Genomic Plasticity of Multidrug-Resistant NDM-1 Positive Clinical Isolate of *Providencia rettgeri*

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Abstract

We performed a detailed whole-genome sequence analysis of *Providencia rettgeri* H1736, a multidrug-resistant clinical pathogen isolated in Israel in 2011. The objective was to describe the genomic flexibility of this bacterium that has greatly contributed to its pathogenicity. The genome has a chromosome size of 4,609,352 bp with 40.22% GC content. Five plasmids were predicted, as well as other mobile genetic elements (MGEs) including phages, genomic islands, and integrative and conjugative elements. The resistome consisted of a total of 27 different antibiotic resistance genes including bla_{NDM-1}, mostly located on MGEs. Phenotypically, the bacteria displayed resistance to a total of ten different antimicrobial classes. Various features such as metabolic operons (including a novel carbapenem biosynthesis operon) and virulence genes were also borne on the MGEs, making *P. rettgeri* H1736 significantly different from other *P. rettgeri* isolates. A large quantity of the genetic diversity that exists in *P. rettgeri* H1736 was due to extensive horizontal gene transfer events, leading to an enormous presence of MGEs in its genome. Most of these changes contributed toward the pathogenic evolution of this bacterium.

Key words: comparative genomics, virulence, antibiotic resistance, carbapenem biosynthesis, pathogenicity..

Introduction

Providencia rettgeri is a pathogenic bacterium that belongs to *Proteeae* bacteria, which also includes *Proteus* and *Morganella* genera. *Providencia rettgeri* causes a variety of infections, especially catheter-related urinary tract infections among others (Tada et al. 2014). *Providencia rettgeri* is known to harbor various virulence and antibiotic resistance (AR) genes (Gefen-Halevi et al. 2013). Most of these genes have contributed significantly to its pathogenicity; they could be easily mobilized due to their locations on mobile genetic elements (MGEs).

A significant amount of bacterial genetic diversity is amassed from other unrelated bacteria through horizontal gene transfer (HGT) (Rodríguez-Blanco et al. 2012; Diene et al. 2013). HGT has remained the key driver of bacterial evolution by allowing bacteria to rapidly acquire intricate new traits such as virulence and AR with the help of MGEs. Pathogens' repeated encounters with other microbes in their host or in the environment provide a platform for the exchange and acquisition of these MGEs (Jackson et al. 2011). These biological processes are known to contribute to virulence and disease (Burrus and Waldor 2004; Rodríguez-Blanco et al. 2012).

The aim of this study was to describe the genetic flexibility of a clinical multidrug-resistant (MDR) *P. rettgeri* H1736 isolate.

Materials and Methods

Bacterial Strain

Providencia rettgeri H1736 was isolated from the rectal swab of 74-year-old man in May 2011 in Israel. He presented with uncontrolled diabetes, hypertension, and an indwelling urinary catheter (Lachish et al. 2012).

Genome Sequencing and Assembly of *P. rettgeri* H1736

Genome sequencing of *P. rettgeri* H1736 was done with both a 454-Titanium instrument (454 Life Sciences, Branford, CT) and SOLiD (Applied Biosystems, Foster City, CA) and

assembled as previously described (Olaitan et al. 2014). Genome annotation was performed using the MicroScope platform available on www.genoscope.cns.fr (last accessed April 8, 2015).

Analysis of AR Genes, Mobilomes, Regions of Genomic Plasticity, and Virulence-Associated Genes

AR-encoding genes were analyzed using ARG-ANNOT database (Gupta et al. 2014). Regions of genomic plasticity (RGPs) and pan-genome computation were done using the genoscope pipeline (www.genoscope.cns.fr). Comparative analysis of *P. rettgeri* H1736 was done with *P. rettgeri* Dmel1 and DSM 1131 genomes retrieved from GenBank. Other genomic analyses were performed using other bioinformatic tools.

Results

Genome Features

The draft genome of *P. rettgeri* H1736 has a genome size of 4,609,352 bp (excluding the plasmidic sequence), 40.22% GC content with 4,525 predicted coding sequences (CDSs). We predicted the bacterium harbored at least five different plasmids. The whole-genome sequence of *P. rettgeri* H1736 has been deposited in the EMBL database (accession numbers: CVLT01000001–CVLT01000099).

Resistome

The *P. rettgeri* H1736 genome harbored a total of 27 AR genes; 19 (70.37%) on the chromosome and 8 (29.63%) on plasmids (table 1). Six efflux pump systems known to be involved in drug resistance were also identified (table 1). The plasmid-mediated quinolone-resistance gene *qnrD* was located on a small nontransmissible plasmid of 2,683 bp. The 48.5-kb region containing bla_{NDM-1} gene showed 100% identity to the same region of bla_{NDM-1} harboring plasmids which includes pPrY2001 from *P. rettgeri* (GenBank accession number: KF295828.1).

Mobilomes

In addition to the five predicted plasmids present in *P. rettgeri* H1736, there were eight prophage sequences, totaling 272 kb and accounting for 5.9% of the genome. Three integrative and conjugative element (ICE) regions were identified in the chromosome of H1736, each harboring one or more AR genes (table 2). A total of 33 insertion sequences were predicted in the H1736 genome. In contrast, 17 insertion sequences were predicted in *P. rettgeri* DMS 1131 and 7 in *P. rettgeri* Dmel1 (fig. 1).

Toxin–Antitoxin System

We identified eight toxin/antitoxin modules associated with various MGEs in H1736 genome (table 3). Intriguingly, the *mazF/mazE* was only present on another plasmid bearing bla_{NDM-1}, pPrY2001, from *P. rettgeri* 09ACRGNY2001.

Virulence

The genome contained a flagellar operon of 47 genes involved in flagellar formation and chemotaxis. Two copies of a urease gene cluster, *ureDABCEFG*, were found in the genome. *Providencia rettgeri* H1736 has two clusters of type 3 secretion systems (T3SS). One of these is similar to the singular T3SS present in *P. rettgeri* Dmel1 and DSM 1131, whereas the other was likely laterally acquired. Type 4 and type 6 secretion system-encoding genes were also identified in H1736.

Region of Genomic Plasticity

RGPs are regions of a genome structurally not present in related genome(s), which could harbor potentially horizontal transferred genes. A total of 47 RGPs were identified in *P. rettgeri* H1736, totaling 888,453 bp (19.28% of the genome). Features associated with these regions include phages, ICEs, fimbrial proteins, capsular polysaccharide synthesis genes, T3SS, an urease operon, a mannose operon (*aga* gene cluster), an arsenic operon (arsenic detoxification), AR genes, and a novel carbapenem biosynthesis operon.

The novel cluster of carbapenem biosynthesis genes comprised eight genes, *cpmA–cpmH* (fig. 2*a*), with a total size of 6,127 bp and GC% of 35.4. It displayed a nucleotide sequence similarity to the carbapenem biosynthesis operon found in *Photorhabdus luminescens* strain TT01. The operon was absent in the *P. rettgeri* DSM 1131 and Dmel1 genomes (fig. 2*b*).

Pan-Genome

The pan-genome of the three *P. rettgeri* (H1736, DSM 1131, and Dmel1) consists of 7,039 gene families. The core genome consisted of 2,658 gene families which were shared by the three genomes. Out of these, 834 were shared with *E. coli* K12 (after including the *E. coli* K12 genome in the analysis; fig. 3). *Providencia rettgeri* H1736 showed the highest percentage of strain-specific CDS (34.055%) in contrast to 21.045% and 25.263% displayed by Dmel1 and DSM 1131, respectively. When compared with Dmel1 and DSM 1131, 917 genes were obtained as specific genes present in H1736 (supplementary table S1, Supplementary Material online).

Discussion

The genome of the MDR clinical pathogen *P. rettgeri* H1736 reveals that this bacterium exhibits significant genomic variation. This indicates the plethora of changes the bacteria has undergone which has shaped its pathogenic nature. The contribution of MGEs, which are mostly mediated by HGT,

Table 1

Resistome of *Providencia rettgeri* H1736

Antibiotic Class	Resistance Gene	%Identity	Accession Number	Genomic Location
Aminoglycoside resistance: amikacin,	*aadA11*	100	AFV27505	Chromosome (ICE)
tobramycin, kanamycin, gentamicin	*aadB*	100	YP_009078767	Chromosome (ICE)
	aac(6′)-Ia	100	P10051	Chromosome (ICE)
	aphA-6	100	YP_005351836	Plasmid
	aadA1	100	YP_007878589	Chromosome (ICE)
	aadA	62	WP_004260670	Chromosome
	aac	65	Q52424	Chromosome
Beta-lactam resistance: amoxicillin, amoxi-	*blaOXA-10*	100	WP_020442392	Chromosome (ICE)
cillin–clavulanic acid, ticarcillin–clavulanic	*blaNDM-1*	100	YP_005351834	Plasmid
acid, cefalotine, cefoxitin, cefotaxime,	*blaPSE4*	100	WP_025441399	Chromosome (ICE)
ceftriaxone, ceftazidime, cefepime,	*ampH*	95	EKT58778	Chromosome
piperacillin, piperacillin–tazobactam,	*ampC*	84	EFE52534	Chromosome
imipenem				
Quinolone/fluoroquinolone resistance:	*qnrD*	100	YP_002504364	Plasmid
ofloxacin, ciprofloxacin	GyrA: S83I, D87E	—	—	Chromosome
	ParC: A56N, S57T, S80I			Chromosome
Macrolide resistance: erythromycin	*mel*[a]	100	NP_775053	Plasmid
	mph2	100	YP_003754029	Plasmid
	mph(A)	99	WP_001584719	Plasmid
Phenicol resistance	*catB2*	100	YP_009080018	Chromosome (ICE)
	catB8	100	AHY82877	Chromosome (ICE)
	cat3	71	EFE52240	Chromosome
Sulfonamide resistance: sulfamethoxazole	*sul1*	100	NP_052895	Plasmid
Trimethoprim resistance: trimethoprim	*dhfr*	100	AEH59665	Chromosome (ICE)
	dfrA1	100	YP_758710	Plasmid
Tetracycline resistance: tetracycline	*tetA*[a]	99	BAG66128	Chromosome (ICE)
	tetR	100	BAG66129	
Fosfomycin resistance: fosfomycin	*fosA*	82	WP_004263671	Chromosome
Streptothricin resistance	*sat-1*	100	ABQ52459	Chromosome (ICE)
Polymyxins[b]: colistin	*pmrAB*, *phoPQ*, *arnT* operon			Chromosome
Other efflux pump systems				
MFS[1]	*emrB*	99	WP_042848401	Chromosome
	emrA	100	WP_042848400	Chromosome
RND[2]	*acrB*	100	WP_042848167	Chromosome
	acrA	99	WP_042848165	Chromosome
	acrR	100	WP_042848164	Chromosome
RND[3]	*mdtA*	99	WP_042843526	Chromosome
	mdtB	99	WP_042843525	Chromosome
	mdtC	99	WP_042843524	Chromosome
ABC[4]	*macA*	100	WP_042846520	Chromosome
	macB	100	WP_042846519	Chromosome

NOTE.—AR associated with efflux pumps: [1]Nalidixic acid, [2]tetracycline, chloramphenicol, ampicillin, nalidixic acid, and rifampin, [3]novobiocin, [4]macrolides. RND, resistance–nodulation–division; MFS, major facilitator superfamily; ABC, ATP-binding cassette.

[a]Efflux pump.

[b]Intrinsic resistance; not counted.

Table 2

ICE-Like Elements Present in *Providencia rettgeri* H1736 Genome

ICE-Related Region	Best BLAST Hit	Accession No.	% ID	Associated AR Gene
1	ICEPdaSpa1: *Photobacterium damselae* subsp. *piscicida*	AJ870986	98	*aadA11, aac(6′)-Ia ,aadB, blaOXA-10,* catB8
2	*Escherichia coli* strain BEN374 AGI-5	GU725392	100	*aadA1, catB2, dfrA1, Sat-1*
3	*Alteromonas macleodii* str. Aegean Sea MED64	CP004848	98	*blaPSE4*

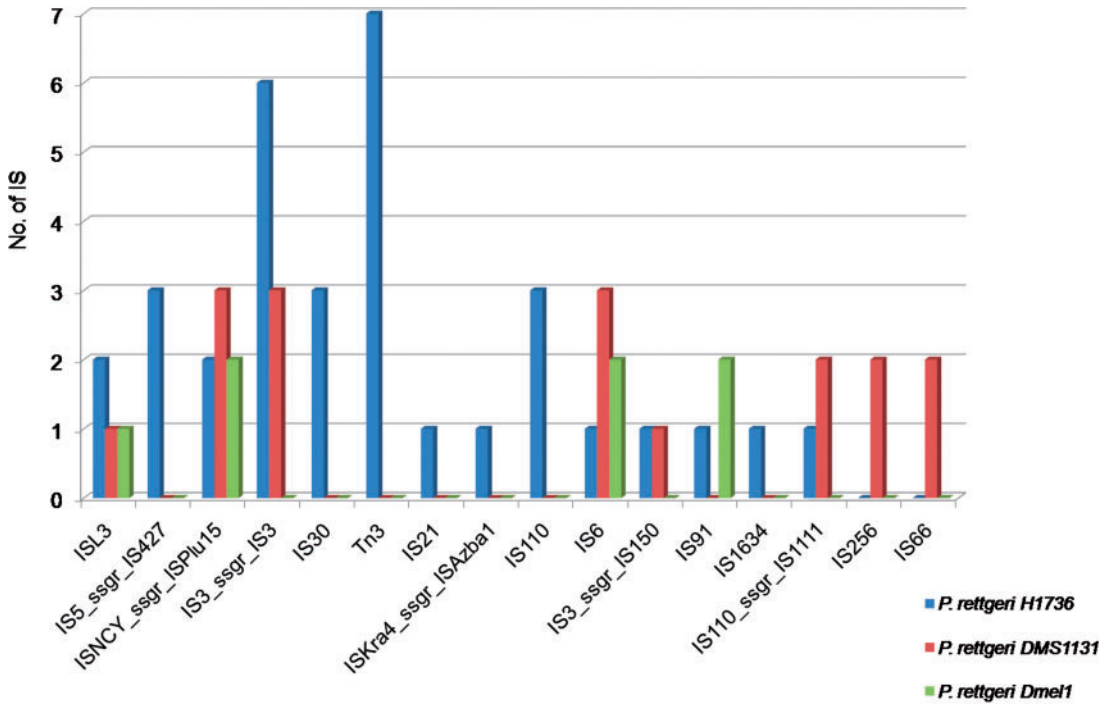

Fig. 1.—Comparison of the total insertion sequences found in *P. rettgeri* H1736 genome to that of *P. rettgeri* DSM 1131 and Dmel1 genomes.

Table 3

Biological Features of the Toxin and Antitoxin Systems Found in *Providencia rettgeri* H1736

TA_no.	T/A	Family	Length (aa)	Associated Features
TA_1	T	HipA	237	Associated with
	AT	HipB	83	ICE_2 region
TA_2	T	YhaV	154	—
	AT	PrlF	112	
TA_3	T	Doc	99	Complete prophage
	AT	Phd	59	within the flagella operon
TA_4	T	COG5654 like_domain	153	—
	AT	COG5642 like_domain	153	
TA_5	T	MosT	312	Associated with
	AT	MosA	277	ICE_1 region
TA_6	T	MazF	110	Plasmid
	AT	MazE	81	
TA_7	T	ParE	42	Plasmid
	AT	ParD	54	
TA_8[a]	T	CptA	136	—
	AT	CptB	88	
TA_9	T	RatA	144	—
	A	RatB	101	

Note.—T, toxin; AT, antitoxin.
[a]Type IV TA module.

to the "flexible gene pool" of bacterial pathogens, has been widely recognized (Jackson et al. 2011; Diene et al. 2013).

ICEs and plasmids are known to contribute to the genomic plasticity of bacteria (Burrus and Waldor 2004) and have both played a major role in the dissemination of AR genes. The sheer number of AR genes harbored by *P. rettgeri* H1736 was greatly influenced by these two MGEs (ICEs and plasmids). Both accounted for 70.37% (19/27) of the AR genes found in *P. rettgeri* H1736. The ICEs present in *P. rettgeri* H1736 shared similarities with other ICEs from other bacteria genera (table 2), indicating the promiscuity of *P. rettgeri* H1736.

Furthermore, plasmids have played a major role in the dissemination of AR genes, including bla_{NDM-1} (Poirel et al. 2011). The plasmid bearing bla_{NDM-1} in H1736 may be prominent in clinical strains of *P. rettgeri*. This is because of the high degree of genetic similarities and composition of this plasmid to pPrY2001 from *P. rettgeri* 09ACRGNY2001 (an NDM-1 positive isolate). The *qnrD* gene is widespread in *Proteeae*, carried on a nontransmissible plasmid and *Proteeae* are believed to be the reservoir of *qnrD* gene (Guillard et al. 2014).

Toxin and antitoxin modules are known to help in the maintenance and stabilization of MGEs. Other biological attributes of toxin/antitoxin modules include bacterial dormancy or death due to phage infection, persistence which could enhance drug-tolerant and biofilm formation; most of these play a role in bacterial pathogenicity (Wen et al. 2014). In fact, the number of toxin/antitoxin systems in pathogenic

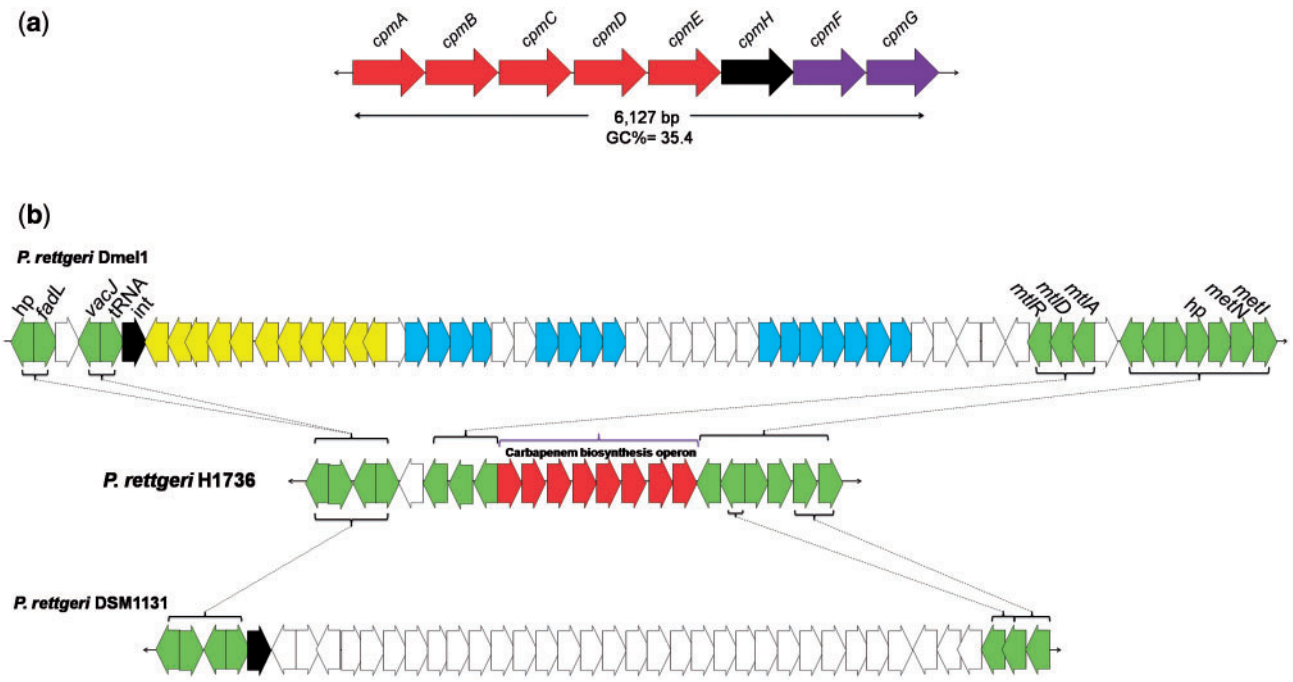

Fig. 2.—(*a*) Organization of the carbapenem biosynthesis gene cluster found in *P. rettgeri* H1736 genome. Genes involved in biosynthesis are depicted in red and those involved in intrinsic resistance mechanisms are depicted in purple, whereas the function of the gene in black is unknown. (*b*) Comparison of the genetic organization of the region where the carbapenem biosynthesis operon was found in *P. rettgeri* H1736 to other *P. rettgeri* genomes. Similar genes are depicted in green, phage-related genes in yellow, the integrase gene in black, genes involved in type 6 secretion systems in blue, and carbapenem biosynthesis genes in red, whereas dissimilar genes are shown in white.

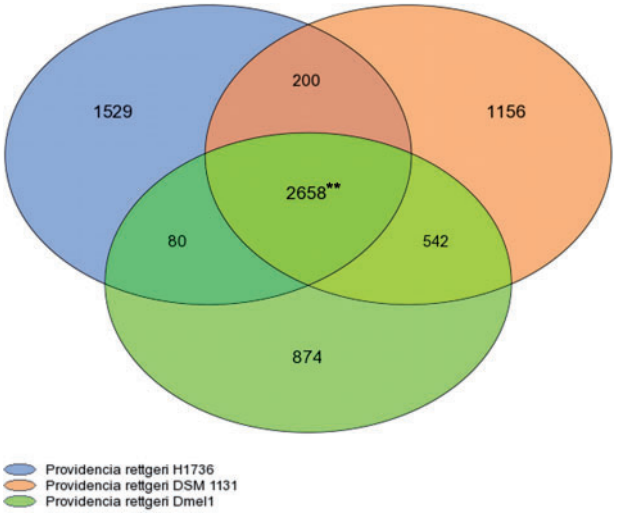

Fig. 3.—Venn diagram showing the numbers of orthologous genes in the genomes of *P. rettgeri* H1736, *P. rettgeri* DSM 1131, and *P. rettgeri* Dmel1. **Among the 2,658 gene families shared by the three *Providencia rettgeri* (core genes), 834 gene families were shared with the *E. coli* K12 genome, leaving 1,824 gene families as core genes specific for *P. rettgeri*.

bacteria has been correlated with the degree of virulence (Georgiades and Raoult 2011).

The abundant insertion sequences in H1736 could partly be attributed to its high HGT due to its abundant MGEs and may be needed for host adaptation. Insertion sequence elements can play a significant role in bacterial pathogenicity and evolution by turning on the expression of nearby genes such as genes for catabolic pathways and AR (Moran and Plague 2004; Jackson et al. 2011).

Finally, the operon for the biosynthesis of carbapenem antibiotic (1-carbapen-2-em-3-carboxylic acid) has been reported among a limited tiny subset of bacterial species: *Pectobacterium carotovorum* subsp. *carotovorum* ATCC39048, *Serratia* sp. strain ATCC39006, and *Ph. luminescens* strain TT01 (Parker et al. 1982; McGowan et al. 1996; Derzelle et al. 2002). Analysis showed that the operon was horizontally acquired. In *Ph. luminescens* strain TT01, the operon was suggested to be part of an MGE, and the synthesized antibiotic was found to be active against Gram-negative bacteria, especially Enterobacteriaceae (Derzelle et al. 2002). This is the first report of the presence of a carbapenem biosynthesis operon in an NDM-1-producing *P. rettgeri* clinical pathogen. This is very intriguing and detailed experimental studies are underway to fully characterize this operon.

Conclusion

The genomic analysis of MDR *P. rettgeri* H1736 fully revealed the enormous plasticity of this pathogen. We believed that the presence of various MGEs in this genome has significantly shaped and contributed to its virulence and pathogenicity owing to the bacterial sympatric lifestyle, this includes ability to resist numerous classes of antibiotics. It is very interesting that this pathogen, which is an NDM-1-producing bacterium, possesses an operon for the biosynthesis of carbapenem, which was acquired through HGT.

Supplementary Material

Supplementary table S1 is available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

Acknowledgments

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