

Article

Whole-Genome Sequencing of Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* Isolated from Human Bloodstream Infections

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Academic Editors: Gabriele Bianco, Sara Comini and Claudio Farina

Received: 27 December 2024

Revised: 11 February 2025

Accepted: 13 February 2025

Published: 20 February 2025

Citation: Sabença, C.; Rivière, R.; Costa, E.; Sousa, S.; Caniça, M.; Silva, V.; Igrejas, G.; Torres, C.; Poeta, P. Whole-Genome Sequencing of Extended-Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* Isolated from Human Bloodstream Infections. *Pathogens* **2025**, *14*, 205. <https://doi.org/10.3390/pathogens14030205>

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Abstract: *Klebsiella pneumoniae* is a Gram-negative bacterium commonly associated with bloodstream infections (BSIs), which can lead to severe clinical outcomes, especially in immunocompromised individuals or patients with underlying health conditions. The increasing prevalence of *K. pneumoniae* that produces extended-spectrum β -lactamases (ESBL) poses a significant challenge for treatment and infection control, necessitating a swift diagnostic approach and tailored antimicrobial therapy to improve patient outcomes. A total of 32 *K. pneumoniae* isolates were recovered from BSIs from December 2021 to August 2022. Whole-genome sequencing (WGS) was performed on the 14 ESBL-producing isolates. All ESBL isolates carried the *bla*_{CTX-M-15} gene, together with other β -lactamase-encoding genes (*bla*_{TEM-1}, *bla*_{SHV-28}, *bla*_{SHV-26}, or *bla*_{OXA-1}). Three of the isolates also carried the *bla*_{KPC-3} gene. Resistance genes to quinolones, sulfonamides, tetracycline, aminoglycosides, and chloramphenicol were also detected. We can conclude that the presence of ESBL-producing isolates among *K. pneumoniae* of BSIs raises concerns, since these enzymes limit the available treatment options, and future research must include studies on alternative therapies for dealing with resistant bacterial infections and developing new approaches to disease treatment.

Keywords: extended-spectrum β -lactamases; *Klebsiella pneumoniae*; bloodstream infections; whole-genome sequencing

1. Introduction

In recent decades, antibiotic-resistant bacteria have become an urgent public health problem. This fact has increased attention to the extended-spectrum β -lactamase (ESBLs) group of enzymes in strains of the order Enterobacterales. These enzymes can be acquired via plasmid transfer, favoring the resistance of their host strains to β -lactam antibiotics and, in particular, the third-generation cephalosporins [1–3]. ESBLs are a diverse group of β -lactamases, and their classification is based on their substrate profile and genetic relatedness. The most common types of ESBLs include CTX-M enzymes, as well as some variants of TEM and SHV. The CTX-M enzymes, in particular, have emerged as the most prevalent in recent years, with the CTX-M-15 variant being widely distributed [4]. ESBLs confer resistance by hydrolyzing third-generation cephalosporins but generally do not affect carbapenems [5].

Strains producing ESBLs together with other mechanisms of resistance for non-beta-lactams have increased, what facilitate the use carbapenems for treatment. However, the emergence of carbapenemases remains a significant public health concern [6,7]. ESBL-encoding genes are often localized on mobile genetic elements, such as plasmids, facilitating horizontal gene transfer between bacterial genera and species [8]. This ability to spread across diverse bacterial species and genera complicates treatment and increases the likelihood of outbreaks. The transfer of these genes can occur within a single bacterial species and between Enterobacterales and non-Enterobacterales members, such as *Pseudomonas* or *Acinetobacter*, further exacerbating the challenge of multidrug resistance [9,10].

According to the 2023 annual epidemiological report from the European Centre for Disease Prevention and Control (ECDC) in Portugal, 50.7% of invasive *Klebsiella pneumoniae* isolates and 17.2% of *Escherichia coli* isolates were resistant to third-generation cephalosporins, while 13.1% of *K. pneumoniae* and 0.5% of *E. coli* were resistant to carbapenems, reaching higher values since 2006 for *K. pneumoniae* and 2001 for *E. coli* (<https://atlas.ecdc.europa.eu/>; accessed on 14 January 2025) [11]. The emergence and subsequent surveillance of strains producing ESBLs are necessary for good hospital management and patient therapy, particularly for those with a clinical diagnosis of septicemia.

Klebsiella pneumoniae is one of the species that have been most frequently isolated from clinical specimens and is a cause of sepsis, meningitis, and bacteremia. Many *K. pneumoniae* strains are resistant to a broad set of antimicrobial drugs, and their capacity to produce ESBLs is an essential mechanism of bacterial resistance, which increases in both the hospital and community environment [12–14].

Two previous studies have been conducted in a hospital located in North Portugal, which showed the predominance of CTX-M-15 among ESBL-producing *K. pneumoniae* isolates alongside SHV-type β -lactamases like SHV-27 and SHV-12 in isolates obtained during 2016–2018 from different origins, including bloodstream infections (BSIs) [15,16]. Both studies reported carbapenem-resistant isolates, and some *K. pneumoniae* strains showed a cooccurrence of ESBL and carbapenemase-encoding genes, highlighting the alarming levels of multidrug resistance within this species [15,16]. A recent study was also performed in the same hospital with *K. pneumoniae* isolates recovered from different origins during a nine-month period in 2021–2022, analyzing their content in β -lactamases by PCR [17]. The present study is focused on the characterization by whole-genome sequencing (WGS) of a collection of 14 ESBL-producing *K. pneumoniae* isolates recovered from BSIs during 2021–2022 in the same hospital in order to deepen in the molecular characterization of *K. pneumoniae* isolates that circulate in this hospital in invasive infections.

2. Materials and Methods

2.1. Bacterial Isolates and Identification

Thirty-two *K. pneumoniae* isolates were recovered from BSIs in the Hospital Center of Trás-os-Montes and Alto Douro between December 2021 and August 2022 and were identified by VITEK® 2 Compact (BioMérieux, Auvergne-Rhône-Alpes, France). Fourteen of these isolates were ESBL producers (43.7%) and were selected for further characterization by WGS. The type of β -lactamases of two of these 14 ESBL-producer strains were already studied by PCR in a previous study [17].

2.2. Whole-Genome Sequencing of ESBL-Producing Isolates

WGS was performed in the 14 ESBL-producing *K. pneumoniae* isolates recovered from BSIs in the studied period. Initially, the strains were seeded in the differential medium, MacConkey, and isolated in nutrient agar. According to the manufacturer's protocol, the genomic DNA was extracted using the Magna Pure 96 system (Roche, Basel, Switzerland). The DNA concentration was measured using a Qubit™ 4 fluorometer (Thermo Scientific, Waltham, MA, USA). Sequencing libraries were prepared using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA) and sequenced using Illumina MiSeq with 150 bp paired-end reads. Raw reads were then submitted for bioinformatics analysis using multiple tools to process the WGS data. De novo assembly, species confirmation, and sequence-type raw data quality control were performed using INNUca (v 4.2.2-02) (<https://github.com/B-UMMI/INNUca> accessed on 23 September 2024). The assessment of read quality, trimming, and estimated genome completeness were performed using FastQC (v 0.11.5) (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>; accessed on 23 September 2024), Trimmomatic (v 0.38) [18], and BUSCO (v 5.5.0_cv1) (<https://gitlab.com/ezlab/busco#how-to-cite-busco>; accessed on 23 September 2024), respectively. The identification of the species was confirmed by calculating the average nucleotide identity (ANI) using FastANI (v 1.33) [19], where the query genome is compared against the complete assembled reference genomes downloaded from the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=570>; accessed on 23 September 2024). For ARGs detection, abriTAMR (v 1.0.14) and ABRicate (v 1.0.1) were used (<http://github.com/tseemann/abricate>; accessed on 23 September 2024) [20]. In ABRicate, the following public databases were used: argannot, resfinder, ncbi, card, plasmidfinder, and vfdb. Multilocus Sequence Typing (MLST) was employed to categorize the isolates into sequence types.

3. Results

3.1. Genomic Characterization by Whole-Genome Sequencing

3.1.1. β -Lactamase and Other Antimicrobial Resistance Genes

The analysis of the genomes of the 14 ESBL-producing *K. pneumoniae* isolates allows us to deepen in the molecular characteristics of these isolates. Three sequence types (ST) were identified in these 14 ESBL-positive isolates: ST15 (8 isolates), ST307 (4 isolates), and ST34 (2 isolates) (Table 1). The *bla*_{CTX-M-15} gene was found in all 14 isolates, and other β -lactamases genes were also identified, such as *bla*_{TEM-1}, *bla*_{SHV-28}, *bla*_{SHV-26}, and *bla*_{OXA-1}. Moreover, the *bla*_{KPC-3} gene was identified in three of these isolates that also showed imipenem resistance (Table 1). Regarding other resistance genes, it was found that the *aac*(3)-IIe, *aph*(3'')-Ib, *aph*(6)-Id, and *aadA16* genes confer resistance to aminoglycosides, as well as the *aac*(6')-Ib-cr gene that confers simultaneous resistance to aminoglycoside and fluoroquinolone antibiotics. Concerning resistance to fluoroquinolone antibiotics, the *qnrB1* and *qnrB6* genes were detected, but efflux pump conferring resistance to this family of antibiotics, such as *oqxA*, *oqxA10*, *oqxB19*, and *oqxB20*, was also identified. The *sul1*,

sul2, *dfrA14*, and *dfrA27* genes were detected, which confer resistance to trimethoprim-sulfamethoxazole. The *tetD* gene, responsible for conferring resistance to tetracycline, was the only associated gene identified. Lastly, three different genes were found to confer resistance to chloramphenicol: *catA1*, *catA2*, and *catB3* (Table 1).

Table 1. Molecular characterization of 14 ESBL-producing *K. pneumoniae* isolates of BSIs.

Isolate	MLST	β -Lactamase Genes	Carbapenemase Genes	Other Resistance Genes	Plasmids	Virulence Genes and Others
HS107	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	<i>bla</i> _{KPC-3}	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB20</i> , <i>sul2</i> , <i>dfrA14</i> , <i>catB3</i>	IncFII, IncFIA(HI1), IncFIB(K), IncR, Col440I	<i>ybtP</i> , <i>ybtQ</i> , <i>fieF</i> , <i>hsp20</i>
HS119	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB20</i> , <i>sul2</i> , <i>dfrA14</i> , <i>catA1</i> , <i>catB3</i>	IncFIA(HI1), IncFIB(K), IncR, Col440I Col(MG828)	<i>ybtP</i> , <i>ybtQ</i> , <i>fieF</i> , <i>hsp20</i>
HS143	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB20</i> , <i>sul2</i> , <i>dfrA14</i> , <i>catA1</i> , <i>catB3</i> ,	IncFII, IncFIA(HI1), IncFIB(K), IncR, Col440I	<i>ybtP</i> , <i>ybtQ</i> , <i>fieF</i> , <i>hsp20</i>
HS203	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB20</i> , <i>sul2</i> , <i>dfrA14</i> , <i>catA1</i> , <i>catB3</i>	IncFII, IncFIA(HI1), IncFIB(K), IncR	<i>ybtP</i> , <i>ybtQ</i> , <i>fieF</i> , <i>hsp20</i>
HS255	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	<i>bla</i> _{KPC-3}	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB20</i> , <i>sul2</i> , <i>dfrA14</i> , <i>catA1</i> , <i>catB3</i>	IncFII, IncFIA(HI1), IncFIB(K), IncR, Col440I	<i>ybtP</i> , <i>ybtQ</i> , <i>tsh</i> , <i>fieF</i> , <i>hsp20</i>
HS265	ST307	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1}	-	<i>aac</i> (3)-IIe, <i>aadA16</i> , <i>aac</i> (6')-Ib-cr, <i>qnrB6</i> , <i>sul1</i> , <i>dfrA27</i> , <i>tetD</i> , <i>catA2</i>	IncFIA(HI1), IncFIB(K), IncR	<i>fieF</i> , <i>hsp20</i>

Table 1. Cont.

Isolate	MLST	β -Lactamase Genes	Carbapenemase Genes	Other Resistance Genes	Plasmids	Virulence Genes and Others
HS269	ST307	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28}	-	<i>aac</i> (3)-IIe, <i>aadA</i> 16, <i>aac</i> (6')-Ib-cr, <i>qnrB</i> 6, <i>sul</i> 1, <i>dfrA</i> 27, <i>tetD</i> , <i>catA</i> 2	IncFIA(HI1), IncFIB(K), IncR	<i>fieF</i> , <i>hsp20</i>
HS286	ST34	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-26} , <i>bla</i> _{OXA-1}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB</i> 1, <i>oqx</i> A10, <i>oqx</i> B19, <i>sul</i> 2, <i>dfrA</i> 14, <i>catB</i> 3	IncFII *, IncFIA(HI1), IncFIB(K), ColRNAI	<i>fieF</i> , <i>hsp20</i> , <i>shsP</i> , <i>yfdX</i> 1, <i>yfdX</i> 2, <i>hdeD</i> -GI, <i>trxLHR</i> , <i>kefB</i> -GI, <i>psi</i> -GI
HS289	ST307	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-28}	-	<i>aadA</i> 16, <i>aac</i> (6')-Ib-cr, <i>qnrB</i> 6, <i>dfrA</i> 27, <i>tetD</i> , <i>catA</i> 2	IncFIA(HI1), IncFIB(K), IncR	<i>fieF</i> , <i>hsp20</i>
HS290	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28} , <i>bla</i> _{OXA-1}	<i>bla</i> _{KPC-3}	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>oqx</i> A, <i>oqx</i> B20, <i>sul</i> 2, <i>dfrA</i> 14, <i>catB</i> 3	IncFII, IncFIB(K), IncR, Col440I	<i>ybtP</i> , <i>ybtQ</i> , <i>fieF</i> , <i>hsp20</i>
HS292	ST307	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-28}	-	<i>aadA</i> 16, <i>aac</i> (6')-Ib-cr, <i>qnrB</i> 6, <i>dfrA</i> 27, <i>tetD</i> , <i>catA</i> 2	IncFIA(HI1), IncFIB(K), IncR	<i>fieF</i> , <i>hsp20</i>
HS302	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>qnrB</i> 1, <i>oqx</i> A, <i>oqx</i> B20, <i>sul</i> 2, <i>dfrA</i> 14	IncFII, IncFIA(HI1), IncFIB(K), Inc- FIB(pKPHS1), IncR	<i>fieF</i> , <i>hsp20</i>
HS321	ST15	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-28}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>sul</i> 2, <i>qnrB</i> 1, <i>oqx</i> A, <i>oqx</i> B20, <i>dfrA</i> 14	IncFII, IncFIA(HI1), IncFIB(K), Inc- FIB(pKPHS1), IncR	<i>fieF</i> , <i>hsp20</i>
HS331	ST34	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1} , <i>bla</i> _{SHV-26} , <i>bla</i> _{OXA-1}	-	<i>aac</i> (3)-IIe, <i>aph</i> (3'')-Ib, <i>aph</i> (6)-Id, <i>aac</i> (6')-Ib-cr, <i>qnrB</i> 1, <i>oqx</i> A10, <i>oqx</i> B19, <i>sul</i> 2, <i>dfrA</i> 14, <i>catB</i> 3	IncFII *, IncFIA(HI1), IncFIB(K), Col440I, Col440II, ColRNAI *	<i>fieF</i> , <i>hsp20</i> , <i>shsP</i> , <i>yfdX</i> 1, <i>yfdX</i> 2, <i>hdeD</i> -GI, <i>trxLHR</i> , <i>kefB</i> -GI, <i>psi</i> -GI

* Two copies were detected.

3.1.2. Plasmid Replicons

Both an incompatibility (Inc) group and colicinogenic (Col) plasmids were detected in the genomes of the 14 sequenced *K. pneumoniae* isolates. Inc plasmids were present in all isolates; however, Col plasmids were only detected in 7 isolates. The most detected plasmid was IncFIB(K) (n = 14), followed by IncFIA(HI1) (n = 13) and IncR (n = 12). Among

the Col plasmids, the most frequently detected was Col440I, present in six isolates. In two isolates, we identified two copies of the IncFII and ColRNAI plasmids (Table 1).

3.1.3. Virulence Genes

The 14 sequenced genomes were screened for genes related to virulence factors. Consequently, the *ybtP* and *ybtQ* virulence genes were detected in six isolates. Also, we detected the *tsh* virulence gene in one isolate, which encodes temperature-sensitive hemagglutinin. We similarly detected other genes that are not virulence genes but may have a role in helping bacterial survival, such as ferrous iron efflux (*fieF*), and eight heat resistance genes (*hsp20*, *psi-GI*, *kefB-GI*, *trxLHR*, *hdeD-GI*, *yfdX1*, *yfdX2*, and *shsP*) (Table 1).

4. Discussion

The purpose of the present study was to analyze the WGS of the ESBL-producing *K. pneumoniae* isolates recovered at the hospital level (hospital center of Trás-os-Montes and Alto Douro) of BSIs during a period of nine months. *K. pneumoniae* is frequently resistant to a wide range of antibiotics, including different β -lactams by β -lactamases [21]. In detail, infections caused by ESBL-producing Enterobacterales represent a worldwide issue concerning public health, especially given their association with poor outcomes, growing community-onset, and high ecological treatment costs [22]. Although the ESBL family is heterogeneous, the global pandemic of plasmids carrying CTX-M-type genes, which started mainly in the 2000s, is the primary driver of ESBL dissemination and has replaced other ESBL enzymes [23].

For this study, 14 ESBL-producing *K. pneumoniae* isolates recovered from BSIs from a Portuguese hospital were analyzed. One previous study was conducted on *K. pneumoniae* isolates of BSIs in the same hospital, but the study was performed four years before [15], and another one was conducted in isolates from the same period as the present study [17]. However, neither of these studies included the characterization of isolates by WGS. Concerning *K. pneumoniae* BSIs and the significant impact of the latter infections in the clinical setting drove our interest in studying these infections. BSIs due to *K. pneumoniae* are associated with higher mortality rates than infections at other body sites [24]. Consequently, filling this gap in the literature is imperative to improve patient outcomes and provide evidence-based treatment options for such serious infections. A study by Gorrie et al. conducted a year-long prospective surveillance study of *K. pneumoniae* clinical isolates in hospital patients and reported that 10% of patients had *K. pneumoniae* isolated from disseminated infections (bloodstream and/or cerebral spinal fluid), a similar percentage to ours [25]. This consistency across studies suggests that a range of infections caused by *K. pneumoniae* progress to BSIs, suggesting that bacterial or host factors may contribute to the potential to disseminate *K. pneumoniae*.

Regarding ESBL production, the reported ESBL production rates in BSIs caused by Enterobacterales are highly variable. The European Centre for Disease Prevention and Control (ECDC), in its 2023 annual epidemiological report, revealed that, in Portugal, the percentage of invasive *K. pneumoniae* isolates resistant to third-generation cephalosporins was 50.7% (<https://atlas.ecdc.europa.eu/>; accessed on 14 January 2025) [11]. This ECDC value represents the highest recorded percentage for third-generation cephalosporins since surveillance began in 2006 in Portugal and is a little higher than the one found in this study (43.7%). This upward trend in resistance poses a substantial challenge to public health, as it limits the effectiveness of commonly used antibiotics and complicates treatment options for infections caused by *K. pneumoniae* [26]. In Ethiopia, 67.3% of BSI isolates were ESBL producers [27]. A study conducted in Egypt reported lower percentages of ESBL-producing isolates than the previous study at 48.93% [28]. Also, a study in India demonstrated that

43.5% of Gram-negative isolates were β -lactamase producers [29]. The high prevalence of CTX-M among *K. pneumoniae* isolates is an important finding for applying rapid diagnostic methods. For example, rapid immunochromatographic or molecular methods capable of detecting CTX-M could be implemented in areas with a high prevalence of CTX-M ESBL [30]. These rates of ESBL occurrence represent a worryingly high prevalence amongst BSIs. This paper also underlines once more the call to strengthen surveillance and detection mechanisms in hospitals towards BSIs caused by ESBL-producing Enterobacterales.

Concerning the analysis of the whole genomes, three different sequence types were detected among the 14 ESBL-producing *K. pneumoniae* sequenced isolates: ST15 (57.1%), ST307 (28.6%), and ST34 (14.3%). Various studies have described different STs among the BSI *K. pneumoniae* isolates, where ST15 frequently emerges alongside other common types, such as ST11 and ST23 [31–34]. The prominence of ST15 in our isolates is notable, as it is associated with multidrug resistance and often implicated in healthcare-associated infections, indicating a potentially high transmission and adaptation rate in clinical settings. However, less commonly found, both ST34 and ST307 have also been reported [35–38]. The identification of these sequence types in our study highlights the diversity and adaptability of *K. pneumoniae* strains contributing to BSIs.

Regarding β -lactamase genes, we verified a predominance of the *bla*_{CTX-M-15} gene, which was also predominant in the previous studies conducted by our group [15]. Still, in some strains, this gene was associated with a carbapenemase gene (*bla*_{KPC} or *bla*_{OXA-48}). It is interesting to show that, in four years, the genetic lineages seemed to differ in both studies. While our study reported isolates belonging to ST15, ST307, and ST34, the previous one reported isolates belonging to ST348, ST11, and ST15. ESBLs encoded by *bla*_{CTX-M-15} are one of the most commonly found among *K. pneumoniae* isolates causing BSIs. Multiple studies have reported a high prevalence of this gene; for example, in South Korea, a study conducted between 2017 and 2019 verified that the dominant ESBL type in *K. pneumoniae* blood isolates was CTX-M-group 1 (78.5%), which included CTX-M-15 (n = 407) and CTX-M-55 (n = 5), while CTX-M-group 9 was less frequently identified (14.0%) but included CTX-M-14 (n = 60) and CTX-M-27 (n = 8) [39]. Also, in Italy, among the *K. pneumoniae* isolates, all encoded CTX-M-15 [40], and in Algeria, CTX-M-15 ESBL-*K. pneumoniae* strains infected 8.2% of neonates [41]. Regarding carbapenemase-producing enzymes, we detected the *bla*_{KPC-3} gene (in addition to *bla*_{CTX-M-15}) in three isolates analyzed by WGS. The *bla*_{KPC} gene is frequent in BSI isolates of *K. pneumoniae*. In this sense, a prevalence of this gene of 33.3% has been identified in China [42] and 67.4% in carbapenem-resistant Enterobacterales isolates in India [43]. In Brazil, the prevalence of the *bla*_{KPC} gene in carbapenem-resistant *K. pneumoniae* BSI isolates was 90.4% [44]. It has been shown that the presence of *bla*_{KPC} genes increases the total mortality in BSI patients [42,44]. In the case of Enterobacterales, KPC-2 and -3 are the most commonly detected variants [35,39,45,46]. In Portugal, CTX-M-15 and KPC-3 are predominant, which is in line with our results. In the last five years, multiple studies have reported the presence of these enzymes, in particular KPC-3, among Enterobacterales isolates, underscoring their significance in antimicrobial resistance profiles within the region [15,16,47–52]. However, the genetic lineages identified in our study appear to diverge from those previously reported in Portugal. While ST15 is frequently encountered in Portuguese clinical bacterial isolates, the other two sequence types have been less commonly documented in healthcare settings [15,16,48,50,52–55]. The persistence of these resistance mechanisms reflects local and global trends in antimicrobial resistance and highlights the need for ongoing surveillance and stringent infection control measures.

Besides ESBLs, several other AMR genes were identified in BSI *K. pneumoniae* isolates, including those conferring resistance to aminoglycoside, fluoroquinolone, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol. Among the commonly detected

genes, those that encode aminoglycoside resistance included *aac(3)-Ile*, *aph(3'')-Ib*, *aph(6)-Id*, and *aadA16*; fluoroquinolones resistance included *qnrB1*, *qnrB6*, and co-resistance to fluoroquinolones *aac(6')-Ib-cr* genes; trimethoprim-sulfamethoxazole included *sul1*, *sul2*, *dfrA14*, and *dfrA27*; tetracyclines included *tetD* genes; and chloramphenicol included *catA1*, *catA2*, and *catB3*. Also, genes for the efflux pumps, such as *oqxA*, *oqxA10*, *oqxB19*, and *oqxB20*, which further facilitated the ability of bacteria to efflux a wide range of antibiotics, were detected. Other studies have also identified several genes that confer resistance to these antibiotic classes, for example, a study conducted in the Eastern Democratic Republic of Congo reported similar results by finding that the *K. pneumoniae* isolates carried at least one gene linked to resistance to aminoglycosides and/or quinolones genes (*aac(3)-IId*, *aac(3)-Ile*, *aadA1*, *aadA16*, *aadA2*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *aac(6')-Ib-cr*, or *aac(6')-Ib-cr5*), as well as to phenicols (*catA1*, *catA2*, and *catB*); sulfonamides (*sul1* and *sul2*); tetracycline (*tetA* or *tetD*); trimethoprim (*dfrA1*, *dfrA12*, *dfrA14*, *dfrA15*, *dfrA25*, and *dfrA27*); plasmid-mediated quinolone resistance genes (*qnrB*, *qnrB1*, *qnrB2*, *qnrB6*, and *qnrS1*); and also efflux pump genes (*oqxA*, *oqxB*, *oqxB5*, *oqxB19*, *oqxB21*, and/or *oqxB32*) [56]. The diverse AMR genes that underpin its complex resistance mechanisms stress the need for intensive surveillance and targeted antimicrobial strategies in controlling this pathogen.

Regarding detected plasmids, we have identified either Inc plasmids or Col plasmids. Among the Inc plasmids, IncFIB(K), IncFIA(HI1), and IncR were the most detected ones, and regarding Col plasmids, we have detected Col440I, Col440II, and ColRNAI. Plasmid incompatibility groups, especially Inc-type plasmids, are known to play a significant role in the spread of antimicrobial resistance genes. IncF and IncR plasmids, for example, are frequently associated with resistance genes and have been widely documented in *K. pneumoniae* BSI isolates [57]. The high prevalence of IncFIB(K) and IncR plasmids is consistent with reports linking these plasmids to multidrug-resistant strains and their capacity to carry multiple resistance genes, thus enhancing the potential for resistance dissemination within hospital settings. While Inc plasmids are generally more common than Col plasmids in *K. pneumoniae*, Col-type plasmids like Col440I and ColRNAI can also contribute to survival under antibiotic pressure by facilitating resistance gene transfer, albeit on a smaller scale [58–61]. Their presence suggests that Col plasmids might play a secondary yet complementary role in the persistence and adaptability of *K. pneumoniae* in clinical environments. Understanding the roles these plasmids play in resistance gene transmission is crucial, as they represent key targets for surveillance and control measures aimed at reducing the spread of resistant strains in healthcare settings.

Regarding virulence genes, the detection of *ybtP* and *ybtQ* virulence genes was made in six isolates. These genes belong to the *ybtPQXS* operon, making part of a yersiniabactin biosynthesis and transport system. This operon is critical for acquiring and transporting iron through a siderophore-mediated mechanism, where yersiniabactin acts as the siderophore [62–64]. We also detected the temperature-sensitive hemagglutinin gene (*tsh*) in one isolate. This virulence factor promotes adherence to host cells, a critical first step in colonization and infection, and degrades the host proteins, which may facilitate tissue invasion and nutrient acquisition [65,66]. Apart from the virulence-associated genes identified, we also found in our isolates other genes that are not categorized under the traditional virulence factors but may considerably enable bacteria to survive against various environmental stresses. For instance, the product of the *fieF* gene is a ferrous iron efflux pump that helps bacteria regulate and export excessive iron ions. This function helps reduce metal-induced stress, supporting bacterial growth and contributing to the bacteria's ability to survive in metal-rich environments, including during host infection [67,68]. We also found eight genes related to heat resistance: *hsp20*, *psi-GI*, *kefB-GI*, *trxLHR*, *hdeD-GI*, *yfdX1*, *yfdX2*, and *shsP*. These same eight genes have also been reported in a study performed on

E. coli from cattle [69]. These heat resistance genes are crucial factors for bacterial survival in extreme environments. For example, the *hsp20* gene encodes for a small-sized heat shock protein that protects bacterial cells under various stressful conditions, such as heat shock, osmotic shock, and oxidative stress [70,71]. Another example is the locus of heat resistance (LHR), a genomic island that confers exceptional heat resistance in Enterobacteriales, including *E. coli* and *Salmonella*. It includes genes such as *yfdX1*, *yfdX2*, and *hdeD*, which are necessary for high-level heat resistance [72–74]. Altogether, these genes confer an impressive armamentarium on bacteria to resist stressing conditions and raise their survival capability outside their host and in environmental reservoirs. This indirectly contributes to the persistence and spread of antibiotic-resistant bacteria, as such genes support their populations in persisting and expanding under the variability of environmental conditions, challenges by antibiotics, or host immune defenses. These survival genes are important in understanding bacterial resilience, though virulence-related, because they can enable the long-term persistence of pathogenic and antibiotic-resistant strains in the ecosystem.

5. Conclusions

Conclusively, the results of this research paper highlight important microbiological aspects of BSIs due to ESBL-producing *K. pneumoniae* in a Portuguese hospital. Crucial findings from this study include ESBL production, with most strains carrying CTX-M-15 as their major ESBL genotype. Further significant aspects involved the identification of the *bla*_{KPC-3} carbapenemase gene. High levels of ESBL-producing isolates raise concerns, since these enzymes limit the available treatment options. To this end, future research must include studies on alternative therapies for dealing with resistant bacterial infections and the development of new approaches to disease treatment. This also calls for the continuous monitoring of resistance patterns and emergence of new mechanisms, provision of point-of-care tests for better management of infections through correct drug application, more stringent efforts related to infection control and the prevention of resistant bacteria spreading, and finally, prompt action taken by every institution towards the optimal use of antibiotics due to global changes in resistance levels that are impermanent and the coexistence of which, with a watchful selection of antimicrobials, is imperative to retaining all available options for the treatment and management of severe infections. A combined strategy of surveillance, hygienic practices, diagnostics, and antibiotic optimization programs is necessary to combat the menace presented by antibiotic-resistant *K. pneumoniae*.

Author Contributions: Conceptualization, C.S.; methodology, C.S., E.C., S.S. and R.R.; investigation, C.S., E.C., S.S. and R.R.; data curation, C.S.; validation, M.C. and V.S.; writing—original draft preparation, C.S.; writing—review and editing, C.S., R.R., E.C., S.S., M.C., V.S., G.I., C.T. and P.P.; supervision, G.I., C.T. and P.P.; funding acquisition, P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the projects UI/00772 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT) and by the FCT/MCTES (LA/P/0008/2020 DOI:10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI: 10.54499/UIDP/50006/2020, and UIDB/50006/2020 DOI: 10.54499/UIDB/50006/2020) through national funds.

Institutional Review Board Statement: Ethical review and approval were waived for this study due to our study did not involve direct interaction with human participants, or human data. The bacterial isolates used in our research were obtained from the Hospital of Trás-os-Montes and Alto Douro, and these were provided to us anonymously by the hospital.

Informed Consent Statement: Patient consent was waived due to the bacterial isolates were collected and provided by the hospital as part of their routine clinical procedures, and since no personal or identifiable data was used in this study.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgments: National funds were provided to Carolina Sabença through the FCT under the Ph.D. grant 2020.06967.BD.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Nagshetty, K.; Shilpa, B.M.; Patil, S.A.; Shivannavar, C.T.; Manjula, N.G. An Overview of Extended Spectrum Beta Lactamases and Metallo Beta Lactamases. *Adv. Microbiol.* **2021**, *11*, 37–62. [[CrossRef](#)]
2. Gelalcha, B.D.; Kerro Dego, O. Extended-Spectrum Beta-Lactamases Producing Enterobacteriaceae in the USA Dairy Cattle Farms and Implications for Public Health. *Antibiotics* **2022**, *11*, 1313. [[CrossRef](#)] [[PubMed](#)]
3. Dikoumba, A.-C.; Onanga, R.; Boundenga, L.; Bignoumba, M.; Ngougou, E.-B.; Godreuil, S. Prevalence and Characterization of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae in Major Hospitals in Gabon. *Microb. Drug Resist.* **2021**, *27*, 1525–1534. [[CrossRef](#)] [[PubMed](#)]
4. Yu, K.; Huang, Z.; Xiao, Y.; Gao, H.; Bai, X.; Wang, D. Global spread characteristics of CTX-M-type extended-spectrum β -lactamases: A genomic epidemiology analysis. *Drug Resist. Updates* **2024**, *73*, 101036. [[CrossRef](#)] [[PubMed](#)]
5. Belley, A.; Morrissey, I.; Hawser, S.; Kothari, N.; Knechtle, P. Third-generation cephalosporin resistance in clinical isolates of Enterobacterales collected between 2016–2018 from USA and Europe: Genotypic analysis of β -lactamases and comparative in vitro activity of cefepime/enmetazobactam. *J. Glob. Antimicrob. Resist.* **2021**, *25*, 93–101. [[CrossRef](#)] [[PubMed](#)]
6. de Sousa Coelho, F.; Mainardi, J.-L. The multiple benefits of second-generation β -lactamase inhibitors in treatment of multidrug-resistant bacteria. *Infect. Dis. Now* **2021**, *51*, 510–517. [[CrossRef](#)]
7. Lomovskaya, O.; Rubio-Aparicio, D.; Nelson, K.; Sun, D.; Tsivkovski, R.; Castanheira, M.; Lindley, J.; Loutit, J.; Dudley, M. In Vitro Activity of the Ultrabroad-Spectrum Beta-Lactamase Inhibitor QPX7728 in Combination with Multiple Beta-Lactam Antibiotics against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **2021**, *65*, e00210-21. [[CrossRef](#)] [[PubMed](#)]
8. Jian, Z.; Zeng, L.; Xu, T.; Sun, S.; Yan, S.; Yang, L.; Huang, Y.; Jia, J.; Dou, T. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *J. Basic Microbiol.* **2021**, *61*, 1049–1070. [[CrossRef](#)] [[PubMed](#)]
9. Tanner, W.D.; Atkinson, R.M.; Goel, R.K.; Toleman, M.A.; Benson, L.S.; Porucznik, C.A.; VanDerslice, J.A. Horizontal transfer of the blaNDM-1 gene to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in biofilms. *FEMS Microbiol. Lett.* **2017**, *364*, fnx048. [[CrossRef](#)] [[PubMed](#)]
10. Olesen, A.K.; Pinilla-Redondo, R.; Hansen, M.F.; Russel, J.; Dechesne, A.; Smets, B.F.; Madsen, J.S.; Nesme, J.; Sørensen, S.J. IncHI1A plasmids potentially facilitate horizontal flow of antibiotic resistance genes to pathogens in microbial communities of urban residential sewage. *Mol. Ecol.* **2022**, *31*, 1595–1608. [[CrossRef](#)] [[PubMed](#)]
11. European Centre for Disease Prevention and Control. *Antimicrobial Resistance in the EU/EEA (EARS-Net)-Annual Epidemiological Report 2023*; European Centre for Disease Prevention and Control: Stockholm, Sweden, 2024.
12. Ballén, V.; Gabasa, Y.; Ratia, C.; Ortega, R.; Tejero, M.; Soto, S. Antibiotic Resistance and Virulence Profiles of *Klebsiella pneumoniae* Strains Isolated From Different Clinical Sources. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 738223. [[CrossRef](#)]
13. Mukherjee, S.; Mitra, S.; Dutta, S.; Basu, S. Neonatal Sepsis: The Impact of Carbapenem-Resistant and Hypervirulent *Klebsiella pneumoniae*. *Front. Med.* **2021**, *8*, 634349. [[CrossRef](#)] [[PubMed](#)]
14. Effah, C.Y.; Sun, T.; Liu, S.; Wu, Y. *Klebsiella pneumoniae*: An increasing threat to public health. *Ann. Clin. Microbiol. Antimicrob.* **2020**, *19*, 1. [[CrossRef](#)]
15. Carvalho, I.; Chenouf, N.S.; Carvalho, J.A.; Castro, A.P.; Silva, V.; Capita, R.; Alonso-Calleja, C.; de Lurdes Nunes Enes Dapkevicius, M.; Igrejas, G.; Torres, C.; et al. Multidrug-resistant *Klebsiella pneumoniae* harboring extended spectrum β -lactamase encoding genes isolated from human septicemias. *PLoS ONE* **2021**, *16*, e0250525. [[CrossRef](#)] [[PubMed](#)]
16. Carvalho, I.; Carvalho, J.A.; Martínez-Álvarez, S.; Sadi, M.; Capita, R.; Alonso-Calleja, C.; Rabbi, F.; de Lurdes Nunes Enes Dapkevicius, M.; Igrejas, G.; Torres, C.; et al. Characterization of ESBL-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Clinical Samples in a Northern Portuguese Hospital: Predominance of CTX-M-15 and High Genetic Diversity. *Microorganisms* **2021**, *9*, 1914. [[CrossRef](#)]
17. Sabença, C.; Costa, E.; Sousa, S.; Barros, L.; Oliveira, A.; Ramos, S.; Igrejas, G.; Torres, C.; Poeta, P. Evaluation of the Ability to Form Biofilms in KPC-Producing and ESBL-Producing *Klebsiella pneumoniae* Isolated from Clinical Samples. *Antibiotics* **2023**, *12*, 1143. [[CrossRef](#)] [[PubMed](#)]
18. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [[CrossRef](#)] [[PubMed](#)]
19. Jain, C.; Rodriguez-R, L.M.; Phillippy, A.M.; Konstantinidis, K.T.; Aluru, S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat. Commun.* **2018**, *9*, 5114. [[CrossRef](#)]

20. Sherry, N.L.; Horan, K.A.; Ballard, S.A.; Gonçalves da Silva, A.; Gorrie, C.L.; Schultz, M.B.; Stevens, K.; Valcanis, M.; Sait, M.L.; Stinear, T.P.; et al. An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. *Nat. Commun.* **2023**, *14*, 60. [[CrossRef](#)] [[PubMed](#)]
21. Jiang, W.; Yang, W.; Zhao, X.; Wang, N.; Ren, H. *Klebsiella pneumoniae* presents antimicrobial drug resistance for β -lactam through the ESBL/PBP signaling pathway. *Exp. Ther. Med.* **2020**, *19*, 2449. [[CrossRef](#)]
22. Boattini, M.; Bianco, G.; Ghibaudo, D.; Comini, S.; Corcione, S.; Cavallo, R.; De Rosa, F.G.; Costa, C. Impact of NG-Test CTX-M MULTI Immunochromatographic Assay on Antimicrobial Management of *Escherichia coli* Bloodstream Infections. *Antibiotics* **2023**, *12*, 473. [[CrossRef](#)]
23. Comini, S.; Bianco, G.; Boattini, M.; Banche, G.; Ricciardelli, G.; Allizond, V.; Cavallo, R.; Costa, C. Evaluation of a diagnostic algorithm for rapid identification of Gram-negative species and detection of extended-spectrum β -lactamase and carbapenemase directly from blood cultures. *J. Antimicrob. Chemother.* **2022**, *77*, 2632–2641. [[CrossRef](#)] [[PubMed](#)]
24. Papadimitriou-Olivergeris, M.; Bartzavali, C.; Georgakopoulou, A.; Kolonitsiou, F.; Papamichail, C.; Spiliopoulou, I.; Christofidou, M.; Fligou, F.; Marangos, M. Mortality of Pandrug-Resistant *Klebsiella pneumoniae* Bloodstream Infections in Critically Ill Patients: A Retrospective Cohort of 115 Episodes. *Antibiotics* **2021**, *10*, 76. [[CrossRef](#)] [[PubMed](#)]
25. Gorrie, C.L.; Mirčeta, M.; Wick, R.R.; Judd, L.M.; Lam, M.M.C.; Gomi, R.; Abbott, I.J.; Thomson, N.R.; Strugnell, R.A.; Pratt, N.F.; et al. Genomic dissection of *Klebsiella pneumoniae* infections in hospital patients reveals insights into an opportunistic pathogen. *Nat. Commun.* **2022**, *13*, 3017. [[CrossRef](#)] [[PubMed](#)]
26. Majumder, M.A.A.; Rahman, S.; Cohall, D.; Bharatha, A.; Singh, K.; Haque, M.; Gittens-St Hilaire, M. Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. *Infect. Drug Resist.* **2020**, *13*, 4713–4738. [[CrossRef](#)]
27. Seman, A.; Mihret, A.; Sebre, S.; Awoke, T.; Yeshitela, B.; Yitayew, B.; Aseffa, A.; Asrat, D.; Abebe, T. Prevalence and Molecular Characterization of Extended Spectrum β -Lactamase and Carbapenemase-Producing Enterobacteriaceae Isolates from Bloodstream Infection Suspected Patients in Addis Ababa, Ethiopia. *Infect. Drug Resist.* **2022**, *15*, 1367–1382. [[CrossRef](#)]
28. Abdallah, H.M.; Wintermans, B.B.; Reuland, E.A.; Koek, A.; al Naiemi, N.; Ammar, A.M.; Mohamed, A.A.; Vandembroucke-Grauls, C.M.J.E. Extended-Spectrum β -Lactamase- and Carbapenemase-Producing Enterobacteriaceae Isolated from Egyptian Patients with Suspected Blood Stream Infection. *PLoS ONE* **2015**, *10*, e0128120. [[CrossRef](#)] [[PubMed](#)]
29. Jena, L.; Swain, B.; Jain, S. Antimicrobial resistance pattern of bacterial isolates and genetic resistance determinants of carbapenemase producers in bloodstream infections. *CHRISMED J. Health Res.* **2020**, *7*, 56.
30. Boattini, M.; Bianco, G.; Comini, S.; Iannaccone, M.; Casale, R.; Cavallo, R.; Nordmann, P.; Costa, C. Direct detection of extended-spectrum- β -lactamase-producers in Enterobacterales from blood cultures: A comparative analysis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2022**, *41*, 407–413. [[CrossRef](#)] [[PubMed](#)]
31. Ma, Y.; Bao, C.; Liu, J.; Hao, X.; Cao, J.; Ye, L.; Yang, J. Microbiological characterisation of *Klebsiella pneumoniae* isolates causing bloodstream infections from five tertiary hospitals in Beijing, China. *J. Glob. Antimicrob. Resist.* **2018**, *12*, 162–166. [[CrossRef](#)]
32. Wyres, K.L.; Nguyen, T.N.T.; Lam, M.M.C.; Judd, L.M.; van Vinh Chau, N.; Dance, D.A.B.; Ip, M.; Karkey, A.; Ling, C.L.; Miliya, T.; et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med.* **2020**, *12*, 11. [[CrossRef](#)]
33. Jia, X.; Li, C.; Chen, F.; Li, X.; Jia, P.; Zhu, Y.; Sun, T.; Hu, F.; Jiang, X.; Yu, Y.; et al. Genomic epidemiology study of *Klebsiella pneumoniae* causing bloodstream infections in China. *Clin. Transl. Med.* **2021**, *11*, e624. [[CrossRef](#)]
34. Zhang, N.; Qi, L.; Liu, X.; Jin, M.; Jin, Y.; Yang, X.; Chen, J.; Qin, S.; Liu, F.; Tang, Y.; et al. Clinical and Molecular Characterizations of Carbapenem-Resistant *Klebsiella pneumoniae* Causing Bloodstream Infection in a Chinese Hospital. *Microbiol. Spectr.* **2022**, *10*, e01690-22. [[CrossRef](#)] [[PubMed](#)]
35. Cejas, D.; Elena, A.; Guevara Nuñez, D.; Sevillano Platero, P.; De Paulis, A.; Magariños, F.; Alfonso, C.; Berger, M.A.; Fernández-Canigia, L.; Gutkind, G.; et al. Changing epidemiology of KPC-producing *Klebsiella pneumoniae* in Argentina: Emergence of hypermucoviscous ST25 and high-risk clone ST307. *J. Glob. Antimicrob. Resist.* **2019**, *18*, 238–242. [[CrossRef](#)] [[PubMed](#)]
36. Le, M.N.-T.; Kayama, S.; Wyres, K.L.; Yu, L.; Hisatsune, J.; Suzuki, M.; Yahara, K.; Terachi, T.; Sawa, K.; Takahashi, S.; et al. Genomic epidemiology and temperature dependency of hypermucoviscous *Klebsiella pneumoniae* in Japan. *Microb. Genom.* **2022**, *8*, 000827. [[CrossRef](#)] [[PubMed](#)]
37. Falcone, M.; Tiseo, G.; Galfo, V.; Giordano, C.; Leonildi, A.; Marciano, E.; De Simone, P.; Biancofiore, G.; Boggi, U.; Barnini, S.; et al. Bloodstream infections in patients with rectal colonization by *Klebsiella pneumoniae* producing different type of carbapenemases: A prospective, cohort study (CHIMERA study). *Clin. Microbiol. Infect.* **2022**, *28*, e1–e298. [[CrossRef](#)]
38. Pristas, I.; Ujevic, J.; Bodulić, K.; Andrijasevic, N.; Bedenic, B.; Payerl-Pal, M.; Susic, E.; Dobrovic, K.; De Koster, S.; Malhotra-Kumar, S.; et al. The Association between Resistance and Virulence of *Klebsiella pneumoniae* in High-Risk Clonal Lineages ST86 and ST101. *Microorganisms* **2024**, *12*, 1997. [[CrossRef](#)]
39. Kim, D.; Yoon, E.J.; Hong, J.S.; Choi, M.H.; Kim, H.S.; Kim, Y.R.; Kim, Y.A.; Uh, Y.; Shin, K.S.; Shin, J.H.; et al. Major Bloodstream Infection-Causing Bacterial Pathogens and Their Antimicrobial Resistance in South Korea, 2017–2019: Phase I Report from Kor-GLASS. *Front. Microbiol.* **2022**, *12*, 799084. [[CrossRef](#)]

40. Arena, F.; Menchinelli, G.; Di Pilato, V.; Torelli, R.; Antonelli, A.; Henrici De Angelis, L.; Coppi, M.; Sanguinetti, M.; Rossolini, G.M. Resistance and virulence features of hypermucoviscous *Klebsiella pneumoniae* from bloodstream infections: Results of a nationwide Italian surveillance study. *Front. Microbiol.* **2022**, *13*, 983294. [[CrossRef](#)]
41. Mairi, A.; Meyer, S.; Tilloy, V.; Barraud, O.; Touati, A. Whole Genome Sequencing of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* Isolated from Neonatal Bloodstream Infections at a Neonatal Care Unit, Algeria. *Microb. Drug Resist.* **2022**, *28*, 867–876. [[CrossRef](#)] [[PubMed](#)]
42. Xu, M.; Fu, Y.; Kong, H.; Chen, X.; Chen, Y.; Li, L.; Yang, Q. Bloodstream infections caused by *Klebsiella pneumoniae*: Prevalence of *bla*KPC, virulence factors and their impacts on clinical outcome. *BMC Infect. Dis.* **2018**, *18*, 358. [[CrossRef](#)] [[PubMed](#)]
43. Shanmugam, P.; Meenakshisundaram, J.; Jayaraman, P. *bla*KPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae in a Tertiary Care Hospital. *J. Clin. Diagn. Res.* **2013**, *7*, 2736–2738. [[PubMed](#)]
44. Soares de Moraes, L.; Gomes Magalhaes, G.L.; Material Soncini, J.G.; Pelisson, M.; Eches Perugini, M.R.; Vespero, E.C. High mortality from carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection. *Microb. Pathog.* **2022**, *167*, 105519. [[CrossRef](#)]
45. Fox, V.; Mangioni, D.; Renica, S.; Comelli, A.; Teri, A.; Zatelli, M.; Orena, B.S.; Scuderi, C.; Cavallero, A.; Rossi, M.; et al. Genomic characterization of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) strains circulating in three university hospitals in Northern Italy over three years. *Antimicrob. Resist. Infect. Control* **2024**, *13*, 70. [[CrossRef](#)]
46. Liu, C.; Du, P.; Yang, P.; Yi, J.; Lu, M.; Shen, N. Emergence of Extensively Drug-Resistant and Hypervirulent KL2-ST65 *Klebsiella pneumoniae* Harboring *bla* KPC-3 in Beijing, China. *Microbiol. Spectr.* **2022**, *10*, e03044-22. [[CrossRef](#)] [[PubMed](#)]
47. Novais, Â.; Ferraz, R.V.; Viana, M.; da Costa, P.M.; Peixe, L. NDM-1 Introduction in Portugal through a ST11 KL105 *Klebsiella pneumoniae* Widespread in Europe. *Antibiotics* **2022**, *11*, 92. [[CrossRef](#)] [[PubMed](#)]
48. Aires-de-Sousa, M.; Lopes, E.; Gonçalves, M.L.; Pereira, A.L.; Machado e Costa, A.; de Lencastre, H.; Poirel, L. Intestinal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae at admission in a Portuguese hospital. *Eur. J. Clin. Microbiol. Infect. Dis.* **2020**, *39*, 783–790. [[CrossRef](#)] [[PubMed](#)]
49. Perdigão, J.; Modesto, A.; Pereira, A.L.; Neto, O.; Matos, V.; Godinho, A.; Phelan, J.; Charleston, J.; Spadar, A.; de Sessions, P.F.; et al. Whole-genome sequencing resolves a polyclonal outbreak by extended-spectrum beta-lactam and carbapenem-resistant *Klebsiella pneumoniae* in a Portuguese tertiary-care hospital. *Microb. Genom.* **2021**, *7*, 349. [[CrossRef](#)]
50. Elias, R.; Spadar, A.; Hendrickx, A.P.A.; Bonnin, R.A.; Dortet, L.; Pinto, M.; Phelan, J.E.; Portugal, I.; Campino, S.; da Silva, G.J.; et al. Emergence of KPC-3- and OXA-181-producing ST13 and ST17 *Klebsiella pneumoniae* in Portugal: Genomic insights on national and international dissemination. *J. Antimicrob. Chemother.* **2023**, *78*, 1300–1308. [[CrossRef](#)] [[PubMed](#)]
51. Spadar, A.; Phelan, J.; Elias, R.; Modesto, A.; Caneiras, C.; Marques, C.; Lito, L.; Pinto, M.; Cavaco-Silva, P.; Ferreira, H.; et al. Genomic epidemiological analysis of *Klebsiella pneumoniae* from Portuguese hospitals reveals insights into circulating antimicrobial resistance. *Sci. Rep.* **2022**, *12*, 13791. [[CrossRef](#)]
52. Perdigão, J.; Caneiras, C.; Elias, R.; Modesto, A.; Spadar, A.; Phelan, J.; Campino, S.; Clark, T.G.; Costa, E.; Saavedra, M.J.; et al. Genomic Epidemiology of Carbapenemase Producing *Klebsiella pneumoniae* Strains at a Northern Portuguese Hospital Enables the Detection of a Misidentified *Klebsiella variicola* KPC-3 Producing Strain. *Microorganisms* **2020**, *8*, 1986. [[CrossRef](#)] [[PubMed](#)]
53. Mendes, G.; Ramalho, J.F.; Duarte, A.; Pedrosa, A.; Silva, A.C.; Méndez, L.; Caneiras, C. First Outbreak of NDM-1-Producing *Klebsiella pneumoniae* ST11 in a Portuguese Hospital Centre during the COVID-19 Pandemic. *Microorganisms* **2022**, *10*, 251. [[CrossRef](#)] [[PubMed](#)]
54. Guerra, A.M.; Lira, A.; Lameirão, A.; Selaru, A.; Abreu, G.; Lopes, P.; Mota, M.; Novais, Â.; Peixe, L. Multiplicity of Carbapenemase-Producers Three Years after a KPC-3-Producing *K. pneumoniae* ST147-K64 Hospital Outbreak. *Antibiotics* **2020**, *9*, 806. [[CrossRef](#)] [[PubMed](#)]
55. Faria, N.A.; Touret, T.; Simões, A.S.; Palos, C.; Bispo, S.; Cristino, J.M.; Ramirez, M.; Carrico, J.; Pinto, M.; Toscano, C.; et al. Genomic insights into the expansion of carbapenem-resistant *Klebsiella pneumoniae* within Portuguese hospitals. *J. Hosp. Infect.* **2024**, *148*, 62–76. [[CrossRef](#)]
56. Ireng, L.M.; Ambrose, J.; Bearzatto, B.; Durant, J.-F.; Bonjean, M.; Gala, J.-L. Genomic Characterization of Multidrug-Resistant Extended Spectrum β -Lactamase-Producing *Klebsiella pneumoniae* from Clinical Samples of a Tertiary Hospital in South Kivu Province, Eastern Democratic Republic of Congo. *Microorganisms* **2023**, *11*, 525. [[CrossRef](#)] [[PubMed](#)]
57. Shankar, C.; Muthuirulandi Sethuvel, D.P.; Neeravi, A.R.; Venkatesan, M.; Devanga Ragupathi, N.K.; Anandan, S.; Veeraraghavan, B. Identification of plasmids by PCR based replicon typing in bacteremic *Klebsiella pneumoniae*. *Microb. Pathog.* **2020**, *148*, 104429. [[CrossRef](#)] [[PubMed](#)]
58. Lipworth, S.; Vihta, K.-D.; Chau, K.; Barker, L.; George, S.; Kavanagh, J.; Davies, T.; Vaughan, A.; Andersson, M.; Jeffery, K.; et al. Ten-year longitudinal molecular epidemiology study of *Escherichia coli* and *Klebsiella* species bloodstream infections in Oxfordshire, UK. *Genome Med.* **2021**, *13*, 144. [[CrossRef](#)] [[PubMed](#)]
59. Posteraro, B.; De Maio, F.; Motro, Y.; Menchinelli, G.; De Lorenzis, D.; Marano, R.B.M.; Aljanazreh, B.; Errico, F.M.; Massaria, G.; Spanu, T.; et al. In-depth characterization of multidrug-resistant NDM-1 and KPC-3 co-producing *Klebsiella pneumoniae* bloodstream isolates from Italian hospital patients. *Microbiol. Spectr.* **2024**, *12*, e0330523. [[CrossRef](#)]

60. Gaibani, P.; Bussini, L.; Amadesi, S.; Bartoletti, M.; Bovo, F.; Lazzarotto, T.; Viale, P.; Ambretti, S. Successful Treatment of Bloodstream Infection due to a KPC-Producing *Klebsiella pneumoniae* Resistant to Imipenem/Relebactam in a Hematological Patient. *Microorganisms* **2022**, *10*, 778. [[CrossRef](#)]
61. Shelenvov, A.; Mikhaylova, Y.; Yanushevich, Y.; Samoilov, A.; Petrova, L.; Fomina, V.; Gusarov, V.; Zamyatin, M.; Shagin, D.; Akimkin, V. Molecular Typing, Characterization of Antimicrobial Resistance, Virulence Profiling and Analysis of Whole-Genome Sequence of Clinical *Klebsiella pneumoniae* Isolates. *Antibiotics* **2020**, *9*, 261. [[CrossRef](#)] [[PubMed](#)]
62. Diamant, I.; Adani, B.; Sylman, M.; Rahav, G.; Gal-Mor, O. The transcriptional regulation of the horizontally acquired iron uptake system, yersiniabactin and its contribution to oxidative stress tolerance and pathogenicity of globally emerging *Salmonella* strains. *Gut Microbes* **2024**, *16*, 2369339. [[CrossRef](#)] [[PubMed](#)]
63. Chaaban, T.; Mohsen, Y.; Ezzeddine, Z.; Ghssein, G. Overview of Yersinia pestis Metallophores: Yersiniabactin and Yersinopine. *Biology* **2023**, *12*, 598. [[CrossRef](#)] [[PubMed](#)]
64. Klebba, P.E.; Newton, S.M.C.; Six, D.A.; Kumar, A.; Yang, T.; Nairn, B.L.; Munger, C.; Chakravorty, S. Iron Acquisition Systems of Gram-negative Bacterial Pathogens Define TonB-Dependent Pathways to Novel Antibiotics. *Chem. Rev.* **2021**, *121*, 5193–5239. [[CrossRef](#)] [[PubMed](#)]
65. Zhou, Y.; Zhou, Z.; Zheng, L.; Gong, Z.; Li, Y.; Jin, Y.; Huang, Y.; Chi, M. Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. *Int. J. Mol. Sci.* **2023**, *24*, 10537. [[CrossRef](#)]
66. Azam, M.W.; Zarrilli, R.; Khan, A.U. Updates on the Virulence Factors Produced by Multidrug-Resistant Enterobacterales and Strategies to Control Their Infections. *Microorganisms* **2023**, *11*, 1901. [[CrossRef](#)] [[PubMed](#)]
67. Grass, G.; Otto, M.; Fricke, B.; Haney, C.J.; Rensing, C.; Nies, D.H.; Munkelt, D. FieF (YiiP) from *Escherichia coli* mediates decreased cellular accumulation of iron and relieves iron stress. *Arch. Microbiol.* **2005**, *183*, 9–18. [[CrossRef](#)]
68. Huang, K.; Wang, D.; Frederiksen, R.F.; Rensing, C.; Olsen, J.E.; Fresno, A.H. Investigation of the Role of Genes Encoding Zinc Exporters zntA, zitB, and fieF during *Salmonella* Typhimurium Infection. *Front. Microbiol.* **2018**, *8*, 2656. [[CrossRef](#)] [[PubMed](#)]
69. Fang, Y.; Tran, F.; Stanford, K.; Yang, X. Stress Resistance and Virulence Gene Profiles Associated with Phylogeny and Phenotypes of *Escherichia coli* from Cattle. *J. Food Prot.* **2023**, *86*, 100122. [[CrossRef](#)]
70. Ventura, M.; Canchaya, C.; Zhang, Z.; Fitzgerald, G.F.; van Sinderen, D. Molecular Characterization of hsp20, Encoding a Small Heat Shock Protein of *Bifidobacterium breve* UCC2003. *Appl. Environ. Microbiol.* **2007**, *73*, 4695–4703. [[CrossRef](#)] [[PubMed](#)]
71. Qi, Y.; Liu, D.; Yu, H.; Zhang, G.; Fan, M. Identification and Characterization of the Small Heat Shock Protein Hsp20 from *Oenococcus oeni* SD-2a. *Curr. Microbiol.* **2020**, *77*, 3595–3602. [[CrossRef](#)] [[PubMed](#)]
72. Mercer, R.G.; Zheng, J.; Garcia-Hernandez, R.; Ruan, L.; Gänzle, M.G.; McMullen, L.M. Genetic determinants of heat resistance in *Escherichia coli*. *Front. Microbiol.* **2015**, *6*, 932. [[CrossRef](#)] [[PubMed](#)]
73. Mercer, R.; Nguyen, O.; Ou, Q.; McMullen, L.; Gänzle, M.G. Functional Analysis of Genes Comprising the Locus of Heat Resistance in *Escherichia coli*. *Appl. Environ. Microbiol.* **2017**, *83*, e01400-17. [[CrossRef](#)]
74. Zhu, T.; Wang, Z.; McMullen, L.M.; Raivio, T.; Simpson, D.J.; Gänzle, M.G. Contribution of the Locus of Heat Resistance to Growth and Survival of *Escherichia coli* at Alkaline pH and at Alkaline pH in the Presence of Chlorine. *Microorganisms* **2021**, *9*, 701. [[CrossRef](#)] [[PubMed](#)]

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