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Analysis of a Worldwide Collection of *Klebsiella pneumoniae* CC258 with Reference to Carbapenemase Production Using the 1928 Core Genome (cg) Multilocus Sequence Type (MLST) Reveals Endemicity and the Global Dissemination of Clones

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Introduction

- *Klebsiella pneumoniae* has emerged in recent years as a major hospital associated pathogen.
- *K. pneumoniae* clonal complex (CC) 258 constitutes an international epidemic clone responsible for the spread of extended spectrum beta-lactamases (ESBL) and carbapenemases, including KPC enzymes.
  - ST258 capsular polysaccharide contributes to evasion of innate host defense leading to higher virulence.
  - Infections caused by these pathogens are difficult to treat as CC258 can be resistant to virtually all clinically useful antibiotics; as a result, mortality rates in these infections are high.
- We explored phylogenetic relationships, resistance genes and plasmid profiles within CC258 from a global collection of *K. pneumoniae* isolates using the 1928 bioinformatic cloud platform.

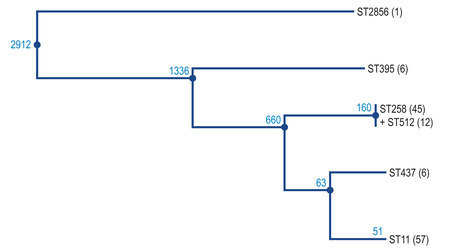
Materials and Methods

- A total of 155 *K. pneumoniae* clinical isolates collected from 19 countries during 2018 as part of the SENTRY Antimicrobial Surveillance Program were analyzed.
  - These isolates included 129 isolates belonging to clonal complex 258 (CC258) and 26 belonging to other MLST types that were used as controls.
  - Selected isolates were collected from 51 medical centers located in North America (20), Latin America (10), Europe (16), and Asia Pacific (5).
- Carbapenem resistant (n=120) and non-carbapenem resistant (n=35) isolates were included in the analysis.
- Whole genome sequencing was performed on MiSeq (Illumina, San Diego, California, USA).
  - High quality genomic DNA was extracted using KingFisher Cell and Tissue DNA kit (Thermo Scientific, Waltham, MA USA) in a robotic workstation KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific).
  - DNA libraries were prepared using the Nextera XT® DNA Library Preparation Kit (Illumina) and sequenced with a target depth of coverage >30X.
  - Each raw data set was quality assured, error corrected, and *de novo* assembled using SPAdes v. 3.11.1.
- FASTQ files were uploaded to the 1928 pipeline for analysis (v. 2020-03.4).
  - MLST and cgMLST data was analyzed against resistance mechanisms and demographic information.
  - Isolates within MLST types were analyzed for subgroups (clades) based on allelic distances.

Results

- Most CC258 isolates belonged to ST258, ST11, and ST512 (Table 1).
  - CC258 isolates clustered apart from non-CC258 STs by an allelic distance (ad) of >2000.
  - Most CC258 MLST types were within an allelic distance of 660 (Figure 1), with exceptions listed below:
    - A single ST2856 isolate showed allelic distance of 2912 from CC258.
    - Six isolates belonging to ST395 (Russia, Spain) showed an allelic distance of 1336 from CC258.
- cgMLST grouped together isolates from the same STs. Those isolates from similar geographies had greater homology, except isolates from the US showed heterogenicity (allelic distance 620).
  - ST258 isolates (n= 45) were limited to four countries while ST11 isolates (n= 57) were from 13 different countries (Table 1).
- Applying an allelic distance cutoff of <100, ST258 isolates were classified in 4 clades with a predominance of either KPC-2 or -3 (Figure 2) in each clade.
  - IncFIB and IncFII plasmid types were predominant in all clades.
  - Tn*4401a* was predominant in clades I and II while 6/11 clade III isolates carried Tn*4401b* (Figure 2C).
- An allelic distance cutoff of <200 identified 7 clades within ST11 that were related by geography and resistance genes (Figure 3).
  - Greater carbapenemase diversity was observed within ST11 (Table 1).
  - NDM-1 was detected in isolates from Greece, Poland, Mexico, Russia, and Argentina.
  - All ST11 from Brazil (clade IV) produced KPC-2.
  - USA ST11 isolates did not carry carbapenemases.
- Among CC258 isolates KPC was the major carbapenemase (58.1%) and associated with Tn*4401* (*a* or *b* in 84% KPC).
  - KPC-2-producing *K. pneumoniae* was more prevalent in Latin America; these isolates were closely related among ST258 (allelic distance 104), compared to ST11 (allelic distance 355).
  - KPC-3-producing CC258 isolates showed allelic distance of 164 and were from Italy, Russia, Greece, and the US.
  - NDM-1 was detected (8.5%) only in ST11 and ST395.
  - CTX-M-14 was prevalent in ST258 while CTX-M-15 was common in other STs, except for in the case of ST512, which carried no CTX-M despite clustering within ST258.
- CC258 isolates producing KPC-2 (allelic distance 630) and KPC-3 (allelic distance 164) showed phylogenetic proximity despite geographic diversity.
  - KPC-2 (n= 46) isolates were from USA, Argentina, Brazil, Greece, Korea, and Taiwan.
  - KPC-3 (n= 29) isolates were from USA, Italy, Greece, and Russia.
- Majority of non-CC258 isolates were from Europe (18/26).
  - These isolates predominantly carried OXA-48-like enzymes (12 isolates) or no carbapenemase encoding genes (9 isolates).
  - CTX-M-15 (n= 21) and/or OXA-1 (n= 22) were the most common ESBL enzymes detected.

Figure 1 Allelic distance dendrogram of CC258 isolates



Numbers in parentheses indicate number of isolates belonging to the ST.

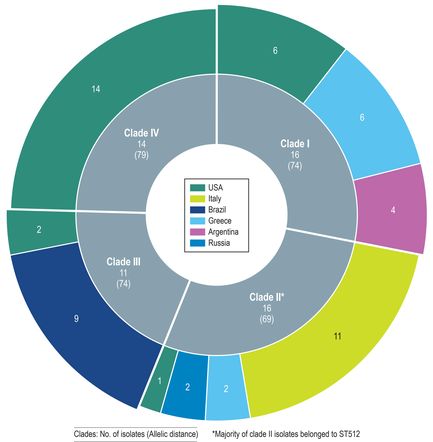
Table 1 Characteristics of major *K. pneumoniae* ST types from the global collection of SENTRY isolates

ST type (No. isolates)	Allelic distance		Countries (No. isolates)	Major beta-lactamases detected (No. isolates)	
	Within ST	Between STs (compared to)		Carbapenemases[a]	ESBL
258[b] (45)	160	660 (ST11)	USA (23), Brazil (10), Greece (8), Argentina (4)	KPC-2 (24), KPC-3 (15)	CTX-M-14 (10), CTX-M-15 (1), OXA-10 (2)
11 (57)	519	631 (ST437)	Brazil (15), Poland (9), USA (6), Greece, Korea, Russia & Taiwan (4 each), Argentina & Chile (3 each), Spain (2), Mexico, Panama, Thailand (1 each)	KPC-2 (19), NDM-1 (10), OXA-48-like (4)	CTX-M-14 (3), CTX-M-15 (30), OXA-10 (1)
512[c] (12) Clustered within ST258	41	56 (ST258)	Italy (10), Russia (2)	KPC-3 (12)	OXA-10 (1)
437 (6)	107	631 (ST11)	Brazil (3) and Costa Rica, Poland, USA (1 each)	KPC-2 (3), OXA-48-like (1)	CTX-M-15 (5)
395 (6)	236	1336 (CC258)	Russia (5), Spain (1)	NDM-1 (1), OXA-48-like (5)	CTX-M-15 (6)
2856 (1)	NA	2909 (all other STs)	Mexico (1)	None	CTX-M-15
307 (10; non-CC258)	63	2763 (CC258)	USA (2) and Australia, Brazil, Costa Rica, Germany, Ireland, Italy, Mexico, Spain (1 each)	KPC-2 (1), KPC-3 (1)	CTX-M-15 (10; CTX-M-15 +OXA-1/-30 [8])
Other non-CC258[d]	NA	NA	Russia (7), Turkey (4), Argentina, Germany, Italy, Poland, and Thailand (1 each)	KPC-2 (2), NDM-1 (1), OXA-48-like (12)	CTX-M-14 (1), CTX-M-15 (11), OXA-1 (3)

[a] OXA-48-like included OXA-48, OXA-181 or OXA-232.
[b] One ST418 isolate clustered within ST258.
[c] One ST3498 isolate clustered within ST512.
[d] Other non-CC258 types (101, 147, 15, 152, 2096, 23, and 2731) were detected in 1–5 isolates each.
ESBL, extended-spectrum β-lactamase
NA, Not applicable

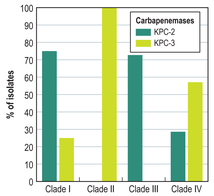
Figure 2 Characteristics of ST258 clades identified based on allelic distance cutoff of <100

A. Allelic distance and geographic distribution of clades identified within ST258 isolates



Clades: No. of isolates (Allelic distance) *Majority of clade II isolates belonged to ST512

B. Carbapenemases detected among ST258 clades



C. Tn*4401* variant associated with bla_{KPC} among isolates in ST258 clades

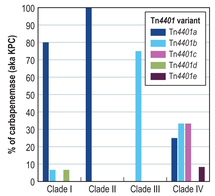
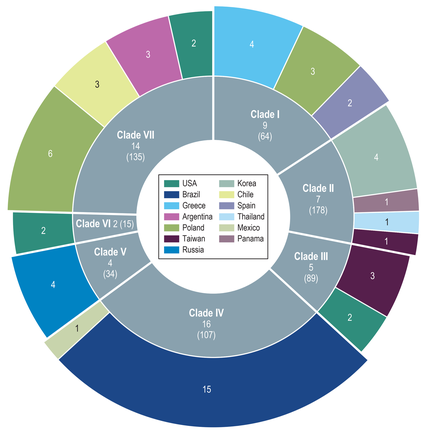


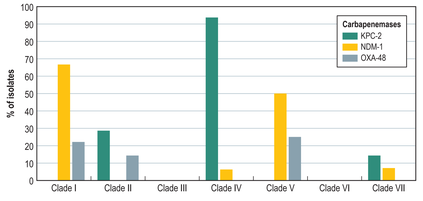
Figure 3 Characteristics of ST11 clades identified based on allelic distance cutoff of <200

A. Allelic distance and geographic distribution of clades identified within ST11 isolates



Clades: No. of isolates (Allelic distance)

B. Diversity of carbapenemases detected among isolates in ST11 clades



Conclusions

- cgMLST analysis generated by the 1928 cloud platform showed good correlation with MLST in classifying all *K. pneumoniae* isolates.
- ST258 isolates showed tight clustering, distribution in the Americas, and none carried NDM genes.
- ST11 showed global dissemination and diversity of carbapenemases.
- CC258 isolates carried major carbapenemases (KPC, NDM) while OXA-48-like enzymes were common among non-CC258.
- Understanding the dissemination of CC258 and having the correct tools to perform this analysis in a timely manner is of utmost importance to prevent further dissemination of these high-risk clones.

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