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Short Communication

Easy analysis of bacterial whole-genome sequencing data for clinical microbiologists using open-source Galaxy platform: Characterization of ESBL-producing *Enterobacterales* from bloodstream infections

Aimé Berwa^{a,b}, Yvan Caspar^{a,b,*}^a Laboratoire de Bactériologie-Hygiène Hospitalière, CHU Grenoble Alpes, Grenoble, France^b Univ. Grenoble Alpes, CNRS, CHU Grenoble Alpes, CEA, IBS, Grenoble, France

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ABSTRACT

Objectives: Clinical microbiologists require easy-to-use open access tools with graphical interfaces to perform bacterial whole-genome sequencing (WGS) in routine practice. This study aimed to build a bioinformatics pipeline on the open-source Galaxy platform, facilitating comprehensive and reproducible analysis of bacterial WGS data in a few steps. We then used it to characterize our local epidemiology of ESBL-producing *Enterobacterales* isolated from patients with bacteremia.

Methods: We built a bioinformatics pipeline consisting of the following sequential tools: Fastp (input data trimming); FastQC (read quality control); SPAdes (genome assembly); Quast (quality control of genome assembly); Prokka (gene annotation); Staramr (ResFinder database) and ABRicate (CARD database) for antimicrobial resistance (AMR) gene screening and molecular strain typing. Paired-end short read WGS data from all ESBL-producing *Enterobacterales* strains isolated from patients with bacteremia over one year were analysed.

Results: The Galaxy platform does not require command line tools. The bioinformatics pipeline was constructed within one hour. It only required uploading fastq files and facilitated systematization of *de novo* assembly of genomes, MLST typing, and AMR gene screening in one step. Among the 66 ESBL-producing strains analysed, the two most frequent ESBL genes were *bla*_{CTX-M-15} (62.1%) and *bla*_{CTX-M-27} (13.6%).

Conclusions: The open-access Galaxy platform provides a graphical interface and easy-to-use tools suitable for routine use in clinical microbiology laboratories without bioinformatics specialists. We believe that this platform will facilitate fast and low-cost analysis of bacterial WGS data, especially in resource-limited settings.

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1. Introduction

The progressive spread of antimicrobial resistance (AMR) genes has increased the inappropriate empirical antimicrobial treatment rates for bloodstream infections (BSI), which are directly corre-

Abbreviations: amr, Antimicrobial resistance; ast, Antibiotic susceptibility testing; bsi, Bloodstream infections; ddst, Double-disk synergy test; esbl, Extended-spectrum beta-lactamase; iqr, Interquartile range; mic, Minimum inhibitory concentration; mlst, Multi-locus sequence typing; MH, Mueller Hinton; NGS, next-generation sequencing; PCB, positive blood cultures; PMQR, plasmid-mediated quinolone resistance; QRDR, quinolone resistance-determining region; WGS, whole-genome sequencing.

* Corresponding author: Yvan CASPAR, Laboratoire de Bactériologie-Hygiène hospitalière, Institut de Biologie et de Pathologie, CHU Grenoble Alpes, CS10217, Grenoble Cedex 9.

E-mail address: YCaspar@chu-grenoble.fr (Y. Caspar).

lated with increased 30-day mortality in patients [1]. In 2019, an estimated 1.27 million deaths and 47.9 million disability-adjusted life-years were directly attributable to AMR, with BSI being the second deadliest syndrome [2]. In particular, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* have reached concerning prevalence rates, threatening the use of 3rd generation cephalosporins (3GC) as empirical treatments for BSI caused by gram-negative bacteria [2,3]. Recent technological advances have enabled the development of rapid molecular or immunochromatographic methods from positive blood cultures (PCB); however, they only allow the rapid detection of *bla*_{CTX-M} ESBL genes to date, whereas >150 variants of TEM, SHV, and CTX-M ESBLs have been described [4–6].

Next-generation sequencing (NGS) has reduced the cost of sequencing and dramatically increased throughput, facilitating routine sequencing of the whole genome of bacterial pathogens in

a few hours and obtaining a rather comprehensive analysis of acquired AMR genes [7,8]. However, bacterial whole-genome sequencing (WGS) is rarely used for clinical strains in routine diagnostic strategies, except in reference laboratories. This partly relies on the fact that most clinical microbiologists are not trained to run bioinformatics analyses directly from a command line console and because bioinformaticians are rare, especially in public hospital settings or in low- and middle-income countries, which slows the introduction of these technologies in routine clinical microbiology.

Galaxy is a web browser that provides user-friendly tools and graphical interfaces, allowing users to share, analyse, and visualize large datasets, such as NGS data [9]. It provides access to extensive computational power, a large diversity of bioinformatics open-source software, and many tutorials that describe how to use these tools efficiently. The platform facilitates easy construction of bioinformatics pipelines that may be shared between users to run standardised analyses. As an example, detailed in this study, we used the Galaxy platform to build a reproducible open-source bioinformatics pipeline suitable for routine diagnosis. We used this pipeline to characterize all ESBL-producing *Enterobacteriales* strains isolated from patients with BSI at the Grenoble Alpes University Hospital in 2018.

2. Materials and methods

2.1. Bacterial strains

This study included all ESBL-producing *Enterobacteriales* strains isolated from PCBs between January and December 2018 at Grenoble Alpes University Hospital (France). Written consent was not required for this study, in accordance with national legislation.

2.2. Phenotypic ESBL detection and confirmation

Upon the initial isolation of the strain in routine diagnosis, ESBL production was determined using phenotypic assays. Antibiotic susceptibility testing of the strain was performed using disk diffusion on Mueller Hinton (MH) agar (bioMérieux, Marcy-l'Étoile, France) directly from a PCB bottle or BD Phoenix™ automated susceptibility testing system (Becton Dickinson, Le Pont-de-Claix, France) from isolated colonies after subculture, based on the recommendations from the Comité de l'Antibiogramme de la Société Française de Microbiologie-European Committee on Antimicrobial Susceptibility Testing (CASFM- EUCAST) [10]. Differentiation between ESBL production and hyperexpression of the *ampC* gene was performed phenotypically with a double-disk synergy test (DDST) and a cloxacillin-containing MH media. If synergy was observed between the 3GC disks and the disks containing clavulanic acid, the strains were considered ESBL-positive [10]. For some strains, the minimum inhibitory concentration (MIC) of temocillin, ceftazidime/avibactam, and ceftolozane/tazobactam were determined using MIC strips (Etest, bioMérieux, Marcy-l'Étoile, France).

2.3. DNA extraction, whole-genome sequencing, and bioinformatics

Bacteria were grown from the frozen cryotubes conserved at -80°C and incubated for 24 h at 36°C on Tryptic Soy Agar plates. Bacterial identification was confirmed by MALDI-TOF mass spectrometry on a Microflex LT Biotyper (Bruker Daltonics, Champs-sur-Marne, France) and ESBL production was confirmed using β -LACTA™ tests (Bio-Rad, Marnes-la-Coquette, France) or DDST. Subsequently, bacterial DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Les Ulis, France) according to the manufacturer's instructions. Paired-end 2×150 bp WGS was performed on a NextSeq NGS platform using a Nextera™ kit (Illumina, San Diego,

CA, USA) to obtain a theoretical 200X coverage depth for each genome (ENA project accession number PRJEB72649). Sequenced genomes were analysed using the online open-source bioinformatics platform Galaxy (<https://usegalaxy.org>) [9]. A snapshot of the bioinformatics pipeline created for this study in 2019 is shown in Fig. S1. The following tools were used: Fastp (Galaxy Version 0.20.1+ galaxy0) to trim input data for quality (Q30 threshold), FastQC (Galaxy Version 0.72+ galaxy1) for read quality control [11], SPAdes (Galaxy Version 3.12.0+ galaxy1) for genome assembly [12], Quast (Galaxy Version 5.0.2+ galaxy1) for quality control of genome assembly, Prokka (Galaxy Version 1.14.5+ galaxy0) for gene annotation [13], Staramr (Galaxy Version 0.7.1+ galaxy1) (PlasmidFinder and ResFinder database) [14], and ABRicate (Galaxy Version 1.0.1) (CARD database) for antimicrobial resistance (AMR) gene screening and molecular strain typing [15]. ESBL activity of the identified genes was confirmed using the Beta-Lactamase Database (<http://blddb.eu/>) [16].

2.4. Detection of mutations in quinolone resistance-determining regions

Mutations in the quinolone resistance-determining regions (QRDRs) were identified manually using multiple sequence alignment in Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The protein sequences of the topoisomerase subunits GyrA, GyrB, ParC and ParE of the isolates were aligned and compared with reference sequences from *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, *Klebsiella oxytoca* and *Serratia marcescens* reference strains provided by the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) and Uniprot (<https://www.uniprot.org/>) databases (see Supplementary Material).

2.5. Statistical analyses

Qualitative variables are described as percentages and quantitative variables as median and interquartile range (IQR). Graphs were created using GraphPad Prism 9.3.1 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Genome sequencing and assembly performances

The bioinformatics pipeline built on the Galaxy platform allowed us to perform all the analyses in one step for each strain (Figs. 1 and S1). In total, 66 strains from 60 patients with BSI due to ESBL-producing *Enterobacteriales* that were isolated at the Grenoble Alpes University Hospital in 2018 and preserved at -80°C were analysed (Fig. 2A and Tables S1–S3). The analysed strains comprised 34 *E. coli*, 24 *K. pneumoniae*, six *E. cloacae* complex, one *K. oxytoca* and one *S. marcescens* strains. Two patients had co-infections with two different ESBL-producing gram-negative bacilli and four had two episodes of bacteremia due to the same pathogens. The sequencing and assembly results for *Enterobacteriales* species are presented in Fig. S2 and show the high quality of the assemblies. The median sequencing coverage was 192X (IQR: 166–222). The median number of reads with a Phred quality score ≥ 30 and of contigs of at least 1000 bp were 7.95 million (IQR: 6.89–9.06) and 85 (IQR: 74–91), respectively, per strain.

3.2. Epidemiology of ESBL-producing enterobacteriales and molecular characterization of AMR genes

The most frequent sequence types were ST131 for *E. coli* strains (50%), ST307 for *K. pneumoniae* isolates (25%), and ST66 for *E. cloacae* complex isolates (33.3%) (Fig. 2B–D). The single *K.*

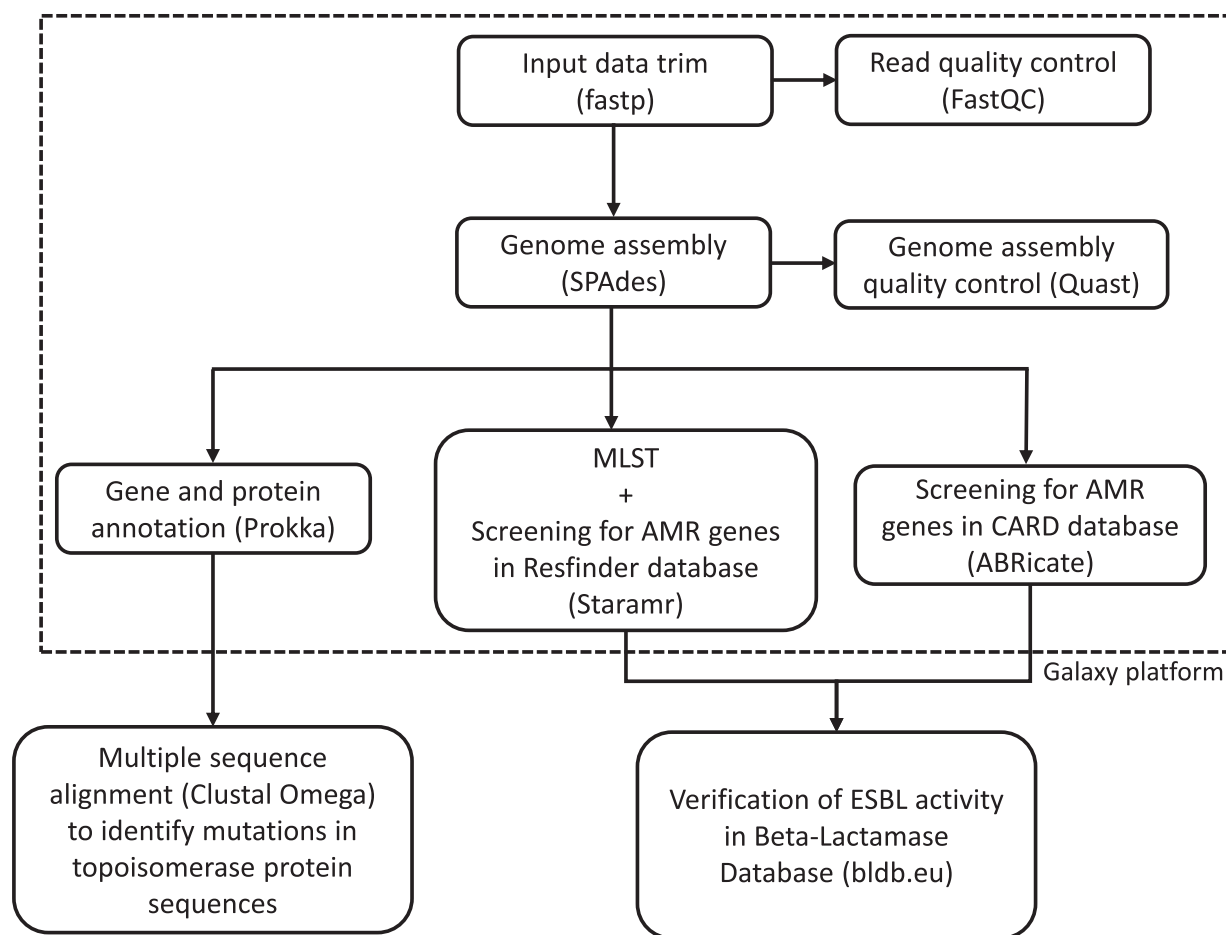


Fig. 1. Summary of the bioinformatics pipeline built for whole genome sequencing analysis of ESBL-producing *Enterobacteriales* isolates. The workflow performed on the Galaxy platform is indicated within the dashed line.

oxytoca isolate belonged to ST2, whereas the sequence type of the single *S. marcescens* strain could not be determined. WGS identified nine different ESBL genes in the 66 strains studied (Fig. 3A). The most common ESBL genes were *bla*_{CTX-M-15} (62.1%), *bla*_{CTX-M-27} (13.6%), and *bla*_{CTX-M-14} (10.6%). Two isolates (3%) harboured *bla*_{CTX-M-1} and *bla*_{CTX-M-3}. The genes *bla*_{CTX-M-9}, *bla*_{SHV-2}, *bla*_{SHV-12} and *bla*_{SHV-165} were found in only one isolate (1.5%). No ESBL genes were identified in one sample (1.5%). Fifty-three *Enterobacteriales* strains (80.3%) carried at least one AME gene (Fig. 3B); *aph [6]-Id* was identified in 54.2% of *K. pneumoniae*, 35.3% of *E. coli*, and 100% of *E. cloacae* complex samples, whereas *aph(3'')-Ib* was found in 54.2% of *K. pneumoniae*, 32.3% of *E. coli* and 100% of *E. cloacae* complex samples. Notably, the aminoglycoside-resistance methylase *armA* gene, which confers resistance to all aminoglycosides, was identified in one *K. pneumoniae* strain. Moreover, 36/66 strains (54.5%) had at least one chromosomal mutation in the QRDR of one or more of the DNA topoisomerase subunits: GyrA, GyrB, ParC and ParE (Fig. 3C). Combinations of point mutations, the most common of which were GyrA_S83L, D87N, ParC_S80I, E84V and ParE_I529L, were detected in 17 *E. coli* isolates. The second most common combination was GyrA_S83I and ParC_S80I, which was found in eight *K. pneumoniae* isolates. Forty isolates (60.6%) in the present study contained plasmid-mediated quinolone resistance (PMQR) genes (Fig. 3D). The most prevalent PMQR gene was *aac(6')-Ib-cr*, which was detected in 58.3% of *K. pneumoniae*, 20.6% of *E. coli*, and 100% of *E. cloacae* complex strains. All *K. pneumoniae* isolates harboured *oqxA* and *oqxB*, whereas *qnrB1* was present in 33.3% of *K. pneumoniae* and 100% of *E. cloacae* complex strains.

4. Discussion

In 2018, 1512 BSI episodes were diagnosed at our university hospital involving 1611 pathogens. Among these, 43.6% were *Enterobacteriales* strains, 9% were *E. coli* isolates, 9.6% were *E. cloacae* complex isolates, and 22.2% of *K. pneumoniae* isolates, and were identified as ESBL producers based on phenotypic assays. Analysis of WGS data using the Galaxy platform revealed that the most prevalent ESBL genes in our hospital and community were *bla*_{CTX-M-15}, *bla*_{CTX-M-27} and *bla*_{CTX-M-14}. The bioinformatics pipeline used to analyse the WGS data did not require command line tools and was built within one hour (Fig. S1). The workflow only required uploading the paired-end fastq files of the strain to the web server, while facilitating the *de novo* assembly of genomes, MLST typing, and identification of antibiotic resistance determinants present in the strain. Therefore, running the pipeline is quite feasible for clinical microbiologists and could potentially provide results within a few hours for each strain, depending on the availability of the web server.

Although NGS has revolutionised many aspects of medicine and diagnosis, the use of WGS in routine clinical microbiology is still often restricted to reference centres, identification of rare resistance mechanisms, research purposes, or retrospective epidemiological surveillance. However, although phenotypic antibiotic susceptibility testing (AST) methods remain the gold standard, the recent drop in sequencing costs and availability of extensive AMR databases make WGS a powerful tool that will have many cost-effective uses in routine clinical microbiology. Various accessible

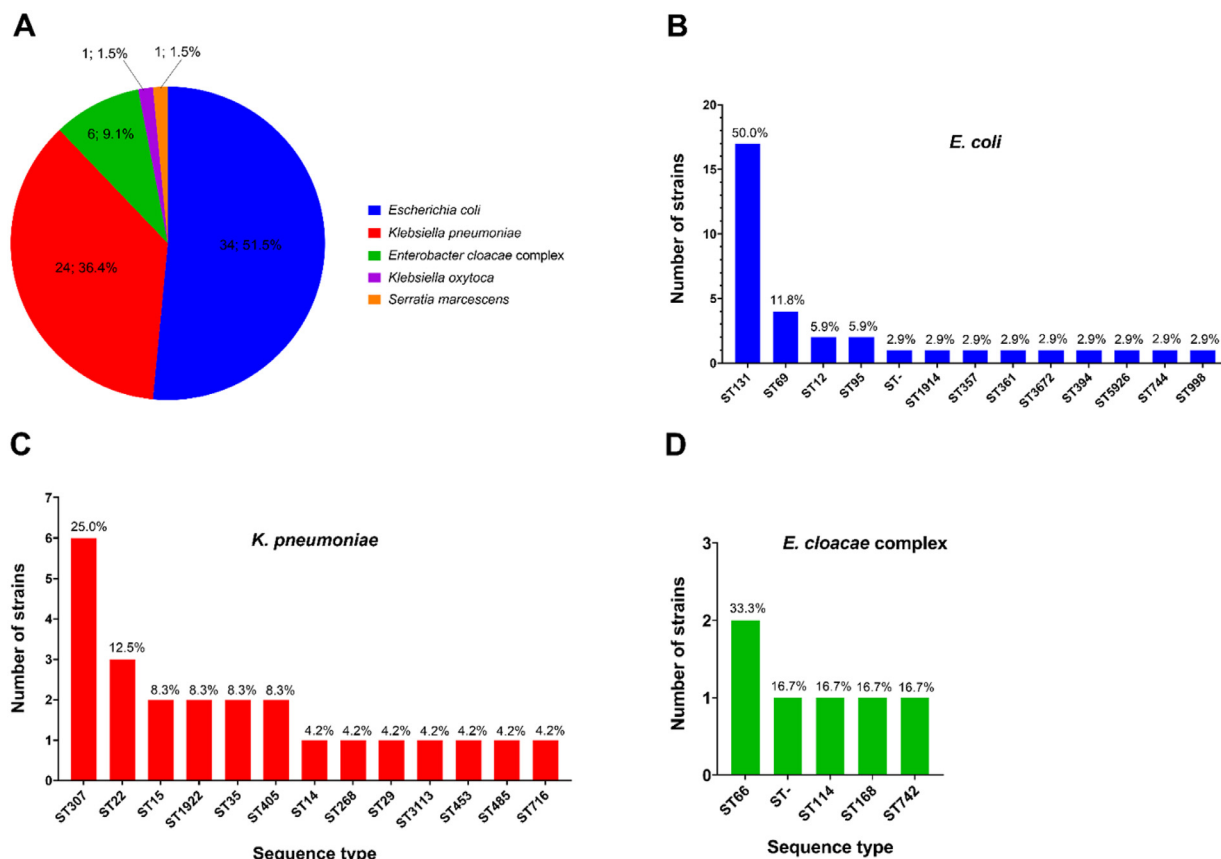


Fig. 2. Distribution and multi-locus sequence typing results of ESBL-producing *Enterobacteriales*. Isolates distribution is presented by species (A), as n; %. N = 66. Number of strains and frequencies of each sequence type for (B) *Escherichia coli* (n = 34), (C) *Klebsiella pneumoniae* (n = 24) and (D) *Enterobacter cloacae* complex (n = 6) strains.

tools can be used to analyse WGS data and predict AMR determinants; however, most analysis pipelines either require bioinformatics skills to choose between the large diversity of open-access tools and run them efficiently or the use of commercial solutions (e.g., BioMerieux Episeq Platform, Qiagen CLC Genomic Workbench, Ridom SeqSphere and Geneious Prime). The Resfinder web server launched in 2012 and the Comprehensive Antibiotic Research Database published in 2013 have paved the way for users without specialised bioinformatics skills, facilitating the detection of AMR genes through free online submission of WGS data [8,17,18]. The Galaxy platform is one of the rare open-access solutions providing graphical interfaces, while still allowing the user to select individual tools to build a customised bioinformatics pipeline (e.g., selecting assembly tools and the AMR database to analyse the data) and select a large variety of parameters. Pipelines can be shared between users, and each tool can be easily updated when a new version is released. However, this platform does not always provide the last version of all bioinformatics tools as they require additional time to implement them on the platform. Another easy-to-use graphical interface is provided by the BV-BRC platform (<https://www.bv-brc.org/app/ComprehensiveGenomeAnalysis>). It facilitates comprehensive analysis of bacterial genomes; however, it uses an internal pipeline without the possibility of selecting individual tools or parameters.

Analysis of the local epidemiology of ESBL-producing *Enterobacteriales* isolated from BSI was concordant with the existing literature, with the three main CTX-M ESBL variants identified in our study (CTX-M-15, CTX-M-14 and CTX-M-1) being particularly common worldwide [5,6]. ESBL genes have been identified in 8.2%, 15.4% and 30.3% in a clinical collection of 15 449 non-carbapenem-

resistant *E. coli* and *K. pneumoniae* isolates collected in Europe, Asia-Pacific and Latin America, respectively [5]. Most of these isolates harboured the *bla*_{CTX-M} gene (95.1% in Europe, 85.2% in Asia-Pacific and 98.1% in Latin America), with genes affiliated with CTX-M group 1 (*bla*_{CTX-M-15} and *bla*_{CTX-M-3}) and CTX-M group 9 (*bla*_{CTX-M-14}) being the most prevalent. Notably, >90% of ESBL-producing *Enterobacteriales* in our study could have been identified using molecular or immunochromatographic assays to detect *bla*_{CTX-M} genes or CTX-M enzymes from PCBs within 30 min to 2 h, whereas disk diffusion methods may take 24 to 48 h to confirm ESBL production and can lead to misinterpretation [4,10]. Indeed, in one *K. pneumoniae* isolate that was initially phenotypically characterised as expressing ESBL, we could not identify any ESBL genes by WGS. The observed phenotype was due to the overexpression of chromosomal beta-lactamase, which was incorrectly categorised during initial testing. All *K. pneumoniae* strains carry a chromosomal SHV allele that confers narrow spectrum resistance to beta-lactams, and the presence of *bla*_{SHV} genes does not automatically confer ESBL status [19]. In our study, in addition to the presence of the CTX-M enzyme, 12 *K. pneumoniae* isolates were initially categorised according to the BL database as harbouring a second ESBL gene because they carried *bla*_{SHV-27}, *bla*_{SHV-28} or *bla*_{SHV-99} genes, which have been reported as ESBLs [16,20]. However, according to Lam et al., these three genes lack the functional mutations necessary for ESBL activity, emphasising the importance of database curation for accurate AMR gene prediction [19].

Phenotypic AST will continue to be performed to detect new resistance mechanisms for which sequencing data are not yet available in the AMR databases. However, a high concordance now exists between the presence of known AMR genes or mutations and minimal inhibitory concentrations. The fourth version of Res-

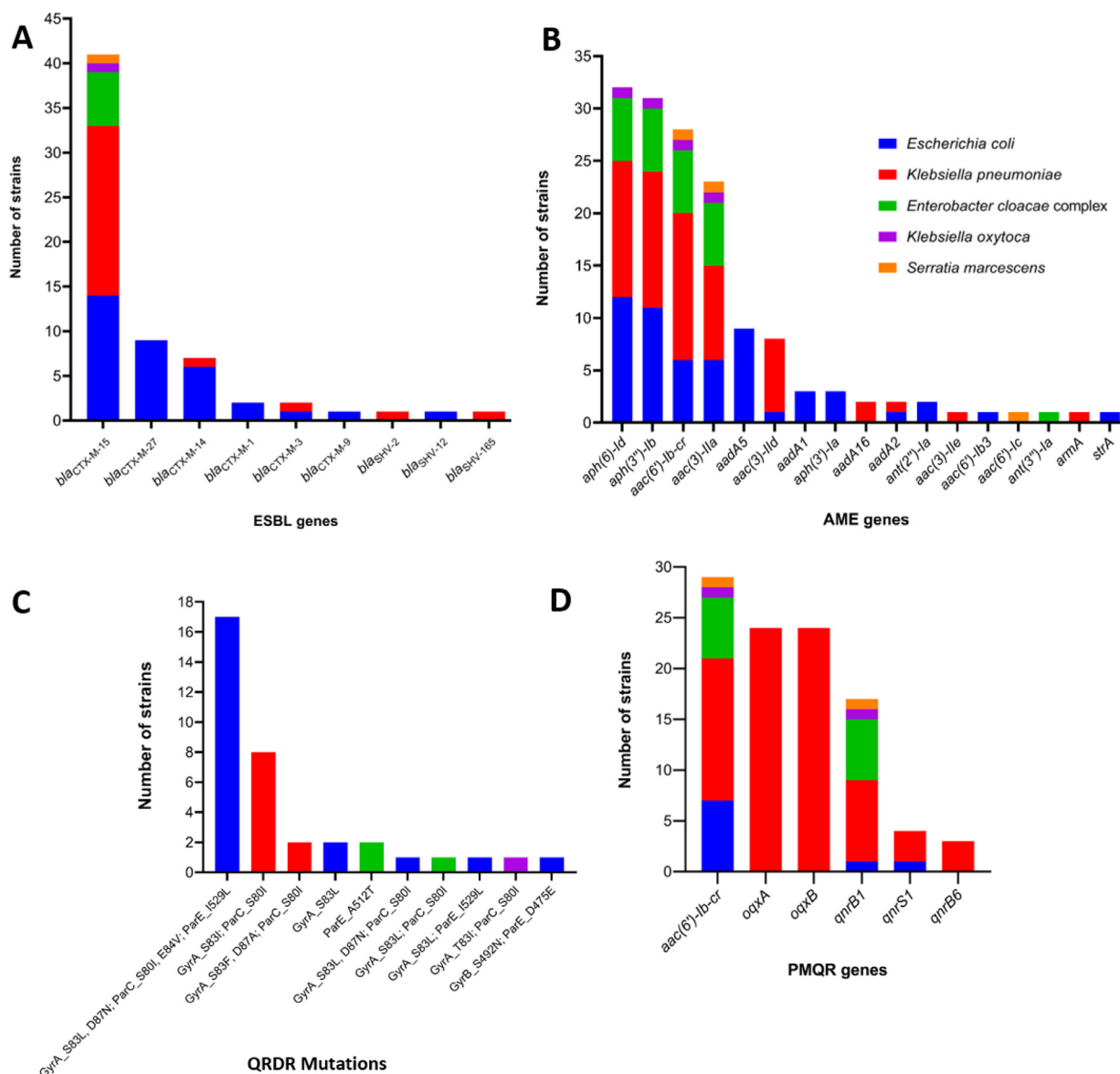


Fig. 3. Distribution of antimicrobial resistance genes in all ESBL-producing *Enterobacteriales*. ESBL genes (A), aminoglycoside-modifying enzyme (AME) genes (B), mutations in the quinolone resistance determining region (QRDR) of topoisomerase subunits GyrA, GyrB, ParC and ParE (C), and plasmid-mediated quinolone resistance (PMQR) genes.

finder allows phenotypic prediction of antimicrobial susceptibility based on genotypic data [8]. Moreover, WGS also has other advantages. WGS facilitates the identification of variants that differ by a few point mutations, such as *bla*_{CTX-M-27} and *bla*_{CTX-M-14} which differ by only one amino acid residue and may be difficult to distinguish using PCR and single-gene sequencing. Finally, whole-genome MLST and in-depth characterization of AMR gene content based on WGS data provides assessment of strain relatedness and to reach definitive conclusions regarding potential cross-transmissions. In particular, strain relatedness can be checked using additional tools on the Galaxy platform (e.g., the snippy tool that looks for high-quality single nucleotide polymorphisms between two genomes), which can be extremely helpful to infection prevention and control teams. Such new tools are expected to be implemented in routine practice to rapidly confirm or eliminate potential cross-transmissions when the strains are not genetically related despite exhibiting similar phenotypic antimicrobial resistance patterns.

Our study has some limitations. It was carried out at a single centre, and the project and bioinformatics tools have evolved

since we compiled the data. Moreover, at the time of the study, we chose to manually identify mutations in the QRDRs using multiple sequence alignment in Clustal Omega, as the PointFinder tool was not available for *Enterobacteriales* species except *E. coli*; however, this tool has been improved.

5. Conclusion

As NGS and bioinformatics technologies evolve, AMR prediction and WGS-based diagnostic tools will be of extremely helpful in the future to tackle the spread of resistant bacteria and rapidly identify multidrug-resistant bacteria, helping shorten the time to effective therapy. Galaxy platforms facilitate bacterial WGS data analysis by clinical microbiologists lacking advanced bioinformatics skills. Its use can facilitate the use of WGS approach in many clinical microbiology settings at a low cost without requiring extensive training.

Author contributions

Conceptualization and methodology: YC. Investigation and formal analysis: AB. Data curation: YC. Writing, review and editing: AB and YC. Supervision: YC. Funding acquisition: YC.

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Declaration of competing interests

All authors have no conflict of interest to declare for this study. Y. Caspar declares study grants from QIAGEN, Becton Dickinson and BAIO-Dx to Grenoble University Hospital and Congress, travel and meeting support from Becton Dickinson France SAS, MENARINI FRANCE and MSD France outside the submitted work.

Ethical approval: Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jgar.2024.08.012](https://doi.org/10.1016/j.jgar.2024.08.012).

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