IRSTI 76.29.50:03.36 https://doi.org/10.26577/appmed2023v4i2a7

O. Gizinger

Peoples' Friendship University of Russia named after Patrice Lumumba, Moscow, Russia
e-mail: ogizinger@gmail.com

KLEBSIELLA PNEUMONIAE: ASPECTS OF POPULATION GENOMICS

Abstract. Klebsiella pneumoniae is one of the causes of antimicrobial-resistant opportunistic infections. Klebsiella pneumoniae is naturally resistant to penicillins and many antimicrobials. Knowledge about the ecology, population structure, or pathogenicity of K. pneumoniae is limited by laboratory technology. K. pneumoniae is a threat for public health due to an increase in healthcare-associated infections caused by multidrug-resistant strains that produce extended-spectrum β-lactamases and/or carbapenemases. The phenomenon of severe community-acquired infections caused by particularly virulent K. pneumoniae is associated with strains expressing acquired virulence factors. The presence of such serious biomedical problems makes sustained interest in research on K. pneumoniae using genetic methods. In this article, we discuss the possibilities of genomic approaches, their role in understanding the taxonomy, ecology, and evolution of K. pneumoniae, its diversity, and the distribution of clinically relevant determinants of pathogenicity and antimicrobial resistance. A detailed consideration of the structure and diversity of the K. pneumoniae population is important for understanding research results, interpreting clinical data, and developing and implementing methods to combat this pathogen.

Key words: Klebsiella pneumoniae, antimicrobial-resistant, ecology, population, opportunistic infections, genomics.

Introduction

+ manifestations (subsp. rhinoscleromatis which causes a progressive and chronic granulomatous infection known as rhinoscleroma, and subsp. ozaenae which causes atrophic rhinitis or ozena) actually represent CGs of K. pneumoniae (CG3 and CG90). Like hvKp clones, these strains also express specific capsule types (K3, K4 and K5) alongside aerobactin and another acquired siderophore, yersiniabactin (Ybt) [9].

Due to its clinical importance and increasing AMR, K. pneumoniae is increasingly the focus of surveillance efforts and molecular epidemiology studies. The sheer volume of clinically relevant molecular targets renders whole-genome sequencing (WGS) the most cost-efficient characterization approach, however extracting and interpreting clinically important features is challenging. To address this, scientists have developed Kleborate, a genotyping tool designed specifically for K. pneumoniae and the associated species complex, which consolidates detection and genotyping of key virulence and AMR loci alongside species, lineage (ST) and predicted K and O antigen serotypes directly from genome assemblies [10]. First, show that Kleborate can rapidly recapitulate and augment the key findings from a recent large-scale European genomic surveillance study. Next, apply Kleborate to a curated collection of 13,156 publicly available WGS to further showcase its utility and derive novel insights into the global epidemiology of Klebsiella AMR, virulence and convergence. Finally, Kleborate can also be applied to detect clinically relevant genotypes from metagenome-assembled genomes (MAGs) [11].

Material and Methods.

To conduct the literature review we used three stages: planning the review, developing the research question, conducting the review, selecting and assessing the quality of studies included in the analysis, and reporting the results. There were 38 articles have been selected.

The research question included the study of the awareness of genomics in understanding the structure of the K.pneumonia population, transmission, pathogenicity, antimicrobial resistance.

The literature search was made using the following bibliographic databases: PubMed, Cochrane Database of Systematic Reviews and Scopus. The search strategy included controlled vocabulary, such as the National Library of Medicine grid (medical headlines), and keywords: Klebsiella pneumoniae, antimicrobial resistance, ecology, population, opportunistic infections, genomics.

At the first stage, the headings and annotations were evaluated, after which full articles were selected for in-depth study of the research problem. An assessment of the compliance of the full articles with the inclusion criteria was carried out.

The studies included in this review met the following inclusion criteria:

Studies on multidrug-resistant clones klebsiella pneumoniae, plasmid diversity in klebsiella pneumoniae, pathogenicity factors k. pneumoniae; a study that allows comparing the results.

After eliminating repetitive work (duplicates), a total of 20 studies were identified, where genomics plays a major role in understanding the structure of the K.pneumonia population, transmission, pathogenicity, and antimicrobial resistance.

Results and Discussion

Typical K. pneumoniae genomes are ~5–6 Mbp in size, encoding ~5,000–6,000 genes. Approximately 1,700 genes are conserved in all members of the species (core genes), whereas the remainder are variably present (accessory genes). The total pan-genome (the sum of all core and accessory genes) is extremely diverse and likely exceeds 100,000 protein coding sequences. The majority of accessory genes are rare in the population, that is, they are present in <10% of genomes. Taxonomic and GC content analyses suggest that these genes are shared with a wide range of other bacterial species, most commonly other Klebsiella species followed by other Enterobacteriales but also including more distant orders [12].

Phylogenetic analyses based on 1,000–2,000 core or common chromosomal genes show that the K. pneumoniae population comprises hundreds of deep-branching lineages that differ from each other by ~0.5% nucleotide divergence. These lineages correspond closely to the clonal groups (CGs) defined by core-genome multilocus sequence typing (cgMLST) as subsets of isolates that each share ≥594 of 694 cgMLST alleles with at least one other member of the group. Whether defined on the basis of core-genome phylogeny (lineages) or cgMLST (CGs), the resulting groups are typically referred to as clones and are identified and labelled based on the dominant seven-gene multilocus sequence type, allowing backwards comparison with the original seven-gene MLST (which covers the entire KpSC). Note that the seven-gene MLST scheme can also be used alone to define CGs, but sometimes fails to correctly distinguish groups whose recent ancestry is affected by chromosomal recombination. K. pneumoniae clones can be distinguished from one another on the basis of accessory gene content. This may be explained by clone-specific niche adaptation through horizontal gene transfer (HGT), so long as migration between niches occurs, and there is evidence that it does (discussed below). However, there is also ample evidence of between-clone HGT, largely driven by chromosomal recombination and plasmid-mediated conjugation, although phage-mediated transduction and integrative conjugative elements (ICEs) also play a role [13].

Homologous recombination between chromosomes is dominated by exchange of capsule biosynthesis loci (a key pathogenicity determinant) and can result in the acquisition of regions of DNA exceeding 1Mbp in length. Many diverse plasmids have been sequenced from K. pneumoniae, ranging considerably in terms of length and incompatibility types, although IncFIIK and IncFIBK are the most prevalent. It seems that some K. pneumoniae strains may be particularly permissive for plasmid uptake and/or maintenance, resulting in plasmid loads that are typically greater than those reported for Escherichia coli and other Gram-negative ESKAPE pathogens. For example, it is not uncommon for a K. pneumoniae isolate to carry between four and six different plasmids, with up to 10 reported. Most complete K. pneumoniae genomes carry multiple prophages, and multiple distinct K. pneumoniae phages have been isolated and sequenced, including for potential use as therapeutic agents. However, there have not yet been any systematic studies of prophage diversity within the population. CRISPR–Cas9 systems and restriction-modification systems are variably present in the K. pneumoniae population, however, how these relate to plasmid and phage diversity is not yet clear [10].

Multidrug-resistant clones Klebsiella pneumoniae

MDR, which is defined as resistance to ≥3 antimicrobial classes in addition to ampicillin to which all K. pneumoniae infections are intrinsically resistant, has evolved many times in hundreds of distinct K. pneumoniae lineages. Some of these lineages emerge to cause localized problems, spreading within a single hospital or health-care network, for example, MDR clones sequence type 70 (ST70) and ST323 that caused outbreaks in Kilifi, Kenya and Melbourne, Australia, respectively. Such events likely represent chance emergence in a given time and place, and the factors influencing their frequency, likelihood and duration of persistence are not known. Many will remain localized problems, causing no or limited infections elsewhere, but a subset of the most highly resistant lineages (for example, those resistant to third-generation cephalosporins and/or carbapenems) have become global problems. These include the well-studied CG258 alongside CG15, CG20 (CG17), CG29, CG37, CG147, CG101 (CG43) and CG307, which are not related to one another but are each widely geographically distributed and common causes of MDR HAIs and/or outbreaks. Important-

ly, although many studies do attribute the majority of third-generation cephalosporin-resistant and/or CRKp infections to a small number of clones (57% of CRKp infections in the recent Europe-wide study EuSCAPE were ST11, ST15, ST101 or ST258/ST512), in many regions the burden attributed to sporadic MDR strains or local problem clones remains substantial (for example, >33% of strains in a recent national survey of CRKp in the UK) [14].

Wyres K.L. et al present the definition of K. pneumoniae clones and a phylogenetic tree obtained using the maximum probability for K. pneumoniae genomes selected from the curated collection of authors / the figure shows a diagram of 509 different types of chromosomal multilocus sequences with 7 genes. Phylogenetic clusters (monophyletic groups) were defined using patristic distance (cutoff = 0.04). Clusters corresponding to clones included in the comparative analysis are marked; blue, hypervirulent; grey, unassigned; red, multidrug resistant. b) Total number of genomes included in the comparative analysis, stained for clone type as above. Note that the sample sizes exceed the number of isolates shown in the tree for the respective clones. c) Distribution of virulence and resistance determinants by clones. The intensity of the box shading indicates the proportion of genomes containing key virulence loci (blue) or acquired genes conferring resistance to different antimicrobial classes (red), according to inset labels. Hypervirulent (Hvir) clones were determined by hierarchical clustering of virulence locus data. Clones with multiple drug resistance (MDR) were determined by hierarchical grouping data on resistance. AMR, antimicrobial resistance; rmpA/A2, mucoid phenotype regulators; ESBL, extended spectrum beta-lactams; MLS, macrolides, lincosamide and antibiotics streptogramin B [15], Fig 1.

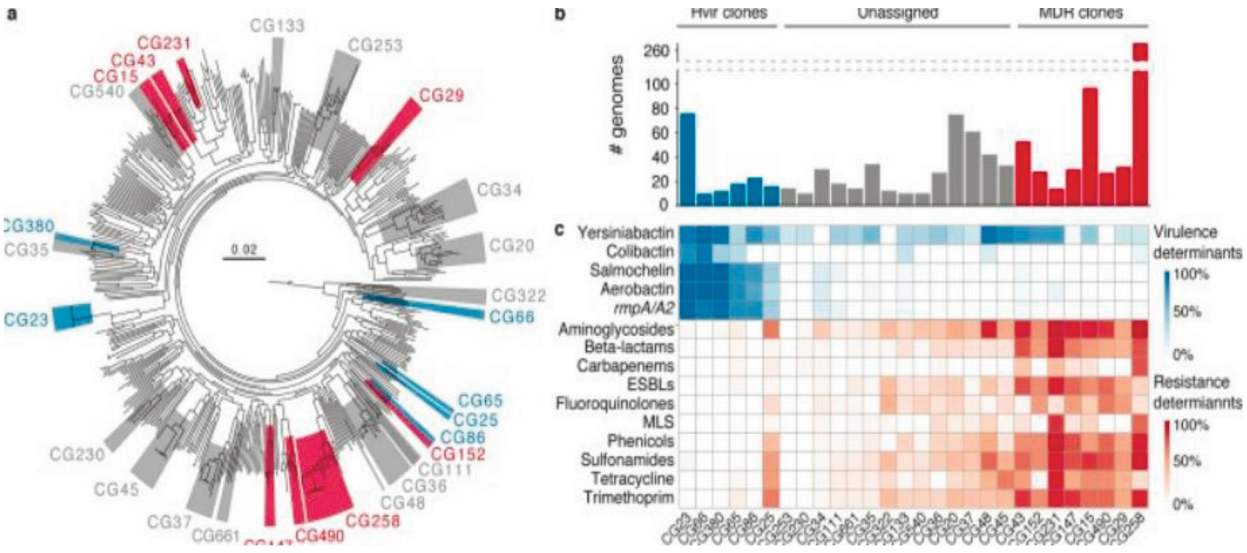

Figure 1- Definition of K. pneumoniae clones investigated in this study [15].

Plasmid diversity in Klebsiella pneumoniae

Plasmids are important vehicles for the transfer of antimicrobial resistance (AMR), virulence and other accessory genes between bacterial cells. In K. pneumoniae, the majority of horizontally acquired AMR genes are carried on large conjugative (self-transmissible) plasmids belonging to a small number of incompatibility groups, which have been recently reviewed (IncFII, IncN, IncR and IncX3), although small (mobilizable but not self-transmissible) plasmids can also harbour AMR genes in K. pneumoniae [16]. Most available data on plasmid diversity and distribution in the K. pneumoniae population come from studies utilizing short-read whole-genome sequencing to survey populations, yielding information on repliconmarkers or mob (relaxase) gene variants rather than whole plasmids (although this is changing with increased use of long-read sequencing). Using these techniques, plasmid load and diversity have been shown to be significantly higher in multidrug-resistant clones than hypervirulent clones or other clonal groups. The FIBK replicon is associated with both multidrug resistant and virulence plasmids, conjugative and non-conjugative, and is common in all clones. Also common are incompatibility type FIIK and R replicons (associated with large conjugative plasmids) and small (Col) plasmids, followed

by other F plasmid variants (FII, FIA and FIB) and incompatibility types X3, N, HI1B and AC/2. Notably, although there can be significant plasmid variation within a single clone, phylogenomic evolutionary analyses suggest that some problem clones have maintained specific plasmids throughout decades of clonal expansion, punctuated by occasional instances of rearrangement and/or gene deletions. For example, sequence type 258 (ST258) has carried FIBK blaKPC plasmid pKpQIL and ST307 has carried a FIIK blaCTX-M-15 plasmid since each emerged in the mid-1990s; and the FIBK K. pneumoniae virulence plasmid (KpVP-1) has been present in ST23 dating back to the 1870s [17].

Pathogenicity factors K. pneumoniae

All K. pneumoniae strains harbour a subset of core chromosomally encoded pathogenicity factors that form the basic requirements for establishing opportunistic infections in mammalian hosts. These include the core locus ent encoding biosynthesis of the siderophore enterobactin (Ent), the core fim and mrk loci encoding type 1 and type 3 fimbriae, respectively, as well as the variable capsular polysaccharide (K antigen) and LPS (O antigen) biosynthesis loci. Ent is required for growth in most niches; the other factors mediate processes at the cell surface involved in the earlier stages of infection and/or evasion of host defense mechanisms. Large-scale genomic comparisons have revealed substantial allelic and gene-content heterogeneity at the capsule (K antigen) and LPS (O antigen) biosynthesis loci, which comprise ~10% of the pan-genome [18].

The capsule is produced through a Wzy-dependent process for which the conserved machinery is encoded by genes that are present in most K-antigen biosynthesis loci (wzi, wza, wzb, wzc, wzx and wzy). However, the capsule-specific sugar synthesis machinery is encoded by a set of highly diverse genes that are variably present and frequently reassorted in the population. To date, >138 distinct combinations have been identified, each defining a distinct K-locus thought to encode a distinct capsule type, but only 77 of these have been distinguished by traditional serological typing. Capsule types K1 and K2 are associated with invasive disease and enhanced pathogenicity in murine models30 and are highly conserved in hypervirulent clones (K1 in CG23 and K2 in the others). Capsule type K5 is also associated with liver abscess in diverse strain backgrounds, and K3 is restricted to the rare rhinoscleromatis lineage (ST67). Little is understood about the relative virulence of other capsule types, and most non-hypervirulent clones, including the global MDR clones, exhibit substantial K-locus diversity. A notable exception is CG307, which so far always carries the K-locus 102 (KL102) locus, as well as an additional putative capsule synthesis locus that has a distinct structure to the K-locus and is rare in the broader K. pneumoniae population [12].

Similar gene-content variation has been used to define 12 distinct O-loci. Unlike the K-loci, however, the genes responsible for defining the nine recognized LPS O serotypes and >5 subtypes include those at the O-locus and also other regions of the genome (locations are type dependent). Serotypes O1 and O2 are most common among clinical K. pneumoniae isolates and may provide comparatively enhanced protection against phagocytosis. There are no firm data as yet on whether K-locus or O-locus variation is related to niche or host specialization, as is the case in other bacterial species [19].The extent and clinical impact of allelic diversity at the fim and mrk loci remain relatively unexplored; however, there is evidence that both types of fimbriae contribute to intestinal colonization, biofilm formation on catheters and the ability to cause pneumonia and urinary tract infections. Notably, both loci also play a role in adhesion to plant cells [20].

Conclusion

The main role in understanding of K.pneumonia population structure, transmission, pathogenicity, antimicrobial resistance belongs to genomics. The application of genomics has revealed remarkable genetic diversity of the microorganism. The emerging population genomic structure has helped clarify different points of confusion related to taxonomy, resistance and virulence; for example, by highlighting those AMR and virulence determinants that are core to all strains and revealing how clonal spread can confound association analyses. it is becoming apparent that there is no such thing as ‘typical’ K. pneumoniae, as gene-content turnover is extensive and even the ‘core’ loci harbour widespread genetic variation of clinical relevance. Experimental studies could benefit from taking this into account, by including multiple diverse strains and exploring the functional dependence of specific genes on factors that vary substantially with genetic background, including both allelic and gene-content variation. Ideally, such studies would also report the genome sequences of strains, enabling others to interpret the findings and make comparisons with their own strain collections.

References

Effah C.Y., Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020 Jan 9;19(1):1. doi: 10.1186/s12941-019-0343-8.

Navon-Venezia S., Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252–275.

Wasfi R, Elkhatib W.F., Ashour H.M. Molecular typing and virulence analysis of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from Egyptian hospitals. Sci Rep. 2016 Dec 22;6:38929. doi: 10.1038/srep38929.

Brisse S., Verhoef J. Phylogenetic diversity of Klebsiella pneumoniae and Klebsiella oxytoca clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping. Int J. Syst Evol Microbiol 51, 915–924, doi: 10.1099/00207713-51-3-915 (2001).

Fuursted K, Schøler L, Hansen F., Dam K., Bojer M.S., Hammerum A.M., Dagnæs-Hansen F, Olsen A, Jasemian Y, Struve C. Virulence of a Klebsiella pneumoniae strain carrying the New Delhi metallo-beta-lactamase-1 (NDM-1). Microbes Infect. 2012 Feb;14(2):155-8. doi: 10.1016/j.micinf.2011.08.015.

Wyres, K.L., Lam, M.M.C. Holt, K.E. Population genomics of Klebsiella pneumoniae. Nat Rev Microbiol 18, 344–359 (2020).

Peng, X., Cheng, L., You, Y. . Oral microbiota in human systematic diseases. Int J Oral Sci 14, 14 (2022). https://doi.org/10.1038/s41368-022-00163-7

Wyres K.L, Lam M.M.C, Holt K.E. Population genomics of Klebsiella pneumoniae. Nat Rev Microbiol. 2020 Jun;18(6):344-359. doi: 10.1038/s41579-019-0315-1.

Krapp F., Morris A.R., Ozer E.A., Hauser A.R.. Virulence Characteristics of Carbapenem-Resistant Klebsiella pneumoniae Strains from Patients with Necrotizing Skin and Soft Tissue Infections. Sci Rep. 2017 Oct 19;7(1):13533. doi: 10.1038/s41598-017-13524-8.

Holt K,E,, Wertheim H,, Zadoks R,N,, Baker S,, Whitehouse C,A, Dance D, Jenney A, Connor T,R, Hsu L.Y., Severin J., Brisse S., Cao H, Wilksch J, Gorrie C., Schultz M.B., Edwards D.J., Nguyen K.V., Nguyen T.V., Dao T.T., Mensink M., Minh V.L., Nhu N.T., Schultsz C., Kuntaman K., Newton P.N., Moore C.E., Strugnell R.A., Thomson N.R.. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A. 2015 Jul 7;112(27):E3574-81. doi: 10.1073/pnas.1501049112.

Bialek-Davenet S., Criscuolo A., Ailloud. F, Passet V., Jones L, Delannoy-Vieillard AS, et al. Genomic definition of hypervirulent and multidrug-resistant Klebsiella pneumoniae clonal groups. Emerg Infect Dis. 2014;20: 1812–1820. 10.3201/eid2011.140206

Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017 May 1;41(3):252-275. doi: 10.1093/femsre/fux013.

Effah C.Y, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020 Jan 9;19(1):1. doi: 10.1186/s12941-019-0343-8.

Saleem A.F., Qamar F.N., Shahzad H, Qadir M, Zaidi A.K. Trends in antibiotic susceptibility and incidence of late-onset Klebsiella pneumoniae neonatal sepsis over a 6-year period in a neonatal intensive care unit in Karachi, Pakistan. Int J Infect Dis. 2013;17(11):e961–e965.

Wyres KL, Wick RR, Judd LM, Froumine R, Tokolyi A, Gorrie CL, Lam MMC, Duchêne S, Jenney A, Holt KE. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of Klebsiella pneumoniae. PLoS Genet. 2019 Apr 15;15(4):e1008114. doi: 10.1371/journal.pgen.1008114.

Padilla E., Llobet E., Doménech-Sánchez A., Martínez-Martínez L., Bengoechea J.A, Albertí S. Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2010 Jan;54(1):177-83. doi: 10.1128/AAC.00715-09.

Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998 Oct;11(4):589-603. doi: 10.1128/CMR.11.4.589.

Su H.Y, Hussain B., Hsu B.M, Lee K.H, Mao Y.C., Chiang L.C., Chen J.S. Bacterial community analysis identifies Klebsiella pneumoniae as a native symbiotic bacterium in the newborn Protobothrops mucrosquamatus. BMC Microbiol. 2023 Aug 8;23(1):213. doi: 10.1186/s12866-023-02936-4.

Egan SL, Loh S.M, Banks P.B, Gillett A, Ahlstrom L, Ryan U.M., Bacterial community profiling highlights complex diversity and novel organisms in wildlife ticks. Ticks Tick Borne Dis. 2020;11(3):101407. doi: 10.1016/j.ttbdis.2020.101407

Ursell L.K., Metcalf J.L., Parfrey L.W., Knight R. Defining the human microbiome. Nutr Rev. 2012;70 Suppl 1:S38–44. doi: 10.1111/j.1753-4887.2012.00493.x.