Genomic Epidemiology of *Pseudomonas aeruginosa* Sequence Type 111

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Abstract

Purpose: *Pseudomonas aeruginosa* ST111 is a global multidrug resistant (MDR) high-risk clone and comprehensive data about its molecular epidemiology is limited in Canada. Comprehensive data about the evolution of ST111 clades is limited. We characterized a Canadian collection of ST111 causing bloodstream infections and investigated the genomic relationship between Canadian and global ST111.

Material and methods: We used long and short read WGS to characterize Canadian ST111 (n = 10 from 2010–18). We performed phylogenetic analysis on a global collection of ST111 (n = 969) and investigated the evolutionary history of clades using BEAST.

Results: ST111 belonged to 3 clades (A, B, C) and two subclades (C1, C2). ST111-A was the ancestral clade while clades B, C1 and C2 emerged during the 1700s and 1800s. ST111-C2 dominated the global ST111 population. Serotype switching from O4 to O12 and the acquisition of Tn*21*, *gyrA*_T83I, *parC*_S87L, In*59* with *bla*$_{\text{VIM-2}}$ and *aacA29* over time, were important in the evolution of ST111-C2. The Calgary ST111 strains consisted of a diverse collection that belonged to ST111-A (O4), ST111-C1 (O4) and ST111-C2 (O12) with different transposon structures.

Conclusions: We provided details on the emergence and evolution of different ST111 clades over time and highlighted the roles of serotype switching and the acquisition of certain AMR determinants and transposon structures in the evolution of ST111-C2.

Keywords: Genomic epidemiology; MDR P. aeruginosa clone; ST111

Introduction

The spread of antimicrobial resistance (AMR) genes within or between bacterial populations, is due to the persistance of certain successful global multidrug resistant (MDR) clones and/or the movement of AMR genes within and between diverse strains or lineages [1, 2]. Successful MDR clones (also known as high-risk clones) are not directly responsible for the movement of AMR genes, but they act as important "hoarders and spreaders" of AMR genes [3]. MDR global successful clones are found among various bacterial pathogens especially within *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and the *Enterobacterales* [4,5,6].

P. aeruginosa has a panmictic/heterogenous population structure that consists of certain high-risk clones among MDR populations (e.g., ST111, ST175, ST233, ST235, ST357) [7]. *P. aeruginosa* ST111 is linked to several carbapenemases (i.e., VIMs, IMPs, KPCs, GES, GIMs) and has been pivotal in the global spread of the metallo-β-lactamase VIM-2 [8]. This MDR high-risk clone has been responsible for several global nosocomial outbreaks since the mid-2000s [5].

Comprehensive data about the evolution of *P. aeruginosa* ST111 clades is limited [7]. We characterized a Canadian collection of ST111 and performed phylogenetic analysis on a global ST111 collection to investigate the genomic relationship between Canadian and global ST111.

Material and Methods

Overview of the Study

The study consisted of two parts: The first part used long and short read whole genome sequencing (WGS) to characterize a Canadian ST111 collection (n = 10) causing bloodstream infections [9] and compared this data to previously published ST111 strains (n = 35) from previous published studies (USA, France, South Korea, Germany, Taiwan, Australia, Poland, Costa Rica, Colombia, UK, Belgium, the Netherlands) that contained sufficient data to analyse different transposon structures [10,11,12]. The second part performed phylogenetic analysis on a global collection of GenBank ST111 sequences (n = 969) and then investigated the evolutionary history of this MDR clone over time using genomes with known dates of isolation (n = 952).

Bacterial Isolates

We included a Canadian clinical *P. aeruginosa* ST111 collection that consisted of unique blood isolates obtained from Calgary, Alberta, Canada (n = 10) during 2010 (n = 4), 2014 (n = 4) and 2018 (n = 2). The clinical, epidemiological and microbiology data were previously published [9].

Genomic Analysis

The ST111 isolates underwent short read WGS (Nextera XT DNA sample preparation kit, Illumina, San Diego, CA, USA) using procedures described previously [13,14,15]. Samples were multiplexed and sequenced on an Illumina NovaSeq for 300 cycles (151 bp paired end). We also performed long read WGS (MinION, Nanopore Technologies, Oxford, UK). Samples for

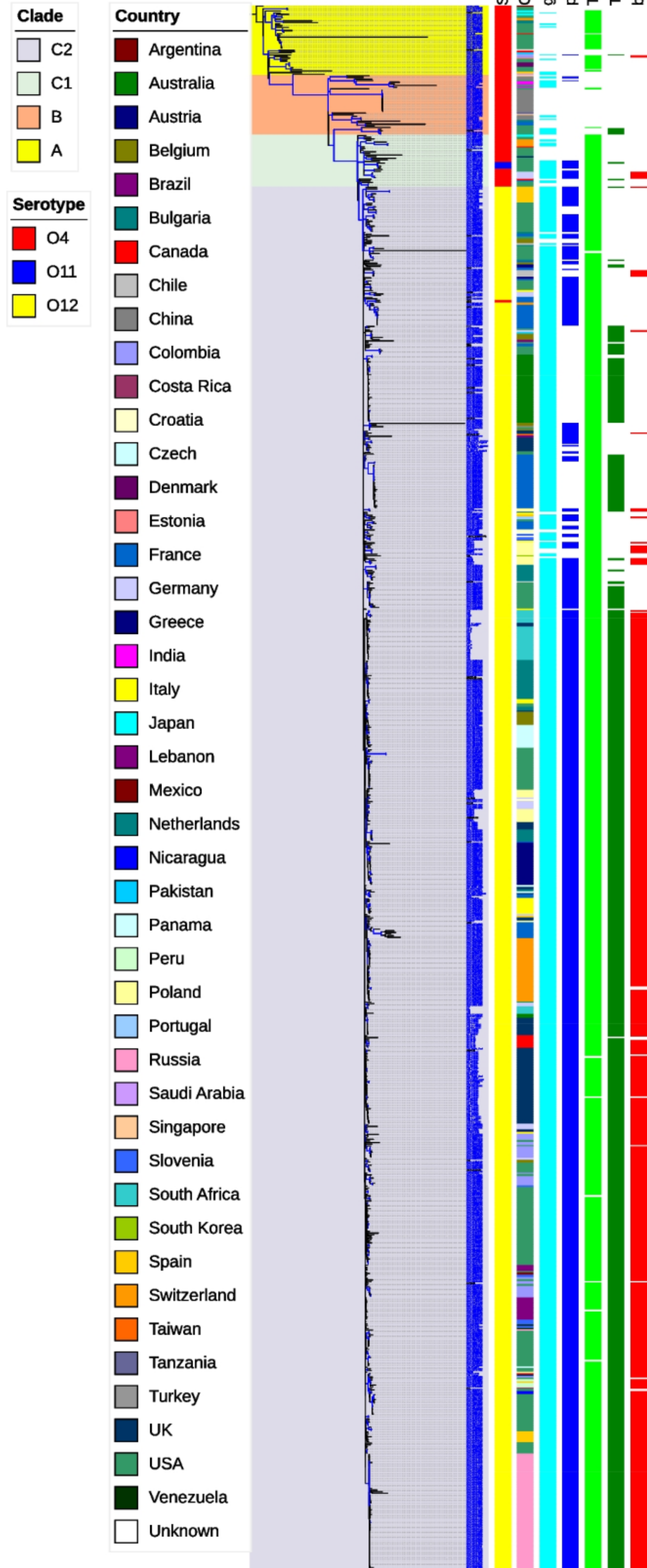
long read WGS were prepped were prepped using Lucigen Masterpure™ Complete DNA & RNA Purification Kit (LGC Biosearch Technologies, Teddington Middlesex, UK) and R9.4 flowcells and run on MinKNOW v5.0.0 with Rapid Barcoding Sequencing Kit (SQK-RBK004), ligation Sequencing Kit 1D (SQK-LSK108) and Native Barcoding Expansion kit (EXP-NBD104).

Basecalling was done by Guppy v.3.1.5 which is part of the MinKNOW software. Hybrid assembly was performed using Unicycler v0.4.9 [15].

AMR genes and chromosomal mutations associated with AMR were detected using AMRFinderPlus [16]. *P. aeruginosa* serotyper (PAst 1.0) was used to identify serotypes [17], and multilocus sequence typing was done in silico with https://pubmlst.org/organisms/pseudomonas-aeruginosa [18].

All *P. aeruginosa* genome assemblies from NCBI RefSeq database, dated as of 18 November 2024, were downloaded and a total of 35 with complete ST111. Additionally, ST111 draft genomes were also included for a total of 969 genomes (Supplementary data set) [10, 19,20,21]. Overall, the genomic data consisted of Calgary complete genomes (n = 10), global complete genomes (n = 35), South African draft genomes (n = 34), global draft genomes (n = 890). Sequences were obtained from the following countries: Australia, Argentina, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Czechia, Denmark, Estonia, France, Germany, Greece, India, Italy, Japan, Lebanon, Mexico, The Netherlands, Nicaragua, Pakistan, Poland, Portugal, Russia, Saudi Arabia, Singapore, Slovenia, Spain, South Africa, South Korea, Switzerland, Taiwan, Tanzania, UK, USA, and Venezuela. The South African isolates was a collection of different isolates obtained from several hospitals in the Gauteng region between 2017 and 2018. We created a core single-nucleotide polymorphism (SNP)–based phylogenetic tree and identified SNPs by mapping the reads or aligning the genomes against the reference strain FRD1 (GenBank Accession no. CP010555.1) using the RedDog pipeline (https://github.com/katholt/RedDog). We included recombination-free, core SNPs that were present in > 90% of genomes to create a maximum-likelihood tree using RAxML with the general time-reversible plus gamma substitution model [22]. Recombination sites and prophage regions were identified using Gubbins v3.3.0 [23] and PHASTER [24]. We visualized the tree by using iTOL version 6 (https://itol.embl.de/). To identify clades within ST111, we used the hierarchal Bayesian Analysis of Population structure clustering analysis [25] was conducted with 2 nested levels with the number of initial clades set at 20. We defined clades within ST111 by using the first level of clustering [14].

We used Bayesian inference (BEAST) to deduce the temporal phylogenetic signal and molecular evolution of ST111 over time by using genomes with known dates of isolation (n = 952). We used a recombination-corrected tree from Gubbins v3.3.0 [23] output and time of isolation as the inputs in BEAST v2.7.7 [26, 27]. We used general time-reversible substitution, a strict molecular clock, and a Bayesian skyline population model. Triplicate runs with 700 million iterations were used to ensure that the Markov chain Monte Carlo was run for long enough for convergence (the effective sample size for all parameters were > 200). We removed the first 10% of steps as burn-in and combined the three log files. A maximum clade credibility tree with median heights was generated using TreeAnnotator v2.7.7.



Tree scale: 0.01
Clade
C2
C1
B
A
Serotype
O4
O11
O12
Country
Argentina
Australia
Austria
Belgium
Brazil
Bulgaria
Canada
Chile
China
Colombia
Costa Rica
Croatia
Czech
Denmark
Estonia
France
Germany
Greece
India
Italy
Japan
Lebanon
Mexico
Netherlands
Nicaragua
Pakistan
Panama
Peru
Poland
Portugal
Russia
Saudi Arabia
Singapore
Slovenia
South Africa
South Korea
Spain
Switzerland
Taiwan
Tanzania
Turkey
UK
USA
Venezuela
Unknown
Serotype
Country
gyrA_T83I
parC_S87L
Tn4661
Tn21
blaVIM-2

Fig. 1. Phylogenetic analysis of publicly available global *Pseudomonas aeruginosa* ST111 genomes (n = 969). This maximum-likelihood phylogram is based on a total of 10,165 core SNPs. The tree is rooted by using clade A isolate AZPAE14832. Branches that had > 90% bootstrap support from 100 replicates were highlighted in blue. Complete genomes and genomes assembled with long read sequencer were marked in blue

Results

Global P. aeruginosa ST111 with complete (n = 45) and draft genomes (n = 924) belonged to three clades (e.g., A, B, C,) and two subclades (e.g., C1, C2). Core SNP phylogenetic analysis of publicly available genomes divided ST111 into three clades and two subclades namely, ST111-A (n = 43), ST111-B (n = 37), ST111-C1 (n = 32) and ST111-C2 (n = 857), (Fig. 1, Supplemental Table 1).

Some AMR determinants were found over 90% of ST111 strains and included $bla_{\text{OXA-395}}$, *gyrA*_T83I, *aph(3')-llb, catB7*, *crpP* and *fosA* (Supplemental Table 1). The chromosomal class C β-lactamase, named PDC, were found in all ST111 isolates: PDC-1 was limited to clades A while PDC-3 was found in ST111-B, ST111-C1, ST111-C2 (Supplemental Table 1). Certain AMR determinants clustered within ST111-C2 that include *parC_S87L, aac(6')–29,* $bla_{\text{VIM-2}}$ and *sul1* (Supplemental Table 1).

All the clade A, B strains and > 80% of ST111-C1 (28/32) contained the O4 serotype while all subclade C2 (except for 2 isolates) carried the O12 serotype (Fig. 1, Supplemental Table 1). The *gyrA*_T83I mutation was less common in ST111-A (7/43 [16%]), ST111-B (12/37 [32%]) and more common in ST131-C1 (16/32 [50%]) and ST111-C2 (843/857 [98%]) (Fig. 1, Supplemental Table 1). Of interest, a different quinolone resistant determining region (QRDR) mutation namely *parC*_S87L was rare in ST111-A (1/43 [2%]), ST111-B (2/37[5%]) and ST131-C1 (10/32 [31%]) but more frequent in ST111-C2 (726/857 [85%]) (Fig. 1, Supplemental Table 1). The VIM-2 gene was absent among ST111-B, rare in ST111-A (1/43 [2%]) and ST111-C1 (4/32 [13%]) but numerous in ST111-C2 (604/857 [70%]) (Fig. 1, Supplemental Table 1).

P. aeruginosa ST111 isolates with complete genomes (n = 45) contained various Tn4661 transposon structures (Fig. 2, Supplemental Figure 1).

- One genome (PA-18–54 from Canada) lacked Tn4661, Tn21 or Tn402-like structures (Fig. 1).
- One genome (no F30658 from the USA) contained Tn21-like structure (ΔTn21(*tnpARM*)-class I integron[*aac(3)-Ia-aac(6')-II*]-*orf5*-ΔTn21(*urf2-merEDACPTR*) but lacked Tn4661 and Tn402 (Fig. 2).
- One genome (no RW109, country of origin not stated) contained partial Tn4661 with Tn402 tniA and intI inserted separately (Fig. 2).
- Fifteen genomes contained Tn4661 but lacked Tn21-like or Tn402 structures. This structure was named Tn4661_structure-A and was inserted into chromosomal gene PA2229 of PAO1 (Fig. 2 and Supplemental Figure 1). One genome (no 2881 from France) contained two copies of Tn4661_structure-A: 1 copy was inserted in was inserted into chromosomal gene PA2229 while another copy was harboured on an untypable 142kb plasmid (Fig. 2).

- Nine genomes contained composite transposons with Tn4461, Tn21-like and Tn402 structures that were also inserted in chromosomal gene PA2229 of PAO1 and possessed gene cassettes other than *bla*$_{VIM-2,}$ and *aac(6')–29*. These structures were named Tn4661_structure-B (Fig. 2 and Supplemental Figure 1). Two Tn4661_structure-B variants were identified namely Tn4661_structure-B1 (n = 5) and Tn4661_structure-B2 (n = 4). Tn4661_structure-B1 contained *bla*$_{\text{IMP-4}}$-*qacG2-aac(6')-Ib* gene cassettes while Tn4661_structure-B2 contained *aac(6')-Ib3-bla*$_{\text{OXA-9}}$-*cmIB* gene cassettes (Fig. 2 and Supplemental Figure 1).
- Thirteen genomes contained the VIM-2 gene that was situated within *In*59 and inserted within Tn402 that formed part of Tn21-like transposon. This structure was named Tn4661_structure-C (Fig. 2 and Supplemental Figure 1). Tn21-like was flanked by two copies of Tn4661 as described before by Molina-Mora and colleagues [28]. This composite transposon structure was inserted within the chromosomal gene PA2229 of PAO1 [28] [Fig. 2 and Supplemental Figure 1].
- Five Tn4661_structure-C variants were identified (Fig. 2): i) Tn4661_ structure-C1 (isolate PA-18–66 from Canada) ii) Tn4661_structure-C2 (isolate Carb01-63 from Netherlands), Tn4661_structure-C3 (isolate NMI5500/10 from Poland), Tn4661_structure-C4 (isolate 613.16 from Germany) and Tn4661_structure-C5 (isolate 2879 from France).
- One genome (2024CK-00032 from the USA) contained two different Tn4661 transposon structures namely Tn4661_structure-A that was inserted in PA2229 of PAO1 and Tn4661_structure-C that was harboured on an untypable 159kb-plasmid (Fig. 2).
- **Fig. 2**

P. aeruginosa ST111 clades evolved during the 1700 and 1800s. BEAST estimated that the most recent common ancestor (MRCA) of ST111 appeared in 1661 [95% confidence interval [CI], 1608 to 1717) (Fig. 3). Clade A evolved from this ST111 MRCA in 1749 (95%CI, 1703 to 1793) while clade B diverged from ST111-A in 1757 (95%CI, 1721–1794]) (Fig. 3). ST111-C1 emerged from the ST111-B in 1863 (95%CI, 1863 to 1849) while ST111-C2 evolved from ST111-C1 around in 1874 (95%CI, 1861 to 1855) (Fig. 3). In59 that contained *bla*$_{\text{VIM-2}}$ became established among ST111-C2 strains from the 1960s onwards (Fig. 3).

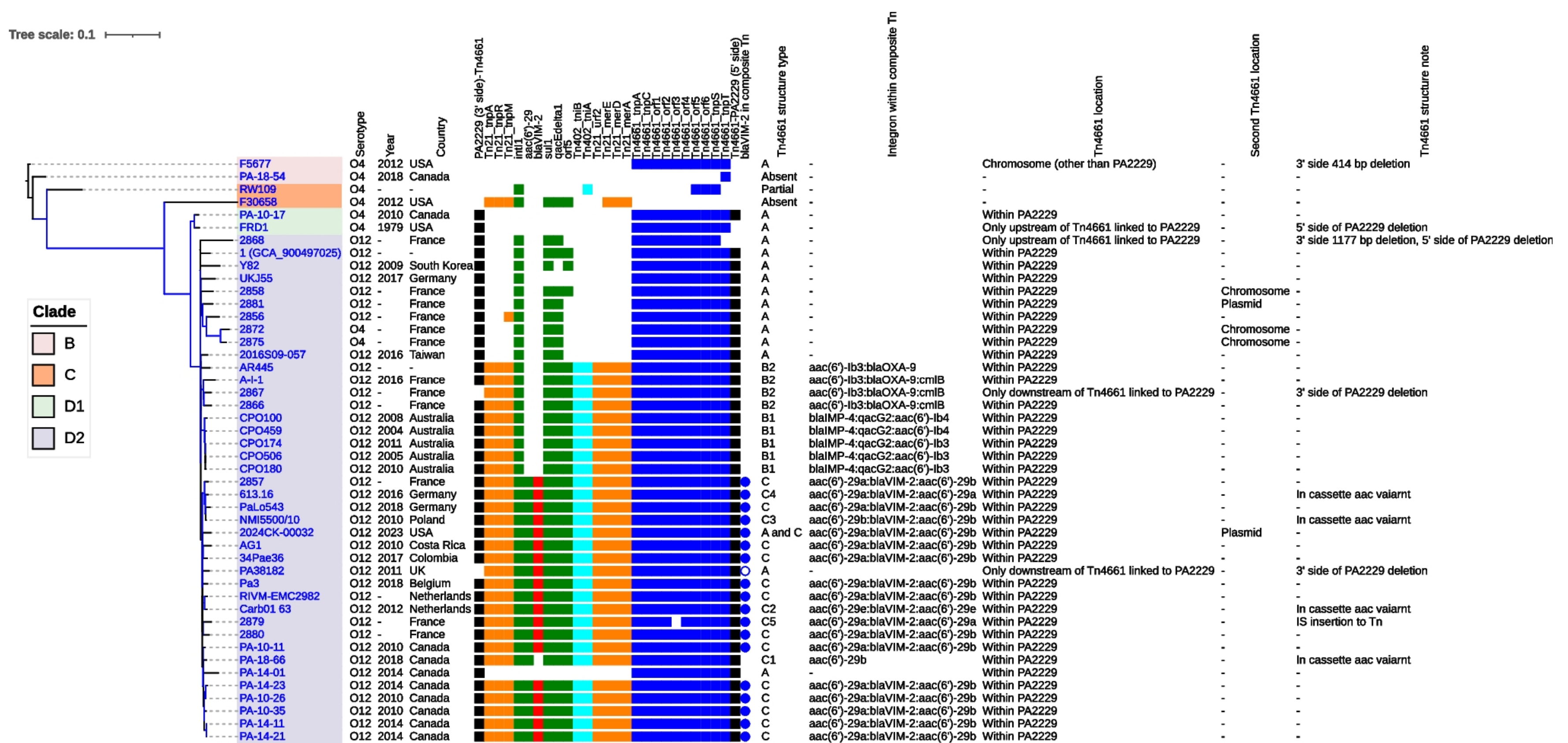


Fig. 2. Tn4661-related transposon structures among Canadian (n = 10) and global *Pseudomonas aeruginosa* ST111 (n = 35) that contained complete sequences. This maximum-likelihood phylogram is based on a total of 1,830 core SNPs. The tree is rooted by using clade B isolate F5677. Branches that had > 90% bootstrap support from 100 replicates were highlighted in blue

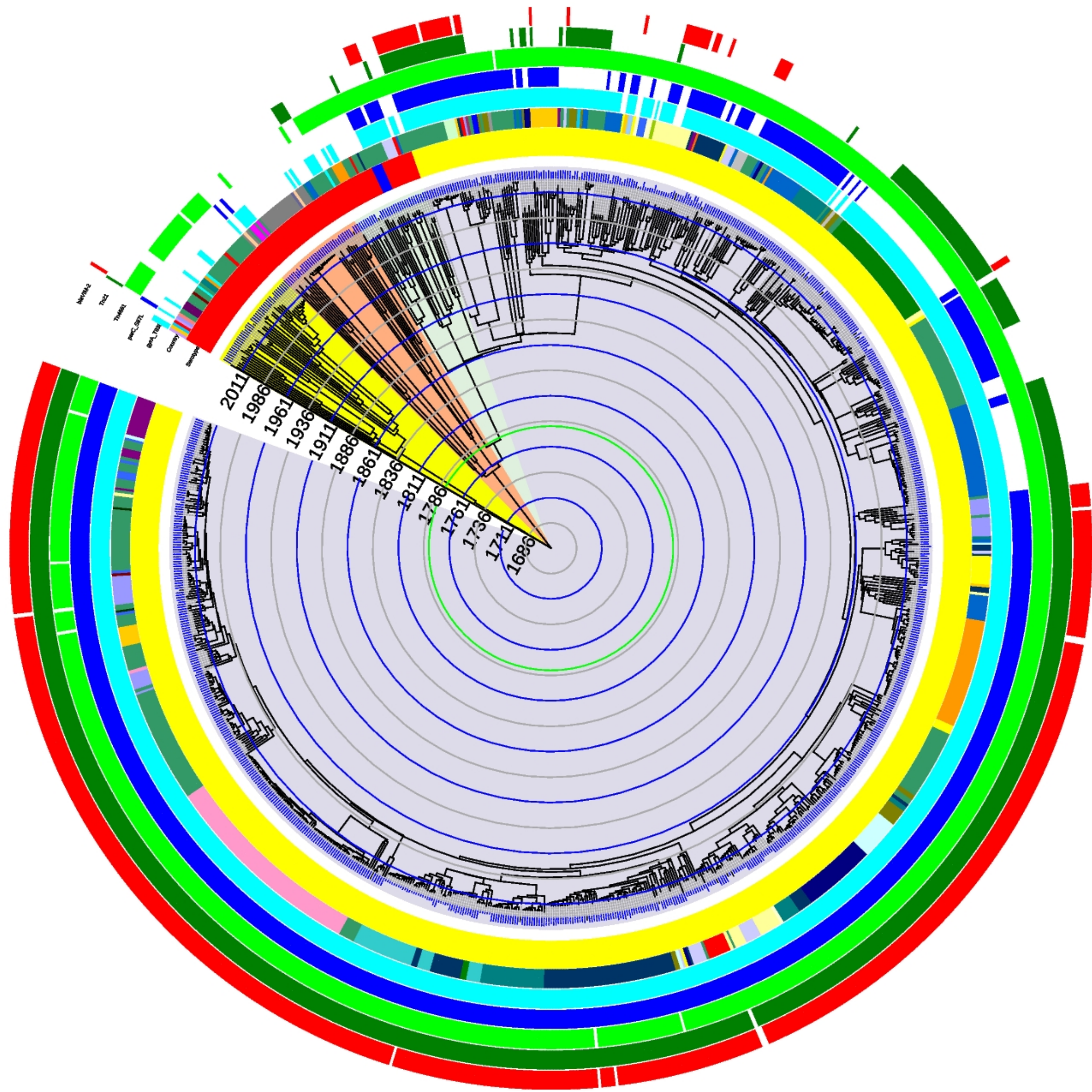

Clade
A
B
C1
C2
Serotype
O4
O11
O12
Country
Argentina
Australia
Austria
Belgium
Brazil
Bulgaria
Canada
Chile
China
Colombia
Costa Rica
Croatia
Czech
Denmark
Estonia
France
Germany
Greece
India
Italy
Japan
Lebanon
Mexico
Netherlands
Nicaragua
Pakistan
Panama
Peru
Poland
Portugal
Russia
Saudi Arabia
Singapore
Slovenia
South Africa
South Korea
Spain
Switzerland
Taiwan
Tanzania
Turkey
UK
USA
Venezuela
Unknown
2011
1986
1961
1936
1911
1886
1861
1836
1811
1786
1761
1736
1711
1686

Fig. 3. Molecular evolution of *Pseudomonas aeruginosa* ST111 over time using genomes (n = 952) with known dates of isolation. A general time-reversible substitution, a strict molecular clock, and Bayesian skyline population models were used

Discussion

The O-polysaccharide is an important *P. aeruginosa* virulence factor responsible for serotype specificity [29] and most global *P. aeruginosa* ST111 isolates contained serotype O12 [7]. A previous study showed that ST111 with the O4 serotype is likely the ancestral form that acquired O12 serotype through a large homologous recombination event that also involved the acquisition of the *gyrA*_T83I mutation [20]. That study did not perform phylogenetic analysis or investigated the evolutionary history of ST111 clades.

Our genomic analysis divided ST111 into 3 clades (e.g., A, B, C) and two subclades (e.g., C1, and C2). ST111-C2 was the most dominant clade. ST111-A was the ancestral clade while clades B, C1 and C2 emerged during the 1700s and 1800s. Our results supported the previous findings that serotype switching from O4 to O12 and the acquisition of *gyrA*_T83I occurred in the ancestral form of ST111 [20]. However, our analysis also indicated that the additional acquisition of *parC*_S87L, Tn21 and $bla_{\mathrm{VIM-2}}$ were likely important in the evolution of subclade ST111-C2. Serotypes switching and the gradual acquisition of different QRDR mutations and carbapenemase genes over time, are shared with MDR *Escherichia coli* high-risk clones such as ST131 [30], ST410 [31], ST1193 [32] as well as MDR *Klebsiella pneumoniae* clones ST147 and ST307 [6]. Serotype switching followed by the stepwise acquisition of QRDR mutations and different β-lactamases genes seem to be pivotal in the success of MDR high-risk Gram-negative clones [3, 33].

The chromosomal AmpC β-lactamases named PDC was present in the majority of ST111 strains. However, PDC-1 was restricted to clade A while PDC-3 was limited to ST111-B and ST111-C. PDC-3 differs from PDC-1 with a T105A mutation that enhances the catalytic activity of PDC-3 to imipenem and oxyimino-cephalosporins such as ceftazidime [34]. Such a mutation could have provided clades B and C strains with selective advantages over clade A, in the presence of imipenem and ceftazidime.

During 2003–2004 Calgary, Canada experienced several nosocomial outbreaks at a tertiary care hospital due to clonally related VIM-2 producing *P. aeruginosa* (named pulsotype MBLV)[35, 36] that lead to the closure of intensive care units for a 3-week period [37]. These nosocomial outbreaks were curtailed by replacing the contaminated faucets and sinks in the intensive care and bone marrow units [37]. *P. aeruginosa* pulsotype MBLV from the 2003–4 outbreak belonged to ST111-C2 (Pitout, unpublished data). The inclusion of all Calgary *P. aeruginosa* isolates causing BSIs (irrespective of antimicrobial susceptibility profiles) [9] provided a diverse collection of ST111 strains that belonged to antimicrobial sensitive ST111-A (serotype O4), antimicrobial sensitive ST111-C1 (serotype O4) and MDR ST111-C2 (serotype O12).

In summary, our study provided details on the emergence and evolution of different ST111 clades over time and highlighted the roles of serotype switching and the acquisition of certain transposon structures and AMR determinants in the evolution of ST111-C2.

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Contributions

All authors designed the study and approved the manuscript. GP, YM, MK performed WGS, bioinformatics, and statistical analysis. JP combined the clinical and genomic data. JP wrote the 1st draft of the manuscript.

Ethics declarations

Ethics approval

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB17-1010).

Consent to participate

Not applicable.

Consent for publication

This manuscript has not been published and is not being considered for publication elsewhere.

Conflicts of interest

The authors declare no competing interests.

Data Availability

The sequencing data was deposited in the NCBI database (BioProject PRJNA988909).

Code availability

The sequencing data was deposited in the NCBI database (BioProject PRJNA988909.

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