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Genomic analysis of a multidrug-resistant clinical *Providencia rettgeri* (PR002) strain with the novel integron *In*1483 and an A/C plasmid replicon

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Whole-genome sequence analysis was performed on a multidrug-resistant *Providencia rettgeri* PR002 clinical strain isolated from the urine of a hospitalized patient in Pretoria, South Africa, in 2013. The resistome, mobilome, pathogenicity island(s), as well as virulence and heavy-metal resistance genes of the isolate, were characterized using whole-genome sequencing and bioinformatic analysis. PR002 had a genome assembly size of 4,832,624 bp with a GC content of 40.7%, an A/C_2 plasmid replicase gene, four integrons/gene cassettes, 17 resistance genes, and several virulence and heavy metal resistance genes, confirming PR002 as a human pathogen. A novel integron, *In*1483, harboring the gene *bla*$_{OXA-2}$, was identified, with other uncharacterized class 1 integrons harboring *aacA4cr* and *dfrA1*. *Aac(3′)-IIa* and *bla*$_{SCO-1}$, as well as *bla*$_{PER-7}$, *sul2*, and *tet*(B), were found bracketed by composite Tn*3* transposons, and IS*91*, IS*91*, and IS*4* family insertion sequences, respectively. PR002 was resistant to all antibiotics tested except amikacin, carbapenems, cefotaxime-clavulanate, ceftazidime-clavulanate, cefoxitin, and fosfomycin. PR002 was closely related to PR1 (USA), PRET_2032 (SPAIN), DSM_1131, and NCTC7477 clinical *P. rettgeri* strains, but not close enough to suggest it was imported into South Africa from other countries. Multidrug resistance in *P. rettgeri* is rare, particularly in clinical settings, making this case an important incident requiring urgent attention. This is also the first report of an A/C plasmid in *P. rettgeri*. The array, multiplicity, and diversity of resistance and virulence genes in this strain are concerning, necessitating stringent infection control, antibiotic stewardship, and periodic resistance surveillance/monitoring policies to preempt further horizontal and vertical spread of these resistance genes.

Keywords: *Providencia rettgeri*; IncA/C2 plasmid; resistome; mobilome; virulence; South Africa

Introduction

Providencia rettgeri is a Gram-negative opportunistic human pathogenic bacterium that belongs to Proteae bacteria, which comprises the Morganella and Proteus genera. It is found in hospital settings and is mainly associated with urinary tract infections (UTI).[1] *P. rettgeri* has also been reported to cause other infections, such as bacteremia, eye infections, meningitis, endocarditis, pneumonia, UTI, and diarrhea.[2,3] *P. rettgeri* is intrinsically resistant to colistin and tigecycline,[4] which can be transferred horizontally to *Escherichia coli,* and generally resistant to gentamycin, tobramycin, aminopenicillins, and first-generation cephalosporins.[3] It is known to harbor various virulence and resistance genes, which could easily be mobilized owing to their locations on mobile genetic elements (MGEs). These MGEs, specifically plasmids, integrons, insertion sequences (ISs), and transposons, contribute significantly to its pathogenicity and resistance.[3,5]

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Horizontal gene transfer (HGT) allows a significant genetic diversity to be amassed from other unrelated bacteria as opposed to vertical gene transfer.[6–8] It has been shown that HGT remains the most effective means of bacterial evolution, as it allows bacteria to rapidly acquire new genomic traits, such as virulence and resistance determinants with the help of MGEs.[9] This acquisition of genetic material can lead to immune suppression, colonization, host cell invasion, immune evasion, as well as other genetic factors involved in persistence and infection.[10] Host and pathogens repeated interactions with other microbes or the environment provide a platform for the exchange and acquisition of these MGEs that are known to influence microbial virulence and diseases.[11,12] To date, 24 *P. rettgeri* genomes from diverse sources and countries have been deposited in public databases (National Center for Biotechnology Information (NCBI)), including our PR002 strain. We report the draft genome sequence of a clinical multi-drug resistant (MDR) *P. rettgeri* PR002 strain isolated from a patient in South Africa in 2013, a strain harboring a diverse resistome and mobilome. We also show the phylogenomic relationship between all the deposited *P. rettgeri* genomes at the NCBI, facilitating the progress toward creating a typing scheme for this opportunistic pathogen, which is on a global rise.

The rarity and MDR nature of this pathogen, particularly in a clinical environment, make this case very concerning, as it is also intrinsically resistant to last-resort antibiotics, such as colistin and tigecycline. It further suggests the need for periodic monitoring for such emerging MDR pathogens.

Methods and materials

Ethical approval

The Faculty of Health Sciences Research Ethics Committee of the University of Pretoria approved this study under the reference number 109/2014.

Isolation of bacterial strains, culture conditions, and antibiotic susceptibility testing

P. rettgeri PR002 was isolated from the urine sample of a 46-year-old male patient in South Africa in 2013. The identification and susceptibility were performed on the Microscan® automated instrument (BD, San Jose, CA) and Vitek II (Biomerieux, Johannesburg, South Africa); broth microdilution was performed for only colistin. The MASTDISCS *Combi* tests (Mast Group, Johannesburg, South Africa) were used to phenotypically confirm ESBL and AmpC phenotype. The susceptibility breakpoints were interpreted using the CLSI version 7.1 (CLSI, 2018) and EUCAST (for Colistin and tigecycline) breakpoints.[13–15]

Genomic sequencing assembly and gene annotation

The whole genome sequencing was performed on the Ion Proton instrument (Thermo Fisher Scientific, Waltham, MA), with a 97× coverage.

Raw sequence reads of the isolates were adaptor- and quality-trimmed using Trimmomatic.[16] The raw reads were *de novo* assembled using the SPAdes assembler.[17] The resulting FASTA file was deposited at GenBank under the bioproject PRJNA355910 with accession number NXKD00000000. NCBI's Prokaryotic Genome Annotation Pipeline (PGAP)[18] and SEED subsystems in the RAST server (rapid annotation using subsystem technology)[19] were used to annotate this genome. The size, GC content, number of contigs, N50, L50, average coverage, as well as the number of RNAs, tRNAs, and protein-coding sequences obtained for each isolate, can be found in Table 1. The Tandem Repeat Finder[20] was used to predict repeats in the genome. The CRISPRFinder[21] was used to ascertain the number of CRISPR arrays in the draft genome. The NCBI average nucleotide identity (ANI) and the 16s rRNA–based species identification tool (SpeciesFinder (v1.2))[22] were used to confirm the species of the draft genome (PR002).

Resistome annotation, virulome predictions, and identification of MGEs/genetic support

Antimicrobial resistance genes of the strain were predicted using the GoSeqIt tool[23] and confirmed with the ResFinder program.[24] Identification of the genetic context of resistance was ascertained using PGAP and ISfinder (https://www-is.biotoul.fr/).[25] PlasmidFinder was used for the *in silico* detection of plasmid replicon types in the isolate.[26] Fluoroquinolone resistance mechanisms were further investigated by determining mutations in DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) using fluoroquinolone-susceptible *P. rettgeri* DSM 1131 (accession no. ACCI00000000) as the reference/wild-type strain to call single-nucleotide polymorphisms (SNPs) with tBLASTn.[27] The same process was followed to determine the presence of

Table 1. Genomic features of *P. rettgeri* PR002

Attribute	Value
Sequencing platform	Ion Proton and MinIon
Assembler	SPAdes and Unicycler
Assembly accession	NXKD00000000
Topology	Circular
No. of contigs	113 (from Ion Proton)
Genome size (bp)	4,832,624
DNA G + C (%)	40.7
Genome coverage (X)	97.0
Number of RNAs genes	62
Number of tRNAs genes	50
23S rRNAs	5
16S rRNAs	1
5S rRNAs	2
N50	189,689
L50	10
Number of subsystems	463
Number of CDSs	4560
Genes assigned to COGs	4273
Pseudo genes	287 (6.29%)
Confirmed CRISPRs	3
Tandem repeats	12,012 bp (0.25%)
Number of suspected plasmids	1–5
Number of phage sequences	6

colistin resistance-conferring mutations in *mgrB, pmrAB,* and *phoPQ*.

Integrons in the genomes predicted by PGAP and RAST subsystems were blasted on the INTEGRALL database to find the actual integrons[28] Novel integrons were sent for curation and naming by INTEGRALL curators. Prophage sequences in the genomes were checked for using PHAge Search Tool (PHAST), available at http://phast.wishartlab.com/.[29] PathogenFinder was also used for predicting the pathogenicity of the isolate toward humans.[30] The virulence factor database (VFDB) was further used to ascertain all the pathogenicity islands (PAIs) associated with the genome of the PR002 strain.[31] Homologues of other reported important virulence factors, which are vital for the survival of *P. rettgeri,* were searched by blasting assembled genomes to a pseudomolecule generated by concatenating a set of target genes using the NCBI in-house BLASTN tool.

To determine if the resistance genes and their associated MGEs were plasmid-borne or chromosomal, the contigs bearing these resistance genes were BLASTed on BLASTn to determine if the closest nucleotide homologies were chromosomal or plasmids.

Phylogenomic analysis of reported P. rettgeri *strains*

The whole genome sequences of all 24 available genomes at GenBank (Table S1, online only) were downloaded and run through Pasnp,[32] using the –c 1000 –C flag, to generate a core-genome SNP-based phylogenetic tree. The generated tree was viewed with Gingr[32] and edited with Figtree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The associated metadata (Table S1 and File S1, online only) of each genome was used to annotate the tree in Figtree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

General genomic characteristics of PR002

The *P. rettgeri* (PR002) genomic features are shown in Table 1. The size, GC content, number of contigs, N50, and L50 of the PR002 genome were 4.83 Mbp, 40.70%, 113, 189,689, and 10, respectively. Annotation with RAST and PGAP resulted in 4273 protein-coding genes, 287 (6.29%) hypothetical proteins, 62 RNAs, and 50 tRNAs. The CRISPRFinder predicted three clustered regularly interspaced short palindromic repeat (CRISPR) arrays on the nodes/contigs 16 and 23 (Table 1). The protein-coding genes (CDSs) and nonprotein-coding genes were assigned to 26 COG functional categories: amino acids and derivatives (429 genes), carbohydrates (342 genes), protein metabolism (252 genes), cofactors, vitamins, prosthetic groups, pigments (205 genes), membrane transport (180 genes), and DNA metabolism (162 genes) were the most abundant functional categories in the strain (Table S2, online only). Tandem repeats finder predicted 12 kbp repeats in 68 regions of the PR002 strain, making a total of 0.25% of the entire genome (Table 1). The PR002 strain was confirmed as a *P. rettgeri* strain with the 16S rRNA-based species identification tool, SpeciesFinder (v1.2) and ANI.

Antibiotic susceptibility profiling and resistome annotation

Antibiotic susceptibility testing showed aminoglycoside, β-lactam, quinolone/fluoroquinolone, rifampicin, sulphonamide, trimethoprim, tetracycline, and chloramphenicol resistance, which agreed with the resistome annotation predicted with GoSeqIt and ResFinder (Table 2). However, there

Table 2. Phenotypic antibiotic susceptibility and corresponding resistance genes of *P. rettgeri* PR002

Antibiotics (abbreviation)	MicroScan MIC (mg/L)	Vitek MIC (mg/L)	Broth microdilution MIC (mg/L)	Resistance gene(s)	Genomic location
Penicillins					
Ampicillin (AM)	>16 (R[a])	≥32 (R)	ND[b]	bla_{DHA-1}	Plasmid/ chromosomal
Ampicillin/sulbactam (A/S)	>16/8 (R)	ND	ND	bla_{SCO-1}	
Amoxicillin/clavulanate (AUG)	16/8 (R)	ND (susceptible on disk diffusion plate)	ND	bla_{PER-7} bla_{OXA-2}	
Piperacillin (PI)	>64 (R)	ND	ND	bla_{OXA-1}	
Piperacillin/tazobactam (P/T)	≥64 (R)	≤4 (S[c])	ND	bla_{OXA-10}	
Monobactams					
Aztreonam (AZT)	16 (R)	ND	ND		
Cephalosporins					
Cefepime (CPE)	>16 (R)	≤1 (S)	ND		
Cefotaxime (CFT) ESBL	>32 (R)	2 (R)	ND		
Cefotaxime/clavulanate (CFT/CA)	≤0.5 (S)	ND	ND		
Ceftazidime (CAZ) ESBL	16 (R)	16 (R)	ND		
Ceftazidime/clavulanate (CAZ/CA)	≤0.25 (S)	ND	ND		
Cefoxitin (CFX)	≤8 (S)	≤1 (S)	ND		
Cefuroxime axetil/sodium (CRM)	>16 (R)	8 (R)	ND		
Cephalothin (CF)	>16 (R)	ND	ND		
Carbapenems					
Doripenem (DOR)	≤1 (S)	ND	ND	ND	ND
Ertapenem (ETP)	≤0.5 (S)	0.5 (S)	ND		
Meropenem (MER)	≤1 (S)	≤0.25 (S)	ND		
Imipenem (IMP)	2 (S)	ND	ND		
Aminoglycosides					
Gentamicin (GM)	>8 (R)	≥16 (R)	ND	*aac(3)-IIa*	Plasmid
Tobramycin (TO)	>8 (R)	ND	ND	*aac(6′)Ib-cr*	
Amikacin (AK)	≤8 (S)	≤2 (S)	ND	*aadA1, aacA4cr*	
Quinolones					
Norfloxacin (NXN)	>1 (R)	ND	ND	*aac(6′)Ib-cr, aacA4cr*	Plasmid
Levofloxacin (LVX)	≤1(S)	ND	ND	*QnrB4*	Plasmid (*qnrB4*)
Ciprofloxacin (CP)	>2 (R)	2 (R)	ND	*GyrA*: S115I	Chromosome (*gyrA* and *parC*)
Nalidixic acid (NA)	>16 (R)	ND	ND	*ParC*: S84I, A621T, T682A	
Tetracyclines					
Tetracycline (TE)	>8 (R)	ND	ND	*tet(B)*	Plasmid
Minocycline (MIN)	>8 (R)	ND	ND	*tet(R)*	
[d]Tigecycline (TGC) (glycylcyclines)	≤1 (S)	2 (R)	ND	ND	ND
Sulfonamides					
Trimethoprim/sulfamethoxazole (T/S)	>4/76 (R)	≥320 (R)	ND	*sul1* and *sul2, dfrA1*	Plasmid
Polymyxins					
[d]Colistin (CL)	>4 (R)	≥16 (R)	≥64 (R)	*pmrB*: V234I, N280K	Chromosome
Phenicol					
Chloramphenicol (C)	>16 (R)	ND	ND	*catB3, cmiA1*	Plasmid
Fosfomycin					
Fosfomycin (FOS)	≤32 (S)	ND	ND	*FosA*	Chromosome

Continued

Table 2. ***Continued***

Antibiotics (abbreviation)	MicroScan MIC (mg/L)	Vitek MIC (mg/L)	Broth microdilution MIC (mg/L)	Resistance gene(s)	Genomic location
Rifamycins					
Rifampicin (RF)	ND	ND	ND	ARR-3	Chromosome
Other efflux pump systems					
ABC[e]	ND	ND	ND	*macA*	Chromosome
	ND	ND	ND	*macB*	
MFS[f]	ND	ND	ND	*MdtG*	
RND[g]	ND	ND	ND	*AcrB*	
	ND	ND	ND	*TolC*	
	ND	ND	ND	*MtrF*	

[a]Resistant.
[b]Not detected or undertaken.
[c]Susceptible.
[d]Providencia is intrinsically resistant to colistin and tigecycline.
AR associated with efflux pumps:
[e]macrolides;
[f]tetracyclines, ciprofloxacin (CP), levofloxacin (LVX), norfloxacin (NXN), and nalidixic acid (NA);
[g]tetracycline, chloramphenicol, ampicillin, rifampicin, ciprofloxacin (CP), levofloxacin (LVX), norfloxacin (NXN), and nalidixic acid (NA); ABC, ATP-binding cassette; MFS, major facilitator superfamily; RND, resistance–nodulation–division.

were discrepancies in the phenome and genome with regard to fosfomycin, amikacin, cefotaxime-clavulanate, and ceftazidime-clavulanate resistance in that the isolate was susceptible to these agents, while it harbored *fosA* and $bla_{\text{DHA-1}}$. Moreover, the isolate failed the disk-diffusion-based AmpC test but passed the ESBL test, which might suggest that $bla_{\text{DHA-1}}$ is silent (not expressed), making the isolate susceptible to cephalosporin–clavulanate combinations. The suggested nonexpression of $bla_{\text{DHA-1}}$ is further confirmed by PR002's susceptibility to cefoxitin, a second-generation cephamycin to which AmpCs are resistant.[33] The contig on which $bla_{\text{DHA-1}}$ is located has very close nucleotide homology to plasmids R16a, pPSI, suggesting that it could be plasmid-borne (Table 3).

Likewise, the *fosA* gene might be silent, making the isolate susceptible to fosfomycin, which is an important antibiotic indicated for UTI; notably, the patient was also having UTI, and the isolate was isolated from his urine. The phenome–genome correlation is, however, quite complicated with regard to amikacin, to which the isolate was susceptible, even in the presence of *aac(3)-IIa, aac(6′)Ib-cr, aadA1,* and *aacA4cr,* which confer resistance to aminoglycosides, such as gentamycin, streptomycin, amikacin, and tobramycin. While the isolate was resistant to other aminoglycosides, it was susceptible to amikacin, an observation that we fail to explain with the available data.

The diverse resistance mechanisms, such as β-lactamases, aminoglycoside acetyl transferases, chromosomal mutations, and efflux, involved in PR002 resistance indicate the various processes the organism express to survive antibiotics' effects (Table 2). The *P. rettgeri* PR002 genome harbored a total of 17 antibiotic resistance (AR) genes, most of which were plasmid-borne (Table 2), indicating the possibility of horizontal transfer of resistome between the PR002 and other species.

Mutations in GyrA (S115I) and ParC (S84I, A621T, and T682A) and plasmid-mediated quinolone resistance genes (*aac(6′)Ib-cr* and *QnrB4*) were implicated in fluoroquinolone resistance. Mutations in *mgrB, pmrA*, and *phoPQ* reported to confer resistance to colistin[4] were not found in the isolate, albeit *pmrB* mutations (V234I and N280K), which might be responsible for the high colistin MIC of >64 mg/L (Table 2), were present. Further complementation and gene expression studies will be required to substantiate the effect of the observed mutations on intrinsic colistin resistance in this species.[4] Suffice to say that the observed colistin resistance is not surprising owing to the intrinsic resistance to colistin in Proteeae.

Table 3. ISs and transposons found in the genetic environment of resistance genes found in PR002

Contig (length, bp)	Resistance gene/s (position on contig)	Mobile genetic element (MGE)	Genetic environment synteny (position on contig)	Closest plasmid/chromosomal nucleotide homology (species: accession number)
27 (52,288)	*Sul2* (3862..4677)	IS*91* family insertion sequence	IS*Vsa3*::*sul2*(3862..4677)	Plasmids pR16a (*E. coli*: KX156773.1), pPSI (*P. stuartii*: CP017055.1), pASP-a58 (*A. veronii*: CP014775.1), and AR_0156 (*P. mirabilis*: CP021853.1)
37 (16,016)	bla_{DHA-1} (1362..2501), *qnrB4* (6625..7269)	–	bla_{DHA-1}(1362..2501):qnrB4 (6625..7269)	Plasmids pR47-309 (*C. freundii*: CP040696.1) and pIMP26 (*E. cloacae*: MH399264.1)
39 (11,577)	bla_{SCO-1} (734..1600), *aac(3)-IIa* (10488..11348)	Tn*3* composite transposon	IS*15DII*(IS*6* transposase): TnEC1(resolvase): bla_{SCO-1} (734..1600):::TnEc1:::IS*Kpn12*(IS*5* transposase)::IS*Kpn11*(IS*3* transposase)::*aac(3)-IIa*(10488..11348)::IS*15DII*	Plasmids unnamed 1 (*E. coli*: CP042868.1), unnamed 1 (*E. coli*: CP042866.1), and P1 (*Klebsiella spp.*: CP037442.1)
43 (6759)	*Tet*(B) (4212..5417)	Composite transposon: IS*4* family	IS*Vsa5*::*tetR*:*tet(B)*(4212..5417) :*tet(C)*::IS*Vsa5*	Plasmids pR16 (*E. coli*: MK758104.1), pNUH15_ECL035 (*E. asburiae*: APO19388.1), and pCV839-06-p1 (CP025751.1)
46 (5011)	bla_{PER-7} (2983..3909), bla_{OXA-2} (4110..4937)	IS*91* family insertion sequence and class 1 integron	IS*Vsa3*:::bla_{PER-7} (2983..3909)::bla_{OXA}-2(4110..4937): *attC*(4077–4146)	Chromosome of *A. baumannii* 10CHAB005133 (CP026750.2) and TG22182 (CP039993.1); plasmid pA681-IMP (MF344570.1)
51 (3641)	*cmlA1* (260..1519), bla_{OXA-10} (1784..2584), *aadA1* (2601..3392), *qacE* (3516..3641)	–	*cmlA5*(260..1519):bla_{OXA-10} (1784..2584): *aadA1*(2601..3392):*qacE*(3516..3641)	Plasmids plasmid2 (*C. freundii*: LS992184.1), tig00000006 (*K. pneumoniae*: CP021961.1), and unitig_1_pilon (CP021958.1)
56 (1609)	*Aac(6′)-Ib-cr2* (22..540), bla_{OXA-1} (671..1501)	–	*Aac(6′)-Ib-cr2*(22..540): bla_{OXA-1} (671..1501)	Chromosomes of *K. pneumoniae* (LR596811.1), *P. vulgaris* (MH160822.1); plasmids pA1705-NDM (*K. pneumoniae*: MH909349.1), pIBAC_IncA/C (*C. freundii*: MH594477.1)
60 (1134)	*Sul1* (271..1110)	–	*QacE*:*sul1*(271..1110)	*E. coli* Plasmids pD8-1_2 (CP042951.1), pCTXM15_000200 (CP022227.3), and unnamed 3 (CP042870.1)
65 (819)	*catB3* (103..735)	–	*catB3*(103..735)	*Salmonella* Indiana plasmids p13520 (CP041182.1), p87912 (CP041180.1), and pSH16G4525 (MH522424.1)
78 (617)	*dfrA1* (74..547)	Class 1 integron	*dfrA1*(74..547):*attC*	Chromosome of *P. mirabilis* PmSC1111 (CP034090.1); *K. pneumoniae* plasmids p4_1_2.2 (CP023841.1) and pDA33140-96 (CP028553.2)
79 (606)	*Arr-3* (117..569)	–	*Arr-3*(117..569)	Plasmids pOXA58_010030 (*Acinetobacter defluvii*: CP029396.2), pCTXM27_020046 (*K. pneumoniae*: CP028782.2), and pCTXM15_020019 (*K. variicola*: CP028553.2)
93 (278)	*aacA4cr*	Class 1 integron	*qacE*(54–154):*aacA4*cr(155–278)	Plasmids pCTXM27_020046 (*K. pneumoniae*: CP028782.2), pCTXM15_020019 (*K. variicola*: CP028553.2), and pMG334 (*E. coli*: MK878894.1)

However, the *P. rettgeri* PR002 strain was susceptible to all the tested carbapenems. The six efflux pump systems known to be involved in drug resistance were also identified. They belonged to three main efflux systems namely: ABC, ATP-binding cassette; MFS, major facilitator superfamily; and RND, resistance–nodulation–division (Table 2). Although these efflux pumps might play important roles in PR002's AR, as has been reported in other Enterobacteriaceae, their expression levels will need to be ascertained to confirm this, as efflux pump hyperexpression, and not its mere presence, is implicated in resistance.[34]

Mobilome associated with PR002 strain

The A/C_2 plasmid replicase gene (100% homology to *Klebsiella pneumoniae* strain Kp7 plasmid pNDM-KN) was identified by the PlasmidFinder

Table 4. Class 1 integrons and gene-cassette structures in PR002

Integron number (contig)	Sequence size	*Int*I1	*attI1*	Array of gene cassettes	5′CS	CDS[a] of GC[b]	*attC*	3′CS
None[c] (78)	617 bp	Missing	1–60	$dfrA1t_{(56–567)}$-$attC_{(542–567)}$-(partial)	1–53	$dfrA1t_{(74–547)}$	$attC_{dfrA1\ (54–59;\ 542–567)}$ truncated	Missing
None (93)	278 bp	Missing	1–60	$qacE^{101}{}_{(54–154)}$-$aacA4cr_{(155–278)}$-(partial)	1–53	$aacA4cr_{(164–278)}$ partial	$attC_{aacA4cr(155–160)}$ partial	Missing
None (64)	936 bp	$IntI1_{P32\_H39}$ 139–936 (partial)	1–58	Missing	3–936	Missing	Missing	Missing
1843 (46)	5011 bp	Missing	1–60	$bla_{OXA-2(4083–4958)}$-$attC_{(4077–4146)}$-(partial)	4959–5011	$bla_{OXA-2(4110–4937)}$	$attC_{OXA-2(4083–4146;\ 4953–4958)}$	Missing

[a]Coding sequence.
[b]Gene cassette.
[c]Where the integron sequences were fragmented during the sequencing and assembling process, it was impossible to get the full sequence and determine the integron number. In this case, it is denoted as *none*.

v1.3 database, with a length of 417 bp (File S1, online only). The A/C_2 plasmid is a broad-host range plasmid that is able to reside in several species.[8] It is notable for usually harboring integrons with the theta replicon and for having large sizes with lower copy numbers. Their association with β-lactamases, such as the bla_{NDM}, has described in *K. pneumoniae, E. coli,* and *Providencia stuartii* isolates from countries as diverse as India, Canada, Kenya, New Zealand, Australia, and the United States.[35–37] Although the contig bearing the A/C_2 replicase gene did not harbor the class 1 integrase and associated gene cassettes, we would not be surprised if further genomic sequencing proves this to be the case. Notably, A/C plasmids have not been reported in *P. rettgeri* as far as we are aware, although it has been previously described in *P. stuartii* in Tunisia, Mexico, and other countries.[1,38] Particularly, the ability of this plasmid group to mobilize many ARGs and transfer them between different bacterial species, including commensals and pathogens, and from animals to humans makes this finding worrying.[39] We thus suggest that further studies be undertaken in South Africa to trace the epidemiology of this plasmid and its association with ARGs.

Class 1 integrons have been associated with multiple classes of antimicrobial resistance genes, including aminoglycosides, quinolones, and β-lactams worldwide.[40] Four class 1 integrons were identified on four different contigs in PR002 (Table 4). However, the full complement of these integrons and gene cassettes could not be ascertained as they were truncated during the assembly process into different contigs; the class 1 integron 797 bp *IntI1* integrase, on NODE_64, had no associated resistance genes. *dfrA1* and *aacA4cr* were also found on uncharacterized class 1 integrons on contigs 78 and 93, respectively. A new integron, *In*1843, was found on contig 46 harboring bla_{OXA-2}; this integron was associated with an IS*91* (IS*Vsa3*) IS (Table 3). The relationship between the *IntI1* integrases in PR002 and other closely related *IntI1* integrases in diverse species was shown as a phylogenetic tree by the RAST SEEDVIEWER (Fig. 1). Interestingly, the *Intl1* of the PR002 strain was similar the one found in *Salmonella enterica* subsp. enterica serovar Choleraesuis str. SC-B67 and *Corynebacterium diphtheriae* NCTC 13129 with a 100% identity as depicted by the bootstrap values (100) (Fig. 1). This indicates a possible sharing of plasmids, specifically the A/C_2 plasmid, bearing the *IntI1* integrase and other potential resistance gene cassettes between species. Unfortunately, all the integrase genes were not full/complete in the respective contigs (Table 4), making it difficult to determine the full functionality of the integrase genes.

Sul2, bla_{SCO-1}, *tet(B),* bla_{PER-7}, and bla_{OXA-2} were found in close synteny with ISs and transposons (Table 3), with IS*Vsa3*:bla_{PER-7}::bla_{OXA-2}:*attC* having both an IS and an integron bracketing it. The PR002 genome was also found to contain six prophage sequences/appendanges totaling 373.3 kb and accounting for 7.7% of the genome. The prophage regions contained four intact, one incomplete, and one questionable prophage (Fig. S1, online only). The ability of bacteriophages to package and transfer bacterial genomes has enabled the evolution and adaptation of bacterial species to different environments. The conduction process,

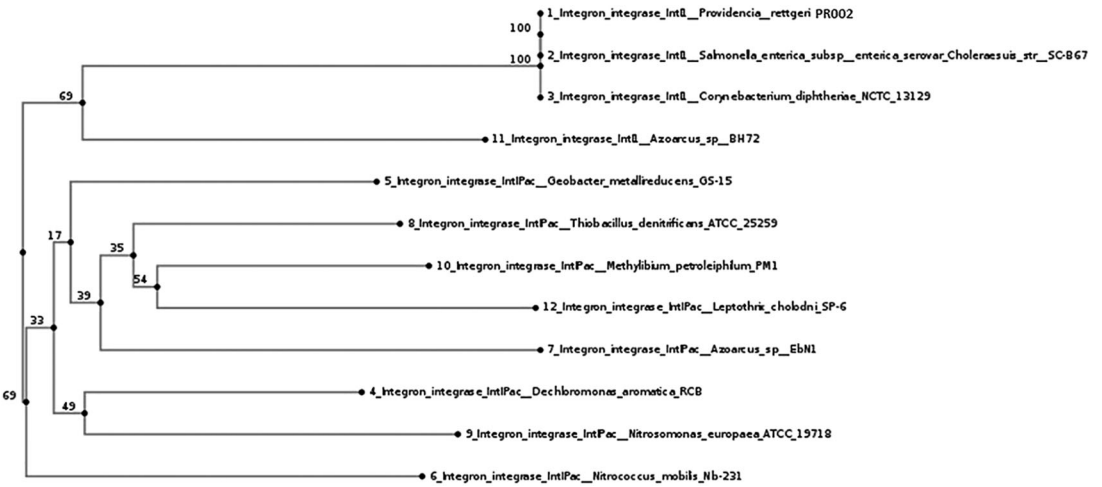

Figure 1. **A phylogenetic tree showing the association between *IntI1* of *P. rettgeri* PR002 and other predicted closely related *IntI1* integrases in diverse species.**

therefore, leaves phage elements in the bacterial genome, as not all phage DNA are mobilized into the phage head during the lytic cycle.[41]

Pathogenicity and virulence-related genes

The Pathogenicity Island DataBase (PAIDB) predicted seven PAIs of which three were linked with virulence genes, two with resistance genes, and two with unknown functions (Table S3, online only). PR002 had a 0.69 probability of being pathogenic to humans and was found to match, with 34 pathogenic families in PathogenFinder 1.1 (data not shown). All 34 pathogenic families were members of the Enterobacteriaceae family, of which *S. enterica* subsp. enterica serovar Newport strain (Identity: 100%) was the organism with the highest pathogenic similarity.

In silico whole genome virulome analysis using the VFDB showed 171 virulence encoded pathogenesis-associated proteins from different sources. These virulence factors were predominantly made up of invasion flagella (*flhC, flhA,flhD flgI,flgG, flgE, flgB, flgR, flgF, fliC, fliN, fliI, fliR, fliG, fliZ, fliP, fliA, fliM, fliE*), and Types III (*vcrD, spa24, yscV/IcrD, mxiA, ssaV, spaP, cdsV, pcrD, escR, escV, mxiK, invA*), IV (*mavN*), and VI (*clpB/vasG, vipB/mglB, Hcp-2, vsaE, vgrG-2*) secretion systems, adherence endotoxin Type IV pili (*pilF*), endotoxin (*rfaE*), adherence heat shock protein (Hsp60-htpB), stress protein (katA), quorum sensing system (luxS), serum resistance (OmpA), O-antigen (gmd), hyaluronic acid capsule (hasB), magnesium, and iron uptake system (mgtB and ccmC) (Table S4, online only). The invasion flagella are required for motility and efficient cellular macrophage invasion.[42] The type III secretion system (T3SS) employs a multifaceted mechanism to cause the cytotoxicity of host cells, involving the induction of autophagy, cell rounding, and cell lysis.[43]

The endotoxin promotes evasion of antigen-specific host immune defenses and allows colonization of different host microenvironments.[44] The adherence heat shock protein mediates a complement-independent attachment to mammalian and amoebal host cells.[45] The stress protein detoxifies H_2O_2 and protects against reactive oxygen species, while the quorum sensing system facilitates bacterial interspecies communications and biofilm formation.[46,47] All these virulence factors with high-degree similarity to those from *Vibrio cholerae, Legionella pneumophila, Haemophilus influenzae, Yersinia enterocolitica, S. enterica, Streptococcus pyogenes,* and *Neisseria* suggest they were possibly obtained horizontally. These further strengthen the call for increased studies into *P. rettgeri* as a rising human pathogen. It also shows the competency of *P. rettgeri* in receiving genetic elements from different bacterial species and strains.

The presence of several resistance and virulence genes might affect the fitness of the bacteria such that some of the genes might not be expressed or the bacteria might be physiologically weakened.[48]

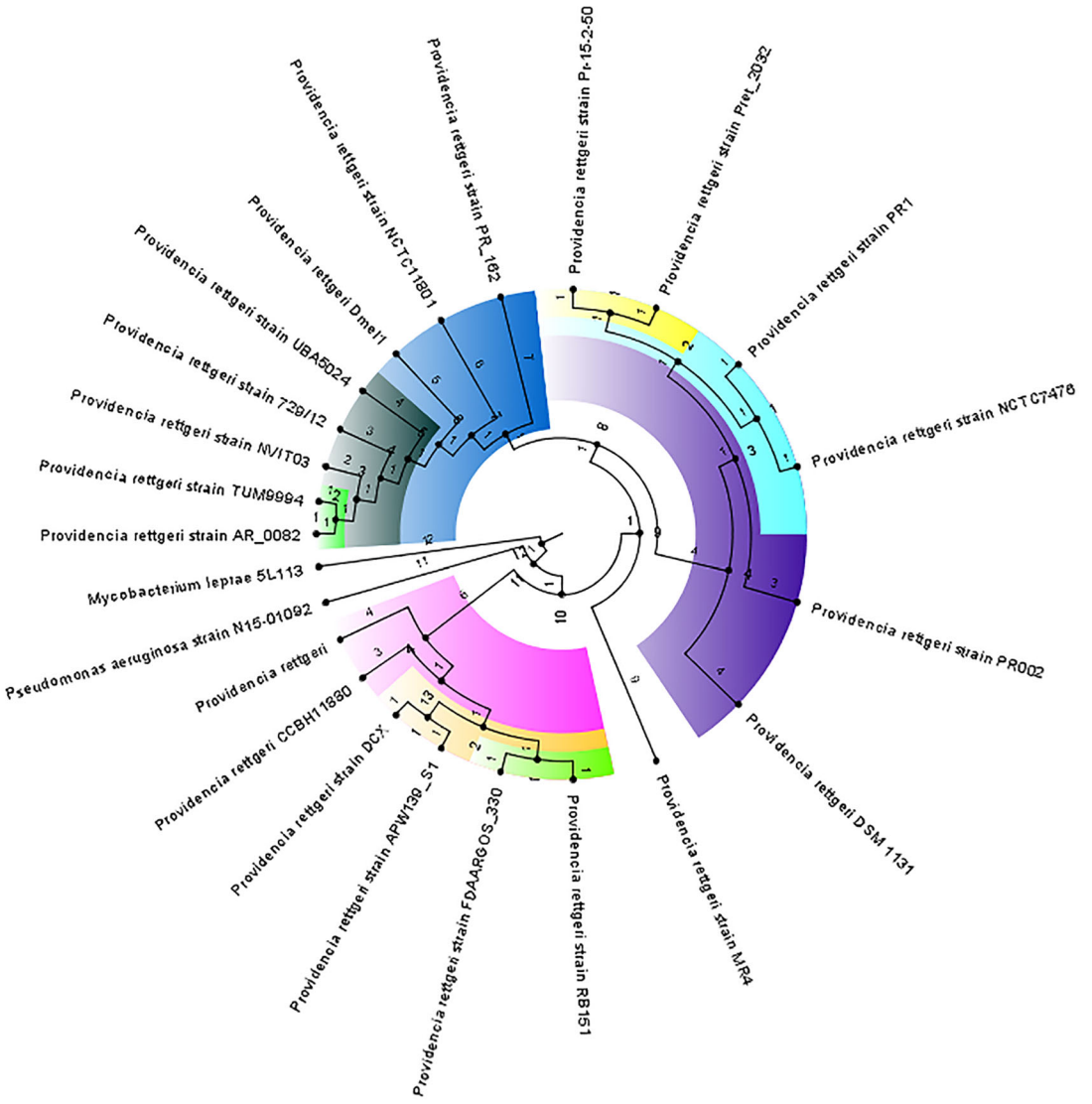

Figure 2. **Phylogenomic tree showing the relationships between PR002 and other international *P. rettgeri* strains.**

Although outside the scope of this work, it will be interesting to know the fitness cost and expression levels of all these genes to the PR002 strain.

Diversity of metal resistance genetic determinant from PR002 genome

The RAST server predicted 23 different genetic determinants involved in resistance to six metals: copper (Cu), cobalt (Co), Zinc (Zn), cadmium (Cd), mercury (Hg), and arsenic (As) (Table S5, online only). Interestingly, copper had the highest number of resistance determinants ($n = 13$ genes). Arsenic had only one resistance gene (*ArsD*). Resistance to arsenic has been described in contaminated soil and has been found to be plasmid mediated.[49] Cadmium resistance genes identified in this isolate have also been described in *Klebsiella* and *Pseudomonas* isolates from hospital effluent in Algeria.[50] Furthermore, the genetic determinants in Co-Zn-Cd resistance were the same, with efflux pumps being primarily involved in their resistance (Table S5, online only). This coresistance is often described in isolates that are also coresistant to antimicrobials, suggesting HGT of multiple genes

through MGEs.[50] The genetic linkage of heavy metals with resistance genes has been reported in the literature.[51] Mercury has been shown to have a genetic linkage with antimicrobial resistance,[52] indicating that the selection for resistance genes is favored by coselection to heavy metals in bacteria.[53]

Resistance to heavy metals in Gram-negative bacteria has been mainly reported in environmental isolates,[19] which makes these findings significant and revealing as this strain was obtained from the urine of a patient and not from the hospital environment. However, there is a possibility that this was a hospital environmental strain that subsequently infected the patient during his stay at the hospital.

Global phylogenomic analysis of reported P. rettgeri *strains*

The global relationship and epidemiological distribution of all deposited *P. rettgeri* genomes on GenBank are depicted in Figure 2, in which isolates of the same clone and clade are highlighted in the same color for easy identification. Even though the isolates were from different geographical sources or countries, continents, clinical, and environmental samples (Table S1 and File S1, online only), some were very closely related (as in belonging to the same clade). This suggests the possible international spread and subsequent evolution of certain *P. rettgeri* strains/clones and clades. However, the PR002 strain was obtained locally as evinced on the phylogeny tree. The strains also clustered according to the β-lactamase enzyme as strains harboring the pathogenic NDM-1 were clustered differently from those with other β-lactamases (IMP-4 and DHA-1), as well as isolates with no β-lactamases. Of note, the human NDM-1 containing strains (RB151, CCBH11880, and H1736) clustered differently from the human strains (PR1 and PR002) harboring other β-lactamase enzymes (IMP-27, OXA-10/2/1, and so on). Interestingly, strains from nonhuman hosts, that is, animals, water, fomite, and plants, were nonpathogenic and did not harbor any β-lactamase enzyme, ruling out the possibility of them acquiring β-lactamases from these sources (Table S1, online only). Although the strains did not cluster according to continents, two human *P. rettgeri* isolates from South America harbored NDM-1 (Fig. 2).

Conclusions

The pathogenomics of MDR *P. rettgeri* PR002 strain reveals the significant mobilome of this pathogen, a finding similar to that of a sequenced Israeli clinical isolate reported in the literature.[5] Many resistance genes of this pathogen were associated with MGEs, viz., plasmids, integrons, and ISs.[9] The repertoire of virulence and resistance genes, as well as of MGEs, shows the potential, although not yet substantiated, acquisition of diverse genetic material from different bacteria, such as *S. enteritica* var Cholerasuis and *C. diphtheriae*. Bacterial evolution and adaption are accelerated by horizontal genes acquisition from other species or strains through plasmids and integrative conjugative elements.[54,55]

This is the first report on the pathogenomics of a MDR *P. rettgeri* clinical strain isolated from Africa and the first report of an A/C_2 plasmid group in a *P. rettgeri* isolate. It offers valuable insights into the resistome, virulome, and pathogenicity of *P. rettgeri*. PR002 shows a large repertoire of antimicrobial resistance, pathogenic, and virulence genes, which could have been attained vertically and/or by HGT (mediated by MGEs), enabling it to evolve into an effective clinical pathogen.

Availability of data and materials

The draft whole-genome sequences of strain *Providencia rettgeri* PR002 have been deposited at NCBI/GenBank under accession number NXKD00000000. The version described in this article is the first version NXKD01000000. The datasets supporting the conclusions of this article are included within the article and its additional file(s).

Author contributions

N.M. contributed in the execution, interpretation, analysis, and write-up of the article; J.O.S. helped in the interpretation, data analysis, bioinformatics of MGEs (resistance genes environment), phylogenomics, write-up, editing, and formatting of the manuscript; D.G.A. was involved in the interpretation, data analysis, and write up of the article; N.E.M. performed data analysis, interpretation, and write-up of the article; and S.E. and C.F. conceptualized the study and design.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. Prophages of *P. rettgeri* PR002 were identified using the PHAge Search Tool (PHAST). PR002 contained four intact prophages (red color), one incomplete prophage (gray color), and one questionable prophage (green color).

Table S1. Metadata (biosample data) of all sequenced *P. rettgeri* isolates from NCBI GenBank database

Table S2. Genes associated with general COG functional categories in the genome of *P. rettgeri* PR002.

Table S3. Pathogenicity islands (PAIs) predicted for PR002 from VFDB (http://www.paidb.re.kr/about_paidb.php).

Table S5. Diversity of metal resistance gene determinants found in PR002 genome.

File S1. Metadata of isolates used in phylogenomics.

Competing interests

The authors declare no competing interests.

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