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ORIGINAL ARTICLE

Molecular epidemiology of virulence and antimicrobial resistance determinants in *Klebsiella pneumoniae* from hospitalised patients in Kilimanjaro, Tanzania

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Abstract

This study aimed to use whole-genome sequencing to determine virulence and antimicrobial resistance genes in *K. pneumoniae* isolated from patients in a tertiary care hospital in Kilimanjaro. *K. pneumoniae* isolates from patients attending Kilimanjaro Christian Medical Centre between August 2013 and August 2015 were fully genome-sequenced and analysed locally. Sequence analysis was done for identification of virulence and AMR genes. Plasmid and multi-locus sequence typing and capsular or capsular (K) typing were performed and phylogeny was done to ascertain *K. pneumoniae* relatedness. Stata 13 (College Station, TX, 77845, USA) was used to determine Cohen's kappa coefficient of agreement between the phenotypically tested and sequence-predicted resistance. A total of 16 (47.1%) sequence types (STs) and 10 (29.4%) K types were identified in 30 (88.2%) and 17 (50.0%) of all analysed isolates, respectively. *K. pneumoniae* ST17 were 6 (17.6%). The commonest determinants were $bla_{CTX\text{-}M\text{-}15}$ in 16 (47.1%) isolates, bla_{SHV} in 30 (88.2%), $bla_{OXA\text{-}1}$ in 8 (23.5%) and $bla_{TEM\text{-}1}$ in 18 (52.9%) isolates. Resistance genes for aminoglycosides were detected in 21 (61.8%) isolates, fluoroquinolones in 13 (38.2%) and quinolones 34 (100%). Ceftazidime and ceftriaxone showed the strongest agreement between phenotype- and sequence-based resistance results: 93.8%, kappa = 0.87 and $p = 0.0002$. Yersiniabactin determinant was detected in 12 (35.3%) of *K. pneumoniae*. The proportion of AMR and virulence determinants detected in *K. pneumoniae* is alarming. WGS-based diagnostic approach has showed promising potentials in clinical microbiology, hospital outbreak source tracing virulence and AMR detection at KCMC.

Keywords *K. pneumoniae* · Whole-genome sequencing · Virulence · Antimicrobial resistance · Tanzania

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Introduction

Klebsiella pneumoniae is a major cause of hospital acquired infections including pneumonia, bloodstream and new-borns infection [1–4]. Treating infections caused by *K. pneumoniae* and other *Enterobacteriaceae* has been challenging worldwide due to emergence and rapid spread of resistant strains [5–9]. Initiatives towards monitoring of AMR should nonetheless go hand-to-hand with identification strategies of hypervirulent *K. pneumoniae* (hvKP) strains. Because roles played by virulence factors (VFs) in hvKP like yersiniabactin [10], colibactin [11], aerobactin and salmochelin [12] in enhancing severity of infections and increase their survival is significant. The global dissemination of hvKP clones [13–15] do pose a serious public health threat, thus underscoring a necessity to characterise VFs *in K. pneumoniae*. Treatment failures and possibly many deaths could now be attributed to *K. pneumoniae* infections [16–18]. Reports have generally described that treating bacterial infections in low- and middle-income countries (LMICs) is adding more burden on the already disease-burdened communities [19–21] both psychosocially and economically. In some countries, it is believed that even carbapenems will no longer treat more than a half of *K. pneumoniae* infections [22]. Having a bacterial infection will soon mean death if measures against irrational prescription and misuse of antibiotics are not taken seriously [23–25]. Antibiotics have been over prescribed and over-consumed especially in LMICs [26, 27]. These practices have been implicated to be fuelling up drug resistance selection pressure [28, 29].

In LMICs like Tanzania, lack of efficient clinical laboratory diagnostic systems is one of the factors often leading to empirical treatment. We previously reported the huge potential for whole-genome sequencing (WGS) to improve clinical diagnostics and infection control at a tertiary hospital in Tanzania where clinical laboratories lack access to molecular-based methodologies for regular typing of bacterial isolates [30, 31]. In this report, we used WGS to determine molecular relatedness, antimicrobial resistance genes, virulence genes and plasmids diversity in *K. pneumoniae* isolates from patients at KCMC, which is a tertiary care hospital in Kilimanjaro, Tanzania.

Materials and methods

Study design, participants and specimen collection

A hospital-based prospective cross-sectional study was conducted at KCMC hospital from 2013 to 2015. Part of the study's methods has been described in details by Kumburu et al. [32]. Geographically, KCMC is located in Moshi municipality in Kilimanjaro and it is one of the biggest referral hospitals in Tanzania. It serves as a zonal referral hospital for a catchment area of around 15 million people. The hospital has a bed capacity of 650 with approximately 500 outpatients seeking medical services daily. This study was granted ethical approval by the KCMUCo Research Ethics Committee and the National Institute for Medical Research in Tanzania. A written informed consent was obtained from each participant or from parents or guardians of children before enrolment into the study. A convenient sampling method was used to recruit the study participants. It included participants suspected to have bacterial infection and admitted in medical and surgical wards. Specimens collected for bacterial culture included sputum, wound or pus swab and stool samples**.** Bacteria culture, isolation and identification were performed following in-house standard operating procedures as well as the Clinical and Laboratory Standards Institute (CLSI) guidelines. Sequentially, all *K. pneumoniae* isolates recovered over the study period were included for whole-genome sequencing and analysis. Over a 2-year period, 590 samples were collected without apriori knowledge of the infecting agent. A total of 377 bacterial strains were isolated, and whole genome sequenced. A number of isolates from this collection were randomly selected for antimicrobial susceptibility testing. A total of 34 *K. pneumoniae* collected sequentially were included in this study; amongst which, 16 *K. pneumoniae* isolates had phenotype-based antimicrobial susceptibility results.

Genomic DNA isolation, whole genome sequencing and analysis

For all *K. pneumoniae* isolates, genomic DNA (gDNA) was purified and its concentration was determined using the Easy-DNA Extraction Kit (Invitrogen®) and the Qubit dsDNA Assay Kit (Invitrogen®), respectively. The gDNA library preparation was performed following Nextera® XT DNA Sample Preparation Guide [33]. In brief, each gDNA was tagmented (tagged and fragmented) by the Nextera® XT transposome. The transposome simultaneously fragments the input DNA and adds adapter sequences to the fragment ends. Then, a limited-cycle PCR amplification followed, whereby indexes required for cluster formation were added to each DNA piece. Then, each gDNA library was normalised to ensure equal representation during sequencing. Equal volumes of the normalised library were combined, diluted in hybridization buffer and heat denatured prior to sequencing on the Illumina MiSeq platform (Illumina Inc.). The sequencer output was analysed using the standard WGS pipeline at KCRI, which is based on local implementations of the bioinformatics services available at https://cge.cbs.dtu.dk/services/. Quality control of the reads was performed using FastQC 0.11.4 [34]. De novo assembly

was performed with SPAdes 3.11.1 [35], and quality assessed using QUAST 4.5 [36]. For this article's purpose, the analyses included resistance gene identification using ResFinder 2.1 [37], multi-locus sequence typing (MLST) determination using MLST 1.8 [38], plasmid and plasmid MLST determination using PlasmidFinder 1.3 and pMLST 1.4 [39] and virulence gene determination using VirulenceFinder 1.4 [40]. Phylogeny reconstruction was done using CSI Phylogeny [41] (reference NTUH-K2044). The 34 assembled *K. pneumoniae* genomes of the present study have been submitted to the European Nucleotide Archive (ENA) with project accession number PRJEB26616. Stata 13 (College Station, TX, 77845, USA) was used to determine Cohen's kappa coefficient of agreement between the phenotype- and whole-genome sequence-based antimicrobial resistance results.

Results

Study participants and *Klebsiella* isolates

A total of 34 *K. pneumoniae* isolates were recovered: 9 (26.5%) in 2013, 17 (50.0%) in 2014 and 8 (23.5%) in 2015. Out of 34 *K. pneumoniae*, 25 (73.5%) isolates were from wound or pus swabs, 5 (14.7%) from sputum, 3 (8.8%) from stool and 1 (2.9%) from throat swab. Sixteen (47.1%) of *K. pneumoniae* were isolated from surgical wards, 3 (8.8%) from surgical ICU, 12 (35.3%) were isolated from patients admitted in medical wards, 1 (2.9%) from medical ICU and 2 (5.9%) were isolated from outpatients. A total of 11 (32.4%) *K. pneumoniae* were isolated from participants with infected wounds. The proportion of *K. pneumoniae* from participants with cough was 6 (17.6%), burn 6 (17.6%), wounds 6 (17.6%) and diabetes 6 (17.6%) (Table 1).

Table 1 Characteristics of participants from which *K. pneumoniae* were isolated

ID	Age (years)	Gender	Wards	Comorbidity	Specimen	Collection date
16	38	Male	Surgical ICU	Infected wound	Swab	2013
17	81	Female	Surgical	Infected wound	Swab	2013
29	44	Male	Surgical ICU	Infected wound	Swab	2013
38	41	Male	Surgical	Infected wound	Swab	2013
41	31	Male	Medical	Diabetes	Stool	2013
50	52	Female	Surgical	Burn wound	Swab	2013
51	53	Female	Surgical	Diabetes	Swab	2013
74	6	Male	Surgical	Burn wound	Swab	2013
79	74	Male	Surgical	Bedsore	Swab	2013
109	42	Female	Medical	Cough	Sputum	2014
110	54	Male	Medical	Diarrhoea	Stool	2014
131	53	Male	Surgical	Infected wound	Swab	2014
134	30	Male	Medical	Burn wound	Swab	2014
150	82	Male	Medical	Cellulitis	Stool	2015
152	70	Male	Medical	Bedsore	Swab	2014
198	70	Male	Surgical	Diabetes	Swab	2014
201	49	Male	Medical	Cough	Sputum	2014
248	56	Male	Medical	Infected wound	Swab	2014
284	54	Male	Medical ICU	Diabetes	Swab	2014
293	66	Female	Surgical	Diabetes	Swab	2014
302	80	Female	Medical	Cough	Sputum	2014
315	54	Female	Medical	Infected wound	Swab	2014
320	55	Female	Surgical	Burn wound	Swab	2014
343	23	Male	Surgical	Infected wound	Swab	2014
350	24	Female	Surgical	Burn wound	Swab	2014
365	47	Male	Surgical ICU	Tumour	Swab	2014
404	59	Male	Medical	Cough	Sputum	2015
566	71	Male	Surgical	Diabetes	Swab	2015
567	28	Male	Surgical	Infected wound	Swab	2015
576	36	Male	Surgical	Infected wound	Swab	2015
585	11	Female	Surgical	Burn wound	swab	2015
591	33	Female	Medical	Infected wound	Swab	2015
ADE[a]	40	Female	Other	Cough	Throat swab	2014
KLEB[a]	37	Female	Other	Cough	Sputum	2015

[a] Outpatients

MLST and capsular (K) typing

A total of 16 (47.1%) STs were identified in 30 (88.2%) of the analysed isolates whilst the remaining isolates could not be typed (unknown STs, 4 (11.8%)). A total of 6 (17.6%) were *K. pneumoniae* ST17, of which 4 were recovered from patients in surgical and 2 in medical wards. A total of 4 (10.8%) were *K. pneumoniae* ST392, of which 2 were recovered from patients in medical and 2 in surgical wards. Three (8.8%) were *K. pneumoniae* ST348 and all were recovered from patients in surgical wards. *K. pneumoniae* ST15, ST25, ST299 and ST1562 each was observed in 2 (5.4%) of the isolates (Table 2). A total of 10 (29.4%) different K types were identified including K2, K7, K10, K19, K23, K28, K34, K41, K60 and K80 whilst isolates with unknown K types were 17 (50.0%). All 4 *K. pneumoniae* ST392 were of unknown K types, whereas 5 out of 6 *K. pneumoniae* ST17 were unknown K types (Table 2).

Beta-lactam-resistance determinants

All isolates were carrying at least one beta-lactam-resistance determinant. The commonest detected determinants were $bla_{\text{CTX-M-15}}$ in 16 (47.1%) isolates, bla_{SHV} in 30 (88.2%), $bla_{\text{OXA-1}}$ in 8 (23.5%) and $bla_{\text{TEM-1}}$ in 18 (52.9%) isolates. A variable population of bla_{SHV} genes was found, whereby $bla_{\text{SHV-11}}$ was found in 15 (44.1%) and $bla_{\text{SHV-1}}$ in 7 (20.6%) isolates. Other infrequently bla_{SHV}-detected genes included $bla_{\text{SHV-12}}$, $bla_{\text{SHV-28}}$, $bla_{\text{SHV-61}}$, $bla_{\text{SHV-83}}$, $bla_{\text{SHV-99}}$ and $bla_{\text{SHV-133}}$. At least three beta-lactam- resistance genes were found in 15 (44.1%) isolates. Carriages of four beta-lactam-resistance genes, namely $bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-11}}$, $bla_{\text{OXA-1}}$ and $bla_{\text{TEM-1B}}$, were frequently observed in *K. pneumoniae* ST392 isolates (Table 2).

Aminoglycoside, fluoroquinolone and quinolone determinants

Almost all isolates were carrying at least one resistance determinant for aminoglycosides in 21 (61.8%) isolates, fluoroquinolones in 13 (38.2%) and quinolones 34 (100%). Fluoroquinolones gene, *aac(6')Ib-cr*, was identified in 13 (38.2%) isolates. Determinants for aminoglycoside resistance included *aph(3")-Ib and aph(6)-Id* in 16 (47.1%) and 17 (50.0%) isolates, respectively. Other infrequent determinants for aminoglycosides were *aac(3)-IIa*, *aac(3)-IId*, *aph(3')-Ia*, *aadA16*, *aadA1*, *aadA5*, *aadA24* and *aadB* (Table 3). The quinolone resistance determinants identified were *oqxAB* in all 34 (100%) and *qnrB* in 11 (32.4%) isolates. The variants for *qnrB* gene included *qnrB1*, *qnrB6*, *qnrB49* and *qnrB66*.

Fosfomycin, macrolide and phenicol

The determinants *FosA* and *mph(A)* for fosfomycin and macrolide resistance were identified in 34 (100%) and 4 (11.8%) isolates, respectively. Several gene families for phenicol resistance were detected: *catA2* in 10 (29.4%), *catA1* in 2 (5.9%), *catB4* in 8 (10.8%) and *cmlA1* in 2 (5.9%) isolates.

Rifampicin, sulphonamide tetracycline and trimethoprim

Seven (20.6%) isolates were carrying *arr-3*, a rifampicin resistance gene. Sulphonamide resistance determinants, *sul1* and *sul2*, were detected in 12 (35.3%) and in 22 (64.7%) isolates, respectively. Two tetracycline resistance genes detected were *tet(A)* in 7 (20.6%) and *tet(D)* in 4 (11.8%) isolates. Trimethoprim resistance genes were identified in 23 (67.6%) isolates. A total of 11 (32.4%) isolates were carrying *dfrA14* and 5 (13.5%) carrying *dfrA27*. Other infrequently observed trimethoprim resistance genes detected included *dfrA(1/5/7/15/17/25/30)* and *dfrG* (Table 3).

Comparison of phenotype- and whole-genome sequence-based antimicrobial resistance

Agreement between phenotype- and whole-genome sequence-based antimicrobial resistance was done for 16 out of 34 *K. pneumoniae* isolates (Table 4). On average, agreement across all antibiotics tested was 77.4%. Overall, the phenotypically determined resistance was higher than the whole-genome sequence-based resistance. Nevertheless, all antibiotics but ampicillin showed substantial (61–80%) or strong agreement (81–100%) between phenotype- and sequence-based resistance results. Ampicillin showed moderate agreement: 56.3%, kappa = 0.13 and $p = 0.1508$. The sequence-based analysis predicted that resistance to ampicillin was in 19 (55.9%) isolates, whereas phenotypic testing revealed 15 (93.8%) of isolates to be resistant. Ceftazidime and ceftriaxone showed the strongest agreement: 93.8%, kappa = 0.87 and $p = 0.0002$. Sequence-based analysis predicted resistance to both ceftazidime and ceftriaxone in 16 (47.1%) of isolates, whereas 6 (37.5%) of isolates were resistant phenotypically.

Virulence determinants in *K. pneumoniae*

Further, we analysed virulence determinants in all *K. pneumoniae* strains. Yersiniabactin was detected in a significant proportion, in 12 (35.3%) of isolates out of which, all ($n = 3$) *K. pneumoniae* ST348 were carrying yersiniabactin

Table 2 Beta-lactam-resistance determinants in *K. pneumoniae*

ID	MLST	K type[a]	$bla_{\text{CTX-M-15}}$	$bla_{\text{CTX-M-55}}$	bla_{LEN12}	bla_{LEN16}	bla_{LEN2}	$bla_{\text{OXA-1}}$	$bla_{\text{OXY-1-1}}$	$bla_{\text{OXY-2-8}}$	$bla_{\text{OXY-5-1}}$
248	ST 15	K60	+					+			
591DK	ST 15	K60	+					+			
17	ST 17	NF	+								
284D	ST 17	NF									
29	ST 17	NF	+								
320	ST 17	NF	+								
41	ST 17	NF			+						
566A	ST 17	K23									
109B	ST 20	K28									
110LF	ST 25	K2									
38	ST 25	K2	+								
350	ST 29	K19	+					+			
ADE	ST 37	NF									
74	ST 152	K41									
KLEB	ST 193	NF	+								
16	ST 231	NF	+					+			
79C	ST 297	K10	+								
567B	ST 299	K7									
576	ST 299	K7									
293	ST 348	NF									
50	ST 348	K23									
585	ST 348	K23	+								
131E	ST 392	NF	+					+			
134	ST 392	NF	+					+			
404C	ST 392	NF	+					+			
51 K	ST 392	NF	+					+			
150C	ST 491	K80									
198C	ST 1562	NF				+					
201B	ST 1562	NF				+					
315	ST 2042	NF									
152E	NF	NF					+				
302	NF	K19									
343C	NF	K34		+							
365	NF	K34	+								

ID	bla_{LEN2}	$bla_{\text{SHV-1}}$	$bla_{\text{SHV-11}}$	$bla_{\text{SHV-12}}$	$bla_{\text{SHV-83}}$	$bla_{\text{SHV-99}}$	$bla_{\text{TEM-1B}}$
248							
591DK							+
17			+				+
284D			+				
29			+				+
320			+				+
41							
566A			+				
109B					+		
110LF			+				
38			+				
350					+		+
ADE			+				
74		+					
KLEB							+
16		+					+
79C							+
567B		+					

Table 2 (continued)

576		+				
293			+			+
50				+		+
585			+			+
131E			+			+
134			+			+
404C			+			+
51 K			+			+
150C			+			
198C						
201B						
315					+	
152E	+					
302		+				+
343C		+				+
365		+				+

A plus (+) means beta-lactam resistance determinant was found, and an empty cell means no determinant was found

[a] K type was not found

genes. The ferric uptake operon system (*kfuABC*) was found in 10 (29.4%) of isolates. Different from the rest, isolate 315 (*K. pneumoniae* ST2042) was carrying genes coding for aerobactin, salmochelin and yersiniabactin but lacked the ferric uptake operon system (Table 5).

Plasmid multi-locus sequence typing

Plasmid analysis revealed a high diversity of incompatibility groups (Inc) or plasmid replicons (Table 6). The most frequent replicon was IncFIB(K) that was found in 30 (88.2%) *K. pneumoniae* isolates. Other IncFI members included IncFIB(pKPHS1), IncFIA(HI1), IncFIB(pECLA) and IncFIB(pENTAS01). Another prevalent replicon IncR was identified in 18 (52.9%) of *K. pneumoniae* isolates. The IncFII was another frequently detected replicons. In this group, IncFII(K) was found in 14 (41.2%), and IncFII in 7 (20.6%). Others were IncFII(pCRY), IncFII(pECLA) and IncFII(Y). Plasmid replicons that were infrequently identified include IncHI1B, IncHI2, IncHI2A and IncN3. A high diversity of plasmid multi-locus sequence types (pMLSTs) was identified (Table 6). IncF[F-:A16:B-] was detected in 5 (14.7%) of *K. pneumoniae* isolates, IncF[F-:A-:B10 detected in 4 (11.8%) of *K. pneumoniae* isolates and 9 (26.5%) and 5 (14.7%) of *K. pneumoniae* isolates had unknown pMLSTs belonging to IncF[Unknown ST] and IncHI1[Unknown ST], respectively. Other pMLSTs identified are shown in Table 6.

Phylogenetic analysis

For epidemiological tracking of nosocomial infections, SNP difference was calculated to show how closely or distantly related the isolates are. The observed minimum and maximum number of SNP difference between *K. pneumoniae* isolates were 57 and 42,893, respectively (Supplemental Table 1). The minimum SNP difference was observed between isolates 134 and 131E (both ST392), showing a possibility of nosocomial infections. The maximum SNP difference was observed between *K. pneumoniae* isolates ADE ST37 and 16 ST231, showing that it is unlikely these isolates closely related. The tree topology showed two isolates (79C ST 297 and 302 with undetermined ST) segregating very distinctly from others. The heatmap showed clear patterns of high beta-lactam, aminoglycoside and quinolone resistance gene proportions spreading almost universally across all isolates. Furthermore, an apparent pattern was observed indicating an inverse correlation between yersiniabactin genes across the isolates (Fig. 1).

Discussion

The present study used whole-genome sequence-based approach in characterising clinical *K. pneumoniae* isolated from

Table 3 Antibiotic resistance determinants other than beta-lactamase genes in *K. pneumoniae*

ID	MLST	K type[a]	AMG	FS	MC	PH	FQ	RI	SU	TE	TMP
248	ST15	K60	aph(3'')-Ib aac(3)-IIa aph(6)-Id aadA1	fosA	ere(A)	catB4 cmlA1	aac(6')-Ib-cr oqxA oqxB	ARR-3	sul1 sul2	tet(A)	dfrA14
591DK	ST15	K60	aph(3'')-Ib aph(6)-Id	fosA		catB4	aac(6')-Ib-cr oqxA oqxB		sul2		dfrA14
17	ST17	NF	aac(3)-IIa aph(3'')-Ib aph(6)-Id aadA16	fosA		catA2	aac(6')-Ib-cr oqxA oqxB qnrB6	ARR-3	sul1 sul2		dfrA27
284D	ST17	NF		fosA			oqxA oqxB		sul2	tet(D)	
29	ST17	NF	aac(3)-IIa aadA16 aph(6)-Id aph(3'')-Ib	fosA		catA2	aac(6')-Ib-cr oqxA oqxB qnrB6	ARR-3	sul1 sul2		dfrA27
320	ST17	NF	aac(3)-IId	fosA		catA2	oqxA oqxB		sul2		dfrA30
41	ST17	NF	ant(2'')-Ia aadA1	fosA		catA1	oqxA oqxB		sul1	tet(A)	dfrA1
566A	ST17	K23		fosA			oqxA oqxB				
109B	ST20	K28		fosA			oqxA oqxB				dfrG
110LF	ST25	K2	aph(6)-Id strA	fosA		catA1	oqxA oqxB		sul1 sul2	tet(D)	dfrA15
38	ST25	K2	aph(3'')-Ib aac(3)-IIa aph(6)-Id	fosA		catA2	oqxA oqxB qnrB2		sul1 sul2	tet(D)	dfrA25
350	ST29	K19	aac(3)-IIa aph(3'')-Ib aph(6)-Id	fosA		catB4	qnrB1 aac(6')-Ib-cr oqxA oqxB		sul2	tet(A)	dfrA14
ADE	ST37	NF		fosA			oqxA oqxB				
74	ST152	K41		fosA			oqxA oqxB				
KLEB	ST193	NF	aac(3)-IId	fosA		catA2	oqxA oqxB		sul1 sul2		dfrA7 dfrA30
16	ST231	NF	aph(3')-Ia aph(3'')-Ib aph(6)-Id	fosA		catB4 catA2	aac(6')-Ib-cr oqxA oqxB qnrS1		sul2		dfrA14
79C	ST297	K10	aac(3)-IId	fosA		catA2	oqxA oqxB		sul2		dfrA30
567B	ST299	K7		fosA			oqxA oqxB				
576	ST299	K7		fosA			oqxA oqxB				
293	ST348	NF	aph(3')-Ia aph(3'')-Ib aph(6)-Id	fosA	mph(A)		oqxA oqxB		sul2		dfrA14
50	ST348	K23	aadA16 aph(3')-Ia aph(3'')-Ib aph(6)-Id	fosA	mph(A)		aac(6')-Ib-cr qnrB1 oqxA oqxB	ARR-3	sul1 sul2		dfrA14 dfrA27
585	ST348	K23	aac(3)-IIa aadA1 aph(3')-Ia aph(3'')-Ib aph(6)-Id	fosA	ere(A) mph(A)	catA2 cmlA1	qnrB1 oqxA oqxB	ARR-3	sul1 sul2		dfrA14
131E	ST392	NF	aph(6)-Id aph(3'')-Ib	fosA		catB4	aac(6')-Ib-cr oqxA oqxB		sul2		dfrA14
134	ST392	NF	aac(3)-IIa aph(6)-Id aph(3'')-Ib	fosA		catB4	aac(6')-Ib-cr oqxA oqxB qnrB1		sul2	tet(A)	dfrA14
404C	ST392	NF	aac(3)-IIa aph(3'')-Ib aph(6)-Id	fosA		catB4	aac(6')-Ib-cr oqxA oqxB qnrB1		sul2	tet(A)	dfrA14
51 K	ST392	NF	aac(3)-IIa aph(3'')-Ib aph(6)-Id	fosA		catB4	aac(6')-Ib-cr oqxA oqxB qnrB1		sul2	tet(A)	dfrA14
150C	ST491	K80		fosA			oqxA oqxB				
198C	ST1562	NF		fosA			oqxA oqxB				
201B	ST1562	NF		fosA			oqxA oqxB				
315	ST2042	NF		fosA			oqxA oqxB				

Table 3 (continued)

ID	MLST	K type[a]	AMG	FS	MC	PH	FQ	RI	SU	TE	TMP
152E	NF	NF		*fosA*			*oqxA oqxB*				
302	NF	K19		*fosA*			*oqxA oqxB*		*sul1*	*tet(D)*	*dfrA5*
343C	NF	K34	*aph(6)-Id aac(3)-IId aadA5 aadA16 aph(3")-Ib*	*fosA*	*mph(A)*	*catA2*	*qnrB6 oqxA oqxB aac(6')-Ib-cr*	*ARR-3*	*sul1 sul2*	*tet(A)*	*dfrA17 dfrA27*
365	NF	K34	*aadA16 aph(6)-Id aph(3")-Ib*	*fosA*		*catA2*	*aac(6')-Ib-cr oqxA oqxB qnrB6*	*ARR-3*	*sul1 sul2*		*dfrA27*

AMG aminoglycoside, *FQ* fluoroquinolone and quinolone, *FS* fosfomycin, *MC* macrolide, *PH* phenicol, *RI* rifampicin, *SU* sulphonamide, *TE* tetracycline, *TMP* trimethoprim

[a] K type was not found

hospitalised patients at KCMC hospital in Moshi, Tanzania. All isolates were analysed to determine (1) *K. pneumoniae* subtypes and molecular relatedness for establishing existence of nosocomial transmissions or outbreaks, (2) virulence and antibiotic resistance determinants and (3) types of plasmids. The present study reveals high diversity of *K. pneumoniae* in the hospital. The observed *K. pneumoniae* diversity is plausibly attributed to the fact that specimens were collected from a diverse population as this is a consultant hospital that is serving the northern, eastern and central zones of Tanzania. Nonetheless through MLST, the majority of *K. pneumoniae* that were clonally related were actually isolates from patients admitted to the same wards. For instance, *K. pneumoniae* ST17 with number 17, 29 and 320 were from surgical wards. Also, instance *K. pneumoniae* ST17 with number 41 and 284 were from medical wards. Although few numbers of strains were identified within distinct ST groups (clusters), this may be an indication of nosocomial transmissions or outbreaks within the hospital. Similar to the present report, polyclonal existence of *K. pneumoniae* with predominance of *K. pneumoniae* ST17 in hospital settings was reported in the Netherlands by Souverein et al. [42]. Identification of *K. pneumoniae* clones particularly ST17 and ST348 within surgical wards in the present study compares with the findings in Norway [43] and Mwanza, Tanzania [6]. In both reports, it was shown that *K. pneumoniae* ST17 and ST348 strains were the likely causes of neonatal sepsis and outbreaks in neonatal ICU. Given the superiority of WGS over classical approaches in microbial identification, typing and tracing of outbreak sources [44–46], the possibility that there were sporadic nosocomial transmissions of *K. pneumoniae* in this hospital becomes highly likely.

Our data further suggests that *K. pneumoniae* circulating in the hospital are carrying high proportions of antimicrobial resistance determinants. These findings are in line with findings of study done in Kenya on *K. pneumoniae* isolates from stool [47]. This study identifies multiple carriages of resistance determinants including those for beta-lactams: bla_{SHV}, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$. Despite the fact that we noted bla_{SHV} being the most prevalent determinant, Tellevik et al. [48] and Mshana et al. [6] had earlier reported $bla_{CTX\text{-}M\text{-}15}$ as the most prevalent determinant in Dar es Salaam and Mwanza, respectively. On average, a strong agreement was observed between phenotype- and sequence-based resistance to all antibiotics tested, findings that are consistent with the previous report on *E. coli* that was conducted in the same settings [49]. Nonetheless, ampicillin revealed the lowest but moderate agreement between the two methods. The phenotypically determined resistance to ampicillin was higher than sequence-based resistance. Plausibly, the observed difference could be due to the fact that WGS analysis uses only known resistance genes and it is also true that not all genes involved in resistance mechanisms have been included in these databases.

Table 4 Agreement between phenotypically tested and whole-genome sequence-predicted antimicrobial resistance

Antibiotic name	DST[a]	WGS[b]	Agreement	Kappa	*P* value
Amoxicillin-clavulanate	7 (43.8%)	7 (20.6%)	75.0%	0.46	0.0147
Ampicillin	15 (93.8%)	19 (55.9%)	56.3%	0.13	0.1508
Ceftazidime	6 (37.5%)	16 (47.1%)	93.8%	0.87	0.0002
Ceftriaxone	6 (37.5%)	16 (47.1%)	93.8%	0.87	0.0002
Chloramphenicol	5 (31.2%)	2 (5.9%)	62.5%	− 0.12	0.7569
Ciprofloxacin	5 (31.2%)	12 (35.3%)	87.5%	0.71	0.0023
Gentamycin	3 (18.8%)	9 (26.5%)	87.5%	0.59	0.0092
Trimethoprim aulphamethoxazole	9 (56.2%)	10 (29.4%)	62.5%	0.30	0.0451

[a] Phenotype-based resistance detection on 16 isolates

[b] Whole-genome sequence-based resistance detection on all 34 isolates

The observed multiple carriage of beta-lactam- resistance determinants in this study, particularly amongst *K. pneumoniae* ST17 and ST392 isolates, might substantially be a reason for their persistence in this hospital as also noted elsewhere [6]. Apart from being prevalent in this study, *K. pneumoniae* ST392 appeared to carry multi-resistance determinants. Findings are similar to those reported in a hospital-based study at an Italian hospital [50], which showed that *K. pneumoniae* ST392 strain might become very aggressive. Although we could not identify a single resistance gene for carbapenem in the present study's isolates, *K. pneumoniae* ST15 and ST348 have been reported in Portugal to be the

Table 5 Virulence factors in *K. pneumoniae*

ID	MLST	K type[a]	Aerobactin	Colibactin	Salmochelin	Yersiniabactin	*kfuABC*	*kvgAS*
248	ST15	K60				+	+	
591DK	ST15	K60					+	
17	ST17	NF				+		
284D	ST17	NF						
29	ST17	NF				+		
320	ST17	NF				+		
41	ST17	NF						
566A	ST17	K23						
109B	ST20	K28						
110LF	ST25	K2						
38	ST25	K2						
350	ST29	K19				+		
ADE	ST37	NF						
74	ST152	K41						
KLEB	ST193	NF						
16	ST231	NF				+	+	
79C	ST297	K10					+	
567B	ST299	K7				+		
576	ST299	K7				+		
293	ST348	NF				+		
50	ST348	K23				+		
585	ST348	K23				+		
131E	ST392	NF						
134	ST392	NF						
404C	ST392	NF						
51 K	ST392	NF						
150C	ST491	K80						
198C	ST1562	NF					+	
201B	ST1562	NF					+	
315	ST2042	NF	+	+	+	+		
152E	NF	NF					+	
302	NF	K19				+	+	+
343C	NF	K34				+	+	
365	NF	K34					+	

A plus (+) means a virulence determinant was found, and an empty cell means no determinant was found

[a] K type was not found

Table 6 Plasmid multi-locus sequence typing (pMLST) in *K. pneumoniae*

ID	MLST	K type[a]	Plasmids	pMLST
248	ST15	K60	Col440I ColpVC FIA(pBK30683) IncFIB(K) IncFII IncR	IncF[K9:A13:B-]
591DK	ST15	K60	Col440I FIA(pBK30683) IncFIB(K) IncFII IncR	IncF[K9:A13:B-]
17	ST17	NF	Col(MGD2) FIA(pBK30683) IncFIB(K) IncFII(K) IncR	IncF[K2:A13*:B-]
284D	ST17	NF	IncFIB(K) IncFII(K) IncR IncY	IncF[F-:A-:B18]
29	ST17	NF	Col(MGD2) FIA(pBK30683) IncFIB(K) IncFII(K) IncR	IncF[K2:A-:B58*]
320	ST17	NF	Col440I IncFIB(K) IncFII(K) IncR	IncF[F-:A16:B-]
41	ST17	NF	Col440I IncFIB(K) IncHI1B	IncF[Unknown ST] IncHI1[Unknown ST]
566A	ST17	K23	IncFIB(K) IncFIB(pKPHS1) IncHI1B	IncF[Unknown ST] IncHI1[Unknown ST]
109B	ST20	K28	Col440I ColRNAI IncFIA(HI1) IncFIB(K) IncFII	IncF[K12:A10*:B-]
110LF	ST25	K2	Col440I IncFIB(K) IncHI1B	IncF[Unknown ST] IncHI1[Unknown ST]
38	ST25	K2	Col440I Col440II ColRNAI IncFIB(K) IncFIB(pECLA) IncFII(pECLA) IncHI1B	IncF[Unknown ST] IncHI1[Unknown ST]
350	ST29	K19	Col440I IncFIB(K) IncFII(K)	IncF[F-:A-:B10]
ADE	ST37	NF	Col440I IncFIB(K)	IncF[Unknown ST]
74	ST152	K41	ColRNAI IncFIB(K)	IncF[Unknown ST]
KLEB	ST193	NF	Col440I Col440II IncFIB(K) IncFII(K) IncR	IncF[F-:A-:B10]
16	ST231	NF	Col(MG828) Col440I ColpVC IncFIB(K) IncFIB(pKPHS1) IncFII(K) IncFII(pCRY) IncQ1 IncR	IncF[F-:A-:B5]
79C	ST297	K10	Col440I ColRNAI IncFII(K) IncR	IncF[K5:A-:B-]
567B	ST299	K7	IncFIB(K) IncFII IncR	IncF[F-:A16:B-]
576	ST299	K7	IncFIB(K) IncFII IncR	IncF[K12:A-:B-]
293	ST348	NF	IncFIB(K) IncFIB(pKPHS1) IncFII(K) IncQ1	IncF[F-:A-:B18]
50	ST348	K23	IncFIB(K) IncFII(K) IncQ1 IncR	IncF[K8:A-:B-]
585	ST348	K23	IncFIB(K) IncFII(K) IncQ1 IncR	IncF[F-:A16:B-]
131E	ST392	NF	Col440I IncFIB(K) IncFII(K)	IncF[F-:A16:B-]
134	ST392	NF	Col440I ColRNAI IncFIB(K) IncFII(K)	IncF[F-:A-:B10]
404C	ST392	NF	Col440I FIA(pBK30683) IncFIB(K) IncFIB(pKPHS1) IncR	IncF[F-:A13:B-]
51 K	ST392	NF	Col440I IncFIB(K) IncFIB(pKPHS1) IncFII(K)	IncF[F-:A16:B-]
150C	ST491	K80	ColRNAI IncFIB(K) IncR	IncF[Unknown ST]
198C	ST1562	NF	Col440I IncFIB(K)	IncF[Unknown ST]
201B	ST1562	NF	Col440I IncFIB(K)	IncF[Unknown ST]
315	ST2042	NF	IncFIA(HI1) IncFIB(K) IncFII	IncF[K4*:A-:B10*]
152E	NF	NF		
302	NF	K19	Col440I IncFIB(K) IncFII IncHI1B IncR	IncF[F-:A-:B10] IncHI1 [Unknown ST]
343C	NF	K34	Col(MGD2) FIA(pBK30683) IncR	IncF[F-:A13:B-]
365	NF	K34	Col(MGD2) FIA(pBK30683) IncR	IncF[K12:A-:B-]

[a] K type was not found

cause of KPC outbreaks [51]. The identification of this aggressive ST348 strain in this hospital should at least signal for the emergence and spread of MDR bacteria and that no sooner than later common infections caused by *K. pneumoniae* and other bacteria will become untreatable.

The co-carriage of aminoglycoside, fluoroquinolone and quinolone resistance determinants was very common in almost all *K. pneumoniae.* The fluoroquinolone, aminoglycoside and quinolone resistance genes: *aac(6')Ib-cr*, *oqxA* and *oqxB*, appeared to be associated with the carriage of $bla_{CTX\text{-}M\text{-}15}$ and other beta-lactam- resistance determinants, findings that are consistent with a report [52] on *K. pneumoniae* strains from urban settings in Barcelona.

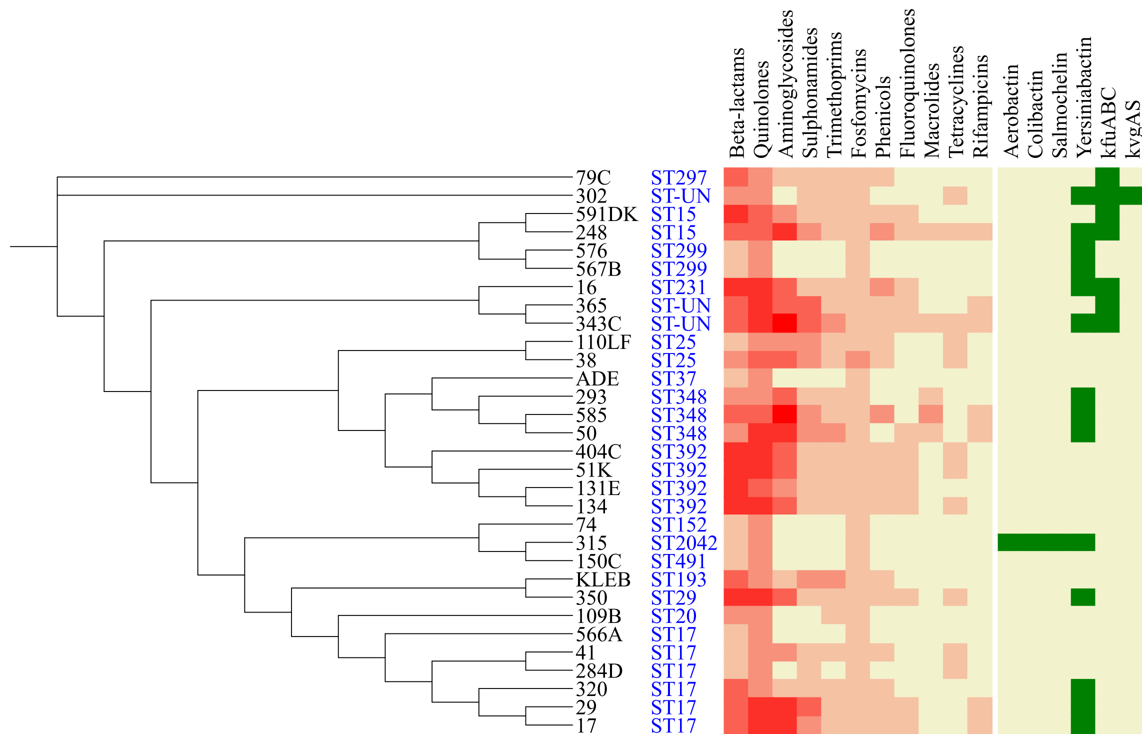

Fig. 1 Phylogenetic analysis of 31 *K. pneumoniae* isolates showing STs, resistance and virulence genes. The heatmap shows the frequency of AMR and virulence genes present in an isolate. The stronger the red colour is, the higher the number of genes identified across antibiotic classes is. The green colour stands for isolates from which a virulence gene was present

The abundance and variability of resistance determinants to sulphonamides, tetracyclines, fluoroquinolones and trimethoprims was found to be high, findings that are in line with those by Taitt et al. [47]. In the current study, we further found a relatively higher proportion of *K. pneumoniae* carrying *arr-3* for rifampicin resistance than the proportion documented by Taitt et al. [47]. Uncontrolled and excessive use of first- and second-line antibiotics for both clinical and veterinary purposes is a plausible explanation to the emergence and spread of these determinants in Enterobacteriaceae [53].

We further observed a significant proportion of yersiniabactin, colibactin, aerobactin and salmochelin in these *K. pneumoniae* strains. However, yersiniabactin was the most prevalent VF and it has been associated with *K. pneumoniae* infection rather than carriage, findings consistent with Holt et al. [54]. Based on the molecular characteristics proposed by Li et al. [55] for a hypervirulent *K. pneumoniae*, isolate 315 (*K. pneumoniae* ST2042) was the likely candidate. It was carrying genes coding for regulators of mucoid phenotype (*rmpA*), aerobactin (*iucABCD* and *iutA*), salmochelin (*iroBCDN*) and yersiniabactin (*ybt*, *fyuA* and *irp1/2*) but lacking the ferric uptake operon (*kfuABC*). Interestingly, we observe that this strain 315 (*K. pneumoniae* ST2042) with high virulence potential has low AMR. Apart from *fosA* and *oqxAB*, which it shares with all other isolates, its only beta-lactam gene is $bla_{\text{SHV-99}}$, which notably none of the other isolates possess. This combination of high virulence and low AMR has been observed elsewhere [54, 56]. Amongst our isolates with many AMR genes, virulence determinants tend to be reduced.

Further analyses revealed that the most frequent plasmid replicon identified was IncF (I/II). Other replicons that were infrequently identified included IncHI1 and IncN3. The plasmids carried by *K. pneumoniae* appeared to be highly diverse. However, the IncFII plasmids seem to be common and correlated with the observed carriage of $bla_{\text{CTX-M-15}}$, similar to a Moroccan study [57] that identified IncFII plasmid as a carrier of $bla_{\text{CTX-M-15}}$ amongst *K. pneumoniae* ST466 strains. For instance *K. pneumoniae* ST15 appeared to carry plasmid ST IncF[K9:A13:B-], and *K. pneumoniae* ST17 was carrying plasmid ST IncF[K2*:A13:B-]. Plasmid ST IncF[K8:A-:B-] was identified in *K. pneumoniae* ST348 and ST231 and plasmid ST IncF[K7:A-:B-] was found in *K. pneumoniae* ST392 and ST29.

We acknowledge the presence of several limitations to this study. First, due to small numbers of isolates, the study lacked epidemiological analysis that might have shown correlation between AMR and virulence genes with patients' demographics (gender, age) and clinical characteristics including admission outcomes, antibiotics use, hospitalisation history and comorbidities. Second, phenotype-based resistance results were available for small numbers of bacterial isolates; this may have impacted on the agreement between phenotype-

and sequence-predicted resistance results. Third, WGS analysis relied on resistance and virulence databases that at time of analysis might comprise of known genes and not all genes involved in resistance or virulence mechanisms have been documented or included in those databases. Further, the existence of genes encoding different resistance and virulence factors do not necessarily indicate gene activity in the isolates. There is therefore a need for future genomics studies to focus on quantifying expression levels of genes encoding different resistance and virulence factors.

Conclusions

In this study, the amount of antimicrobial resistance and virulence determinants detected in *K. pneumoniae* is alarming. Besides its application for research purposes, in resource-limited settings, WGS-based diagnostic approach has showed promising potentials in clinical microbiology, hospital outbreak source tracing, virulence and AMR detection. Having been implemented successfully in Kilimanjaro, WGS can be used as a surveillance tool for infectious agent and AMR detection nationwide. It has the potential of accelerating informed decisions in formulation of pragmatic antimicrobial stewardships, and other infection prevention and control initiatives.

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Authors' contributions TS conceived the initial idea; FA, OL, MA, BTM and GK refined the idea. TS and HK performed the laboratory analyses. TS and MZ analysed the data and prepared the manuscript draft. TS, HK, MZ, FA, OL, MA, BTM and GK read, revised and approved the final manuscript.

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Compliance with ethical standards

Ethical approval and participant's consent This study was granted ethical approval by the KCMUCo Research Ethics Committee and the National Institute for Medical Research with approval numbers 893 and NIMR/HQ/R.8a/Vol.IX/2080, respectively. A written informed consent was obtained from each participant or from parents or guardians of children before enrolment into the study.

Consent for publication Not applicable.

Competing interests The authors declare that they have no conflicts of interest.

Availability of Data and Materials Genome assemblies have been submitted to the European Nucleotide Archive with project accession number PRJEB26616. Other data are available on request to the authors.

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