Journal of Global Antimicrobial Resistance 30 (2022) 384–389

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Plasmid characterization in bacterial isolates of public health relevance in a tertiary healthcare facility in Kilimanjaro region, Tanzania

Lameck Pashet Sengeruan[a,b,*], Marco van Zwetselaar[c], Happiness Kumburu[c], Frank M. Aarestrup[d], Katharina Kreppel[a], Elingarami Sauli[a], Tolbert Sonda[c]

[a] School of Life Sciences and Bioengineering, The Nelson Mandela African Institution of Sciences and Technology, Arusha, Tanzania
[b] Training and Capacity Building Department, Ifakara Health Institute, Bagamoyo, Tanzania
[c] Genomics and Bioinformatics unit, Kilimanjaro Clinical Research Institute, Moshi, Tanzania
[d] DTU-Food, Technical University of Denmark, Lyngby, Denmark

A R T I C L E I N F O

Article history:
Received 7 April 2022
Revised 15 June 2022
Accepted 27 June 2022
Available online 4 July 2022

Editor: Prof Francesco Iannelli

Keywords:
Whole-genome sequencing
plasmid replicon types
antibiotic resistance
virulence
public health
Tanzania

A B S T R A C T

Objectives: Plasmids are infectious double stranded DNA molecules that are found within bacteria. Horizontal gene transfer promotes successful spread of different types of plasmids within or among bacteria species, making their detection an important task for guiding clinical treatment. We used whole genome sequenced data to determine the prevalence of plasmid replicon types in clinical bacterial isolates, the presence of resistance and virulence genes in plasmid replicon types, and the relationship between resistance and virulence genes within each plasmid replicon.
Methods: All bacterial sequences were de novo assembled using Unicycler before extraction of plasmids. Assembly graphs were submitted to Gplas+plasflow for plasmid contigs prediction. The predicted plasmid contigs were validated using PlasmidFinder.
Results: A total of 159 (56.2%) out of 283 bacterial isolates were found to carry plasmid replicons, with *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus* being the most prevalent plasmid carriers. A total of 26 (86.7%) multiple-replicon types were found to carry both resistance and virulence genes compared to 4 (13.3%) single plasmid replicons. No statistically significant correlation was found between the number of antibiotic resistance and virulence genes in multiple-replicon types ($r = -0.14$, $P > 0.05$).
Conclusion: Our findings show a relatively high proportion of plasmid replicon-carrying isolates suggesting selection pressure due to antibiotic use in the hospital. Co-occurrence of antibiotic resistance and virulence genes in clinical isolates is a public health problem warranting attention.
© 2022 The Authors. Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Plasmids are circular, double-stranded DNA molecules that occur naturally in bacterial cells [1], whose genes often provide evolutionary advantages for bacteria, such as antimicrobial resistance and/or virulence [2,3]. Plasmids are important vehicles in disseminating and acquiring antibiotic resistance and virulence, and can thus constitute a major burden on human health [4]. Recent studies have suggested that the prevalence of antimicrobial resistance (AMR) is higher in Low- and Middle-income Countries compared to European countries and the United States [5,6]. There is however, limited knowledge regarding the dissemination of antibiotic resistance genes and virulence among clinical isolates in Sub-Saharan Africa. This study was conducted to determine the proportion of bacterial isolates carrying plasmid replicons, to identify plasmid replicons that mediated resistance and virulence genes, and to investigate the relationship between antibiotic resistance genes and virulence genes within multiple incompatibility groups using whole genome sequence data from bacterial isolates among inpatients admitted at the Kilimanjaro Christian Medical Centre (KCMC) in Tanzania.

* Corresponding author: School of Life Sciences and Bioengineering, The Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania.

E-mail addresses: lameckpashet@gmail.com, lpashet@ihi.or.tz (L.P. Sengeruan).

https://doi.org/10.1016/j.jgar.2022.06.030
2213-7165/© 2022 The Authors. Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Table 1
Demographic and clinical characteristics of the study patients

Patient characteristics	Missing[a] (%)	Total (%)
Number of patients		128 (100)
Mean age in years (SD)		46.2 (18.0)
Gender	4 (3.1)	
Female		47 (36.7)
Male		77 (60.2)
Ward of admission	1 (0.8)	
Surgical		62 (48.4)
Surgical ICU		9 (7.1)
Medical		52 (40.6)
Medical ICU		4 (3.1)
Specimen collected	1 (0.8)	
Blood		8 (6.3)
Sputum		13 (10.2)
Stool		19 (14.8)
Swab		87 (67.9)
Underlying conditions		
Cancer		6 (4.7)
Diabetes		28 (21.9)
HIV		2 (1.5)
Tuberculosis		6 (4.7)
Others		86 (67.2)
Type of wound		
Burn wound		11 (8.6)
Diabetic wound		24 (18.8)
Motor traffic wound		6 (4.6)
Post-surgical wound		10 (7.8)
Others		77 (60.2)
History of hospitalization	8 (6.3)	
No		78 (60.9)
Yes		42 (32.8)
Patient hospital transfer	3 (2.3)	
No		44 (34.4)
Yes		81 (63.3)
Patient ward transfer	5 (3.9)	
No		110 (85.9)
Yes		13 (10.2)
Median time in days stayed in the hospital before survey (IQR)	8 (6.3)	8 (4–11.5)

SD, standard deviation; ICU, intensive care unit; IQR, interquartile range.
[a] Missing values in each variable

2. Materials and methods

2.1. Study setting: Whole-genome sequencing and library preparation

Kilimanjaro Christian Medical Centre (KCMC) is one of Tanzania's five zonal referral hospitals, located in Moshi, northern Tanzania. KCMC has a bed capacity of 650 and serves a catchment area of about 15 million people. It serves around 500 outpatients daily, from different parts of Tanzania [7]. The whole genome sequence data that were analyzed originated from a prospective cross-sectional study conducted at KCMC between August 2013 and August 2015. In this study, a total of 56 stool, 122 sputum, 126 blood, and 286 wound swabs (wound/pus) clinical samples, with patients' clinical and socio-demographic characteristics, were collected from 575 patients admitted to KCMC hospital [8,9]. Written informed consent was obtained from each participant and from parents or guardians of children before enrollment into the study.

Collected specimens were taken to the microbiology unit at Kilimanjaro Clinical Research Institute (KCRI) for culture and identification of bacterial isolates. Of 590 specimens collected, 249 were culture positive, resulting in 377 isolates [8]. All bacterial isolates were sequenced in the KCRI genomics lab, and all sequences were archived on the KCRI compute cluster. In brief, whole genome sequencing was performed for genomic DNA that was extracted from cultures of bacterial isolates using the Easy-DNA Extraction Kit (Invitrogen®). Short-read whole genome sequencing was performed using the Illumina MiSeq platform (Illumina Inc.). Libraries for Illumina sequencing were constructed using the Illumina Nextera XT kit (Illumina Ltd., San Diego, CA) according to the manufacturer's recommendations. The libraries were sequenced on Illumina MiSeq platform using the 2 × 250 bp paired-end protocol, previously reported by Kumburu et al. [8] and Sonda et al. [9]. For this study, a total of 283 bacterial whole genomes isolates with associated metadata were retrieved for analysis. Additional ethical approval was obtained from the Ifakara Health Institute Research Ethics Committee (IHI/IRB/No: 14-2021) for plasmid characterization.

2.2. Bioinformatics analysis

2.2.1. Quality control and trimming of Illumina sequences

The following steps were followed: (i) All bacterial raw reads were submitted to the in-house bacterial analysis pipeline (BAP), available at https://github.com/zwets/kcri-cge-bap. Assembly was performed using SKESA 2.4.0 [10]. (ii) All resulting assemblies were then processed in batches by the Genome Taxonomy Database Toolkit (GTDB-Tk) 0.3.2 [11] for detailed taxonomic assignment. (iii) Metrics produced by the BAP and GTDB-Tk were then used to assess the quality of each assembly. Assessment was based on read counts, coverage depth, assembly structure (contig count, N1, N50, L50), deviation of assembly length from reference, GTDB alignment fraction, and GTDB Multi-Locus Sequence Alignment (MSA) coverage. A six-point scale was used for assembly quality rating: 0 (Unusable), 1 (Mix), 2 (Bad), 3 (Usable), 4 (Good), and 5 (Excellent). (iv) Finally, categories 0 to 2 were excluded, while categories 3 through 5 were used for subsequent analysis. Every assembly in

Table 2
Proportion of bacterial isolates that carried plasmid replicons.

	Isolates		Plasmid replicons	
			Single	Multiple[a]
Species	n	%	n	n
Enterobacter asburiae	1	0.6	1	0
Enterobacter cloacae	3	1.9	1	2
Enterobacter hormaechei	10	6.3	6	4
Enterobacter kobei	1	0.6	0	1
Enterobacter roggenkampii	1	0.6	1	0
Enterobacter soli	1	0.6	1	0
Enterobacter sp. n18-03635	1	0.6	1	0
Enterococcus faecalis	7	4.4	5	2
Enterococcus faecium	3	1.9	2	1
Enterococcus gallinarum	1	0.6	1	0
Escherichia coli	38	23.9	23	15
Klebsiella michiganensis	2	1.3	1	1
Klebsiella oxytoca	2	1.3	1	1
Klebsiella pneumoniae	25	15.7	8	17
Klebsiella variicola	2	1.3	2	0
Micrococcus sp. Kbs0714	1	0.6	1	0
Morganella morganii	4	2.5	4	0
Proteus columbae	1	0.6	1	0
Proteus mirabilis	14	8.8	14	0
Proteus penneri	1	0.6	1	0
Proteus vulgaris	1	0.6	1	0
Pseudomonas aeruginosa	2	1.3	2	0
Shewanella algae	1	0.6	1	0
Staphylococcus aureus	30	18.9	15	15
Staphylococcus capitis	1	0.6	1	0
Staphylococcus epidermidis	1	0.6	1	0
Staphylococcus haemolyticus	3	1.9	3	0
Staphylococcus hominis	1	0.6	0	1

[a] Multiple-replicon plasmid types in an isolate.

these categories was for a single isolate that had (nearly) complete genome coverage, at sufficient sequencing depth.

2.2.2. Plasmid extraction and validation

Raw reads assembly was repeated with Unicycler 0.4.7 [12] for its ability as a “SPAdes optimiser” to produce long and, in the ideal case, circular contigs. A total of 327 contigs from the bacterial analysis pipeline were obtained, and 310 were predicted as circular contigs. Assembly graphs were submitted to Gplas+plasflow for plasmid contigs prediction. Gplas 0.6.1 [13] + Plasflow 1.1 [14] take into account the connected components in the assembly graph when predicting plasmid contigs. The components predicted to be plasmid contigs were extracted from the assemblies and submitted to PlasmidFinder version 1.3 [15] for validation.

2.2.3. Identification of antibiotic resistance genes and virulence genes in plasmid replicon types

To identify antibiotic resistance and virulence genes carried in plasmid replicons, the assembled putative plasmid sequences for each isolate were submitted to Resfinder 4.0 [16] and VirulenceFinder 1.4 [17], respectively. In both Resfinder and VirulenceFinder, 90% identity and 60% coverage settings to call a gene were selected.

2.2.4. Statistical analysis

Stata 14 (STATA Corp., College Station, TX) was used for descriptive statistics and determination of the relationship between antibiotic resistance and virulence genes in plasmid replicons.

3. Results

3.1. Study population

In total, 128 patients whose whole genome bacterial isolates were analyzed were included in this study (Table 1). One hundred twenty-eight patients were plasmid positive isolates. The mean age in years (SD) was 46.2 (18.0). There were 77 male patients (60.2%), 47 (36.7%) female patients, and 4 (3.1%) did not report gender identification. A total of 62 (48.4%) patients were admitted to a surgical ward, 9 (7.1%) to the surgical ICU, 52 (40.6%) to a medical ward, 4 (3.1%) to the medical ICU ward, and 1 (0.8%) was missing ward admission identification. Eighty-seven (67.9%) specimens were swabs, 19 (14.8%) were stool, 13 (10.2%) were sputum, 8 (6.3%) were blood, and 1 (0.8%) specimen was missing identification. A total of 28 (21.9%) patients were diabetic, 6 (4.7%) were cancer patients, 6 (4.7%) were suffering from tuberculosis, and 2 (1.5%) were HIV positive. Of the 86 (67.2%) others, 61 (47.7%) had no underlying conditions, and 25 (19.5%) had other underlying conditions. Of the wound swabs, twenty-four (18.8%) were from patients with diabetic wounds, 11 (8.6%) were from burn wounds, 10 (7.8%) were from post-surgical wounds, and 6 (4.6%) were from motor traffic accident wounds. Of the 77 (60.2%) others, 35 (27.3%) had other wounds, and 42 (32.8%) had no wounds.

Seventy-eight (60.9%) patients had no history of hospitalization, 42 (32.8%) had a hospitalization history, and 8 (6.3%) were missing identification. A total of 81 (63.3%) patients were transferred from another hospital, 44 (34.4%) were not, and 3 (2.3%) were missing identification. Among all participants, the median number of days stayed in the hospital before the survey was 8 (4 -11.5) days (Table 1).

3.2. Proportion of bacterial species carrying plasmid replicon types

A total of 283 whole genome bacterial sequences were analyzed. One hundred fifty-nine (56.2%) bacterial isolates were detected to carry plasmid replicons. Out of 159 plasmid replicons, 93 non-repetitive plasmid replicons were predicted. Of 93 plasmid replicons, 48 (51.6%) occurred in isolates carrying single replicons, and 45 (48.4%) were in multiple-replicon isolates. *Klebsiella pneumoniae* isolates were the most frequent carriers of multiple-replicons (17, 28.3%), followed by *S. aureus* (15, 25.0%) and *E. coli* (15, 25.0%). *Escherichia coli* isolates were the most common single-replicon carriers (23, 23.2%), followed by *S. aureus* (15, 15.2%) and *P. mirabilis* (14, 14.4%) (Table 2).

3.3. Plasmid replicon types mediated resistance and virulence genes concurrently

A total of 30 isolates with plasmid replicons were identified to carry both resistance and virulence genes, of which 26 (86.7%) were multiple-replicon plasmids and 4 (13.3%) were single-replicon

Table 3
Single-replicon plasmid types mediated both resistance and virulence genes

Single-replicon	Resistance genes	Virulence genes
IncFII	*aac(3)-IIa,aadA5, blaCTX-M15, dfrA17, qacE, sul1, tet(B)*	*traT*
IncQ1	*aph(3'')-Ib,aph(6)-Id, blaTEM-1B, dfrA7, qacE, sul1, sul2*	*cea, focCsfaE, focG, focI, iha, ireA, iucC, iutA, mchB, mchC, mchF, mcmA, papA_F48, papC, sat*
IncFIA	*sitABCD, tet(A)*	*iucC, iutA, sitA*
IncFII(pRSB107)	*dfrA5, qacE, sul1, sul2*	*capU, iroN, iss, iucC, iutA, mchB, mchC, mchF, mcmA, vat*

Table 4
Multiple-replicon plasmid types mediated both resistance and virulence genes

IncFIB(K), IncFII(pKP91), IncR	*ARR-2, aac(3)-IIa, aac(6′)-Ib-cr, aadA1, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, catB3, cmlA1, dfrA14, ere(A), qacE, sul1, sul2, tet(A)*	*traT*
IncFIB(K)(pCAV1099-114), IncHI2, IncHI2A, IncX3	*aac(6′)-Ib-cr, aadA5, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, blaTEM-1B, catB3, dfrA17, qacE, qnrB1, sul1, sul2, tet(A), tet(B)*	*terC*
Col156, IncFIA, IncFIB(AP001918), IncFII(pRSB107)	*aac(3)-IIa,aac(6′)-Ib-cr, aadA5, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, catB3, dfrA17, mph(A), qacE, sul1, sul2, tet(A)*	*hra, iha, iucC, iutA, sat, senB, traT*
IncFIB(K), IncFII(K), IncQ1, IncR	*ARR-2, aac(3)-IIa, aadA1, aph(3′′)-Ib, aph(3′)-Ia, aph(6)-Id, blaCTX-M-15, blaTEM-1B, cmlA1, ere(A), qacE, qnrB1, sul1, sul2*	*traT*
IncFIA(HI1), IncFIB(K), IncFII(Yp), IncHI2, IncHI2A, IncN3, pKP1433	*aac(3)-IIa, aac(6′)-Ib-cr, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, blaTEM-1B, catB3, qacE, qnrB1, sul1, sul2, tet(A)*	*terC*
IncFIB(K), IncFII(K)	*aac(3)-IIa, aac(6′)-Ib-cr, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, blaTEM-1B, catB3, dfrA14, qnrB1, sul2, tet(A)*	*traT*
IncHI2, IncHI2A	*aac(3)-IIa, aac(6′)-Ib-cr, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, blaTEM-1B, catB3, dfrA14, qnrB1, sul2, tet(A)*	*terC*
Col156, IncFIA, IncFIB(AP001918), IncFII	*aac(3)-IIa, aac(6′)-Ib-cr, aadA5, blaCTX-M-15, blaOXA-1, catB3, dfrA17, mph(A), qacE, sitABCD, sul1, tet(A)*	*capU, fyuA, irp2, iucC, iutA, senB, sitA, traT*
IncFIB(pECLA), IncFII(pECLA), IncHI2, IncHI2A	*aac(3)-IIa, aac(6′)-Ib-cr, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, catB3, dfrA14, qnrB1, sul2, tet(A)*	*terC*
Col156, IncFIA, IncFIB(AP001918)	*aac(6′)-Ib-cr, aadA5, blaCTX-M-15, blaOXA-1, catB3, dfrA17, mph(A), qacE, sitABCD, sul1, tet(A)*	*iucC,iutA,senB,sitA, traT*
IncFIA, IncFIB(AP001918)	*aac(3)-IIa, aac(6′)-Ib-cr, aadA5, blaCTX-M-15, blaOXA-1, catB3, dfrA17, qacE, sitABCD, sul1*	*fyuA, irp2, iucC, iutA, sitA, traT*
IncFIA, IncFII	*aac(6′)-Ib-cr, aadA5, blaCTX-M-15, blaOXA-1, catB3, dfrA17, mph(A), qacE, sul1, tet(A)*	*afaA, afaC, afaD, iha, iucC, iutA, nfaE, papA_F43, sat, traT*
IncFIA, IncFIB(AP001918), IncFII(pAMA1167-NDM-5)	*aac(3)-IId, aadA2, blaTEM-1B, catA1, dfrA12, mph(A), qacE, qepA4, sul1*	*traT*
IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	*aac(6′)-Ib-cr, aph(3′′)-Ib, aph(6)-Id, blaCTX-M-15, blaOXA-1, blaTEM-1B, catB3, dfrA14, sul2*	*traT*
IncFIA, IncFIB(AP001918), IncFII	*aac(6′)-Ib-cr, aadA5, blaOXA-1, catB3, dfrA17, qacE, sul1, tet(B)*	*traT*
IncFIB(K), IncFII(K), IncQ1	*aph(3′′)-Ib, aph(3′)-Ia,aph(6)-Id, blaTEM-1B, dfrA14, mph(A), sul2*	*traT*
Col156, IncFIB(AP001918), IncFII	*aph(3′′)-Ib, aph(6)-Id, blaTEM-1B, catA1, dfrA7, sul2, tet(D)*	*afaA, afaB, afaC, afaD, afaE, hra, iha, iss, iucC, iutA, papA_F43, sat, senB, traT*
Col156, IncFIA, IncFIB(AP001918), IncQ1	*aph(3′′)-Ib, aph(6)-Id, blaTEM-1B, dfrA17, sul2, tet(B)*	*iha, iucC, iutA, papA_F43, sat, senB*
IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR)	*aph(3′′)-Ib,aph(6)-Id, dfrA15, qacE, sul1, sul2*	*terC*
IncFIB(K), IncFII(K), IncR	*aac(3)-IId, blaCTX-M-15, blaTEM-1B, dfrA30, sul2*	*traT*
IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR), IncR	*blaTEM-1B, dfrA5, qacE, sul1, tet(D)*	*fyuA, irp2, traT*
IncFII(K), IncR	*aac(3)-IId, blaCTX-M-15, blaTEM-1B, dfrA30, sul2*	*traT*
IncFIB(AP001918), IncFII,IncQ1	*aph(3′′)-Ib, aph(6)-Id, blaTEM-1B, dfrA5, sul2*	*cia, cvaC, etsC, hlyF, ireA, iroN, iss, iucC, iutA, mchF, ompT, papA_F11, papC, traT*
IncFIB(K)(pCAV1099-114), IncY	*sul2, tet(D)*	*terC*
IncFIB(pHCM2), IncHI2, IncHI2A	*blaTEM-1B*	*terC*
IncFIB(pB171), IncFII(pCoo)	*mdf(A)*	*eae, espA, espF, nleB, nleC, perA, tir, traT*

plasmids (Tables 3 and 4). All four single-replicon plasmids were carried by *E. coli*. Resistance gene *Sul1* was the most common across three single-replicon plasmid types IncFII, IncQ1, and IncFII(pRSB107). Virulence genes *iucC* and *iutA* were also the most common across three single-replicon plasmid types IncQ1, IncFII(pRSB107), and IncFIA (Table 3).

Among the 26 multiple-replicon plasmids, 11 (42.3%) were carried by *E. coli* isolates, 10 (38.4%) by *K. pneumoniae* isolates, 2 (7.6%) by *E. hormaechei* isolates, 1 (3.9%) by the *E. cloacae* isolate, 1(3.9%) by the *K. oxytoca* isolate, and 1 (3.9%) by the *K. michiganensis* isolate. Virulence gene *traT* was seen in 18 (69.2%) of the 26 multiple-replicon plasmids, followed by *terC,* which was identified in 7 (26.9%) multiple-replicon plasmids. Regarding resistance genes in multiple-replicon plasmids, *sul2* was observed in 17 (65.4%) replicon types, followed by *blaTEM-1B* in 15 (57.7%) replicon types, followed by *blaCTX-M-15* in 14 (57.7%) replicon types, and *blaOXA-1* in 13 (50.0%) replicon types (Table 4).

3.4. Correlation between antibiotic resistance and virulence genes

We explored the relationship between the number of antibiotic resistance genes and virulence genes in 26 multiple-replicon types using Pearson correlation analyses. There was an inconclusive negative relationship between antibiotic resistance and virulence genes existence in multiple-replicon plasmid types (r = -0.14, $P > 0.05$).

4. Discussion

In the present study a high proportion of clinical bacterial isolates from inpatients at KCMC hospital was found to carry plasmid replicons. The present findings are in concordance with previous studies elsewhere [18]. The observed high carriage of plasmid replicons by the analyzed isolates might plausibly be a reflection of resistance selection pressure due to high antibiotic exposure in hospital settings [19].

Escherichia coli isolates were the most prevalent carriers of single-replicon plasmid types followed by *S. aureus* and *P. mirabilis.* On other hand, *K. pneumonia* were the most prevalent carriers of multiple-replicon plasmid types, followed by *S. aureus* and *E. coli*. The present study findings are in line with study results in a tertiary care hospital in South India [18]. A possible explanation could be that the mentioned bacterial species have great medical relevance and thus are relatively highly isolated in hospital settings compared to other species [20,21]. However, the present study findings show a larger proportion of *P. mirabilis* carrying plasmid replicons than the study in South India. This difference might be due to the fact that the majority of the present study isolates

were from wound specimens in which *P. mirabilis* were identified [22,23].

This study identified bacterial species with low plasmid replicon prevalence including *Enterobacter sp. n18-03635, Enterobacter kobei, Klebsiella variicola* and *Klebsiella oxytoca.* The study findings are consistent with other studies conducted in Canada, Greece, and Mexico [24–26]. Interestingly, the study observed other low plasmid replicon prevalence species that were reported elsewhere in soil samples, fish flesh samples [27–29], and pigeon flesh specimens [30], such as *Micrococcus sp. Kbs0714, Enterobacter soli* and *Proteus columbae*. The observed species with low plasmid replicon prevalence might be due to rarity and in most cases misidentification [31,32]. However, reports of bacterial species with low plasmid replicon prevalence indicate the possible emergence and transmission of bacterial pathogens in humans, both in community and hospital settings [26].

Contrary to previous studies reporting IncF plasmid replicon type in *E. coli* to often carry resistance and virulence genes [33], the present study shows IncQ1 replicon type carried the highest number of both resistance and virulence genes in *E. coli*, and the finding is in line with study conducted in Brazil [34,35]. This is possibly due to the fact that IncQ1 replicon type has high-level mobility, stability, and replication at high copy number and is transferred in a wide range of bacterial species through conjugative plasmids [36–39].

In this study it was also identified that there are different multiple-replicon plasmids ranging from two to seven plasmids. This is probably indicative of bacterial evolution to adapt and thrive in hospitals where they are excessively exposed to antimicrobials, antiseptics, and disinfectants [40–42]. A similar distribution of some multiple-replicon plasmids in other regions carrying similar or different antibiotic resistance and virulence genes was noted in the present study. This suggests resistant bacteria arising in one geographical area can spread countrywide/worldwide either by direct exposure or through the food chain,climate change, or the environment [6].

There was no significant relationship found in the present study between numbers of antibiotic resistance and virulence genes in multiple-replicon plasmids ($r = -0.14$, $P > 0.05$), which would indicate that acquisition of antibiotic resistance genes induces the loss of virulence factors. Previous studies support this study finding [43], but do not agree with a study by Dionisio [44]. This discordance might be due to the fact that in other studies the relationship between resistance and virulence genes was determined at the species level and were from gut and environmental samples [33].

4.1. Limitations

We acknowledge there are a number of limitations in the present study that warrant careful interpretation. There was no antimicrobial susceptibility testing done that could provide insights on validating the presence of resistance and virulence genes and actual resistance phenotypes. Bioinformatics analysis was performed on Illumina short reads, which limited the ability to assemble completed plasmid genomes, and consequently the ability to 'tease out' individual plasmids from assembled contigs. We intend to use the short read predictions as an initial screening step for selecting isolates for long read sequencing and plasmid fusion analysis in the future. Assembly graphs were classified by Gplas+PlasFlow for plasmid prediction. As for any machine learning-based approach or indeed any method based on inference from similarity with known sequences, including tools such as PlasmidFinder, the predictive ability of the model is strongly dependent on the data in its reference database or training set. A bias toward plasmid replicon types in well-studied organisms is therefore likely.

4.2. Availability of data

Sequences of the 30 isolates carrying resistance and virulence genes on plasmids have been deposited in the European Nucleotide Archive at EMBL-EBI under accession number PRJEB53343 (https://www.ebi.ac.uk/ena/browser/view/PRJEB53343). Additional data analyzed during the current study are available from the corresponding author on reasonable request.

5. Conclusion

There is a high proportion of isolates carrying resistance and virulence genes in plasmids, indicating a significant concern of AMR development and spread in Tanzanian and other Low- and Middle-income Countries health care settings. With limited resources and health service capacities, the increasing AMR trends are expected to severely affect bacterial-associated mortalities and morbidities.

Funding

This study was financially supported by the Ifakara Health Institute (Training and Capacity Building Department) as part of the MSc in Public Health Research scholarship to Lameck Pashet Sengeruan, and DANIDA through projects No. 20-12-TAN and 12-007 DTU. The funders had no role in the design, analysis, interpretation of the results, and preparation of manuscript or decision to publish.

Ethical approval

This study was approved by the Research Ethical Committee of Ifakara Health Institute in Tanzania [ref. no IHI/IRB/No: 14-2021].

Declaration of Competing Interest

None declared.

Acknowledgements

We thank all patients who consented to participate in this study, and the management of Kilimanjaro Christian Medical Centre. We appreciate the efforts of the Data Management team of Kilimanjaro Clinical Research Institute, in particular Salim Semvua, Lilian Mboya and Krisanta Wilhelm who were the data entry clerks.

References

[1] Dionisio F, Zilhão R, Alves J. Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. Plasmid 2019;102:29–36. doi:10.1016/j.plasmid.2019.01.003.

[2] Smillie C, Garcillán-Barcia MP, Francia MV, Rocha EPC, De Cruz F. Mobility of plasmids. Microbiol Mol Biol Rev 2010;74:434–52. doi:10.1128/MMBR.00020-10.

[3] Brown SP, Cornforth DM, Mideo N. Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. Trends Microbiol 2012;20:336–42. doi:10.1016/j.tim.2012.04.005.

[4] Foley SL, Kaldhone PR, Ricke SC, Han J. Incompatibility Ggroup I1 (IncI1) plasmids: their genetics, biology, and public health relevance. Microbiol Mol Biol Rev 2021;85 e00031-20. doi:10.1128/MMBR.00031-20.

[5] O'Neill, J. Tackling drug resistant infections globally: final report and reommendations. The review on antimicrobial resistance. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf; 2016 accessed 29/11/2021.

[6] Iskandar K, Molinier L, Hallit S, Sartelli M, Catena F, Coccolini F, et al. Drivers of antibiotic resistance transmission in low- and middle-income countries from a "One Health" perspective — A Review. Antibiotics (Basel) 2020;9:372. doi:10.3390/antibiotics9070372.

[7] Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Mmbaga BT, Aarestrup FM, et al. Whole genome sequencing reveals high clonal diversity of Escherichia coli isolated from patients in a tertiary care hospital in 2018. Infection Control 2018;7:72. doi:10.1186/s13756-018-0361-x.
[8] Kumburu HH, Sonda T, Mmbaga BT, Alifrangis M, Lund O, Kibiki G, et al. Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania. Trop Med Int Health 2017;22:454–64. doi:10.1111/tmi.12836.
[9] Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Mmbaga BT, Lund O, et al. Prevalence and risk factors for CTX-M gram-negative bacteria in hospitalized patients at a tertiary care hospital in Kilimanjaro, Tanzania. Eur J Clin Microbiol Infect Dis 2018;37:897–906. doi:10.1007/s10096-018-3196-8.
[10] Souvorov A, Agarwala R, Lipman DJ. Open Access SKESA : strategic k-mer extension for scrupulous assemblies. Genome Biol 2018;219:153. doi:10.1186/s13059-018-1540-z.
[11] Chaumeil P, Mussig AJ, Parks DH, Hugenholtz P. Genome analysis GTDB-Tk : a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 2020;36:1925–7. doi:10.1093/bioinformatics/btz848.
[12] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595. doi:10.1371/journal.pcbi.1005595.
[13] Arredondo-alonso S, Bootsma M, Rogers MRC, Corander J, Willems RJL, Schu AC. Genome analysis gplas: a comprehensive tool for plasmid analysis using short-read graphs. Bioinformatics 2020;36:3874–6. doi:10.1093/bioinformatics/btaa233.
[14] Krawczyk PS, Lipinski L, Dziembowski A. PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. Nucleic Acids Res 2018;46:e35. doi:10.1093/nar/gkx1321.
[15] Carattoli A, Zankari E, García-fernández A, Larsen V, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58:3895–903. doi:10.1128/AAC.02412-14.
[16] Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 2020;75:3491–500. doi:10.1093/jac/dkaa345.
[17] Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol 2014;52:1501–10. doi:10.1128/JCM.03617-13.
[18] Ragupathi NKD, Bakthavatchalam YD, Mathur P, Pragasam AK, Walia K, Ohri VC, Veeraraghavan B. (2019). Plasmid profiles among some ESKAPE pathogens in a tertiary care centre in south India. doi:10.4103/ijmr.IJMR_2098_17
[19] Sonda T, Kumburu H, Van Zwetselaar M, Alifrangis M, Lund O, Kibiki G, et al. Meta-analysis of proportion estimates of producing Enterobacteriaceae in East Africa hospitals. Antimicrob Resist Infect Control 2016;5:18. doi:10.1186/s13756-016-0117-4.
[20] Malachowa N, Deleo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life 2010;67:3057–71. doi:10.1007/s00018-010-0389-4.
[21] Ramirez MS, Iriarte A, Reyes-Lamothe R, Sherratt DJ, Tolmasky ME. Small Klebsiella pneumoniae plasmids: neglected contributors to antibiotic resistance. Front Microbiol 2019;10:1–14. doi:10.3389/fmicb.2019.02182.
[22] Mordi RM, Momoh MI. Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. African J Biotechnology 2009;8:725–30. http://www.academicjournals.org/AJB.
[23] Endimiani A, Luzzaro F, Brigante G, Perilli M, Lombardi G, Amicosante G, et al. Proteus mirabilis bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. Antimicrob Agents Chemother 2005;49:2598–605. doi:10.1128/AAC.49.7.2598.
[24] Tsakris A, Poulou A, Markou F, Pitiriga V, Piperaki ET, Kristo I, et al. Dissemination of clinical isolates of Klebsiella oxytoca harboring CMY-31, VIM-1, and a new OXY-2-type variant in the community. Antimicrob Agents Chemother 2011;55:3164–8. doi:10.1128/AAC.00102-11.
[25] Boyd DA, Lefebvre B, Mataseje LF, Gagnon S, Roger M, Savard P, et al. Enterobacter sp. N18-03635 harbouring blaFRI-6 class A carbapenemase. Canada. J Antimicrob Chemother 2020;75:486–8. doi:10.1093/jac/dkz438.
[26] Rodríguez-Medina N, Barrios-Camacho H, Duran-Bedolla J, Garza-Ramos U. Klebsiella variicola: an emerging pathogen in humans. Emerg Microbes Infect 2019;8:973–88. doi:10.1080/22221751.2019.1634981.
[28] Manter DK, Hunter WJ, Vivanco JM. Enterobacter soli sp. nov.: A lignin-degrading γ-Proteobacteria isolated from soil. Curr Microbiol 2011;62:1044–9. doi:10.1007/s00284-010-9809-9.
[27] Bhassu S, Brahim Eid II, Annie Tan GY. First report of Enterobacter soli associated with gastro-hepato infection of farmed fish. Channa striatain Malaysia. Gastroenter Hepatol Open Access 2019;10:61–5. doi:10.15406/ghoa.2019.10.00357.
[29] Kuo V, Shoemaker WR, Muscarella ME, Lennon JT. Whole-genome sequence of the soil bacterium Micrococcus sp. KBS0714. Genome Announc 2017;5 e00697-17. doi:10.1128/genomeA.00697-17.
[30] Dai H, Wang Y, Fang Y, Xiao T, Huang Z, Kan B, et al. Proteus columbae sp. nov., isolated from a pigeon in Ma'anshan, China. Int J Syst Evol Microbiol 2018;68:552–7. doi:10.1099/ijsem.0.002541.
[31] Potter RF, Lainhart W, Twentyman J, Wallace MA, Wang B, Burnham CA, et al. Population structure, antibiotic resistance, and uropathogenicity of Klebsiella variicola. mBio 2018;9:1–17. doi:10.1128/mBio.02481-18.
[32] Jousset AB, Oueslati S, Bernabeu S, Takissian J, Creton E, Vogel A, et al. False-positive carbapenem-hydrolyzing confirmatory tests due to ACT-28, a chromosomally encoded AmpC with weak carbapenemase activity from Enterobacter kobei. Antimicrob Agents Chemother 2019;63:1–11. doi:10.1128/AAC.02388-18.
[33] Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 2013;26:185–230. doi:10.1128/CMR.00059-12.
[34] de Oliveira Sangtos IC, Albano RM, Asensi MD, D'Alincourt Carvalho-Assef AP. Draft genome sequence of KPC-2-producing Pseudomonas aeruginosa recovered from a bloodstream infection sample in Brazil. J Glob Antimicrob Resist 2018;15:99–100. doi:10.1016/j.jgar.2018.08.021.
[35] Cerdeira LT, Lam MMC, Wyres KL, Ribas RM, Morais MM, Holt KE, et al. Small IncQ1 and Col-like plasmids harboring blaKPC-2 and non-Tn4401 elements (NTEKPC-IId) in high-risk lineages of Klebsiella pneumoniae CG258. Antimicrob Agents Chemother 2019;63 e02140-18. doi:10.1128/AAC.02140-18.
[36] Mcmillan EA, Jackson CR, Frye JG, Thomas CM, Mcmillan EA. Transferable plasmids of Salmonella enterica associated with antibiotic resistance genes. Front Microbiol 2020;11:562181. doi:10.3389/fmicb.2020.562181.
[37] Oliva M, Monno R, D'Addabbo P, Pesole G, Dionisi AM, Scrascia, et al. A novel group of IncQ1 plasmids conferring multidrug resistance Oliva. Plasmid 2017;89:22–6. doi:10.1016/j.plasmid.2016.11.005.
[38] Rawlings DE, Tietze E. Comparative biology of IncQ and IncQ-like plasmids. Microbiol Mol Biol Rev 2001;65:481–96. doi:10.1128/MMBR.65.4.481.
[39] Lima GJ, Scavuzzi AML, Beltrão EMB, Firmo EF, Oliveira ÉM, Oliveira SR, et al. Identification of plasmid IncQ1 and NTE KPC -IId harboring bla KPC-2 in isolates from Klebsiella pneumoniae infections in patients from Recife-PE. Brazil. Rev Soc Bras Med Trop 2020;53:e20190526. doi:10.1590/0037-8682-0526-2019.
[40] Xie M, Li R, Liu Z, Wai E, Chan C, Chen S. Recombination of plasmids in a carbapenem-resistant NDM-5-producing clinical Escherichia coli isolate. J Antimicrob Chemother 2018;73:1230–4. doi:10.1093/jac/dkx540.
[41] McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12:147–79. doi:10.1128/CMR.12.1.147.
[42] Smalla K, Jechalke S, Top EM. Plasmid detection, characterization and ecology. Microbiology Spectrum 2015;3:1–21. doi:10.1128/microbiolspec.plas-0038-2014.
[43] Pan Y, Zeng J, Li L, Yang J, Tang Z, Xiong W, et al. Coexistence of antibiotic resistance genes and virulence factors deciphered by large-scale complete genome analysis. mSystems 2020;5 e00821-19. doi:10.1128/mSystems.00821-19.
[44] Dionisio F. Antibiotic Resistance Gene Diversity and Virulence Gene Microbiomes. mSpehere 2019;4(3) 135–19. doi:10.1128/mSphere.00135-19.