms (SNPs) (Figure 1). Since its identification in India, virtually identical plasmids to pNDM-MGR194 have been described in humans and animals worldwide, [6] carried by *Klebsiella pneumoniae*, *Citrobacter freundii* and a wide variety of *E. coli* sequence types - though not previously ST 2083. There were a number of other plasmid replicons identified in our isolate: IncFI, IncFIA, IncFIB, IncFII and IncI1. The location of the CMY-42 ampC in the genome could not be determined, but did not seem to carried on the same plasmid as the *bla*$_{NDM-5}$ gene.

The admission stool sample was also selectively cultured for ESBL-E using the same protocol; the patient was found to be colonised with an ESBL-producing E coli on admission but the admission isolate was distinct in terms of AMR profile and *E. coli* sequence type. It was meropenem sensitive on antimicrobial sensitivity testing and, following whole-genome sequencing, MLST and identification of AMR genes as above, was found to contain no carbapenemase genes but a *bla*$_{CTX-M-16}$ ESBL gene. It was also not ST 2082 on MLST, but a novel ST, suggesting possible hospital acquisition of the carbapenemase-producing isolate.

The rapid emergence of carbapenem resistance in Malawi soon after the introduction of carbapenems on only a small scale is alarming, and hard to balance with the growing unmet need for access to this class of antimicrobial due to the problem of ESBL-E infection. It is likely that sporadic availability of carbapenems, often for incomplete courses, is creating selection pressure for the dissemination of this resistance type. This report highlights the urgent need for a holistic and context specific approach to both hospital infection prevention and control and antimicrobial stewardship in low-income settings, respecting the need to ensure appropriate access as well as to safeguard watch and reserve antimicrobials.

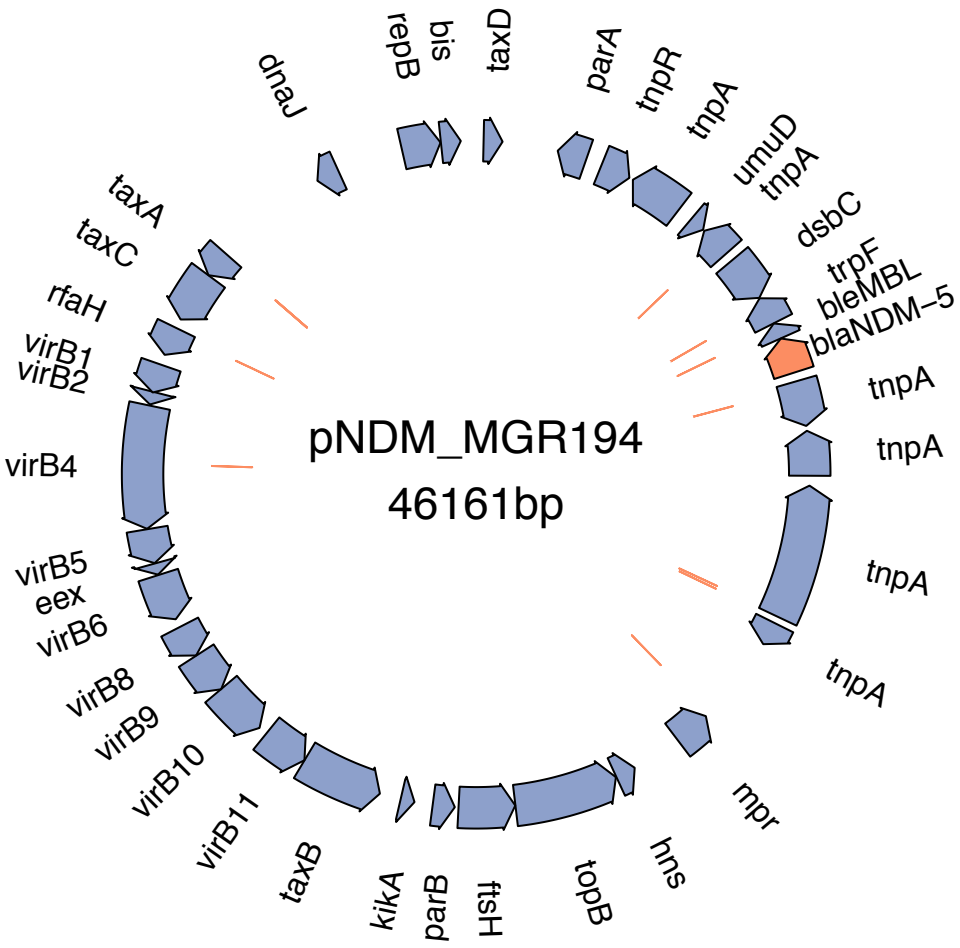

Figure 1: Plasmid pNDM_MGR194 annotated with gene names; location of single nucleotide polymorphisms (SNPs) identified in the Malawian plasmid shown in inner ring as lines. NDM-5 gene highlighted.

Funding

This work was supported by the Wellcome Trust; JL and RL hold Wellcome Clinical PhD Fellowships (109105Z/15/A and the University of Liverpool block award 347 [203919/Z/16/Z] respectively) and the Malawi Liverpool Wellcome Trust Clinical Research Programme and Wellcome Trust Sanger Institute are core funded by the Wellcome Trust.

Transparency Declarations

None to declare.

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