ORIGINAL ARTICLE

Cefoxitin Resistance in Hypermucoviscous *Klebsiella Species* Isolated from Patients with Hospital-Acquired Infections in Mansoura University Hospitals

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ABSTRACT

Background*: AmpC beta-lactamases have a major role in mediating cefoxitin resistance which is escalating worldwide in pathogenic Klebsiella species. Two main pathogenic models of Klebsiella species are classical Klebsiella species and hypervirulent Klebsiella species.* ***Objectives****: The current study aimed to ascertain the risk factors for hypermucoviscous Klebsiella (hmK) species acquisition in patients with healthcare-associated infections (HAIs), the prevalence of cefoxitin-resistant hmK species in patients with HAIs and compare various phenotypic approaches to PCR for precise cefoxitin resistance detection.* ***Methodology****: 67 Klebsiella isolates were identified and classified as non-hmK and hmK phenotypes by string test. The boronic acid assay, Modified three-dimensional test, and AmpC cefoxitin-EDTA test were performed for phenotypic evaluation. Multiplex PCR assay was performed to evaluate the presence of AmpC genes in hypermucoviscous strains with cefoxitin resistance.* ***Results****: In relation to other Klebsiella infections, the prevalence of hmK species was 77.6%. The prevalence of hmK species among HAIs was 46.4%. The prevalence of cefoxitin-resistant hmK species among HAIs was 38.4%. The modified three-dimensional test yielded the highest results concerning the detection rates of the three confirmatory phenotypic techniques. Using multiplex PCR, 69.8% carried AmpC genes while 30.2% carried no genes.* ***Conclusions****: There is an alarming increase in HAIs caused by hmK species. The risk factors for the acquisition of hmK species in patients with HAIs are associated co-morbidities such as DM, liver diseases, malignancy, and history of ICU stays.*

INTRODUCTION

The development of resistance to cefoxitin in pathogenic *Klebsiella* species is escalating worldwide. AmpC beta-lactamases have a major role in mediating cefoxitin resistance[1].

Two main pathogenic models of *Klebsiella species* are noted. They are classical *Klebsiella species* and the emerging model hypervirulent *Klebsiella species*. Hypermucoviscous clones observed in the hypervirulent *Klebsiella* strains are detected by string test[2].

Hypermucoviscous *Klebsiella species* (*hmK)* retain the unique characteristic of inflicting infection in both young and immunocompetent individuals[3].

AmpC beta-lactamases grant resistance against cephalosporins, oxyimino-cephalosporins, cephamycins, and monobactams[4].

Tracking down the AmpC beta-lactamases is tricky as there are no approved guidelines released by the Clinical and Laboratory Standards Institute (CLSI) for systematic discovery of the phenotypic expression of genes encoding the AmpC beta-lactamases[5].

Diagnostic tools for phenotypic identification of AmpC beta-lactamases include initial screening using cefoxitin disc, AmpC Cefoxitin‑EDTA Test, phenylboronic acid-based assays, and modified three-dimensional test (M3DT)[6].

The six families of plasmid-mediated AmpC (p-AmpC) can be evaluated by multiplex polymerase chain reaction (PCR)[1].

The goal of the current study was to detect the prevalence of cefoxitin-resistant *hmK Species* in patients with healthcare-associated infections (HAIs), study different phenotypic methods in comparison to PCR for accurate detection of cefoxitin resistance and determine the risk factors for acquisition of *hmK Species* in patients with HAIs.

METHODOLOGY

Study population:

The current study was a descriptive prospective study with an analytic component performed in Medical Microbiology and Immunology Department, Faculty of

Medicine, Mansoura University, during the period from October 2021 to July 2022, on 112 clinical specimens obtained from patients admitted at Mansoura University Hospitals exhibiting symptoms and signs of HAIs.

Data collection:

The following information was gathered from the patients regarding name, age, gender, underlying disease (such as diabetes, liver disease, and malignancy), duration of hospital stay before the onset of infection, laboratory investigations, invasive procedures, antimicrobial regimen received 15 days before the onset of infection[7], and signs or symptoms that suggested the development of HAIs, whether systemic or local.

Microbiological methods:

The following samples (peripheral blood, wound swab, urine, and sputum) were collected, and processed in MDICU where they were cultured then suspected *Klebsiella* isolates were identified by colony morphology, Gram-stained films, and biochemical reactions[8].

Identification of hypermucoviscous strains:

The string test was used to identify the hypermucoviscous phenomenon. A sterile loop was used to gently touch and stretch *Klebsiella* colonies. A viscous filament measuring at least 5 mm was considered a positive string test[9].

Antimicrobial susceptibility testing:

Antibiotic sensitivity was performed using the disk diffusion method according to the CLSI, (2021) guidelines[10]. Antibiotic discs: Cefoxitin (30 ug), amoxicillin/clavulanate (20/10 µg), cefuroxime (30 ug), ceftazidime (30 ug), cefotaxime (30 µg), aztreonam (30 µg), imipenem (10 µg), gentamicin (10µg), and azithromycin (15µg), amikacin (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), and ciprofloxacin (5µg).

Phenotypic Detection of AmpC β-Lactamase Production:

Phenotypic detection tests for AmpC β-Lactamase production were conducted on the isolates that exhibited reduced sensitivity to cefoxitin and an inhibitory zone of less than 18 mm[11].

The boronic acid assay, M3DT, and AmpC Cefoxitin-EDTA test were performed for phenotypic evaluation.

Boronic acid assay for detection of AmpC β-Lactamases:

Procedure:

The surface of the Mueller–Hinton agar (MHA) plate was inoculated with 0.5 McFarland bacterial suspension made from an overnight blood agar plate. A 30 µg cefoxitin disk was placed on the MHA's inoculated surface. Sterile tips were used to dispense 20 µl of 15 µg/ml phenylboronic acid onto a second cefoxitin disk. The plate was incubated for 24 hours at 35°C[12].

Modified three-dimensional test (M3DT):

Procedure: A freshly grown overnight culture on MHA plate was put into a sterile microcentrifuge tube. For 15 minutes, centrifugation at 3000 rpm was used to pelletize the growth, which was suspended in peptone water. Crude enzyme extract was created through multiple freeze-thawing cycles. On MHA plates, lawn cultures of Escherichia coli ATCC 25922 25922, and cefoxitin (30 µg) discs were arranged. Three-centimeter linear slits were made, 3 mm from the cefoxitin disc's edge, using a sterile surgical blade. Using a sterile glass pipette, tiny circular wells were created on the agar surface at a distance of 5 mm inside the slit's outer edge. The wells were filled with approximately 30–40 µL of enzyme extract, added in batches of 10 µL at a time. The plates were incubated at 37°C overnight[13].

AmpC Cefoxitin EDTA Test:

Procedure: The surface of MHA plate was inoculated using a 0.5 McFarland bacterial suspension of Escherichia coli ATCC 25922. AmpC disks (i.e., filter paper disks containing Tris-EDTA) were rehydrated with 20 µl of saline right before usage, and several colonies of every test organism were placed on a disk. The inoculated surface of the MHA was coated with a 30 µg cefoxitin disk. With its face toward the agar surface, the inoculated AmpC disk was positioned so that it nearly touched the antibiotic disk. The plate was incubated at 35°C for 24 hours[14].

Genotypic detection of AmpC β-Lactamase Genes[15]:

Genomic DNA was extracted by the boiling technique. Two multiplex PCR assays were performed to evaluate the presence of blaACC, blaCIT, blaEBC, blaFOX, blaMOX, and blaDHA genes in hypermucoviscous strains with cefoxitin resistance.

A master mix was prepared in a final volume of 25µL, each 25µL reaction mixture: 12.5µL master Mix, 1µL of each primer (total 6 µL), 5 µL DNA template, 1.5 µL nuclease-free water. All the primers[16] used are listed in Table 1.

Table 1: Primers Used in this Study:

Genes Family	Sequence (5' to 3', as Synthesized)	Amplic-on (bp)
MOXM-F	GCT GCT CAA GGA GCA CAG GAT	520
MOXM-R	CAC ATT GAC ATA GGT GTG GTG C	
CITM-F	TGG CCA GAA CTG ACA GGC AAA	462
CITM-R	TTT CTC CTG AAC GTG GCT GGC	
DHAM-F	AAC TTT CAC AGG TGT GCT GGG T	405
DHAM-R	CCG TAC GCA TAC TGG CTT TGC	
ACCM-F	AAC AGC CTC AGC AGC CGG TTA	346
ACCM-R	TTC GCC GCAATCATC CCT AGC	
EBCM-F	TCG GTAAAG CCG ATG TTG CGG	302
EBCM-R	CTT CCA CTG CGG CTG CCA GTT	
FOXM-F	AAC ATG GGG TAT CAG GGA GAT G	190
FOXM-R	CAAAGC GCG TAA CCG GAT TGG	

Amplification was done by initial denaturation for 3 min at 94 °C, then 25 cycles including: (denaturation at 94 °C for 30 sec, an annealing temperature at 64 °C for 30 sec, an extension at 72 °C for 1 min), then finally a final extension at 72 °C for 7 min. After electrophoresis on a 2% agarose gel stained with ethidium bromide, PCR products were visualized by UV illumination and compared with a 100 bp DNA ladder[17].

Statistical analysis:

The data was analyzed using the Statistical Package for Social Science (SPSS) (Standard version 23). When applicable, the means (SD) or medians were used to characterize the quantitative data. The t-test was utilized to compare groups for the normally distributed variables. The Mann-Whitney test was utilized to compare groups based on non-normally distributed variables. Numbers and percentages were used to describe the qualitative data. Chi-square test, Fisher's Exact test, and Monte Carlo test were used for comparison between groups, as appropriate. The threshold of significance for all of the above-mentioned statistical tests is set at 5%. When $p \leq 0.05$, the results were regarded as significant. The lower the p-value, the more significant the findings. Validity of screening tests was calculated[18].

RESULTS

Sixty-seven out of 112 clinical specimens were identified as *Klebsiella species*. In relation to other *Klebsiella* infections, the prevalence of *hmK species* was 77.6%. The prevalence of *hmK species* among HAIs was (52/112), 46.4%. The prevalence of cefoxitin-resistant *hmK species* among HAIs was 38.4%. These cefoxitin-resistant *hmK species* comprised 14 (32.6%) *K.oxytoca* and 29 (67.4%) *K. pneumoniae*.

Demographic and clinical criteria of the study population:

Forty-two males and ten females, aged from 16 to 87 years with a median of 54, were found to be infected with *hmK isolates*. Eight males and seven females, aged from 15 to 87 years with a median of 57, were infected with non-*hmK isolates*. The hypermucoviscous and non-hypermucoviscous *Klebsiella* isolates were more frequently isolated from males (80.8% & 53.3%, respectively) than females (19.2% & 46.7%, respectively). There was a statistically significant difference in the prevalence of diabetes mellitus (51.9% vs 20.0%; p=0.03), liver disease (36.5% vs 6.7%; p=0.03), and solid malignancy (25.0% vs 0.0%; p=0.03) between patients with *hmK* infections and those with non- *hmK* infections. Hypertension (36.5% vs 26.7%; p=0.5) and renal disease (5.8% vs 0.0%; p=0.99) were more prevalent in patients with *hmK* infections with no statistical significance. In contrast, patients with non-*hmK* infections had higher rates of neurologic diseases (30.8% vs 33.3%; p=0.002). The history of intensive care unit (ICU) stay 3 months before culture was more common in the patients' group of *hmK* (88.5%) than in the patients' group of non-*hmK* (60.0%) with a significant statistical difference (p=0.02).

Antimicrobial resistance pattern of the isolated *Klebsiella species*:

A significant difference (p=0.04) was noticed on comparing the *hmK* isolates (82.7%) to non-*hmK* ones (60.0%) as regards resistance to cefoxitin. The prevalence of cefotaxime resistance was found to be higher in hypermucoviscous isolates (94.2%) compared to non-hypermucoviscous isolates (73.3%); this difference was statistically significant (p=0.02). Antimicrobial resistance pattern of the isolated hypermucoviscous *Klebsiella* in relation to non-hypermucoviscous *Klebsiella* isolates is illustrated in Table 2.

Table 2: Antimicrobial resistance pattern of the isolated hypermucoviscous *Klebsiella* in relation to non-hypermucoviscous *Klebsiella* isolates:

Characteristic	Hypermucoviscous *Klebsiella* (77.6%) N=52	Non-hypermucoviscous *Klebsiella* (22.4%) N=15	Test of significance
Cefoxitin			
Sensitive	(11.5)	(40.0)	Monte Carlo test, p=0.04
Resistant	(82.7)	(60.0)	
Intermediate	(5.8)	(0.0)	
Amoxicillin-clavulanate			
Sensitive	(3.8)	(0.0)	Fisher's Exact test, p=0.99
Resistant	(96.2)	(100.0)	
Cefuroxime			
Sensitive	(1.9)	(0.0)	Fisher's Exact test, p=0.99
Resistant	(98.1)	(100.0)	
Cefotaxime			
Sensitive	(3.8)	(26.7)	Monte Carlo test, p=0.02
Resistant	(94.2)	(73.3)	
Intermediate	(1.9)	(0.0)	
Aztreonam			
Sensitive	(5.8)	(6.7)	Fisher's Exact test, p=0.99
Resistant	(94.2)	(93.3)	
Imipenem			
Sensitive	(30.8)	(20.0)	Fisher's Exact test, p=0.5
Resistant	(69.2)	(80.0)	
Amikacin			
Sensitive	(36.5)	(20.0)	Fisher's Exact test, p=0.4
Resistant	(63.5)	(80.0)	
Gentamycin			
Sensitive	(36.5)	(26.7)	Monte Carlo test, p=0.6
Resistant	(59.6)	(73.3)	
Intermediate	(3.8)	(0.0)	
Azithromycin			
Sensitive	(13.5)	(20.0)	Monte Carlo test, p=0.2
Resistant	(67.3)	(80.0)	
Intermediate	(19.2)	(0.0)	
Ciprofloxacin			
Sensitive	(11.5)	(13.3)	Monte Carlo test, p=0.4
Resistant	(84.6)	(73.3)	
Intermediate	(3.8)	(13.3)	

Detection of AmpC enzymes in the isolated cefoxitin-resistant *hypermucoviscous Klebsiella species* by phenotypic methods:

Phenyl boronic acid-based assay revealed that 34 (79.1%) of the 43 cefoxitin-resistant *hmK* isolates were positive and 9 (20.9%) were negative. Using the M3DT 38 (88.4%) were positive and 5 (11.6%) were negative. By AmpC Cefoxitin EDTA test 35 (81.4%) were positive and 8 (18.6%) were negative. In terms of the three confirmatory phenotypic approaches' detection rates, the modified three-dimensional test showed the highest findings.

Comparison between Phenotypic methods and the gold standard Multiplex PCR in the detection of AmpC enzymes in the isolated cefoxitin-resistant hypermucoviscous *Klebsiella* isolates is illustrated in Table 3, Table 4, Table 5.

Table 3: Comparison between Phenyl boronic acid-based assay and the gold standard Multiplex PCR in the detection of AmpC enzymes in the isolated cefoxitin-resistant hypermucoviscous *Klebsiella* isolates

Phenyl boronic acid-based assay	Multiplex PCR		Total
	Genes present	Genes absent	
Positive	23	11	**34**
Negative	7	2	**9**
Total	**30**	**13**	**43**

Table 4: Comparison between Modified three-dimensional test method and the gold standard Multiplex PCR in the detection of AmpC enzymes in the isolated cefoxitin-resistant hypermucoviscous *Klebsiella* isolates

Modified three-dimensional test	Multiplex PCR		Total
	Genes present	Genes absent	
Positive	28	10	**38**
Negative	2	3	**5**
Total	**30**	**13**	**43**

Table 5: Comparison between AmpC Cefoxitin EDTA test method and the gold standard Multiplex PCR in the detection of AmpC enzymes in the isolated cefoxitin-resistant hypermucoviscous *Klebsiella* isolates

AmpC EDTA Cefoxitin test	Multiplex PCR		Total
	Genes present	Genes absent	
Positive	24	11	**35**
Negative	6	2	**8**
Total	**30**	**13**	**43**

Detection of AmpC enzymes in the isolated cefoxitin-resistant *hypermucoviscous Klebsiella species* by genotypic methods:

By multiplex PCR done for the detection of *AmpC* genes, among 43 cefoxitin-resistant *hmK* isolates, 30 (69.8%) carried *AmpC* genes while 13 (30.2%) carried no genes.

DISCUSSION

The emergence of hypermucoviscous *Klebsiella* (*hmK*) strains is causing an increasing challenge, particularly in countries lacking central surveillance systems for infectious diseases. However, little is known about the prevalence of hypermucoviscous *Klebsiella pneumoniae* (*hmKP)* strains in Egypt. In addition, very limited data are available on cefoxitin-resistant *hmKP*[19].

Fifty-two *Klebsiella* isolates (77.6%) from the current study were identified as *hmK*. As far as we are aware, this is the highest percentage recorded and it also aligns with previous analyses that estimated *hmKP* in 68.2% of isolates[20]. Another investigation carried out in Egypt revealed that approximately 40% of *Klebsiella pneumoniae* isolates were hypermucoviscous[21].

This means that the antimicrobial resistance problem in our locality is increasing probably due to the abuse of antibiotics in agriculture, their needless prescription in medical settings, and unrestricted access to antimicrobials. International travel, spontaneous evolution, mutations, and horizontal gene transfer of resistant genes are also significant contributors to antimicrobial resistance.

In the current study, *hmK* accounted for (46.4%) of HAIs caused by *Klebsiella*. Nonetheless, other studies have shown that (68.8%) of *hmKP* were linked to HAIs[22]. According to another study, hypervirulent *Klebsiella pneumoniae* (*hvKP*) is related to a lower percentage of infections in healthcare facilities (38%)[23]. The differences in the size and composition of the samples under investigation, the technique used to identify the *hmK* isolates, the notification standards applied, and the characteristics of HAI patients can all be used to explain these disparities.

The prevalence of cefoxitin-resistant *hmK* among HAIs was 38.4%. To the best of our knowledge, this is the first report on the prevalence of cefoxitin-resistant *hmK* among HAIs.

There were twenty-nine (67.4%) cefoxitin-resistant *hmKP* and fourteen (32.6%) cefoxitin-resistant hypermucoviscous *K. oxytoca* in the present research. There were 29 (67.4%) cefoxitin-resistant *hmKp* in the current investigation compared to 47.1% in another study[24].

This study is one of the few describing the presence of hypermucoviscous phenotype in *K. oxytoca* isolates and the prevalence of cefoxitin-resistant hypermucoviscous *K. oxytoca*.

The hypermucoviscous and non-hypermucoviscous *Klebsiella* isolates in this study were more frequently isolated from males than females. This is in accordance with research that showed most patients with nosocomial infections (84.9%) and healthcare-associated illnesses (94.7%) caused by *hvKP* were male[25]. In a study involved 120 patients with non-*hmKP* healthcare-associated infections, 56.4% were men; the mean age was 55.63 ± 1.43 (mean ± SE) years, which corresponds to our findings[26].

Diabetes (51.9%), liver disease (36.5%), hypertension (36.5%), neurologic disease (30.8%), cancer (25.0%), and renal disease (5.8%) were the most common comorbidities related to *hmK* isolates in the current investigation. The most common comorbidities associated with non-*hmK* isolates were neurologic disease (33.33%), hypertension (26.67%), diabetes (20%), liver disease (6.67%), while malignancy and renal disease weren't recorded as comorbidities associated with non-*hmK* isolates.

These results correlate with another study that found the *hmKP-infected* patients' group had considerably higher rates of diabetes mellitus (DM) (58.9% vs. 36.3%; p=0.001) than the non-*hmKP* infected patients' group[27].

The results also match findings from another article that showed hypertension was present in 35.3% of *hvKP* cases[28]. Similar results were found in another article, which indicated that 37.5% of *hvKP* and 24.2% of classical *Klebsiella pneumoniae* (*cKP*) had liver illness.

About 25.8% of (*cKP*) had DM, according to the same article[29].

The results of this study are consistent with those of another publication, which found that neurologic dysfunction was present in 30.51% of *hvKP* cases[30]. Comparable data from another publication showed that 8.1% of *cKP* and 7.0% of *hvKP* had kidney damage. Nonetheless, 16.7% of *hvKP* and 8.1% *cKP* exhibited malignant tumors[31].

These findings coincide with what was illustrated in the preceding risk factor studies, that diabetes mellitus, liver disease, and solid malignancy are considered significant risk factors for *hvKP* infections.

The history of ICU stay 3 months before culture was more common in the patients' group of *hmK* (88.5%) than in the patients' group of non-*hmK* (60.0%) with a significant statistical difference (p=0.02). This is in agreement with another study, which found that 76.9% of *hmKP* infected patients had a history of ICU hospitalization[32].

In terms of cefoxitin resistance, a significant difference (p=0.04) was observed when comparing *hmK* isolates to non-*hmK isolates*. Nearly 82.7% of *hmK* isolates were resistant, compared to 60.0% of non-*hmK* isolates. This is analogous to the previously recorded cefoxitin resistance in (89.7%) of *hvKP* isolates[33], also compatible with the witnessed cefoxitin resistance in (64.9%) of *cKP* isolates[34].

The prevalence of cefotaxime resistance was found to be higher in hypermucoviscous isolates (94.2%) compared to non-hypermucoviscous isolates (73.3%); this difference was statistically significant (p=0.02). This is in accordance with separate research that demonstrated cefotaxime resistance in (87.5%) of HAIs caused by *hmKP*[35].

This study is the first that we are aware of that employed the AmpC Cefoxitin EDTA test, the M3DT, and the phenylboronic acid assay to determine the presence of p-AmpC β-lactamases in cefoxitin-resistant *hmK* isolates. Also, the first to compare the outcomes of these phenotypic tests with the PCR results in *hmK* isolates.

Three confirmatory phenotypic approaches were applied to these forty-three *hmK* isolates demonstrating resistance. Thirty-four (79.1%) of the 43 cefoxitin-resistant *hmK* isolates were found to be positive and (20.9%) were negative using the phenylboronic acid assay.

Modified three-dimensional test (M3DT) results showed that 38 (88.4%) of the 43 cefoxitin-resistant *hmK* isolates were positive and 5 (11.6%) were negative. Thirty-five (81.4%) of the 43 cefoxitin-resistant *hmK* isolates that were tested according to the AmpC Cefoxitin EDTA test were positive, whereas 8 (18.6%) were negative. An additional analysis using the AmpC Cefoxitin EDTA test revealed that 7.4% of *hmKP* isolates harbored AmpC[34].

The M3DT showed the highest values in terms of accuracy, PPV, NPV, sensitivity, and specificity among the three phenotypic approaches. Unfortunately, no comparable information on these findings could be provided.

Thirteen (30.2%) of the forty-three cefoxitin-resistant *hmK* isolates displayed no genes, whereas thirty (69.8) possessed *AmpC* genes, according to multiplex PCR used to detect it.

The current findings represent one of the few reports on AmpC genes in *hmK* isolates. It also surpasses those of a study that found 30.26% of *Klebsiella* isolates (both hypermucoviscous and classical) suspected of being AmpC producers, carried AmpC genes[36].

The prevalence and nature of acquired p-AmpCs found are influenced by the study period, the species under investigation, and the place of inquiry. Because of this, comparing the prevalence of acquired AmpCs between researchers is challenging.

CONCLUSION

There is an alarming increase in healthcare-associated infections (HAIs) caused by hypermucoviscous *Klebsiella species* (*hmK*). The risk factors for the acquisition of *hmK* species in patients with HAIs are associated co-morbidities such as DM, liver diseases, malignancy, and history of ICU stays. This manuscript is not currently being considered for publication in any other reviewed medium in any manner, nor has it ever been published before. My contribution to the project suits my inclusion as an author.

Declarations

Ethics Approval and Consent to Participate:
The study protocol was accepted by the Institutional Review Board (IRB) after approval by ethical committees in Faculty of Medicine, Mansoura University; code number: MS.21.09.1646.

Human and Animal Rights: No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

Consent for Publication: Informed written consent was obtained from all participants.

Standards of Reporting: This study was conducted in accordance with the STROBE guidelines.

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Conflict Of Interest: The authors declare no conflict of interest, financial or otherwise.

Abbreviations: *Klebsiella pneumoniae* (*K. pneumoniae*), *Klebsiella oxytoca* (*K.oxytoca*), Hypermucoviscous *Klebsiella species* (*hmK)*, Clinical and Laboratory Standards Institute (CLSI), modified three-dimensional test (M3DT)**,** plasmid-mediated AmpC (p-AmpC),

multiplex polymerase chain reaction (PCR), healthcare-associated infections (HAIs), Mueller–Hinton agar (MHA), Institutional Review Board (IRB), Statistical Package for Social Science (SPSS), hypermucoviscous *Klebsiella pneumoniae* (*hmKP),* hypervirulent *Klebsiella pneumoniae* (*hvKP*), diabetes mellitus (DM), classical *Klebsiella pneumoniae* (*cKP*), intensive care unit (ICU).

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