Trans R Soc Trop Med Hyg 2021; **115**: 1080–1085
doi:10.1093/trstmh/traa173 Advance Access publication 27 January 2021

ORIGINAL ARTICLE

Molecular characterisation of the first New Delhi metallo-β-lactamase 1-producing *Acinetobacter baumannii* from Tanzania

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Received 3 September 2020; revised 1 December 2020; editorial decision 8 December 2020; accepted 22 December 2020

Background: We aimed to characterise the genetic determinants and context of two meropenem-resistant clinical isolates of *Acinetobacter baumannii* isolated from children hospitalised with bloodstream infections in Dar es Salaam, Tanzania.

Methods: Antimicrobial susceptibility was determined by disc diffusion E-test and broth microdilution. Genomes were completed using a hybrid assembly of Illumina and Oxford Nanopore Technologies sequencing reads and characterisation of the genetic context of resistance genes, multi-locus sequence types (STs) and phylogenetic analysis was determined bioinformatically.

Results: Twelve *A. baumannii* were isolated from 2226 blood cultures, two of which were meropenem-resistant. The two meropenem-resistant isolates, belonging to distinct STs, ST374 and ST239, were found to harbour bla_{NDM-1}, which was chromosomally located in isolate DT0544 and plasmid-located in isolate DT01139. The genetic environment of bla_{NDM-1} shows the association of insertion sequence ISAba*125* with bla_{NDM-1} in both isolates. Both isolates also harboured genes conferring resistance to other β-lactams, aminoglycosides and cotrimoxazole.

Conclusions: This is the first report of New Delhi metallo-β-lactamase-producing isolates of *A. baumannii* from Tanzania. The genetic context of bla_{NDM-1} provides further evidence of the importance of ISAba*125* in the spread of bla_{NDM-1} in *A. baumannii*. Local surveillance should be strengthened to keep clinicians updated on the incidence of these and other multidrug-resistant and difficult-to-treat bacteria.

Keywords: *Acinetobacter baumannii*, antimicrobial resistance mechanisms, bloodstream infections, New Delhi metallo-β-lactamase 1, Tanzania

Introduction

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen that can cause infections of multiple body sites, including the bloodstream, lungs and urinary tract.[1–3] *Acinetobacter baumannii* infections are often difficult to treat because of intrinsic and acquired resistance mechanisms and are associated with poor clinical outcomes.[2] Carbapenems are indispensable last-resort antibiotics for severe infections caused by multidrug-resistant bacteria, although they are expensive and largely unavailable in low-income settings. The clinically important β-lactamase New Delhi metallo-β-lactamase 1 (NDM-1), which confers resistance to carbapenems, was first reported in *A. baumannii* in India[4] and NDM-1-producing *A. baumannii* have since been reported from northern and eastern Africa (Algeria, Libya, Egypt, Tunisia, Kenya and Ethiopia) and South Africa.[5–11] To the best of our knowledge, NDM-1-producing *A. baumannii* has not yet been reported in Tanzania. As bla_{NDM-1}-carrying bacteria are often multidrug-resistant, infections due to NDM-1-producing *A. baumannii* may increase the risk of poor clinical outcomes due to a lack of therapeutic options. Therefore,

there is a need to report the detection, spread and molecular epidemiology of multi-drug resistant *A. baumannii*-producing NDM-1 in resource-limited settings.

In a large-scale study to determine the causes of bloodstream infections in children in Dar es Salaam, Tanzania,[12] we detected two carbapenem-resistant isolates of *A. baumannii* in blood cultures from febrile Tanzanian children. This study was conducted to determine the mechanisms responsible for carbapenem resistance. Using whole genome sequencing (WGS) we predicted the resistance genes present in the two *A. baumannii* isolates and compared them with the corresponding phenotypic resistance. Furthermore, we characterised the genetic context of $bla_{\text{NDM-1}}$, determined the sequence types (STs) of both isolates and placed them within the phylogenetic context of other *A. baumannii*-carrying $bla_{\text{NDM-1}}$ previously sequenced.

Materials and methods

Study population, bacteria isolation and identification

A cross-sectional study was conducted from March 2017 to July 2018.[12] We obtained blood cultures from 2226 children aged <5 y hospitalised because of fever at Amana, Temeke and Mwananyamala Regional hospitals and Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania. Blood was cultured using BACTEC FX40 system (Becton-Dickinson, Sparks, MD, USA) and the bacteria isolated were identified by Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometry (MS), using the Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by disk diffusion on Mueller-Hinton agar plates at 35°C and incubated for 16–18 h according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] Antibiotic discs included were piperacillin/tazobactam (TZP), ceftazidime, cefotaxime, meropenem, imipenem, ciprofloxacin, sulphamethoxazole/trimethoprim, gentamicin tetracycline and doxycycline (Oxoid, UK). The minimum inhibitory concentrations (MICs) for TZP, ceftazidime, cefotaxime, meropenem, imipenem, ciprofloxacin, sulphamethoxazole/trimethoprim, gentamicin tetracycline and doxycycline were determined by E-test (bioMérieux, Marcy-I´Etoile, France) following CLSI guidelines.

The MIC of colistin was determined by broth microdilution in cation-adjusted Mueller-Hinton broth according to CLSI guidelines.

WGS and analysis

WGS was performed using HiSeq X10 (Illumina, San Diego, CA, USA) by Microbes NG (UK), who also performed quality filtering and sequencing read trimming and MinION (Oxford Nanopore Technologies [ONT], Oxford, UK) platforms. ONT long reads were de-multiplexed with Porechop (v. 0.2.4; https://github.com/rrwick/Porechop) and filtered with a quality score of 30 using Filtlong (v. 0.2.0; https://github.com/rrwick/Filtlong). Long and short read sequences were assembled using Unicycler (v. 0.4.8.0)[14,15] and the genome was annotated with Prokka (v. 1.14.6).[16]

The $bla_{\text{NDM-1}}$-carrying plasmid from isolate DT01139 and upstream and downstream of $bla_{\text{NDM-1}}$ in the chromosome of isolate DT0544 were annotated manually using a combination of Prokka (v. 1.14.6)[16] BLAST (v. 2.11.0),[17] ResFinder (v. 4.1),[18] UniProt and MobileElementFinder (v. 1.0.3)[18] in SnapGene (v. 3.3.4) from GSL Biotech (available at snapgene.com). Comparison of the annotated plasmid from DT01139 with other $bla_{\text{NDM-1}}$-carrying plasmids from *A. baumannii* was performed using BRIG (v. 0.95).[19] Comparison of upstream and downstream of $bla_{\text{NDM-1}}$ for the isolate DT01139 and DT0544 was produced using EasyFig (v. 2.2.2).[20]

Identification of resistance genes and multi-locus sequence typing

Prediction of antimicrobial resistance genes and multi-locus sequence typing (MLST) were carried out using ResFinder (v. 4.1)[21,22] and MLST (v. 2.19.0; https://github.com/tseemann/mlst), which uses the PubMLST database (https://pubmlst.org).[23]

Phylogenetic analysis

A single nucleotide polymorphism (SNP)-based phylogenetic tree was created using conserved signature inserts phylogeny server (v. 1.4),[24] comparing the two isolates in this study to 27 published $bla_{\text{NDM-1}}$-carrying *A. baumannii* WGS using default parameters. *Acinetobacter baumannii* ab736 [accession number NZ_CP015121] was used as the reference genome for the phylogenetic tree. The phylogenetic tree was annotated using the Interactive Tree of Life (v. 5.6.3).[25]

Results

Characteristics of the two patients with *A. baumannii*-carrying NDM-1 gene

In total, 12 *A. baumannii* isolates were identified, two of which were found to be meropenem-resistant by antimicrobial susceptibility testing and were designated as DT0544 and DT01139. The two meropenem-resistant *A. baumannii* isolates were obtained from blood cultures of neonates. Isolate DT0544 was obtained from a 4-d-old male neonate, admitted as a referral patient from a regional hospital to MNH in October 2017 with a history of fever and convulsions. This patient received ceftriaxone and gentamicin on admission but died the next day. Isolate DT01139 was obtained from a 3-d-old female neonate, admitted from a healthcare centre to Amana Regional Hospital in November 2017 with a history of fever. This patient received amoxicillin-clavulanate and gentamicin on admission, but due to her worsening condition the treatment changed to ceftriaxone and gentamicin. After 7 d she was transferred to another hospital and was lost to follow-up.

Antimicrobial susceptibility testing results

Susceptibility testing results identified the two *A. baumannii* isolates were resistant to imipenem and meropenem as well as numerous other antibiotics, including those prescribed to the

Table 1. Antimicrobial susceptibility results and acquired resistance genes of the two *A. baumannii*

		Disc diffusion		MIC (E-test) μg/ml		Acquired resistance genes	
Antimicrobial class	Antimicrobial agent	DT0544	DT01139	DT0544	DT01139	DT0544	DT01139
Fluoroquinolone	Ciprofloxacin	S (23 mm)	S (27 mm)	0.094	0.064	none	none
β-lactams	Piperacillin/tazobactam	R (13 mm)	R (17 mm)	>256	128	$bla_{CARB-25}$, $bla_{CARB-16}$, $bla_{OXA-259}$ and bla_{NDM-1}	$bla_{CARB-25}$, $bla_{CARB-16}$, bla_{OXA-51} and bla_{NDM-1}
	Ceftazidime	R (0 mm)	R (0 mm)	>256	>256		
	Cefotaxime	R (0 mm)	R (0 mm)	32	32		
	Meropenem	R (14 mm)	R (16mm)	4	4		
	Imipenem	R (11 mm)	R (16 mm)	32	8		
Folate antagonist	Sulphamethoxazole/trimethoprim	R (0 mm)	R (0 mm)	>256	4	*sul2* and *dfrA1*	*sul2*
Tetracyclines	Tetracycline	S (19 mm)	S (21 mm)	4	4	none	none
	Doxycycline	S (21 mm)	S (24 mm)	0.5	0.5		
Aminoglycosides	Gentamicin	R (0 mm)	R (0 mm)	24	128	*aadA1*, *aph (3″)-Ia*, and *ant (2″)-Ia*	*aac (3)-Iid* and *aph (3′) VI*
Polymyxin	Colistin	NA	NA	16*	16*	none	none

Note: NA, test not applicable for that antimicrobial agent; * MIC tested by broth microdilution.

patients, and were susceptible to ciprofloxacin and tetracyclines (Table 1). Both isolates were resistant to imipenem with a MIC of 32 μg/ml (DT0544) and 8 μg/ml (DT01139). Resistance to gentamicin and colistin was also identified in two isolates with a MIC towards gentamicin of 128 and 24 μg/ml for DT01139 and DT054, respectively, while both isolates had a MIC of 16 μg/ml towards colistin.

WGS results

Isolate DT0544 contained two plasmids of approximately 55 and 4 Kb in size, while isolate DT01139 contained three plasmids of 97, 64 and 10 Kb in size. bla_{NDM-1} was predicted to be present in both isolates; the β-lactamase was chromosomally located in isolate DT0544 while for DT01139 it was plasmid-located (Figure 1A,1B). The β-lactamases bla_{ADC-25} and $bla_{CARB-16}$ were also present in both isolates, while $bla_{OXA-259}$, belonging to bla_{OXA-51} type, was present in DT0544; and DT01139 contained bla_{OXA-51}. Several other resistance genes were predicted in the genome of DT0544: *aadA1 aph (3″)-Ia* and *ant (2″)-Ia* (aminoglycosides), *sul2* (sulphonamides) and *dfrA1* (trimethoprim), all located on a 55 Kb plasmid, while DT01139 was predicted to contain *aac (3)-IId* and *aph (3′) VI* (aminoglycosides), *sul2* (sulphonamides) and *floR* (phenicol), all located on the 64 Kb plasmid with bla_{NDM-1}. Predicted resistance genes by WGS were supported by and corresponded to phenotypic susceptibility. However, it is worth noting that no acquired *mcr* gene conferring resistance to colistin was detected.

Genetic environment of bla_{NDM-1} gene

bla_{NDM-1} is located on a composite transposon, Tn*125*, in DT0544 flanked by two copies of the insertion sequence (IS) ISAba*125* orientated in the same direction (Figure 1A). However, in DT01139, only one copy of ISAba*125* is present upstream and the approximately 20 kb region containing bla_{NDM-1} and other resistance genes (for aminoglycosides, sulphonamides and phenicol) is flanked by two copies of ISAba*14* (Figure 1A). Figure 1B shows comparison of the bla_{NDM-1}-carrying plasmid on isolate DT01139 with other reported NDM-1-carrying plasmids (pAB17, pAbNDM-1, pAR_0088, pIEC383, pM131 and pNDM-GJ0; see Supplementary Table 1 for accession numbers) of *A. baumannii*. The bla_{NDM-1}-harbouring plasmid from the isolate DT01139 differs from the other plasmids compared, but has shown some areas of similarity upstream and downstream of bla_{NDM-1}.

STs and phylogenetic analysis

Using the Pasteur MLST scheme, the two isolates were found to belong to two distinct STs, ST374 (DT0544) and ST 239 (DT01139). Figure 1C is a whole genome SNP-based phylogenetic tree containing the two isolates from this study and 27 other *A. baumannii* containing bla_{NDM-1} (see Supplementary Table 1 for accession numbers). We found a clonal diversity among NDM-1-producing *A. baumannii* isolates from different parts of the world and isolate DT0544 from this study was closely related to strain R2090 from Egypt.

Discussion

While NDM-1-producing *A. baumannii* has been reported from other sub-Saharan African countries (e.g. Kenya,[8] Ethiopia[9] and South Africa),[10] this is the first time it has been reported from Tanzania. Contrary to reports from neighbouring countries

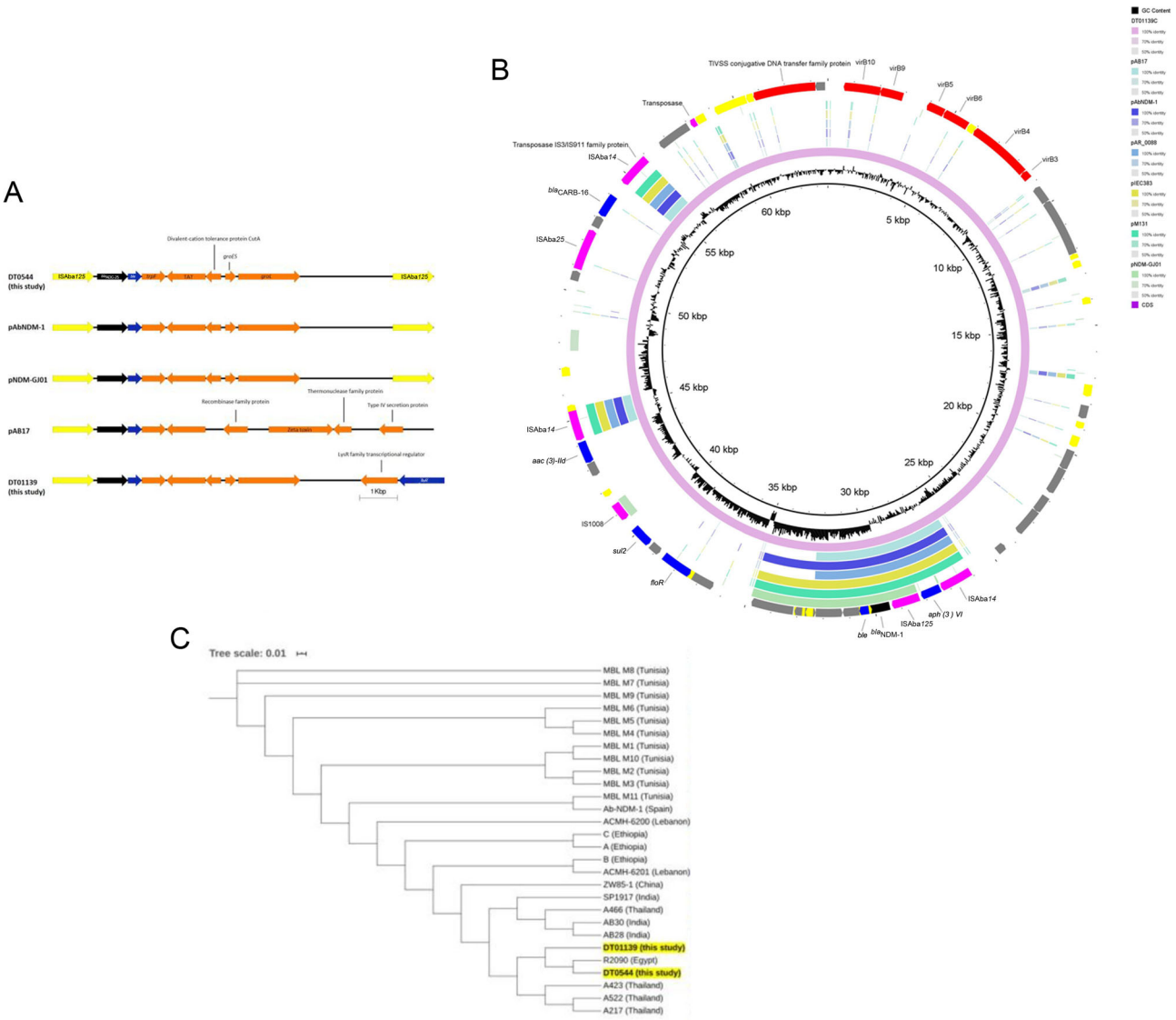

Figure 1. The genetic environment of the bla_{NDM-1} and phylogenetic context of DT0544 and DT01139. (A) Genetic context of bla_{NDM-1} from DT01139 and DT0544 compared with bla_{NDM-1} from *A. baumannii* with a similar genetic arrangement. Arrows represent insertion sequences (yellow), antimicrobial resistance genes (blue), bla_{NDM-1} (black) and other resistant genes (orange). (B) Annotation of the 97 Kb plasmid-containing bla_{NDM-1} from DT01139 comparison with other bla_{NDM-1}-containing plasmids from *A. baumannii*. Arrows represent hypothetical proteins (grey), insertion sequences and transposable elements (purple), antimicrobial resistance genes (blue), bla_{NDM-1} (black), type IV secretion system genes (green) and other genes (red). (C) Whole genome single nucleotide polymorphism (SNP)-based phylogenetic tree compared with other bla_{NDM-1} *A. baumannii*. Country of isolation is in brackets and isolates for this study, DT01139 and DT0544, are highlighted in yellow.

where NDM-1-producing *A. baumannii* originated from samples obtained from axilla, abscesses, peritoneal swabs and the urinary tract,[8–10] our isolates originated from bloodstream infections, emphasising their clinical importance. These findings are of significant public health importance as they show that there is ongoing dissemination of NDM-1-type resistance in an African setting.

The fact that the two neonates, only 3 and 4 d old, were transferred from a health centre and a regional hospital, respectively, and that the bla_{NDM-1}-producing *A. baumannii* isolates were obtained from blood cultures taken on admission at the study hospital, raises concern that they may have acquired these multidrug-resistant bacteria locally at health facilities serving local communities.

The two isolates were susceptible only to ciprofloxacin and tetracyclines, but resistant to all other antibiotics tested. Evidence-based guidelines are vital for the acute management of severe systemic infections before microbiological results are ready. In sub-Saharan Africa, empirical treatment guidelines are even more important, as microbiological laboratory services are impeded by limited infrastructure capacity and funding to perform routine blood cultures and antimicrobial susceptibility

testing. Only major referral hospitals have the capacity to identify specific multidrug-resistant problematic bacteria such as carbapenemase-producing Gram-negatives. The empiric treatment protocol that was used to treat the two patients did not include the antibiotics to which the isolates were sensitive, hence the patients did not receive appropriate treatment, and we know at least one of them died. This highlights the importance of introducing and strengthening antimicrobial resistance surveillance programmes.

Differing antibiograms, resistance gene profiles and STs show the isolates are not clonal. Furthermore, bla_{NDM-1} was carried on a chromosomally located composite transposon Tn*125* in one isolate and on a plasmid with only one ISAba*125* in the other and therefore are not representative of an outbreak.

The two neonates and their parents had no history of travelling outside the country and the travel history of their healthcare providers is unknown. Therefore, to understand in depth the origins and extent of NDM-1-producing *A. baumannii* in the region there is a need for a comprehensive surveillance programme within the healthcare system in the country.

This study has shown further evidence of diversity among the NDM-1-producing *A. baumannii* in different parts of the world, for example, the two isolates belong to ST374 and ST239 (Pasteur MLST), while in the neighbouring countries Kenya and Ethiopia, NDM-1-producing *A. baumannii* belong to ST25 and ST957, respectively, and from Tunisia in northern Africa they belong to ST85.[7–9] In European countries (Switzerland, Slovenia, Germany, France and Belgium), the NDM-1-producing isolates of *A. baumannii* were reported to belong to ST1, ST25, ST85 and ST92.[5,26] While ST1, ST25 and ST85 are now widely reported throughout the globe, the two STs (374 and 239) in our study have been rarely reported. Non-NDM-1-producing *A. baumannii* ST374 isolates have previously been isolated from a wound swab in Kilimanjaro, the northern region of Tanzania (strain KCRI-49 with [accession number GCA_900406775.1]) and from respiratory tract infection in Brazil (strain Ac56 with [accession number WP1Q00000000]). There is one previously reported ST239 *A. baumannii* isolate, H33 from Japan (https://pubmlst.org/bigsdb?page=info&db=pubmlst_abaumannii_isolates&id=1695) and other ST239 have been isolated from pets in France.[27] In addition to a wide variety of STs among NDM-1-producing *A. baumannii*, the phylogenetic analysis (Figure 1C) also showed that the strains of *A. baumannii*-producing NDM-1 are not clonally related between different countries and within one country (e.g. two strains in the current study and three strains from Ethiopia and Thailand). This shows that the spread of NDM-1-producing *A. baumannii* in Africa is not clonal and likely results from the spread of the NDM-1 gene itself, as reported in Europe.[26] A similar situation has been recently reported with NDM-1 in *Klebsiella pneumoniae*.[28]

The genetic environment of bla_{NDM-1} has previously been reported to be on the composite transposon Tn*125*, flanked by ISAba*125*.[29,30] In *A. baumannii* DT0544, Tn*125* harbouring blaNDM-1 is 100% identical to that found in *A. baumannii* VB473 [accession number CP050388] isolated from human sputum in India and is up to 99% identical to many other copies of Tn*125* from various *Acinetobacter* spp.[30] and other bacteria, including *Escherichia coli* and *K. pneumoniae*. The similar genetic organisation of bla_{NDM-1} on a Tn*125* in most *A. baumannii* and other *Acinetobacter* spp. is due to the Tn*125*-linked mobility of bla_{NDM-1}.[31] In *A. baumannii*, DT01139 bla_{NDM-1} is associated with an upstream copy of ISAba*125* in a more complex, plasmid-located arrangement flanked by ISAba*14* (Figure 1B).

This is the first report of NDM-1-producing *A. baumannii* isolated from neonates with bloodstream infections from Tanzania. The genetic context of bla_{NDM-1} provides further evidence of the importance of ISAba*125* in the spread of bla_{NDM-1} in *A. baumannii*. These findings shed light on the epidemiology of carbapenem resistance in Africa and calls for continued and strengthened surveillance to guide clinicians treating severe bacterial infections.

Supplementary data

Supplementary data are available at *Transactions* online.

Authors' contributions: SJM, NL and BB conceived the study. SJM, JM and NSM were involved in data collection. SJM and JM performed the microbiological investigations. SJM, ATMH, RB and APR were involved in WGS and analysis. SJM and APR drafted the manuscript. All the authors contributed to editing the manuscript and they approved the final version.

Acknowledgements: We would like to thank the technical staff at Muhimbili University of Health and Allied Sciences for technical assistance in performing blood cultures. We also thank Helene Heitmann Sandness from the Department of Clinical Science at the University of Bergen for her support in antimicrobial susceptibility testing.

Funding: This work was supported by the University of Bergen, Bergen, Norway. APR would like to acknowledge funding from the AMR Cross-Council Initiative through a grant from the Medical Research Council, a Council of UK Research and Innovation [grant number MR/S004793/1] the Medical Research Council funded LSTM-Lancaster Doctoral Training Partnership [grant no. MR/N013514/1] for supporting RLB and the National Institute for Health Research [grant number NIHR200632].

Competing interests: APR is a policy advisor (drug resistance) for the RSTMH. All other authors have no conflicts of interest to disclose.

Ethical approval: This study was approved by the Senate Research and Publications Committee of Muhimbili University of Health and Allied Sciences, National Health Research Ethics Committee and by the Regional Committee for Medical and Health Research Ethics in western Norway. Written informed consent was obtained from the parents or guardians on behalf of the children.

Data availability: The chromosomal and plasmid sequences of DT0544 and DT01139 were submitted to GenBank with [accession numbers PRJNA679703 and PRJNA679704], respectively.

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