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# Molecular characterization of hypermucoviscous carbapenemase-encoding *Klebsiella pneumoniae* isolates from an Egyptian hospital

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## Abstract

This study aimed to screen antibiotic resistance and virulence genes in carbapenem-resistant hypermucoviscous *Klebsiella pneumoniae* isolates from an Egyptian hospital. Among 38 previously confirmed carbapenem-nonsusceptible *K. pneumoniae* isolates, a string test identified three isolates as positive for hypermucoviscosity. Phenotypic characterization and molecular detection of carbapenemase- and virulence-encoding genes were performed. PCR-based multilocus sequence typing and phylogenetics were used to determine the clonality and global epidemiology of the strains. The coexistence of virulence and resistance genes in the isolates was analyzed statistically using a chi-square test. Three isolates showed the presence of carbapenemase-encoding genes ($bla_{NDM}$, $bla_{VIM}$, and $bla_{IMP}$), adhesion genes (*fim-H-1* and *mrkD*), and siderophore genes (*entB*); the isolates belonged to sequence types (STs) 101, 1310, and 1626. The relatedness between these sequence types and the sequence types of globally detected hypermucoviscous *K. pneumoniae* that also harbor carbapenemases was determined. Our analysis showed that the resistance and virulence profiles were not homogenous. Phylogenetically, different clones clustered together. There was no significant association between the presence of resistance and virulence genes in the isolates. There is a need for periodic surveillance of the healthcare settings in Egypt and globally to understand the true epidemiology of carbapenem-resistant, hypermucoviscous *K. pneumoniae*.

**KEYWORDS**
carbapenemases, Egypt, hypermucoviscosity, *Klebsiella pneumoniae*, virulence genes

## INTRODUCTION

*Klebsiella pneumoniae* is an important Gram-negative bacterium of great concern in healthcare settings.[1,2] This is attributed to its diverse plasmid repertoire that carries various antimicrobial resistance genes and virulence determinants.[3–5] One of the problematic features of *K. pneumoniae* is their resistance to carbapenems, which are the last-resort β-lactams used for treating life-threatening infections.[6] Carbapenem-resistant *K. pneumoniae* is classified as one of the critical priority pathogens in the World Health Organization Global Priority list of antimicrobial-resistant bacteria for the research and development of new antibiotics; these include carbapenem-resistant

Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.[7] Carbapenem resistance depends mainly on the inhibition of cell wall synthesis in bacteria via one or more of the following mechanisms: porin loss, efflux pumps, hyperexpression of extended-spectrum β-lactamases and/or AmpCs, and/or production of carbapenemases.[8,9] The main five carbapenemases $bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP}$, and $bla_{OXA\text{-}48\text{-}like}$ have been extensively disseminated within various species through mobile genetic elements such as plasmids, insertion sequences, transposons, and integrons.[10,11]

Another significant feature of *K. pneumoniae* is its array of virulence factors that contribute greatly to its pathogenicity.[12] Such virulence factors include capsule, lipopolysaccharide, fimbriae (Type 1 and 3), siderophores (enterobactin, yersiniabactin, salmochelin, and aerobactin), outer membrane proteins, allantoin metabolism, and nitrogen source utilization.[2,13] Through these virulence factors, *K. pneumoniae* causes infections such as pneumonia, bacteremia, and urinary tract infections.[12,14]

Some *K. pneumoniae* strains show hypermucoviscosity (hypermucoid) and/or hypervirulence.[14] Hypermucoviscosity is a phenotypic feature confirmed by the string test, that is, the generation of a viscous string that is greater than 5 mm upon stretching the colonies on an agar plate by an inoculation loop.[15] Hypervirulence depends on the ability to cause life-threatening infections at multiple sites or to spread at unusual infection sites, leading to significant morbidity and mortality rates.[14,16] Several infections such as meningitis, endophthalmitis, pneumonia, bacteremia, and liver abscess are caused by hypervirulent strains.[12,17]

The two terms, hypermucoviscosity and hypervirulence, have been used interchangeably in various publications worldwide and this may be attributed to a knowledge gap in giving an accurate definition to both terms. Recently, the synonymous meaning of the two terms has been called into question.[18,19] Hypermucoviscosity is a phenotypic feature that can be observed in the routine work of clinical laboratories in healthcare settings and is a significant sign for the probability of hypervirulence, which necessitates clinical studies and animal models that are not affordable in daily routine work.[14,18]

Even with the absence of hypervirulence, the characterization of hypermucoviscous *K. pneumoniae* is still an attractive research field for different research groups.[20–22] *K. pneumoniae* strains, particularly those cohosting carbapenem resistance and virulence genes, are of significant concern owing to the serious health consequences of infections from them.[23] Our study here reports the phenotypic and genotypic characteristics of three selected hypermucoviscous *K. pneumoniae* isolated from a tertiary hospital in Egypt.

## METHODS

### Isolate collection and confirmation

Thirty-eight carbapenem-nonsusceptible (intermediate or resistant) *K. pneumoniae* were isolated from urine specimens of patients who were admitted to different departments of a tertiary hospital in Cairo, Egypt. The isolates were nonduplicated and nonconsecutive. None of the isolates were collected from the same patient. The isolates were collected from October 2014 to December 2016 and their identities were confirmed using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF/MS) (Vitek MS; BioMérieux, Inc). Hypermucoviscosity was tested using the string test, which is considered positive if the isolate shows the formation of a viscous string greater than 5 mm by stretching colonies on agar by the loop as described previously.[14] Only three isolates, KP3, KP93, and KP393, showed positive string tests and underwent further investigations, as shown in the flowchart (Figure 1).

### Antimicrobial susceptibility testing

Further investigation of the three isolates included antimicrobial susceptibility testing by disc diffusion following the Kirby—Bauer method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-134 S26, 2016).[24] The antimicrobial panel included penicillin/cephalosporin with or without β-lactamases inhibitors: (amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, and cefepime), monobactams (aztreonam), carbapenems (imipenem, meropenem, and ertapenem), aminoglycosides (gentamicin and amikacin), quinolones (ciprofloxacin), folate pathway inhibitor (trimethoprim/sulphamethoxazole), and protein synthesis inhibitor (tigecycline), (Oxoid). *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853 were used as quality control strains. The results were interpreted according to the CLSI guidelines (M100-S26, 2016). The interpretive criteria for tigecycline were according to the Food and Drug Administration recommendations: http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021821s016lbl.pdf

### Phenotypic confirmation of carbapenemases production

Phenotypic screening for carbapenemases production was performed by the modified Hodge test (MHT) according to the CLSI guidelines.[24] The test is recorded as positive if a cloverleaf-like indentation of grown *E. coli* (ATCC 25922) formed along the investigated isolate.

*K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706 were used as positive and negative controls, respectively. Also, the inhibitor-based tests were done for further phenotypic confirmation of carbapenemase production by using MASTDISCS ID Carbapenemase Detection Disc Set D70C (MAST group). The set consists of the following discs: disk A (meropenem, 10 μg); disk B (meropenem + metallo-β-lactamases [MβLs] inhibitor); disk C (meropenem + KPC inhibitor), and disk D, (meropenem + AmpC inhibitor). A temocillin disc (30 μg) was additionally applied to give a preliminary indication of $bla_{OXA\text{-}48\text{-}like}$ production until further confirmation via PCR.

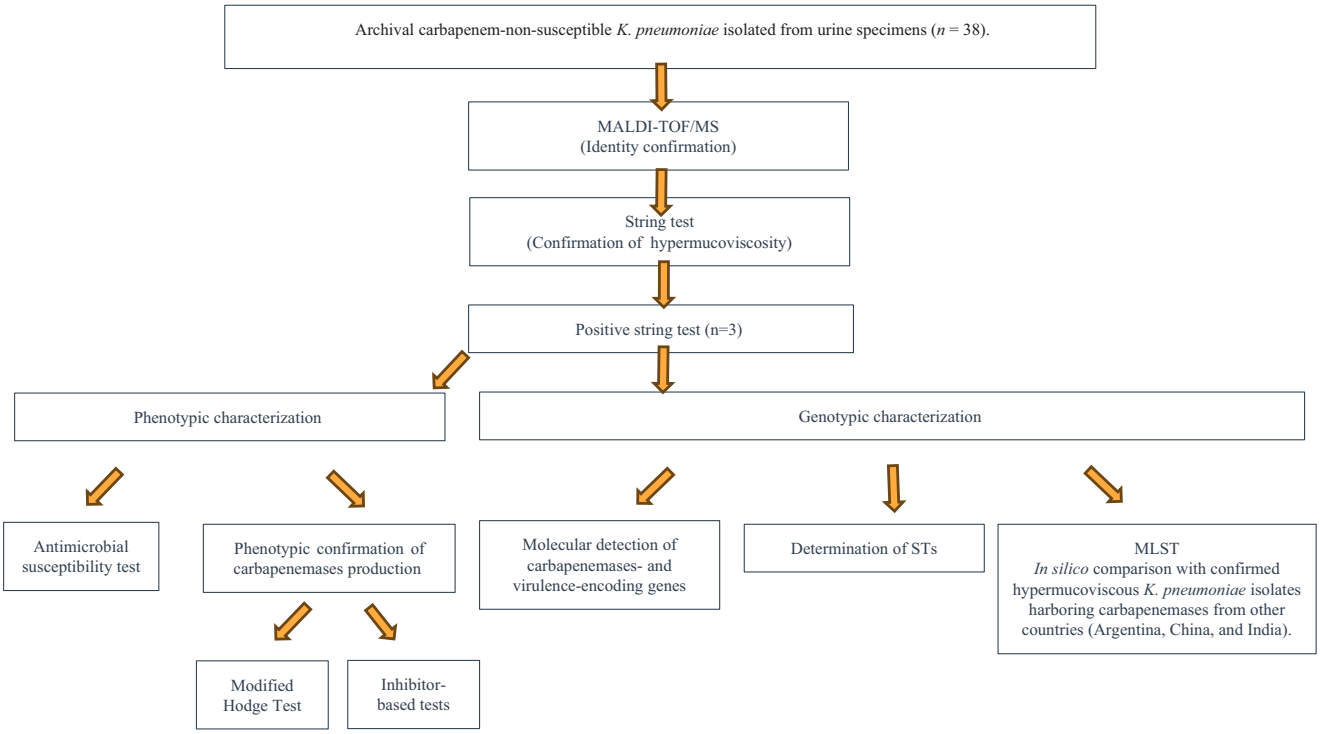


**FIGURE 1** The flowchart of the applied steps in this study.

The inhibitor-based test interpretations depend on the comparison of the zones of inhibition of the meropenem disc and the meropenem disc combined with the carbapenemase inhibitor,[25] according to the following criteria: MβL production was confirmed if only disc B showed a difference in inhibition zone ≥5 mm, KPC production was confirmed if only disc C showed a difference in inhibition zone ≥4 mm, and AmpC activity (with porin loss) was confirmed if both discs C and D showed differences in inhibition zone as ≥4 and ≥5 mm, respectively. Furthermore, the absence of synergy, except for temocillin resistance (inhibition zone <11 mm), was suggested to be an indication for OXA-48-like production till further molecular confirmation.

## Molecular detection of carbapenemase- and virulence-encoding genes

Isolates' total DNA was extracted by the boiling method.[26] PCR reactions were carried out for carbapenemase-encoding genes ($bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP}$, and $bla_{OXA\text{-}48\text{-}like}$) and virulence-encoding genes: K1/K2 capsular serotypes; type 1 and type 3 adhesins (*fimH-1*, *mrkD*); a regulator of mucoid phenotype A (*rmpA*); genes encoding siderophores such as *iutA* for aerobactin, *entB* for enterobactin, and *ybtS* for yersiniabactin. Primer synthesis was by Eurofins MWG Operon. PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, appropriate annealing temperature for 40 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min.

Positive controls were obtained from the archival section of the hospital laboratory and included strains that harbored carbapenemase- and virulence-encoding genes that had been sequenced previously. The primer sequences of investigated genes and the expected amplicon sizes are shown in Table S1.

## Multilocus sequence typing

Genomic DNA was extracted by GeneJet Genomic DNA Purification Kit (K0721, Thermo Scientific) according to the manufacturer's instructions. The sequence type of each isolate was determined according to the *K. pneumoniae* MLST scheme.[35]

Further analysis of the characteristics of three isolates (KP3, KP93, and KP393) was performed by in silico comparison with confirmed hypermucoviscous *K. pneumoniae* isolates harboring carbapenemases from other previously published studies. The strains used for comparison were isolated from Argentina,[36,37] China,[38–40] and India.[41] The following genes were included in the comparative analysis: capsule-related genes (K1, K2, K20, *rmpA*, *magA*, *wcaG*), fimbrial genes (*fimH-1* and *mrkD*), lipopolysaccharide-related genes (*uge*, *wabG*, *ycf*), siderophore-based iron uptake system (enterobactin [*entA*, *entB*, *entD*, *entE*, *entF*], salmochelin [*IroN*], aerobactin [*iucA*, *iucB*, *iucD*, *iutA*], yersiniabactin [*ybtA*, *ybtS*, *ybtU*, *ybtT*, *irp1*, *irp2*, *fyuA*]), ABC transporter-based iron uptake system (*Kfu*), nitrogen source utilization (*ureA*), silver resistance (*SilS*), and tellurite resistance gene (*terW*). The relatedness between the sequence types of KP3, KP93, and KP393 and the

**TABLE 1** The sequence types of the investigated hypermucoviscous *K. pneumoniae* isolates.

| ST | Country | Reference |
|---|---|---|
| 11 | China | 38, 40 |
| | India | 41 |
| 23 | Argentina | 36 |
| | China | 39 |
| 25 | Argentina | 37 |
| 43 | India | 41 |
| 65 | China | 38, 40 |
| 101 | Egypt | The current work. |
| 231 | India | 41 |
| 268 | China | 40 |
| 595 | China | 40 |
| 692 | China | 40 |
| 1310 | Egypt | The current work. |
| 1626 | Egypt | The current work. |
| 1797 | China | 39 |

*Note*: Country refers to the country in which the carbapenemase-encoding hypermucoviscous *Klebsiella pneumoniae* isolates were detected.
Abbreviation: ST, sequence type.

hypermucoviscous *K. pneumoniae* harboring carbapenemases from other studies was compared using phylogenetics.

The seven housekeeping genes of each isolate and the downloaded genome were aligned using MUSCLE prior to drawing the phylogenetic tree on phylogeny.fr (https://www.phylogeny.fr/), using PhyML BioNJ, which uses the neighbor-joining method. Phylogenetic analysis was run with a bootstrap reassessment of 1000. The tree was annotated using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and the bootstrap values were used to determine the relatedness of the isolates. A bootstrap value of >50 on a branch was defined as significant.[42] *E. coli* ST113 was used as the outgroup. Table 1 illustrates the selected sequence types and their source countries. Also, further investigation of the synonymous and nonsynonymous mutations for each housekeeping gene of KP3, KP93, and KP393 was done by implementing the multiple sequence alignment for nucleotides and amino acid sequences using BioEdit 7.2 (https://bioedit.software.informer.com/7.2/).

## Statistical analysis

The presence or absence of resistance and virulence genes was represented as 1 and 0, respectively, in Excel sheets for each isolate. The sum of resistance genes and the sum of virulence genes were separately calculated and used to determine the association of these two genetic traits using the chi-square test in GraphPad Prism 10.1.2. A $p$-value of $< 0.05$ was defined as significant.

# RESULTS

## Antimicrobial susceptibility testing

The isolates were screened phenotypically to determine their resistance profiles and to select those with both carbapenem resistance and hypermucoviscosity. Out of 38 carbapenem-nonsusceptible (intermediate or resistant) *K. pneumoniae*, only KP3, KP93, and KP393 showed positive results for the string test, confirming hypermucoviscosity. The antimicrobial susceptibility testing of the three isolates showed that they were resistant to most of the tested antimicrobial classes (Table 2), making them multidrug-resistant (MDR) with a tendency of being extensively drug-resistant (XDR) according to Magiorakos et al. recommendations.[43]

## MHT and inhibitor-based tests

Phenotypic determination of carbapenemase production was confirmed using the MHT and the disc-based inhibitory test. The three isolates gave the same results for the phenotypic testing of carbapenemases whether for the MHT, which represents nonspecific detection of carbapenemase production, or the inhibitor-based tests, which confirm carbapenemase production. Although the three isolates were negative for the MHT, they were positive for the inhibitor-based test that confirms the production of MβLs. The data of the MHT and the inhibitor-based tests of the three investigated isolates is shown in Table 3.

## PCR of carbapenemase- and virulence-encoding genes

The molecular screening of the carbapenemase-encoding genes ($bla_{KPC}$, $bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$, and $bla_{OXA\text{-}48\text{-}like}$) was performed by standard PCR to confirm the results of the aforementioned phenotypic tests. The three tested isolates showed minor variability in PCR results, including the absence of both $bla_{KPC}$- and $bla_{OXA\text{-}48\text{-}like}$-encoding genes and the coexistence of $bla_{IMP}$- and $bla_{VIM}$-encoding genes in all isolates. Additionally, $bla_{NDM}$-encoding genes existed in only two isolates (KP3 and KP393). The virulotyping of the isolates showed identical results, that is, the absence of all investigated virulence-encoding genes and the coexistence of only *fimH-1*-, *mrkD*-, and *entB*-encoding genes. Figure 2 shows the detected virulence-encoding genes in one of the investigated isolates (KP93).

Tables S2 and S3 show the antimicrobial susceptibility and the carbapenemase- and virulence-encoding genes of the other hypermucoviscous strains that were isolated from other countries, namely, Argentina, China, and India. Among all isolates from other countries ($n = 38$), it was obvious that $bla_{KPC}$ was the sole carbapenemase detected in 35 isolates, three isolates harbored $bla_{OXA\text{-}48\text{-}like}$ and one isolate had $bla_{NDM}$. Also, no isolate harbored either $bla_{VIM}$ or $bla_{IMP}$. The detected virulence-encoding genes from such countries are shown in Table S4.

**TABLE 2** Phenotypic and genotypic features of the three investigated isolates from Egypt.

| | | | Antimicrobial Susceptibility Profile (R, I, or S). | | | | | | | | | | | | | | | | Carbapenemase-encoding genes | | | Virulence-encoding genes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Year isolated | Sequence type (ST) | AUG | TZP | FOX | CTX | CTC | CAZ | CZC | CPM | ATM | IMI | MEM | ETR | GN | AK | CIP | STX | TGC | *bla*$_{NDM}$ | *bla*$_{VIM}$ | *bla*$_{IMP}$ | *fim-H-1* | *mrkD* | *entB* |
| KP3 | 2014 | ST1310 | R | R | R | R | R | R | R | I | I | I | R | R | R | R | S | R | I | + | + | + | + | + | + |
| KP93 | 2015 | ST1626 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | I | R | R | – | + | + | + | + | + |
| KP393 | 2016 | ST101 | R | R | R | R | R | R | R | R | R | R | R | R | S | R | R | R | I | + | + | + | + | + | + |

*Note*: All investigated isolates from Egypt were of urine source.
Penicillins/cephalosporins with or without β-lactamases inhibitors: amoxicillin/clavulanic acid (AUG), piperacillin/tazobactam (TZP), cefoxitin (FOX), cefotaxime (CTX), cefotaxime/clavulanic acid (CTC), ceftazidime (CAZ), ceftazidime/clavulanic acid (CZC), cefepime (CPM). Monobactams: aztreonam (ATM). Carbapenems: imipenem (IMI), meropenem (MEM), ertapenem (ETR). Aminoglycosides: gentamicin (GN), amikacin (AK). Quinolones: ciprofloxacin (CIP). Folate pathway inhibitor: trimethoprim/sulphamethoxazole (STX). Protein synthesis inhibitor: tigecycline (TGC). Abbreviations: I, intermediate; R, resistant; S, sensitive.

**TABLE 3** The interpretation of the modified Hodge test and the inhibitor-based tests of the three investigated isolates from Egypt.

| | | Results of the inhibitor-based tests | | | | | Interpretation of the inhibitor-based tests | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | MHT | A | B | C | D | TEM (mm) | B-A | C-A | D-A | TEM (mm) |
| KP3 | – | 17 | 26 | 17 | 20 | 20 | + | – | – | S |
| KP93 | – | 18 | 26 | 18 | 21 | 17 | + | – | – | S |
| KP393 | – | 6 | 21 | 6 | 8 | 6 | + | – | – | R |

A: meropenem disc (10 μg); B: meropenem disc + EDTA; C: meropenem disc + phenyl boronic acid; D: meropenem disc + AmpC inhibitor; TEM: temocillin disc (30 μg). Abbreviations: R, resistant; S, sensitive.

## Multilocus sequence typing

The genotypic relationship between the isolates and other global isolates was established using MLST and phylogenetics. The isolates from Egypt were of different sequence types: isolate KP3 belonged to ST1310, isolate KP93 belonged to ST1626, and isolate KP393 belonged to ST101. Nevertheless, the isolates from the other countries showed that ST11 was the most common ($n = 18$ isolates), followed by ST65 ($n = 6$ isolates), ST23 ($n = 3$ isolates), and ST1797 ($n = 3$ isolates). As well, two isolates were of ST25 and ST268, whereas single isolates were of ST43, ST231, ST595, and ST692.

The evolutionary relationship between all investigated STs is shown in Figure 3. The phylogenetic tree showed the distribution of 13 STs among two major clades, namely, clades A and B. Clade A branched into three clusters: A1, A2, and A3. Cluster A1 has five isolates that were represented by five STs with various characteristics: ST23, ST25, ST65, ST692, and ST1797. Among this cluster, there was a notable variation in the isolates that belong to it. Both ST65 and ST692 were from China; the former (ST65) comprised six isolates from diverse sources (i.e., blood, sputum, tracheal secretion, and urine), whereas ST692 comprised only one isolate from a sputum sample. Further, the same cluster included other closely related STs, namely, ST23 and ST1797. ST23 comprised three isolates from Argentina and were obtained from blood, sputum, and tracheal aspirate, whereas ST1797 comprised three isolates in China from blood and sputum samples. Also, ST25 was found on a single branch within cluster A but with a significant bootstrap of 0.87 from the branch of ST65 and ST692. ST25 was identified in two isolates in Argentina, and was isolated from bone and bronchoalveolar lavage.

Cluster A2 had four isolates that belonged to distinct sequence types. Both clones ST43 and ST231 were from India and from blood. ST101 was found in Egypt and from urine, whereas ST595 was from China and from sputum. ST43 was found on a single branch with a significant bootstrap of 0.82; the branch of the other three STs (ST101, ST231, and ST595) was supported by low bootstrap values. On the other hand, cluster A3 had two STs, namely, ST11 and ST268, with ST11 comprising 18 reported hypermucoviscous isolates from both China and India; ST11 was from various clinical sources (i.e., blood, pus discharge, sputum, tracheal aspiration, and urine). The other clone was ST268, which comprised two isolates from China; it was isolated from sputum and blood. In addition, clade B had a bootstrap value of 1 and included two STs, namely, ST1310 and ST1626, which were exclusively found in two isolates from urine in Egypt. However, despite the absence of a significant bootstrap among some distinct clusters, both Clade A and Clade B represented significant bootstrap (0.82 and 1.0, respectively) from the ancestor. This may reflect the relatedness of the STs collectively regardless of the isolates' origin and sources.

The multiple sequence alignment of the housekeeping genes of the three isolates' sequences showed the presence of synonymous and nonsynonymous mutations (Table S5 and Figure S1A–S1G). Notable substitutions and deletions were found in *rpoB* (in the three isolates), *phoE* (KP93), *infB*, and *mdh* (isolate KP393), compared to the reference sequence. On the other hand, other genes, namely *inf B* (isolate KP3),

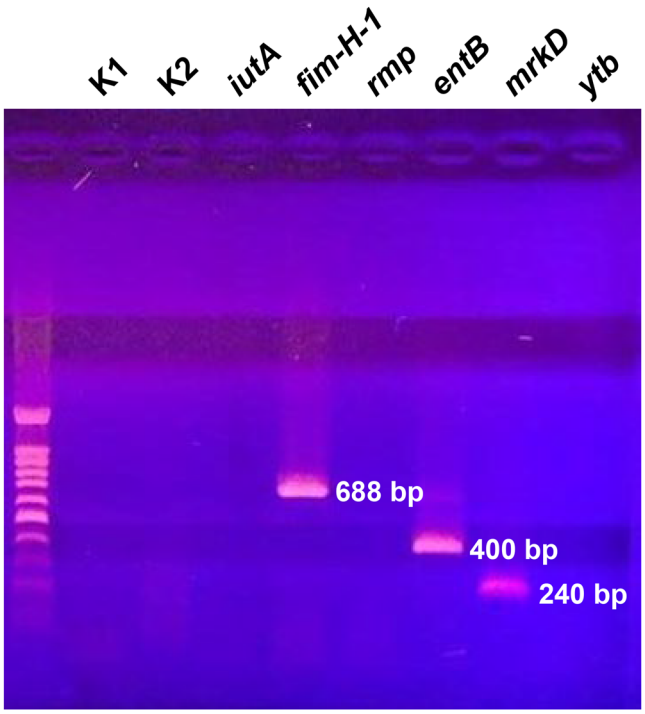


**FIGURE 2** Detection of the virulence gene in isolate 93. The lane on the far left is the molecular size marker. Lane 4 (688 bp positive for *fim-H-1*), Lane 6 (400 bp positive for *entB*), Lane 7 (240 bp positive for *mrkD*), the remaining lanes represent negative results of the other investigated genes (K1, K2, i*utA*, *rmp*, and *ytb*).

*pgi* (isolates KP3 and KP93), and *gap* (isolates KP93 and KP393), did not have mutations.

## Statistical analysis

There was no significant association between the incidence of resistance genes and virulence genes in the isolates. A chi-square test found no significant association between these two traits (Figure 4 and Table S3).

## DISCUSSION

*K. pneumoniae* remains a major pathogen in several clinical settings globally. Some *K. pneumoniae* strains are described as dual-risk strains owing to their vast repertoire of antimicrobial resistance and virulence determinants, which facilitates their pathogenesis.[44,45] Specifically, the presence of mosaic plasmids that harbor both resistance and virulence genes in *K. pneumoniae* accelerates the dissemination of such high-risk strains.[3,5,46] Recently, several studies showed the absence of any relationship between the positive string test (hypermucoviscosity) and hypervirulence, and therefore considered the two terms distinct. Some studies pointed out that the two terms represent different traits by confirming hypervirulence in the absence of hypermucoviscosity phenotype.[47–49] This indicates the importance of using additional techniques to assess hypervirulence instead of only relying on the string test.

In our study, we investigated three hypermucoviscous isolates that were collected at different times from an Egyptian tertiary hospital. The investigated isolates belonged to ST101, ST1310, and ST1626. Both ST1310 and ST1626 are less frequently detected globally, and so far have not been reported to be hypermucoviscous. In contrast, ST101 has been reported globally in many studies to harbor various resistance and virulence characteristics, regardless of the hypermucoviscosity state of the isolates.[50–53]

Based on our literature review, 10 additional STs were found to carry both traits (i.e., carbapenem resistance and hypermucoviscosity) and thus were included for comparative phylogenetic and resistance analyses. Among different sequence types of the investigated isolates that showed hypermucoviscosity, ST11 was the only clone that has also been detected in 18 out of the 41 isolates (43.90%) from the included studies, mainly from various clinical sources in China and India. This clone had substantial resistome and virulome characteristics, as shown in Tables S3 and S4. Generally, the existence of ST11 in different studies either in the same or different countries strongly confirms it as a high-risk clone, particularly if we consider its association with serious infections regardless of the resistance profile of the investigated *K. pneumoniae* strains as reported by other research groups.[54–56] Comparatively, ST23 and ST65 were less frequently detected; ST23 was detected in Argentina ($n$ = 1/41, 2.44%) and China ($n$ = 2/41, 4.88%), and ST65 ($n$ = 6, 14.63%) was reported in two independent studies in China. In contrast, Guo et al.[57] found a higher prevalence of ST23 ($n$ = 19/69, 27.5%) and ST 65 ($n$ = 18/69, 26.1%) in their investigations that identified isolates with resistance to various antimicrobial agents.

The remaining STs with hypermucoviscosity, namely, ST25, ST43, ST231, ST268, ST595, ST692, and ST1797, were solely detected in single studies and may not be as widespread as the previously mentioned STs. These global data show that hypermucoviscous *K. pneumoniae* with carbapenemase production are relatively few and distributed in limited regional clones. Further, the bootstrap values on the phylogenetic tree, most of which were below 0.5 (50%), show that the clones were not closely related, confirming their diverse genotypes. The nonrelatedness of the strains is further evidenced by the limited similarity in their genetic virulence and resistance genes (Figure 4).

Among the sequences of housekeeping genes of the three investigated isolates, there were mutational variations. The mutation analysis showed that isolate KP3 (ST1310) had the least nonsynonymous mutations. Notably, although *rpoB* in all isolates showed nonsynonymous mutations, *tonB* of isolate KP93 (ST1626) showed both types—one synonymous and two nonsynonymous—which contrasts with the study by Liao et al.[58] that showed seven mutations in the investigated *tonB* of ST23 and ST65. Notably, isolate KP393 belonged to ST101, which showed mutations in five housekeeping genes and was also located phylogenetically in a clade that differs from that of the other two isolates, KP3 (ST1310) and KP93 (ST1626).

In our study, we applied two phenotypic tests namely, the MHT and the inhibitor-based test for confirmation of carbapenemase

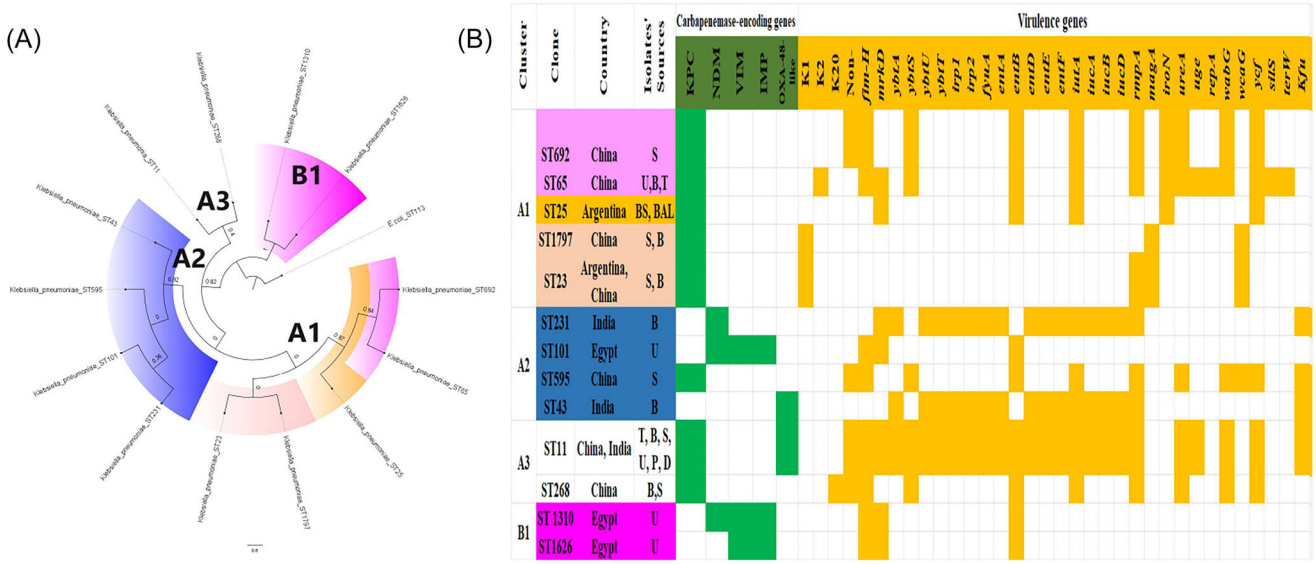


**FIGURE 3** The epidemiological characteristics of the investigated isolates from Egypt and other countries. (A) A phylogenetic tree representing the relationship between the clones/sequence types of three carbapenem-resistant hypermucoviscous *K. pneumoniae* isolates from Egypt (ST101, ST1310, and ST1626) and those from other previously published studies is shown in the figure above. (B) The distribution of various resistance- and virulence-encoding genes of the investigated isolates is shown per the various clades. The bootstrap values are shown on the branches. The tree is drawn to a scale of 0.8 (shown below the tree). Abbreviations: B, blood; BAL, bronchoalveolar lavage; BS, bone sample; D, discharge; P, pus; S, sputum; T, tracheal secretion; U, urine.

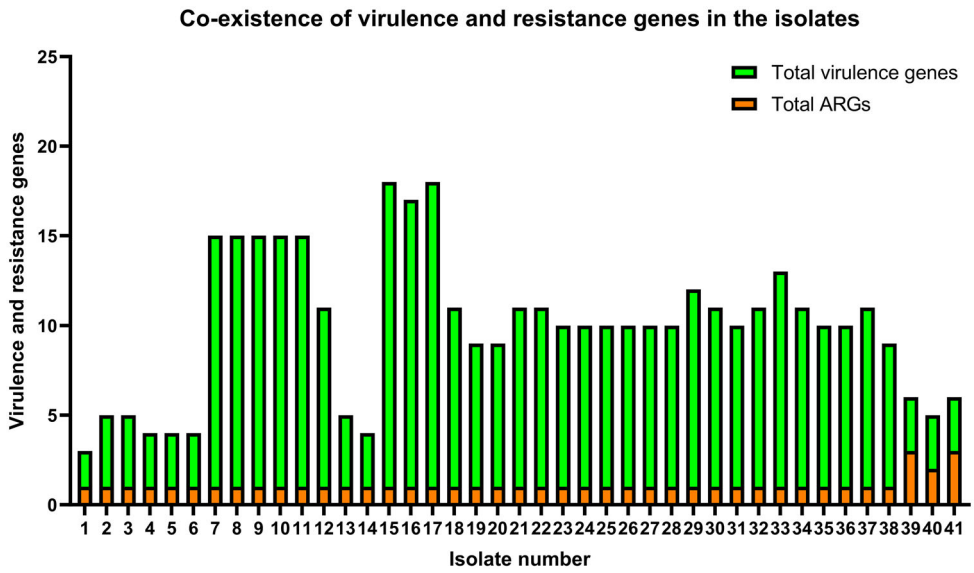


**FIGURE 4** The coexistence of virulence and resistance genes in carbapenemase-producing isolates from Egypt and other countries (Argentina, China, and India). GraphPad prism 10.1.2 was used to analyze and generate the chart. Chi-square test was used to determine the significance of the association between the virulence and resistance genes. The association between the two traits was not significant. Abbreviation: ARG, antimicrobial-resistant genes.

production. All three isolates (viz., KP3, KP93, and KP393) showed negative results for the MHT, indicating nonspecificity of such a test for confirming carbapenemases production. On the other hand, although the three isolates were positive for the inhibitor-based test that confirmed MβLs production, the results did not discriminate between the different MβLs that were specifically detected by PCR: $bla_{NDM}$-, $bla_{VIM}$-, and $bla_{IMP}$-encoding genes in isolates KP3 and KP393, and $bla_{VIM}$- and $bla_{IMP}$-encoding genes in isolate KP93. This gives an indication that molecular tests are a gold standard for evaluations of specific genes detection and allows for confident investigations.[59]

The dissemination of carbapenemase-encoding genes is of great concern worldwide.[60,61] Egyptian healthcare settings differ in the

detected carbapenemases, especially in Enterobacterales.[62–65] Also, several research groups in Egypt have focused on *K. pneumoniae* isolates harboring carbapenemase-encoding genes that show the coexistence of such genes.[66–71] Moreover, colistin-resistant *K. pneumoniae* isolates have been recently reported.[72,73] This may seriously increase the possibility of exacerbation of the resistance profile from MDR or XDR to pandrug resistance.[74]

In our study, the $bla_{NDM}$-encoding gene was present in two isolates (KP3 and KP393) that belonged to ST1310 and ST101, respectively, while $bla_{VIM}$- and $bla_{IMP}$-encoding genes were present in all isolates (KP3, KP93, and KP393); notably, $bla_{KPC}$- and $bla_{OXA\text{-}48\text{-}like}$-encoding genes were absent in all three isolates. Isolate KP393 belongs to ST101, which is a global high-risk clone.[75] This isolate harbored MβL carbapenemase-encoding genes and did not have $bla_{KPC}$- and $bla_{OXA\text{-}48}$-encoding genes, which is contrary to other ST101-related isolates from Spain[76] and Tunisia[77] that showed the presence of $bla_{KPC}$- and $bla_{OXA\text{-}48}$-encoding genes, respectively. Also, isolate KP393 showed the presence of only investigated fimbrial genes (*fim-H-1* and *mrkD*), whereas a pool of virulence-encoding genes that belonged to siderophores and fimbriae clusters and others were detected in ST101-related isolates.[76]

Recently, the problematic features of the ST101 clone have been explained among isolates of human, animal, and environmental origin.[78] On the other hand, ST1310 and ST1626 of *K. pneumoniae* were located independently in one clade. Although both STs were less frequently detected, ST1310 was reported in *K. pneumoniae* isolated in India and showed resistance to cephalosporins, carbapenem (meropenem) aminoglycosides, and quinolones.[79] According to our knowledge, ST1626 has not been documented previously in other research. In general, the presence of newly emerged STs in the epidemiological studies of *K. pneumoniae* demonstrates a source of risk clones.

Regarding other studies, Shankar et al.[41] detected $bla_{OXA\text{-}48\text{-}like}$-encoding genes in two *K. pneumoniae* isolates (B1647 [ST11] and B20038 [ST43]) and a $bla_{NDM}$-encoding gene in only one *K. pneumoniae* isolate B20143 (ST231) in India. Other work from Argentina and China showed a higher presence of $bla_{KPC}$ than other carbapenemase-encoding genes.[36–40] Evidently, this reflects the diversity of the carbapenemase-encoding gene content in hypermucoviscous *K. pneumoniae* isolates and the independence of the two traits, that is, the presence of certain carbapenemase-encoding genes and hypermucoviscosity.

Although the correlation between hypermucoviscosity and antimicrobial resistance is less discussed, there are assumptions that the presence of capsules, which is the basis of hypermucoviscosity, hampers the acquisition of mobile genetic elements that play important roles in the conjugation of antimicrobial resistance genes.[80] In contrast, another point of view suggests that capsules cannot hinder the emergence of carbapenem-resistant hypervirulent strains. This might be attributed to the fusion of antimicrobial resistance–encoding genes and virulence-encoding genes.[81] As the distinction between hypervirulence and hypermucoviscosity has been recently determined, the correlation between hypervirulence (which could sometimes be ascertained by hypermucoviscosity) and carbapenem resistance was investigated. Pooled data from Lan et al.,[82] who reviewed the scenarios or patterns of emerging carbapenem-resistant hypervirulent *K. pneumoniae*, found that hypervirulent phenotypes could be acquired by carbapenem-resistant *K. pneumoniae*, that carbapenem-resistant *K. pneumoniae* could acquire hypervirulence genes, and that *K. pneumoniae* could acquire hybrid plasmids with both carbapenem resistance and hypervirulence.

Zhang et al.[39] observed that carbapenem-susceptible hypermucoviscous strains could become carbapenem resistant upon treatment with a carbapenem (e.g., imipenem).[39] They suggested that this could be due to the acquisition of a plasmid harboring a $bla_{KPC}$-encoding gene or the incorporation of this gene in a virulence plasmid by a transposon.

In our *K. pneumoniae* isolates (KP3, KP93, and KP393), there were no K serotypes, contrary to the study of Zhan et al.,[40] which confirmed the presence of K1, K2, or K20 besides some nontypeable serotypes.[40] This might confirm that hypermucoviscosity and capsule production are independent of each other, as clarified by Walker et al.[83] Additionally, the coexistence of *fim-H-1* and *mrkD* fimbrial-encoding genes was detected in our study and that of Yao et al.,[38] while other studies showed either the absence of both genes[36,39] or the presence of either *fim-H*[40] or *mrkD*.[41]

Hypermucoviscosity was thought to be attributed to mucoviscosity-associated gene A (*magA*) and/or the regulator of mucoid phenotype A (*rmpA*).[84,85] In our isolates from Egypt, both genes were absent, contrary to other studies that detected *rmpA* and found no *magA* in most isolates. This indicates that the presence of these genes is one of the factors of hypermucoviscosity but not the sole one. Other virulence factors such as *wabG*, *ycf*, *entB*, *iutA*, and *ybtS* were not present in all isolates. Interestingly, Shankar et al.[41] identified the notable coexistence of siderophores, which are used by *K. pneumoniae* to acquire free iron and survive during mammalian infection.[2,41,86]

Significantly, the presence of various antimicrobial resistance–encoding genes and virulence factors reflects the screening panel used and does not demonstrate the absence of genes in all cases. Furthermore, some studies used fewer isolates (i.e., one to three isolates) than other studies. Hence, studies with fewer isolates may not reflect the true prevalence or incidence of hypermucoviscous *K. pneumoniae* that harbor carbapenemase-encoding genes. In our work, the coexistence of the tested carbapenemase- and virulence-encoding genes and hypermucoviscosity indicates possible clinical consequences, such as limited treatment options, long hospital stays, or high rate of mortality. However, in our work, the number of isolates that had hypermucoviscosity accompanied by carbapenemase- and virulence-encoding genes was very limited ($n = 3$) and this number may reflect limited incidence.

Our analysis shows no direct association between resistance genes and virulence genes. This is an encouraging finding, as the occurrence of both in bacteria can yield both untreatable and virulent species. Co-occurence of virulence and/or pathogenic factors is often problematic, as demonstrated with the recent COVID-19 pandemic, with tuberculosis, and with other difficult-to-treat infections.[87–89] Bacteria develop resistance in response to exposure to antibiotics, while they develop virulence genes to survive in the host. Whereas

resistance genes are mostly transmitted horizontally through plasmids or other mobile genetic elements, virulence genes are mainly transmitted vertically/clonally. Therefore, the presence or absence of resistance genes need not be biologically related or determined by the presence or absence of virulence genes. Such a stochastic phenomenon is not always measurable statistically (Table S3). The absence of such a correlation was discussed by Candan and Aksöz[90] in a study from Turkey. Furthermore, the absence of a correlation between the carbapenemases and capsular serotypes was documented by an Egyptian study of Taha et al.[91] Although Albasha et al.[92] found no association between antimicrobial resistance and virulence genes in *K. pneumoniae* isolated from Sudan, they identified a significant association between *entB* and $bla_{NDM}$ ($p$-value = 0.005).

In general, several studies have been conducted on various bacterial species using in silico analyses of a large set of retrieved bacterial genomes for studying the relationship or associations between the two traits (i.e., antimicrobial resistance and virulence).[93,94] Pan et al.[93] pointed out the risk of the coexistence of antimicrobial-resistance genes and virulence genes on mobile genetic elements in opportunistic pathogens—the pathogenicity and antibiotic resistance of such pathogens could be enhanced via mobility.[93] On the other hand, Darmancier et al.[94] found the absence of a correlation between the two traits at chromosomal or plasmid levels.[94] Correlation between antimicrobial resistance and virulence needs extensive investigation as it depends mainly on four main factors, namely, the bacterial species, the virulence and resistance mechanisms, the environment, and the host's immune system.[95]

Our study had significant limitations such as the lack of sufficient demographic and clinical data of the investigated patients besides their treatment plans and outcomes. And as there is more interest in carbapenem-resistant traits (than virulence), PCR of all the isolates for different carbapenemase-encoding genes would have been desirable. We focused on two features, namely, hypermucoviscosity and the presence of carbapenemase-encoding genes, and this limited the number of studies we compared.

Limited resources and funding in developing countries such as Egypt hinder the wide application of whole-genome sequencing (WGS) for screening all antimicrobial resistance- and virulence-encoding genes. There have, however, recently been some initial steps to adopt WGS to characterize various *K. pneumoniae* isolated in Egypt. This will be a helpful step for further extensive investigation and epidemiological screening.[96,97]

## AUTHOR CONTRIBUTIONS

S.M.R.: Conceptualization, methodology, data curation, writing the original draft, and formatting the manuscript for publication. J.O.S.: Design, supervision, and undertaking bioinformatic and statistical analyses, image generation, phylogenomic trees annotation, writing up, revision, and formatting of the manuscript for publication.

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## COMPETING INTERESTS

The authors have no competing interests to declare.

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## REFERENCES

1. Podschun, R., & Ullmann, U. (1998). *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews*, *11*, 589–603.
2. Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews*, *80*, 629–661.
3. Ramirez, M. S., Traglia, G. M., Lin, D. L., Tran, T., & Tolmasky, M. E. (2014). Plasmid-mediated antibiotic resistance and virulence in gram-negatives: The *Klebsiella pneumoniae* paradigm. *Microbiology Spectrum*, *2*, 2–5.
4. Wyres, K. L., & Holt, K. E. (2018). *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Current Opinion in Microbiology*, *45*, 131–139.
5. Wyres, K. L., Lam, M. M. C., & Holt, K. E. (2020). Population genomics of *Klebsiella pneumoniae*. *Nature Reviews Microbiology*, *18*, 344–359.
6. Papp-Wallace, K. M., Endimiani, A., Taracila, M. A., & Bonomo, R. A. (2011). Carbapenems: Past, present, and future. *Antimicrobial Agents and Chemotherapy*, *55*, 4943–4960.
7. World Health Organization. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.
8. Nicolau, D. P. (2008). Carbapenems: A potent class of antibiotics. *Expert Opinion on Pharmacotherapy*, *9*, 23–37.
9. Eichenberger, E. M., & Thaden, J. T. (2019). Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. *Antibiotics*, *8*, 37.
10. Reyes, J. A., Melano, R., Cárdenas, P. A., & Trueba, G. (2020). Mobile genetic elements associated with carbapenemase genes in South American Enterobacterales. *Brazilian Journal of Infectious Diseases*, *24*, 231–238.
11. Ragheb, S. M., Govinden, U., & Osei Sekyere, J. (2021). Genetic support of carbapenemases: A One Health systematic review and meta-analysis of current trends in Africa. *Annals of the New York Academy of Sciences*, *1509*(1), 50–73.
12. Gonzalez-Ferrer, S., Peñaloza, H. F., Budnick, J. A., Bain, W. G., Nordstrom, H. R., Lee, J. S., & Van Tyne, D. (2021). Finding order in the chaos: Outstanding questions in *Klebsiella pneumoniae* pathogenesis. *Infection and Immunity*, *89*, e00693-e00620.
13. Li, B., Zhao, Y., Liu, C., Chen, Z., & Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future Microbiology*, *9*, 1071–1081.
14. Shon, A. S., Bajwa, R. P. S., & Russo, T. A. (2013). Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: A new and dangerous breed. *Virulence*, *4*, 107–118.
15. Fang, C.-T., Chuang, Y.-P., Shun, C.-T., Chang, S.-C., & Wang, J.-T. (2004). A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. *Journal of Experimental Medicine*, *199*, 697–705.
16. Russo, T. A., & Marr, C. M. (2019). Hypervirulent *Klebsiella pneumoniae*. *Clinical Microbiology Reviews*, *32*, e00001–e00019.
17. Choby, J. E., Howard-Anderson, J., & Weiss, D. S. (2020). Hypervirulent *Klebsiella pneumoniae*–Clinical and molecular perspectives. *Journal of Internal Medicine*, *287*, 283–300.

18. Catalán-Nájera, J. C., Garza-Ramos, U., & Barrios-Camacho, H. (2017). Hypervirulence and hypermucoviscosity: Two different but complementary *Klebsiella* spp. phenotypes? *Virulence*, *8*, 1111–1123.
19. Park, S., Lee, H., Shin, D., & Ko, K. S. (2020). Change of hypermucoviscosity in the development of tigecycline resistance in hypervirulent *Klebsiella pneumoniae* sequence type 23 strains. *Microorganisms*, *8*, 1562.
20. Scaltriti, E., Piccinelli, G., Corbellini, S., Caruso, A., Latronico, N., & De Francesco, M. A. (2020). Detection of a hypermucoviscous *Klebsiella pneumoniae* co-producing NDM-5 and OXA-48 carbapenemases with sequence type 383, Brescia, Italy. *International Journal of Antimicrobial Agents*, *56*, 106130.
21. Cerdeira, L., Nakamura-Silva, R., Oliveira-Silva, M., Sano, E., Esposito, F., Fuga, B., Moura, Q., Miranda, C. E. S., Wyres, K., Lincopan, N., & Pitondo-Silva, A. (2021). A novel hypermucoviscous *Klebsiella pneumoniae* ST3994-K2 clone belonging to Clonal Group 86. *Pathogens and Disease*, *79*, ftab047.
22. Kong, Z. X., Karunakaran, R., Abdul Jabar, K., Ponnampalavanar, S., Chong, C. W., & Teh, C. S. J. (2021). The detection of hypermucoviscous carbapenem-resistant *Klebsiella pneumoniae* from a tertiary teaching hospital in Malaysia and assessment of hypermucoviscous as marker of hypervirulence. *Microbial Drug Resistance*, *27*, 1319–1327.
23. Karampatakis, T., Tsergouli, K., & Behzadi, P. (2023). Carbapenem-resistant *Klebsiella pneumoniae*: Virulence factors, molecular epidemiology and latest updates in treatment options. *Antibiotics*, *12*, 234.
24. Clinical and Laboratory Standards Institute. (2016). *Performance standards for antimicrobial susceptibility testing. CLSI document M100-S26*. Clinical and Laboratory Standards Institute.
25. Lutgring, J. D., & Limbago, B. M. (2016). The problem of carbapenemase-producing-carbapenem-resistant-Enterobacter iaceae detection. *Journal of Clinical Microbiology*, *54*, 529–534.
26. Vaneechoutte, M., Dijkshoorn, L., Tjernberg, I., Elaichouni, A., De Vos, P., Claeys, G., & Verschraegen, G. (1995). Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. *Journal of Clinical Microbiology*, *33*, 11–15.
27. Akpaka, P. E., Swanston, W. H., Ihemere, H. N., Correa, A., Torres, J. A., Tafur, J. D., Montealegre, M. C., Quinn, J. P., & Villegas, M. V. (2009). Emergence of KPC-producing *Pseudomonas aeruginosa* in Trinidad and Tobago. *Journal of Clinical Microbiology*, *47*, 2670–2671.
28. Poirel, L., Walsh, T. R., Cuvillier, V., & Nordmann, P. (2011). Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic Microbiology and Infectious Disease*, *70*, 119–123.
29. Aktaş, Z., Kayacan, Ç. B., Schneider, I., Can, B., Midilli, K., & Bauernfeind, A. (2008). Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in *Klebsiella pneumoniae* in Istanbul, Turkey. *Chemotherapy*, *54*, 101–106.
30. Turton, J. F., Baklan, H., Siu, L. K., Kaufmann, M. E., & Pitt, T. L. (2008). Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella* sp. and comparison of isolates within these serotypes. *FEMS Microbiology Letters*, *284*, 247–252.
31. Yeh, K.-M., Kurup, A., Siu, L. K., Koh, Y. L., Fung, C.-P., Lin, J.-C., Chen, T.-L., Chang, F.-Y., & Koh, T.-H. (2007). Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in Singapore and Taiwan. *Journal of Clinical Microbiology*, *45*, 466–471.
32. El Fertas-Aissani, R., Messai, Y., Alouache, S., & Bakour, R. (2013). Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. *Pathologie Biologie*, *61*, 209–216.
33. Hennequin, C., & Forestier, C. (2007). Influence of capsule and extended-spectrum beta-lactamases encoding plasmids upon *Klebsiella pneumoniae* adhesion. *Research in Microbiology*, *158*, 339–347.
34. Compain, F., Babosan, A., Brisse, S., Genel, N., Audo, J., Ailloud, F., Kassis-Chikhani, N., Arlet, G., & Decré, D. (2014). Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. *Journal of Clinical Microbiology*, *52*, 4377–4380.
35. Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A. D., & Brisse, S. (2005). Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *Journal of Clinical Microbiology*, *43*, 4178–4182.
36. Cejas, D., Fernández Canigia, L., Rincón Cruz, G., Elena, A. X., Maldonado, I., Gutkind, G. O., & Radice, M. A. (2014). First isolate of KPC-2-producing *Klebsiella pneumonaie* sequence type 23 from the Americas. *Journal of Clinical Microbiology*, *52*, 3483–3485.
37. Jure, M. A., Albarracin, L., Vargas, J. M., Maidana, S. D., Zamar, J. C., Kitazawa, H., & Villena, J. (2021). Draft genome sequences of two hypermucoviscous carbapenem-resistant ST25 *Klebsiella pneumoniae* strains causing respiratory and systemic infections. *Journal of Global Antimicrobial Resistance*, *26*, 174–176.
38. Yao, B., Xiao, X., Wang, F., Zhou, L., Zhang, X., & Zhang, J. (2015). Clinical and molecular characteristics of multi-clone carbapenem-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in a tertiary hospital in Beijing, China. *International Journal of Infectious Diseases*, *37*, 107–112.
39. Zhang, R., Lin, D., Chan, E. W.-C., Gu, D., Chen, G.-X., & Chen, S. (2016). Emergence of carbapenem-resistant serotype K1 hypervirulent *Klebsiella pneumoniae* strains in China. *Antimicrobial Agents and Chemotherapy*, *60*, 709–711.
40. Zhan, L., Wang, S., Guo, Y., Jin, Y. E., Duan, J., Hao, Z., Lv, J., Qi, X., Hu, L., Chen, L., Kreiswirth, B. N., Zhang, R., Pan, J., Wang, L., & Yu, F. (2017). Outbreak by hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a tertiary hospital in China. *Frontiers in Cellular and Infection Microbiology*, *7*, 182.
41. Shankar, C., Nabarro, L. E. B., Devanga Ragupathi, N. K., Muthuirulandi Sethuvel, D. P., Daniel, J. L. K., Doss C, G. P., & Veeraraghavan, B. (2016). Draft genome sequences of three hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolates from bacteremia. *Genome Announcements*, *4*, e01081-e01016.
42. Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, *42*, 182–192.
43. Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*, 268–281.
44. Fasciana, T., Gentile, B., Aquilina, M., Ciammaruconi, A., Mascarella, C., Anselmo, A., Fortunato, A., Fillo, S., Petralito, G., Lista, F., & Giammanco, A. (2019). Co-existence of virulence factors and antibiotic resistance in new *Klebsiella pneumoniae* clones emerging in south of Italy. *BMC Infectious Diseases [Electronic Resource]*, *19*, 1–10.
45. Ahmadi, M., Ranjbar, R., Behzadi, P., & Mohammadian, T. (2022). Virulence factors, antibiotic resistance patterns, and molecular types of clinical isolates of *Klebsiella Pneumoniae*. *Expert Review of Anti-Infective Therapy*, *20*, 463–472.
46. Tang, M., Kong, X., Hao, J., & Liu, J. (2020). Epidemiological characteristics and formation mechanisms of multidrug-resistant hypervirulent *Klebsiella pneumoniae*. *Frontiers in Microbiology*, *11*, 2774.
47. Lin, Y.-C., Lu, M.-C., Tang, H.-L., Liu, H.-C., Chen, C.-H., Liu, K.-S., Lin, C., Chiou, C.-S., Chiang, M.-K., Chen, C.-M., & Lai, Y.-C. (2011). Assessment of hypermucoviscosity as a virulence factor for experimental *Klebsiella pneumoniae* infections: Comparative virulence analysis with hypermucoviscosity-negative strain. *BMC Microbiology*, *11*, 1–8.
48. Zhang, Y., Zeng, J., Liu, W., Zhao, F., Hu, Z., Zhao, C., Wang, Q., Wang, X., Chen, H., Li, H., Zhang, F., Li, S., Cao, B., & Wang, H. (2015). Emergence of a hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolate from clinical infections in China. *Journal of Infection*, *71*, 553–560.
49. Hernández, M., López-Urrutia, L., Abad, D., De Frutos Serna, M., Ocampo-Sosa, A., & Eiros, J. (2021). First report of an extensively

drug-resistant ST23 *Klebsiella pneumoniae* of capsular serotype K1 co-producing CTX-M-15, OXA-48 and ArmA in Spain. *Antibiotics*, *10*, 157.
50. Can, F., Menekse, S., Ispir, P., Atac, N., Albayrak, O., Demir, T., Karaaslan, D. C., Karahan, S. N., Kapmaz, M., Kurt Azap, O., Timurkaynak, F., Simsek Yavuz, S., Basaran, S., Yoruk, F., Azap, A., Koculu, S., Benzonana, N., Lack, N. A., Gönen, M., & Ergonul, O. (2018). Impact of the ST101 clone on fatality among patients with colistin-resistant *Klebsiella pneumoniae* infection. *Journal of Antimicrobial Chemotherapy*, *73*, 1235–1241.
51. Shankar, C., Shankar, B. A., Manesh, A., & Veeraraghavan, B. (2018). KPC-2 producing ST101 *Klebsiella pneumoniae* from bloodstream infection in India. *Journal of Medical Microbiology*, *67*, 927–930.
52. Loconsole, D., Accogli, M., De Robertis, A. L., Capozzi, L., Bianco, A., Morea, A., Mallamaci, R., Quarto, M., Parisi, A., & Chironna, M. (2020). Emerging high-risk ST101 and ST307 carbapenem-resistant *Klebsiella pneumoniae* clones from bloodstream infections in Southern Italy. *Annals of Clinical Microbiology and Antimicrobials*, *19*, 1–10.
53. Chen, H., Jiang, T., Wu, J., Sun, Q., Zha, Q., Jin, S., & Ruan, Z. (2021). Genomic and phylogenetic analysis of a multidrug-resistant mcr-1-carrying *Klebsiella pneumoniae* recovered from a urinary tract infection in China. *Journal of Global Antimicrobial Resistance*, *27*, 222–224.
54. Gu, D., Dong, N., Zheng, Z., Lin, D. I., Huang, M., Wang, L., Chan, E. W.-C., Shu, L., Yu, J., Zhang, R., & Chen, S. (2018). A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: A molecular epidemiological study. *Lancet Infectious Diseases*, *18*, 37–46.
55. Yu, F., Hu, L., Zhong, Q., Hang, Y., Liu, Y., Hu, X., Ding, H., Chen, Y., Xu, X., Fang, X., & Yu, F. (2019). Dissemination of *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in integrated and emergency intensive care units in a Chinese tertiary hospital. *Journal of Medical Microbiology*, *68*, 882–889.
56. Hu, R., Li, Q., Zhang, F., Ding, M., Liu, J., & Zhou, Y. (2021). Characterisation of blaNDM-5 and blaKPC-2 co-occurrence in K64-ST11 carbapenem-resistant *Klebsiella pneumoniae*. *Journal of Global Antimicrobial Resistance*, *27*, 63–66.
57. Guo, Y., Wang, S., Zhan, L., Jin, Y., Duan, J., Hao, Z., Lv, J., Qi, X., Chen, L., Kreiswirth, B. N., Wang, L., & Yu, F. (2017). Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. *Frontiers in Cellular and Infection Microbiology*, *7*, 24.
58. Liao, C. H., Huang, Y. T., Chang, C. Y., Hsu, H. S., & Hsueh, P. R. (2014). Capsular serotypes and multilocus sequence types of bacteremic *Klebsiella pneumoniae* isolates associated with different types of infections. *European Journal of Clinical Microbiology & Infectious Diseases*, *33*, 365–369.
59. Azimi, L., Talebi, M., Owlia, P., Pourshafie, M.-R., Najafi, M., Lari, E. R., & Lari, A. R. (2016). Tracing of false negative results in phenotypic methods for identification of carbapenemase by real-time PCR. *Gene*, *576*, 166–170.
60. Hammoudi Halat, D., & Ayoub Moubareck, C. (2020). The current burden of carbapenemases: Review of significant properties and dissemination among gram-negative bacteria. *Antibiotics*, *9*, 186.
61. Sleiman, A., Fayad, A. G. A., Banna, H., & Matar, G. M. (2021). Prevalence and molecular epidemiology of carbapenem resistant Gram-negative bacilli and their resistance determinants in the Eastern Mediterranean Region over the last decade. *Journal of Global Antimicrobial Resistance*, *25*, 209–221.
62. Poirel, L., Abdelaziz, M. O., Bernabeu, S., & Nordmann, P. (2013). Occurrence of OXA-48 and VIM-1 carbapenemase-producing Enterobacteriaceae in Egypt. *International Journal of Antimicrobial Agents*, *41*, 90–91.
63. Khalifa, H. O., Soliman, A. M., Ahmed, A. M., Shimamoto, T., Hara, T., Ikeda, M., Kuroo, Y., Kayama, S., Sugai, M., & Shimamoto, T. (2017). High carbapenem resistance in clinical gram-negative pathogens isolated in Egypt. *Microbial Drug Resistance*, *23*, 838–844.
64. Abdallah, H. M., Alnaiemi, N., Reuland, E. A., Wintermans, B. B., Koek, A., Abdelwahab, A. M., Samy, A., Abdelsalam, K. W., & Vandenbroucke-Grauls, C. M. J. E. (2017). Fecal carriage of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae in Egyptian patients with community-onset gastrointestinal complaints: A hospital-based cross-sectional study. *Antimicrobial Resistance & Infection Control*, *6*, 1–7.
65. Soliman, A. M., Zarad, H. O., Nariya, H., Shimamoto, T., & Shimamoto, T. (2020). Genetic analysis of carbapenemase-producing Gram-negative bacteria isolated from a university teaching hospital in Egypt. *Infection, Genetics and Evolution*, *77*, 104065.
66. Khalil, M. A. F., Elgaml, A., & El-Mowafy, M. (2017). Emergence of multidrug-resistant New Delhi Metallo-β-lactamase-1-producing *Klebsiella pneumoniae* in Egypt. *Microbial Drug Resistance*, *23*, 480–487.
67. Ramadan Mohamed, E., Ali, M. Y., Waly, N. G. F. M., Halby, H. M., & Abd El-Baky, R. M. (2019). The Inc FII plasmid and its contribution in the transmission of blaNDM-1 and blaKPC-2 in *Klebsiella pneumoniae* in Egypt. *Antibiotics*, *8*, 266.
68. Hassuna, N. A., Abdelaziz, R. A., Zakaria, A., & Abdelhakeem, M. (2020). Extensively-drug resistant *Klebsiella pneumoniae* recovered from neonatal sepsis cases from a major NICU in Egypt. *Frontiers in Microbiology*, *11*, 1375.
69. Ragheb, S. M., Tawfick, M. M., El-Kholy, A. A., & Abdulall, A. K. (2020). Phenotypic and genotypic features of *Klebsiella pneumoniae* harboring carbapenemases in Egypt: OXA-48-like carbapenemases as an investigated model. *Antibiotics*, *9*, 852.
70. Elshamy, A. A., Saleh, S. E., Alshahrani, M. Y., Aboshanab, K. M., Aboulwafa, M. M., & Hassouna, N. A. (2021). OXA-48 carbapenemase-encoding transferable plasmids of *Klebsiella pneumoniae* recovered from Egyptian patients suffering from complicated urinary tract infections. *Biology (Basel)*, *10*, 889.
71. Enany, S., Zakeer, S., Diab, A. A., Bakry, U., & Sayed, A. A. (2022). Whole genome sequencing of *Klebsiella pneumoniae* clinical isolates sequence type 627 isolated from Egyptian patients. *PLoS ONE*, *17*, e0265884.
72. Attia, H., Szubin, R., Yassin, A. S., Monk, J. M., & Aziz, R. K. (2019). Draft genome sequences of four metallo-beta-lactamase-producing multidrug-resistant *Klebsiella pneumoniae* clinical isolates, including two colistin-resistant strains, from Cairo, Egypt. *Microbiology Resource Announcements*, *8*, e01418.
73. Mashaly, G. E.-S., & Mashaly, M. E.-S. (2021). Colistin-heteroresistance in carbapenemase-producing *Enterobacter* species causing hospital-acquired infections among Egyptian patients. *Journal of Global Antimicrobial Resistance*, *24*, 108–113.
74. Kannian, P., Mahanathi, P., Ashwini, V., Vaishnavi, M., & Priya, C. (2021). Carbapenem–resistant gram negative bacilli are predominantly multidrug or pan-drug resistant. *Microbial Drug Resistance*, *27*, 1057–1062.
75. Roe, C. C., Vazquez, A. J., Esposito, E. P., Zarrilli, R., & Sahl, J. W. (2019). Diversity, virulence, and antimicrobial resistance in isolates from the newly emerging *Klebsiella pneumoniae* ST101 lineage. *Frontiers in Microbiology*, *10*, 542.
76. Oteo, J., Pérez-Vázquez, M., Bautista, V., Ortega, A., Zamarrón, P., Saez, D., Fernández-Romero, S., Lara, N., Ramiro, R., Aracil, B., & Campos, J. (2016). The spread of KPC-producing enterobacteriaceae in Spain: WGS analysis of the emerging high-risk clones of *Klebsiella pneumoniae* ST11/KPC-2, ST101/KPC-2 and ST512/KPC-3. *Journal of Antimicrobial Chemotherapy*, *71*, 3392–3399.
77. Ben Sallem, R., Laribi, B., Arfaoui, A., Ben Khelifa Melki, S., Ouzari, H. I., Ben Slama, K., Naas, T., & Klibi, N. (2022). Co-occurrence of genes encoding carbapenemase, ESBL, pAmpC and non-β-Lactam resistance among *Klebsiella pneumonia* and *E. coli* clinical isolates in Tunisia. *Letters in Applied Microbiology*, *74*, 729–740.

78. De Koster, S., Rodriguez Ruiz, J. P., Rajakani, S. G., Lammens, C., Glupczynski, Y., Goossens, H., & Xavier, B. B. (2022). Diversity in the characteristics of *Klebsiella pneumoniae* ST101 of human, environmental, and animal origin. *Frontiers in Microbiology*, *13*, 838207.
79. Singh, S. K., Mishra, M., Sahoo, M., Patole, S., Sahu, S., Misra, S. R., & Mohapatra, H. (2017). Antibiotic resistance determinants and clonal relationships among multidrug-resistant isolates of *Klebsiella pneumoniae*. *Microbial Pathogenesis*, *110*, 31–36.
80. Haudiquet, M., Buffet, A., Rendueles, O., & Rocha, E. P. C. (2021). Interplay between the cell envelope and mobile genetic elements shapes gene flow in populations of the nosocomial pathogen *Klebsiella pneumoniae*. *PLoS Biology*, *19*, e3001276.
81. Lam, M. M. C., Wyres, K. L., Wick, R. R., Judd, L. M., Fostervold, A., Holt, K. E., & Löhr, I. H. (2019). Convergence of virulence and MDR in a single plasmid vector in MDR *Klebsiella pneumoniae* ST15. *Journal of Antimicrobial Chemotherapy*, *74*, 1218–1222.
82. Lan, P., Jiang, Y., Zhou, J., & Yu, Y. (2021). A global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*. *Journal of Global Antimicrobial Resistance*, *25*, 26–34.
83. Walker, K. A., Miner, T. A., Palacios, M., Trzilova, D., Frederick, D. R., Broberg, C. A., Sepúlveda, V. E., Quinn, J. D., & Miller, V. L. (2019). A *Klebsiella pneumoniae* regulatory mutant has reduced capsule expression but retains hypermucoviscosity. *mBio*, *10*, e00089-e00019.
84. Kawai, T. (2006). Hypermucoviscosity: An extremely sticky phenotype of *Klebsiella pneumoniae* associated with emerging destructive tissue abscess syndrome. *Clinical Infectious Diseases*, *42*, 1359–1361.
85. Yu, W.-L., Ko, W.-C., Cheng, K.-C., Lee, H.-C., Ke, D.-S., Lee, C.-C., Fung, C.-P., & Chuang, Y.-C. (2006). Association between rmpA and magA genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. *Clinical Infectious Diseases*, *42*, 1351–1358.
86. Miethke, M., & Marahiel, M. A. (2007). Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews*, *71*, 413–451.
87. Feldman, C., & Anderson, R. (2021). The role of co-infections and secondary infections in patients with COVID-19. *Pneumonia*, *13*, 1–15.
88. Lokate, M., Bathoorn, E., Akkerman, O. W., Gröschel, M. I., Stienstra, Y., De Lange, W., Omansen, T. F., & Van Der Werf, T. S. (2016). Case report: Multidrug-resistant tuberculosis complicated by nosocomial infection with multidrug-resistant enterobacteriaceae. *American Journal of Tropical Medicine and Hygiene*, *94*, 517.
89. Langford, B. J., So, M., Simeonova, M., Leung, V., Lo, J., Kan, T., Raybardhan, S., Sapin, M. E., Mponponsuo, K., Farrell, A., Leung, E., Soucy, J.-P. R., Cassini, A., Macfadden, D., Daneman, N., & Bertagnolio, S. (2023). Antimicrobial resistance in patients with COVID-19: A systematic review and meta-analysis. *Lancet Microbe*, *4*, e179–e191.
90. Candan, E. D., & Aksöz, N. (2015). *Klebsiella pneumoniae*: Characteristics of carbapenem resistance and virulence factors. *Acta Biochimica Polonica*, *62*, 867–874.
91. Taha, M. S., Hagras, M. M., Shalaby, M. M., Zamzam, Y. A., Elkolaly, R. M., Abdelwahab, M. A., & Maxwell, S. Y. (2023). Genotypic characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from an Egyptian University Hospital. *Pathogens*, *12*, 121.
92. Albasha, A. M., Osman, E. H., Abd-Alhalim, S., Alshaib, E. F., Al-Hassan, L., & Altayb, H. N. (2020). Detection of several carbapenems resistant and virulence genes in classical and hyper-virulent strains of *Klebsiella pneumoniae* isolated from hospitalized neonates and adults in Khartoum. *BMC Research Notes*, *13*, 1–7.
93. Pan, Y., Zeng, J., Li, L., Yang, J., Tang, Z., Xiong, W., Li, Y., Chen, S., & Zeng, Z. (2020). Coexistence of antibiotic resistance genes and virulence factors deciphered by large-scale complete genome analysis. *mSystems*, *5*, 10–1128.
94. Darmancier, H., Domingues, C. P. F., Rebelo, J. S., Amaro, A., Dionísio, F., Pothier, J., Serra, O., & Nogueira, T. (2022). Are virulence and antibiotic resistance genes linked? A comprehensive analysis of bacterial chromosomes and plasmids. *Antibiotics*, *11*, 706.
95. Beceiro, A., Tomás, M., & Bou, G. (2013). Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clinical Microbiology Reviews*, *26*, 185–230.
96. Ahmed, M. A. E.-G. E.-S., Yang, Y., Yang, Y., Yan, B., Chen, G., Hassan, R. M., Zhong, L.-L., Chen, Y., Roberts, A. P., Wu, Y., He, R., Liang, X., Qin, M., Dai, M., Zhang, L., Li, H., Yang, F., Xu, L., & Tian, G.-B. (2021). Emergence of hypervirulent carbapenem-resistant *Klebsiella pneumoniae* coharboring a bla NDM-1-carrying virulent plasmid and a bla KPC-2-carrying plasmid in an Egyptian hospital. *mSphere*, *6*, e00088-e00021.
97. Abdelwahab, R., Alhammadi, M. M., Hassan, E. A., Ahmed, E. H., Abu-Faddan, N. H., Daef, E. A., Busby, S. J. W., & Browning, D. F. (2021). Antimicrobial resistance and comparative genome analysis of *Klebsiella pneumoniae* strains isolated in Egypt. *Microorganisms*, *9*, 1880.

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