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First identification of bla_{NDM-5} producing *Escherichia coli* from neonates and a HIV infected adult in Tanzania

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Abstract

Introduction. Carbapenem-resistant members of the family Enterobacteriaceae are emerging as a global public-health threat and cause substantial challenges in clinical practice.

Gap Statement. There is a need for increased and continued genomic surveillance of antimicrobial resistance genes globally in order to detect outbreaks and dissemination of clinically important resistance genes and their associated mobile genetic elements in human pathogens.

Aim. To describe the resistance mechanisms of carbapenem-resistant *Escherichia coli*.

Methods. Rectal swabs from neonates and newly diagnosed human immunodeficiency virus (HIV) infected adults were collected between April 2017 and May 2018 and screened for faecal carriage of carbapenamases and OXA-48 producing members of the family Enterobacteriaceae. Bacterial isolates were identified using matrix assisted laser desorption ionization time of flight mass spectrometry. Antimicrobial susceptibility testing was performed by E-test. Whole genomes of carbapenem-resistant *E. coli* were investigated using a hybrid assembly of Illumina and Oxford Nanopore Technologies sequencing reads.

Results. Three carbapenem-resistant *E. coli* were detected, two from neonates and one from an HIV infected adult. All three isolates carried bla_{NDM-5}. Two *E. coli* from neonates belonged to ST167 and bla_{NDM-5} co-existed with $bla_{CTX-M-15}$ and bla_{OXA-01}, and all were carried on IncFIA type plasmids. The *E. coli* from the HIV infected adult belonged to ST2083, and carried bla_{NDM-5} on an IncX3 type plasmid and bla_{CMY-42} on an IncI type plasmid. All bla_{NDM-5} carrying plasmids contained conjugation related genes. In addition, *E. coli* from the HIV infected adult carried three more plasmid types; IncFIA, IncFIB and Col(BS512). One *E. coli* from a neonate also carried one extra plasmid Col(BS512). All three *E. coli* harboured resistance genes to fluoroquinolone, aminoglycosides, sulfamethoxazole, trimethoprim, macrolides and tetracycline, carried on the IncFIA type plasmid. Furthermore, *E. coli* from the neonates carried a chloramphenicol resistance gene (*catB3*), also on the IncFIA plasmid. All three isolates were susceptible to colistin.

Conclusion. This is the first report, to our knowledge, from Tanzania detecting bla_{NDM-5} producing *E. coli*. The carbapenemase gene was carried on an IncFIA and IncX3 type plasmids. Our findings highlight the urgent need for a robust antimicrobial resistance (AMR) surveillance system to monitor and rapidly report on the incidence and spread of emerging resistant bacteria in Tanzania.

BACKGROUND

Infections caused by carbapenem-resistant members of the family Enterobacteriaceae are emerging as a global public health concern. These infections cause substantial challenges in clinical practice as they are associated with increased morbidity and mortality as well as health care costs [1]. Carbapenems are considered a last resort for treatment of infections with multidrug-resistant members of the family Enterobacteriaceae. The remaining alternative treatment options for infections by carbapenem-resistant members of the family Enterobacteriaceae are colistin and tigecycline [2–5], which are expensive, poorly tolerated and often unavailable in low-and middle-income countries.

Resistance to carbapenems in Enterobacteriaceae is mainly mediated by the production of carbapenemase enzymes, which hydrolyze carbapenems and all other β-lactam antibiotics. Currently, three classes of carbapenemase enzymes of clinical importance have been identified in pathogenic bacteria. These are Ambler class A (KPC, IMI), class B (NDM, IMP, VIM) and class D (OXA-48) [2, 6].

Previously, carbapenem resistance in Gram-negative bacteria was mainly mediated by production of KPC, IMP, VIM and OXA-48 carbapenemases [6, 7]. In recent years, New Delhi metallo-β-lactamase has received global attention due to its world-wide dissemination, rapid evolution, and high levels of resistance to β-lactam antibiotics [5]. Since the first report of bla_{NDM-1} in 2008 [8], 24 variants of NDM have been discovered in Gram-negative bacteria [5]. Of the NDM variants, $bla_{NDM-5,}$ which was first detected in *Escherichia coli* in the United Kingdom in 2011 [9], is notable for its elevated resistance to carbapenems compared with other variants [10]. NDM-5 differs from NDM-1 by substitution of two amino acids (Val88Leu and Met154Leu) [11].

The bla_{NDM-5} is mostly carried on plasmids, which also may carry resistance genes to other antibiotics [12, 13] and catalyse their transfer between bacteria. Several plasmids have been reported to carry bla_{NDM-5} [5], with IncX3 type plasmid being the most commonly reported [4, 14, 15]. Since its discovery, bla_{NDM-5} producing *E. coli* has been reported in Asia, mainly PR China [16, 17], Europe [18, 19] and Africa [20, 21].

Meanwhile, in Tanzania, unpublished data shows increased use of carbapenems, but it is unknown whether this has led to an increased incidence of carbapenem resistance. Three Tanzanian studies have described resistance to carbapenems in clinical isolates, documenting the presence of VIM, IMP, NDM-1 and KPC type carbapenemases [22–24], but only one of these studies found NDM-1 carbapenemases in members of the family Enterobacteriaceae [24]. We performed this study to assess the faecal carriage of carbapenem-resistant members of the family Enterobacteriaceae among newly diagnosed HIV infected adults and children hospitalized with fever in Dar es Salaam, Tanzania. We also identified resistance mechanisms to carbapenems and their genetic context using a whole genome sequencing approach.

METHODS

Study sites and study participants

Newly diagnosed HIV infected adults were recruited consecutively from six HIV care and treatment centres in Dar es Salaam, Tanzania, namely Amana hospital, Mwananyamala hospital, Temeke hospital, PASADA, Mbagala hospital and Mnazi Mmoja hospital, as part of the double blinded randomized clinical trial, CoTrimResist (ClinicalTrials.gov identifier: NCT03087890) between April 2017 to May 2018. At the same time, children admitted with fever were enrolled in a febrile illness study at four hospitals in Dar es Salaam (Amana, Temeke and Mwananyamala Regional hospitals and Muhimbili National Hospital).

Screening for carbapenemase producing *Enterobacteriaceae*

Rectal swabs were collected from each participant and transported in liquid Cary–Blair medium (Faecal transwab, MWE). Screening for faecal carriage of carbapenamases and OXA-48 producing members of the family Enterobacteriaceae was carried out on CHROMID CARBA SMART (BioMérieux) using two drops (0.1 ml) from an overnight culture in brain–heart infusion broth.

Bacterial identification and antimicrobial susceptibility testing

Carbapenem resistant bacterial isolates which grew on the selective media were identified by matrix assisted laser desorption ionization time of flight mass spectrometry using the Microflex LT instrument and matrix assisted laser desorption ionization Biotyper 3.1 software (Bruker Daltonics).

Antibiotic susceptibility testing was determined by E-test (when available), following guidelines from the Clinical and Laboratory Standards Institute [25]. The antimicrobial agents tested were cefotaxime, imipenem, tetracycline, ciprofloxacin, gentamicin and colistin. When E-tests were not available disc diffusion was used to determine meropenem susceptibility according to Clinical and Laboratory Standards Institute guidelines.

Whole genome sequencing

Genomic DNA isolation for short read sequencing was carried out at MicrobesNG (Birmingham, UK). Short read whole genome sequencing was performed using HiSeq X10 (Illumina) by MicrobesNG, which also performed quality filtering and sequencing read trimming.

Genomic DNA for long read sequencing was extracted using a Fire Monkey High Molecular Weight DNA Extraction Kit (RevoluGen). Long read sequencing was carried out using a R9.4.1 flow cell (Oxford Nanopore Technologies) on a MinION sequencer. Base calling of the reads was performed with MinKNOW software (v20.06.4) using the Guppy algorithm (v4.0.9). The long-read sequences were trimmed using Porechop (https://github.com/rrwick/Porechop) and filtered using Filtlong (https://github.com/rrwick/Filtlong) with a minimum length threshold of 1000 bp, keeping 90% of the best reads up to a total of 500000000 bp.

Long and short read sequences were assembled using Unicycler (v0.4.8.0) [26], running in 'normal' mode and the genome was annotated with Prokka (v1.14.6) [27] and the RAST annotation server [28]. Genomes were aligned using both Clinker (v0.0.12) [29] and BRIG (v0.95) [30]. The genomes were visualized using Snapgene (v3.3.4) (https://www.snapgene.com/). All genome assemblies from this study have been deposited in GenBank under the BioProject numbers PRJNA756167 (strain PC-NDM34), PRJNA756168 (strain NDM_12_14482) and PRJNA756169 (strain NDM_11.16372).

Identification of resistance genes and sequence types

For identification of acquired antimicrobial resistance genes, and detection of virulence genes, we used ResFinder v4.1 [31] and virulence Finder 2.0 [32], respectively, from the Centre for Genomic Epidemiology GEE server (http://www.genomicepidemi-ology.org/). For identification of mobile genetic elements and their relation to antimicrobial resistance genes and virulence factors we used Center of Genomic Epidemiology Mobile Element Finder v1.0.3 [33]. For assignment of multilocus sequence typing (MLST) and clonal complexes we used an online MLST database website (https://pubmlst.org/).

Phylogenetic analysis

Phylogenetic analysis of *E. coli* ST167 whole genomic sequence single nucleotide polymorphisms (SNPs) was performed using CSI phylogeny 1.4 [34]. For comparison, two *E. coli* ST167 from this study were compared with 22 globally available complete whole genome sequences of *E. coli* ST167 (bla_{NDM-5}-positive and bla_{NDM-5}-negative) downloaded from NCBI nucleotide GenBank and the European nucleotide archive (NEA). The phylogenetic tree was visualized using the Fig Tree programme version 1.4.4 (https://github.com/rambaut/figtree/releases).

Comparative analysis of IncFIA and IncX3 carrying bla_{NDM-5} plasmids

For comparison of the genetic environments surrounding bla_{NDM-5} on IncFIA and IncX3 plasmids, approximately 20 kb segments were selected including the bla_{NDM-5} gene from each strain, and annotated using Prokka (v1.14.6) [27] and aligned using Clinker (v0.0.12) [29].

IncFIA and IncX3 plasmids were compared with other available plasmids. A total of 17 IncX3 and 25 IncFI carrying bla_{NDM-5} plasmids' genome sequences available globally were downloaded from the NCBI 'nuccore' database using Entrez Direct as assembled genomes in fasta file format. BRIG v0.95 [30] was used for genomic comparison and to produce the visualisations. For the IncF comparisons, the 1446 NDM-5 plasmid sequence was used as reference and for the IncX3 comparisons the PC34 NDM-5 plasmid sequence was used as a reference. ResFinder v4 [31]. was used to annotate the reference genomes with acquired antimicrobial resistance (AMR) genes.

RESULTS

Bacterial isolates

Three carbapenem resistant *E. coli* designated as PC-NDM34 (from a HIV infected adult), NDM_12_14482, and NDM_11.16372 (both from neonates) were isolated from screening of 737 rectal swabs (537 HIV infected outpatient adults and 200 under five years old inpatient children). Isolate PC-NDM34 was from a 49-year-old individual, an outpatient newly diagnosed with HIV at PASADA HIV care and treatment center in May 2018. The patient had a CD4 count of 132 cell μl^{-1} with no recent history of hospitalization or antibiotic use. Isolates NDM_12_14482 (22 Jan, 2018) and NDM_11.16372 (05 Feb 2018) were isolated from two neonate patients aged three days from the same ward (Temeke Hospital) two weeks apart in February 2018.

Antimicrobial susceptibility pattern

Table 1 shows the minimum inhibitory concentrations (MICs) of different antimicrobial agents tested against the three isolates. The MICs for all the three isolates to cefotaxime, gentamicin, ciprofloxacin and tetracycline were more than 256 µg ml^{-1}. Meropenem susceptibility was checked for isolates NDM_11.16372 and NDM_12_14482 by disc diffusion, revealing

Table 1. Susceptibility and MIC of the three carbapenem resistant isolates

Antimicrobial agent	MIC		
	PC-NDM34	NDM_11.16372	NDM_12_14482
Meropenem	6 µg ml^{-1} †	13 mm*	12 mm*
Imipenem	3 µg ml^{-1} †	3 µg ml^{-1}†	1.5 µg ml^{-1}†
Colistin	0.125 µg ml^{-1} †	0.25 µg ml^{-1} †	0.19 µg ml^{-1} †
Cefotaxime	>256 µg ml^{-1} †	>256 µg ml^{-1} †	>256 µg ml^{-1} †
Tetracycline	>256 µg ml^{-1} †	>256 µg ml^{-1} †	>256 µg ml^{-1} †
Ciprofloxacin	>256 µg ml^{-1} †	>256 µg ml^{-1} †	>256 µg ml^{-1} †
Gentamicin	>256 µg ml^{-1} †	>256 µg ml^{-1} †	>256 µg ml^{-1} †

*Meropenem E-tests were unavailable, therefore meropenem disc diffusion assays were used for isolates NDM_11.16372 and NDM_12_14482.
†, AST by E-tests.

zones of inhibition of 13 mm and 12 mm respectively. For isolate PC-NDM34 meropenem susceptibility was determined by E-test and shown to be 6 µg ml^{-1}. Subsequently, the MICs of these three isolates PC-NDM34, NDM_11.16372 and NDM_12_14482 to imipenem were determined to be 3 µg ml^{-1}, 3 µg ml^{-1} and 1.5 µg ml^{-1} respectively. The three isolates were susceptible to colistin at different MIC values as follows; 0.125 µg ml^{-1} for isolate PC-NDM34, 0.25 µg ml^{-1} for isolate NDM_11.16372 and 0.19 µg ml^{-1} for isolate NDM_12_14482.

Resistance determinant genes and association with plasmids

Isolate PC-NDM34 contained six plasmids, IncFIA (109 kb), IncFIB (108 kb), IncI (49 kb), IncX3 (46 kb), Col (BS512) (2 kb), and another unknown plasmid with an approximate size of 5 kb. Isolate NDM_11.16372 contained three plasmids, IncFIA (137 kb), Col (BS512) (2 kb), and another of about 4 kb, and isolate NDM_12_14482 contained one IncFIA plasmid of approximately 137 kb. All three carbapenem resistant *E. coli* carried plasmid-located *bla*$_{NDM-5}$. For isolate PC-NDM34, *bla*$_{NDM-5}$ was located on an IncX3 type plasmid, while for both isolates NDM_11.16372 and NDM_12_14482 it was located on the IncFIA plasmid. All the *bla*$_{NDM-5}$ carrying plasmids also carried genes whose products are predicted to be involved in conjugation.

Other *β*-lactam resistance genes carried by PC-NDM34 were *bla*$_{TEM-1b}$ and *bla*$_{CMY-42}$, located on IncI and IncFIA type plasmids, respectively. The IncFIA type plasmid from P34 harboured other resistance genes including fluoroquinolone-aminoglycosides (*aac(6')-Ib-cr*); sulfamethoxazole (*sul*2); trimethoprim (*dfr*17); aminoglycoside [*aac(3)-IId*, *aph(3')-lb*, *aadA*5]; tetracycline (*tetB*); and macrolides *mph*(A). While *bla*$_{NDM-5}$ on IncFIA plasmid from both NDM_11.16372 and NDM_12_14482 co-existed with other *β*-lactam resistance genes (*bla*$_{TEM-1b}$, *bla*$_{OXA-01}$ and *bla*$_{CTX-M-15}$), other resistance genes present on the IncFIA plasmid from both NDM_11.16372 and NDM_12_14482 conferred resistance to fluoroquinolone-aminoglycosides [*aac(6')-Ib-cr*]; aminoglycosides (*aadA*2, *rmtB*); sulfamethoxazole (*sul*1); trimethoprim (*dfr*12); chloramphenicol (*catB*3); tetracycline (*tet*A); and macrolides *mph*(A). Table 2 shows resistance genes present in the three isolates.

Multi-locus sequence typing (MLST)

MLST analysis revealed that both NDM_12_14482 and NDM_11.16372, the isolates from neonates, harbouring *bla*$_{NDM-5}$ belonged to ST167 (ST10 clonal complex). In contrast, PC-NDM34 *bla*$_{NDM-5}$, isolated from an adult HIV infected patient, belonged to ST2083.

Phylogenetic analysis

A total of 22 *E. coli* ST167 (*bla*$_{NDM-5}$-positive and *bla*$_{NDM-5}$-negative) globally available from GenBank and the European nucleotide archive (see Supplementary file 1, available with the online version of this article, for accession numbers) were compared with two ST167 strains from this study. The SNP analysis of all *E. coli* ST167 differed by between 2 and 3410 SNPs, revealing the high genetic diversity of this high-risk clone. The phylogenetic analysis revealed that *E. coli* ST167 was clustered into two distinct clades with multiple subclades (Fig. 1). The two *E. coli* ST167 from this study had a SNP difference of two SNPs and were clustered in clades with other ST167 from PR China, Italy and Myanmar and two with undetermined origins. In this clade, the SNP difference between this study's ST167 and other ST 167 ranged between 880 and 1312 SNPs.

This analysis reveals that the two isolates (NDM_12_14482 and NDM_11.16372 *E. coli*) which were isolated from neonates, were closely related and probably arose from the same source. Furthermore, this revealed that the isolates from neonates were more distantly related to the ST167 isolates from other part of the world.

Table 2. Genotypic characteristics of the three bla_{NDM-5} containing *E. coli*

Genotypic characteristics	PC-NDM34	NDM_11.16372	NDM_12_14482
Resistance genes			
Beta-lactams	bla_{TEM-1B}	bla_{TEM-1B}	bla_{TEM-1B}
	bla_{NDM-5}	bla_{NDM-5}	bla_{NDM-5}
	bla_{CMY-42}	–	–
	–	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$
	–	bla_{OXA-1}	bla_{OXA-1}
Fluoroquinolone-aminoglycosides	*aac(6')-Ib-cr*	*aac(6')-Ib-cr*	*aac(6')-Ib-cr*
Sulfamethoxazole	*sul*2	*sul*1	*sul*1
Trimethoprim	*dfr17*	*dfr12*	*dfr12*
Aminoglycosides	*aac(3)-IId, aph(3')-lb, aadA5*	*aadA2, rmtB*	*aadA2, rmtB*
Chloramphenicol		*catB3*	*catB3*
Tetracycline gene	tetB	*tetA*	*tetA*
Mdf	*MdfA*	*MdfA*	*MdfA*
Macrolide	*mph*(A)	*mph*(A)	*mph*(A)
Sequence type (ST)	2083	167	167
Plasmid replicon types	IncFIA	IncFIA,	IncFIA
	Col (BS512)	Col (BS512)	
	IncI	–	–
	IncIB	–	–
	IncX3	–	–
	Unknown	Unknown	–
Virulence gene			
Fimbrial protein	yfcV	–	–
EAST-1 heat-stable toxin	astA	–	–
Long polar fimbriae	lpfA	–	–
Glutamate decarboxylase	gad	gad	gad
Increased serum survival	–	iss	iss
OMP complement resistance	trat	trat	trat
Heat resistant agglutinin	–	hra	hra
Hexosyltransferase homology	–	capU	capU
Tellurium ion resistance protein	terC	terC	terC

Genetic environment for the bla_{NDM-5} carrying plasmids

Comparative analysis of plasmids carrying bla_{NDM-5}, revealed the IncFIA type plasmids from NDM_12_14482 and NDM_11.16372 harboured 100% identical sequences and bla_{NDM-5} co-existed with several resistance determinant genes (Fig. 2).

Comparative analysis of the genetic environments of bla_{NDM-5} in IncX3 and IncFIA type plasmids revealed similar genetic context downstream of bla_{NDM-5} flanked by conserved ble_{MBL}–*trpF*–*DsbD*. In the IncX3 plasmid bla_{NDM-5} was flanked upstream by IS5, and in the IncFIA type plasmid it was flanked upstream by IS*26* interrupted by a gene predicted to encode a conserved hypothetical protein (Fig. 3). In the IncX3 type plasmid, bla_{NDM5}–ble_{MBL}–*trpF*–*DsbD* was flanked by *IS5* upstream and IS*26* downstream (Fig. 4).
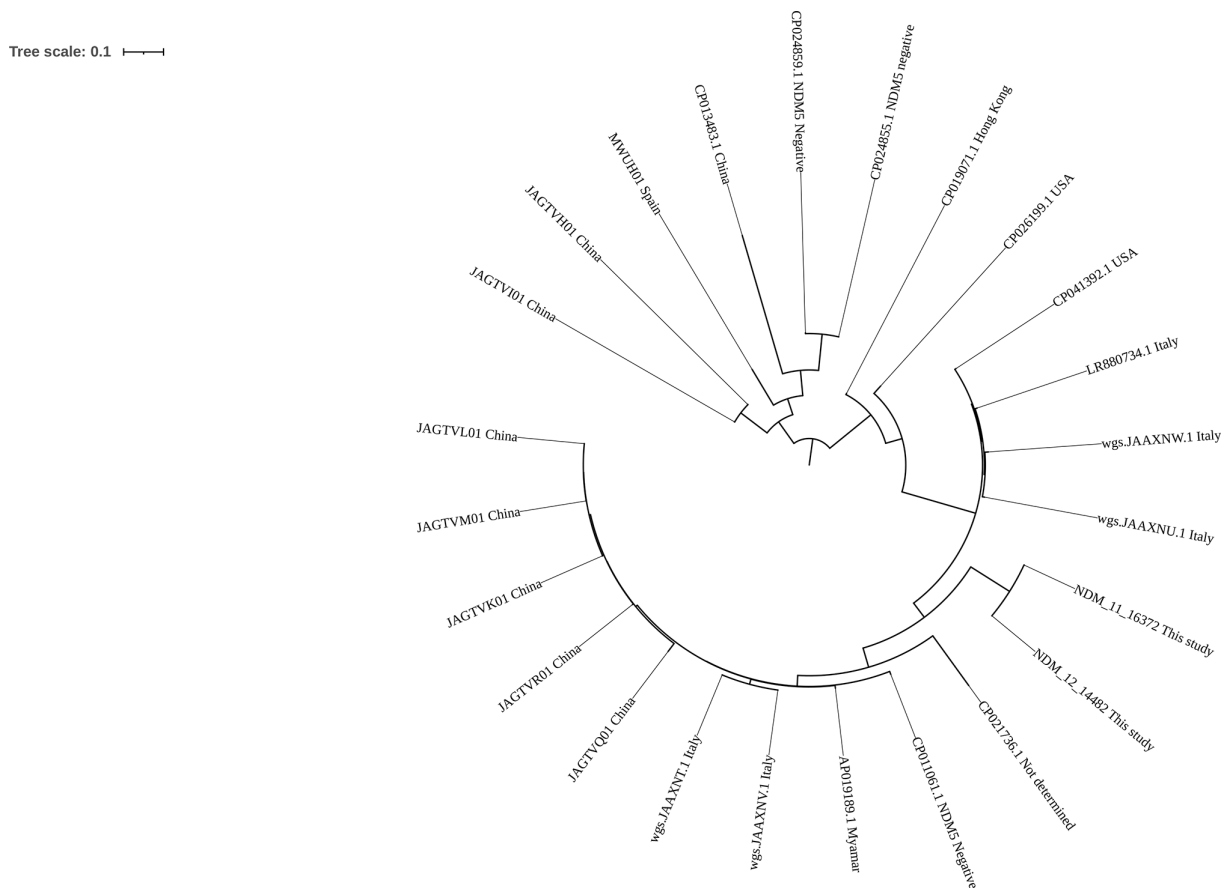
Fig. 1. Phylogenetic tree *E. coli* ST167 (*bla*$_{NDM-5}$-positive and *bla*$_{NDM-5}$-negative). Countries of origin of each *E. coli* ST167 have been highlighted in the tree, not determined indicates that the origin is not known.

In the IncFIA plasmid, further downstream of *bla*$_{NDM5}$–*ble*$_{MBL}$–*trpF*–*DsbD* were a set of several genes including *sul*-2, *ant*(3′)-Ia, *dfrA*12 and *IntI*1. The *bla*$_{NDM-5}$–*IntI*1 genetic complex was flanked on both ends by IS*26* (Fig. 3).

Fig. 5 shows the results of the comparative analysis of the fully sequenced *bla*$_{NDM-5}$ carrying IncFIA plasmids from this study (NDM_12_14482 and NDM_11.16372) and the IncF plasmids (*bla*$_{NDM-5}$-positive and *bla*$_{NDM-5}$-negative) of global representatives (see Supplementary file 1 for accession numbers). Structural similarities (downstream) were observed within the genetic environment surrounding *bla*$_{NDM5}$ (Fig. 5). The NDM_12_14482 and NDM_11.16372 plasmid sequences differ slightly compared with other global IncF plasmids.

Comparative analysis of *bla*$_{NDM-5}$ carrying IncX3 plasmids is depicted in Fig. 6, comparing PC-NDM34 from this study with globally identified IncX3 plasmids (see Supplementary file 1 for accession numbers). The genetic context of *bla*$_{NDM-5}$ on IncX3 plasmids was similar downstream. The genetic sequence of PC-NDM34 IncX3 was almost identical to those of most other global IncX3 plasmids, with slight differences observed (Fig. 6).

DISCUSSION

This is the first report from Tanzania and East Africa, to our knowledge, on the detection of *bla*$_{NDM-5}$ producing *E. coli*. We found three *E. coli* carrying *bla*$_{NDM-5}$, one from a newly diagnosed HIV infected patient and two from admitted neonates.

The comparative genomic analysis of *bla*$_{NDM-5}$ producing *E. coli* revealed that the carbapenemase gene was plasmid located and that these plasmids also carried resistance gene determinants to other antibiotics, including aminoglycosides, fluoroquinolones, macrolides, tetracycline, trimethoprim, sulfamethoxazole and chloramphenicol. The finding of carbapenem resistance conferred by the *bla*$_{NDM-5}$ gene in Tanzania is of the great concern, since these isolates also were resistant to virtually all other antibiotics commonly used in Tanzania. Carbapenems have been used as the last alternative for treatment of severe infections caused by multi-drug resistant Gram-negative bacteria infection, the emergence of carbapenem resistance severely
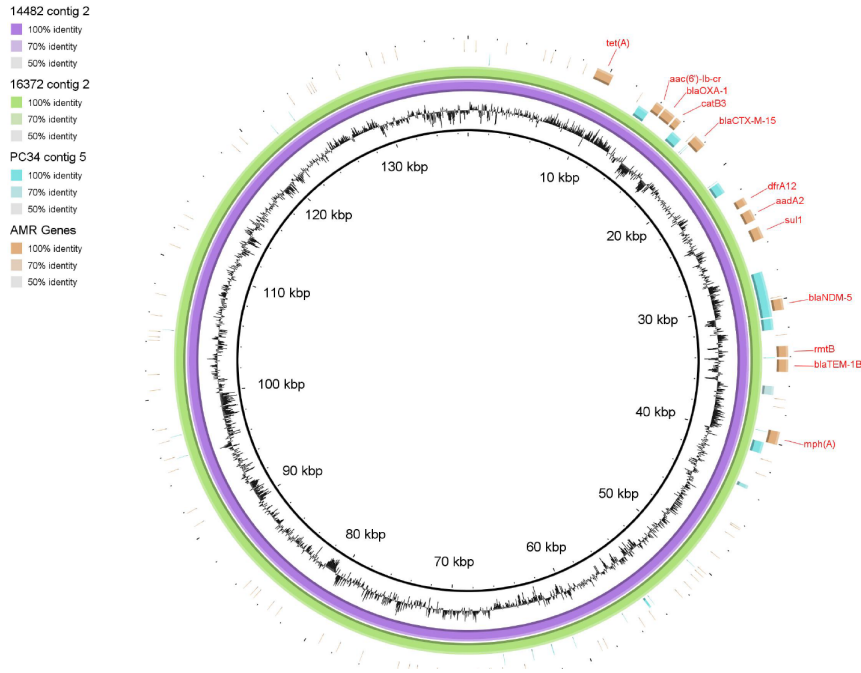
Fig. 2. Comparative analysis of the three plasmids carrying *bla*_{NDM-5}, image produced using BRIG. A comparison between the three *bla*_{NDM-5} positive plasmids across the three strains, each coloured accordingly. The reference genome was the plasmid of the isolate 14482. The inner black ring represents GC content of the reference sequence. AMR genes are labelled. 14482= NDM_12_14482, 16372= NDM_11.16372 and PC34= PC-NDM34.

limits treatment options for these patients. Lately, the use of carbapenems in Tanzania has been frequent [35], and increased use is anticipated in the future due to increased extended spectrum beta-lactamase (ESBL) producing bacterial infections [36]. Increasing carbapenem use will increase selection for horizontal gene transfer events and, therefore, the movement of the gene and human movement will increase dissemination of carbapenem resistant bacteria around the country.

To date, reports on *bla*_{NDM-5} producing Gram-negative bacteria from Africa have been uncommon. In Africa, *bla*_{NDM-5} was first reported in Algeria in three *E. coli* recovered from urine and blood in 2012 [20]. Since then, *bla*_{NDM-5} in humans has been reported in Egypt [37], Angola [38], South Africa [39], Nigeria [40], Chad [15] and Malawi [41, 42]. Recently, *bla*_{NDM-5} producing *E. coli* have been detected in Mali from one outpatient [21] and in Mozambique in a patient with a bloodstream
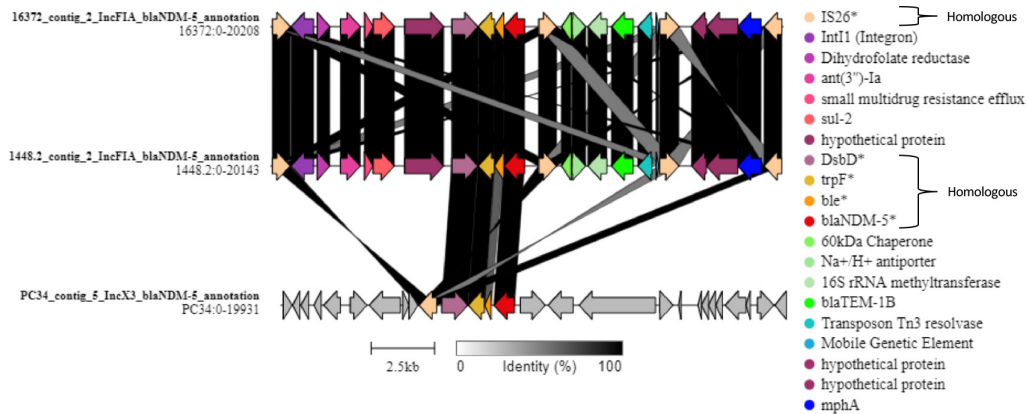

Fig. 3. Comparison of *bla*_{NDM-5} genetic context on 16372 (IncFIA), 14482 (IncFIA) and PC34 (IncX3) plasmids. A sequence comparison between the genetic environments surrounding *bla*_{NDM-5} in the three strains. Approximately 20 kb segments were selected including the *bla*_{NDM-5} gene from each strain, these were annotated using Prokka and aligned using clinker. Unique sequences are coloured according to feature. Those features which are homologous across all three strains are highlighted with asterisks. 14482= NDM_12_14482, 16372= NDM_11.16372 and PC34= PC-NDM34.
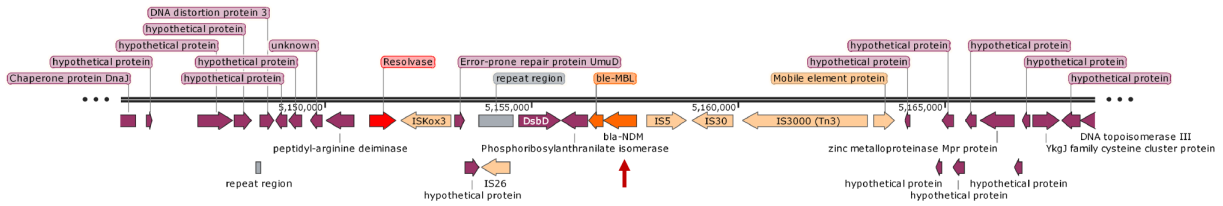
**Fig. 4.** Genetic context of bla_{NDM-5} on the IncX3 plasmid (PC-NDM34).

infection [12]. The reason for increased detection of bla_{NDM-5}-producing Gram-negative bacteria in Africa is not known, it could be independent introduction from the rest of the world. Our three bla_{NDM-5} *E. coli* were isolated from patients with no history of travel beyond Tanzania. It is therefore likely that the patients have acquired the bla_{NDM-5} producing *E. coli* in Tanzania or their resident *E. coli* had acquired bla_{NDM-5} from a transient donor also in Tanzania.

In analysing mobile genetic elements and their relationship with resistance genes, we found that the bla_{NDM-5} producing *E. coli* ST2085 from the HIV infected patient was located on an IncX3 type plasmid. Similarly, a recent published study from Malawi reported detection of blaNDM-5 contained in an IncX3 plasmid from *E. coli* ST2085 isolated from the stool of an HIV-infected adult [42]. The results of previous studies have demonstrated that bla_{NDM-5} is commonly carried on IncX3 type plasmid [13, 15, 43–45]. In addition, the results of previous studies have indicated that the IncX3 type plasmid plays an important role in dissemination of bla_{NDM-5} in members of the family *Enterobacteriaceae* [13, 14, 21]. This hypothesis has been supported by the results of several experimental conjugation studies, where bla_{NDM-5} *E. coli* carrying IncX3 type plasmids were able to be successfully transferred amongst, and between, different species of the family *Enterobacteriaceae* [14, 43]. The bla_{NDM-5} carrying IncX3 plasmid from this study revealed almost identical plasmid sequence to globally identified IncX3 plasmid. Our finding of an IncX3 type plasmid associated with bla_{NDM-5} in a newly diagnosed HIV patient from the community setting in Tanzania is concerning, since this plasmid is epidemic and has been shown to carry multiple carbapenemase genes,
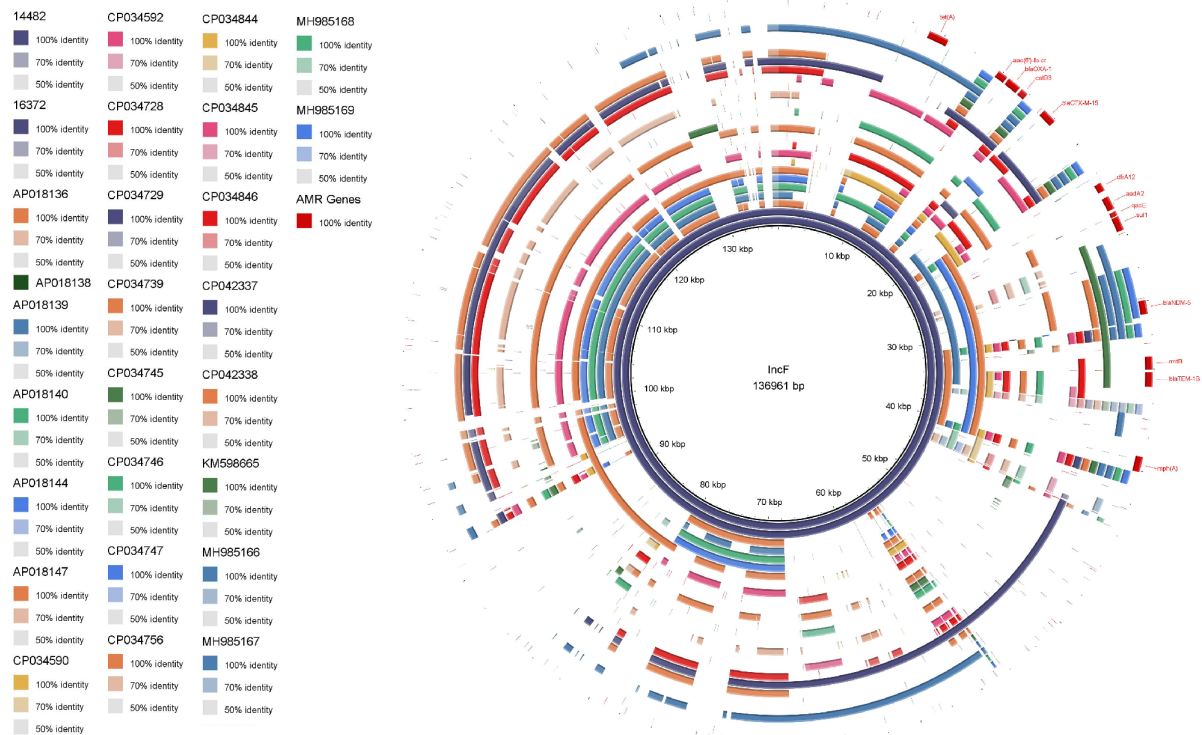
Fig. 5. A comparative analysis of the two IncFIA plasmids (16372 and 14482) containing bla_{NDM-5} against IncF plasmids identified globally. BRIG was used for genomic comparison and visualization. Plasmid (137 Kb) from isolate NDM_12.14482 containing bla_{NDM-5} was used as the reference. Sequences are named using their accession numbers. (16372=NDM_11.16372 and 14482=NDM_12.14482).
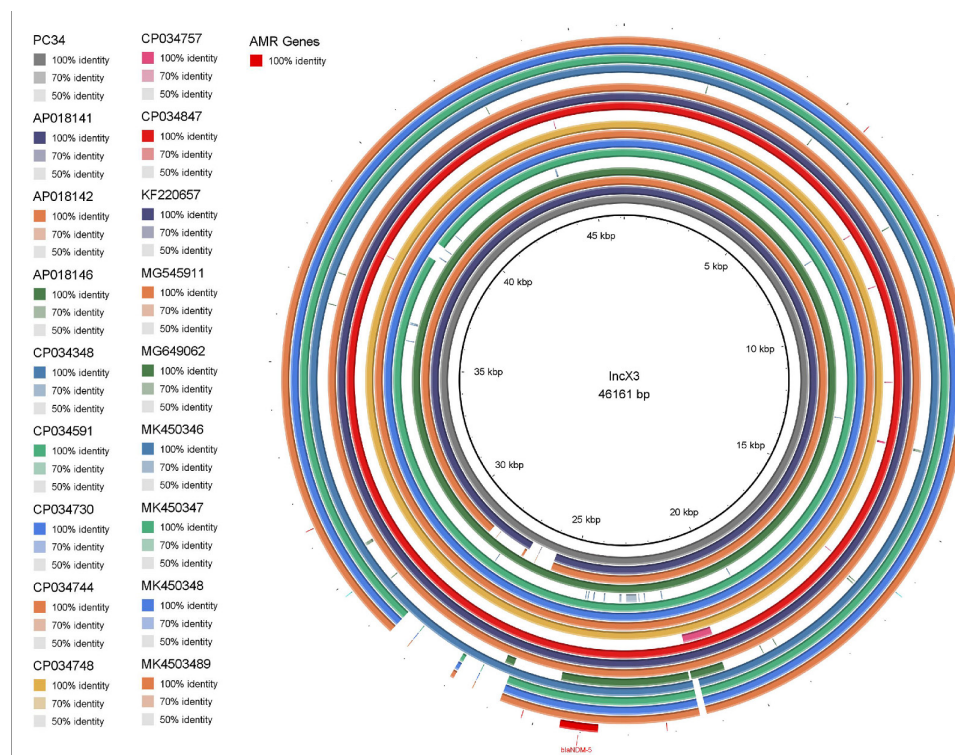
Fig. 6. A comparative analysis of the IncX3 (PC34) plasmid of the isolate PC-NDM34 against IncX3 plasmids identified globally. BRIG was used for genomic comparison and visualization. The plasmid (46 Kb) from isolate PC-NDM34 containing bla_{NDM-5} was used as a reference. All sequences were of plasmids containing IncX3 except for CP034348 which contained an IncX1 plasmid. Sequences are named using their accession numbers.

including bla_{NDM-5}, and has a high potential to efficiently disseminate globally. Spread of bla_{NDM-5} in the community has serious implication since bla_{NDM-5} carrying isolates also display multidrug resistance [13].

IncF type plasmids have also been shown to contribute to the dissemination of bla_{NDM-5} among *E. coli* [17, 18, 46]. In this study, we observed that the bla_{NDM-5} in *E. coli* from neonates was carried on IncFIA type plasmids. The bla_{NDM-5} from these isolates co-existed with $bla_{CTX-M-15}$, bla_{TEM-1B} and bla_{OXA-1} located on the IncFIA plasmid. Other recent studies in Africa and elsewhere have observed co-harbouring of bla_{NDM-5} and bla_{CTX-15}, bla_{TEM-1B}, bla_{OXA-1} in the same plasmid type in *E. coli* [3, 12, 46]. Co-localization of bla_{NDM-5} and extended spectrum β-lactamase ($bla_{CTX-M-15}$) plus other resistance genes in the same plasmid could increase the dissemination of multiple resistances in a single gene transfer event.

Furthermore, the two bla_{NDM-5} producing *E. coli* from neonates had similar resistance gene determinants, virulence genes and ST167, indicating that the isolates were likely to be clonal. Chromosomal DNA SNP analysis of the two isolates also revealed a pairwise distance of two SNPs. This finding indicates that these two isolates were closely related and the source of spread was likely to be the same. It implies there was probably local transmission of bla_{NDM-5} producing *E. coli*. *E. coli* ST167 carrying bla_{NDM-5} has been reported in South Africa in one inpatient with nosocomial infection [39]. Recently, *E. coli* ST167 has been regarded as a high-risk and successful epidemic clone involved in transmission of the bla_{NDM-5} gene [19, 43]. Furthermore, bla_{NDM-5} producing *E. coli* ST167 has the potential for global dissemination due its combination of resistance and virulence genes [43]. The bla_{NDM-5} producing *E. coli* ST167 has been reported in neonatal and adult infections in PR China [3, 16, 17, 47], the USA [48], Europe [18] and Africa [39]. Similar to our finding, an *E. coli* ST167 strain co-producing bla_{NDM-5}, $bla_{CTX-M-15}$ and bla_{OXA-1} has been reported in bacteria causing infection in PR China [3]. Phylogenetic analysis revealed the *E. coli* ST167 strains are clonally diverse and our study isolates were phylogenetically distant from global circulating *E. coli* ST167, (Fig. 1).

Our study revealed in all three *E. coli*, that the bla_{NDM-5} on each plasmid was flanked by highly a conserved region (ble_{MBL}–$trpF$–$DsbD$) downstream, indicating that the region is very probably transferring between replicons (plasmids) and between bacteria as a single unit. The upstream genetic environment of bla_{NDM5} in two plasmids were different. Previous in-depth analysis of the genetic environment of bla_{NDM-5} genes revealed that the bla_{NDM-5} is flanked upstream by IS*Aba125* and downstream by ble_{MBL}–$trpF$–$DsbD$ (IS*3000*–IS5–ΔIS*Aba125*–bla_{NDM-5}–ble_{MBL}–$trpT$–$DsbD$) [21, 49, 50]. In this study we found complete deletion of IS*3000*–IS5–ΔIS*Aba125* upstream

of bla_{NDM-5} in the IncFIA plasmid from the neonates. In the IncX3 plasmid, complete deletion of $\Delta ISAba125$ was observed and the IS*3000*–IS*5* element was interrupted by IS*30*.

CONCLUSION

This is the first detection of bla_{NDM-5} producing *E. coli* in Tanzania, to our knowledge. We found bla_{NDM-5} in *E. coli* ST167 located on an IncFIA plasmid colonizing the gut of two neonates and bla_{NDM-5} producing *E. coli* ST2083 located on an IncX3 plasmid colonizing the gut of an outpatient newly diagnosed with HIV. Our findings highlight the urgent need for a robust AMR surveillance system to monitor and rapidly report on the incidence and spread of emerging resistant bacteria in Tanzania. In addition, long-term infection prevention and control procedures and antimicrobial stewardship policies need to be introduced, optimized and maintained to curb the spread of resistant bacteria within healthcare environments.

Author contributions
B. B., N. L. and S. J. M., conceived the study. J. M., collected study data. J. M., S. M. and U. K., performed the microbiological investigations. S. J. M., R. N. G., E. A., A. T. M. H. and A. P. R., carried out further experimentation and bioinformatics analysis. J. M., drafted the manuscript. S. J. M., B. B., A. T. M. H., N. L. and A. P. R., revised the manuscript. All authors approved the final version.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Approvals to conduct this study were obtained from Muhimbili University of Heath and Allied Sciences Senate Research and Publications Committee (reference number 2015-10-27/AEC/Vol.X/54), National Health Research Ethics Committee (reference number. NIMRlHQ/R. SaJVol. 1X12144), Tanzania Medicines and Medical Devices Authority (reference number TZ16CT007) in Tanzania and Regional Committee for Medical and Health Research Ethics of Western Norway (Ref. No. REK2015/540). Written informed consent were obtained from the patients or parents/guardians or legally authorized persons for participation in the study.

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