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

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**Abstract**

**Objective:** *Klebsiella pneumoniae* (*K. pneumoniae*) involves both community-acquired and nosocomial infections. It is responsible for a wide variety of infections, including infections of the urinary tract, pneumonia, bacteremia, meningitis, wound infection and purulent abscesses. We constructed this study to detect several carbapenems resistant and virulence genes in classical and hyper-virulent strains of *K. pneumoniae* isolated from hospitalized neonates and adults in Khartoum state.

**Results:** Seventy percent of the isolates were resistant to ceftazidime, 18(30%) to ciprofloxacin, 23(38.3%) to chloramphenicol, 24(40%) to gentamicin and 8% to imipenem, 35% were multidrug-resistant, and 7% extensively drug-resistant, all blood isolates (*n* = 14) were resistant to ceftazidime. *entB* was the most predominant virulence gene (93.3%), followed by *mrkD* (78.3%), *kfu* (60%), *K2* (51.7%), *magA* (18.3%) and *rmpA* (5%). *bla*OXA-48 was the most predominant carbapenem-resistant gene (68.3%), followed by *bla*NDM (10%), *bla*KPC (8.3%), and *bla*IMP (3.3%). Eight hyper-virulent strains were positive for *bla*OXA-48 and two for *bla*NDM genes.

**Keywords:** *K. pneumoniae*, MDR, XDR, hvKp, Nosocomial infection and, Sudan

**Introduction**

*Klebsiella pneumoniae* (*K. pneumoniae*) is a non-motile, capsulated gram-negative rod about 1–2 µm long, and is a facultative anaerobe [1]. It is a common cause of urinary tract, soft-tissue, and central nervous system infections, in addition to endocarditis, and cases of severe bronchopneumonia, sometimes with chronic destructive lesions and multiple abscess formation in the lungs. In many cases, localized infections lead to bacteremia [1].

There are two types of *K. pneumoniae* strains “classic” (cKp), usually non-virulent, and drug-resistant gene producer and usually associated with hospital infections, while the other type is a hypervirulent (hvKp) drug-sensitive strain [2]. *K. pneumoniae* possesses different virulence and antimicrobial resistance genes associated with various clinical conditions [3].

Carbapenemase enzymes of *K. pneumoniae* can resist most β-lactam-ring-containing antibiotics, including carbapenems, and thus conferring resistance to these drugs [4]. Ambler molecular class A *K. pneumoniae*
carbapenemase (KPC), class B; Verona integron metallo-beta-lactamases types (VIM), Imipenemase (IMP) and New Delhi metallo-beta-lactamase (NDM) and class D oxacillinase-48 (OXA-48) are frequently isolated from severe hospital infections [5].

Carbapenem-resistant hypervirulent strains of K. pneumonia are one of the most important organisms that cause fatal nosocomial infections [6]. Recently, increasing reports of resistance to carbapenem in healthcare-associated with K. pneumonia infections have been documented in Sudan [7–9]. The mortality rate of carbapenem-resistant K. pneumoniae bacteraemia could reach 50% of cases [10].

However, to date, there are no published data in Sudan about the distribution and epidemiology of various types of Carbapenemases and virulence genes on hvKp and cKp strains circulating in Khartoum hospitals. This information is of great importance to understand their local epidemiology and to establish eradication and prevention procedures. Thus, this study was conducted to detect and to characterize the common virulence and carbapenem-resistant genes of hvKp and cKp strains isolated from hospitalized patients in different hospitals in Khartoum state.

### Methods

A total of 60 isolates of Klebsiella pneumoniae were obtained from hospitalized patients (45 adults, and 15 neonates) in different hospitals of Khartoum State, during the period from January 2017 to March 2017. These isolates were collected and identified at hospitals as a part of their routine clinical procedure.

### Bacterial identification

The isolates were re-identified by gram stain, standard biochemical methods (urease test, indole test, and carbohydrates fermentation test, motility test, and citrate utilization test) [11, 12], and by K. pneumoniae species-specific primers (Table 1) targeting the 16S rRNA gene. Antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method on Mueller–Hinton agar, the following commonly used antibiotics for the treatment of K. pneumonia infection in Sudan were selected; ciprofloxacin (5 mcg), gentamicin (10 mcg), ceftazidime (30 mcg), imipenem (10 mcg), and chloramphenicol (30) (HiMedia Laboratories Pvt. Ltd. Mumbai, India), the results of sensitivity tests were interpreted according to Clinical And Laboratory Standards Institute (CLSI) guidelines [13]. E. coli ATCC 25922 and K. pneumonia (ATCC 700603) were used as quality control strains.

### Table 1 Primers sequences and PCR protocols used in this study

| Protocols | Temperature cycling | Marker | Sequence (5–3) | Amplicons size (bp) | References |
|-----------|---------------------|--------|---------------|-------------------|------------|
| 1st       | 35 cycles at 94 °C for 30 s, 58 °C for 90 s and 72 °C for 90 s | 16 s rRNA | F. ATTGGAAGGTTGCAACGAT R.TTCACTCTTGAAATTTTCGTTTC | 130 | [38] |
| 2nd       | 30 cycles at 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 60 s | mrrD | F. AAGCTATCGTACTTCCCGCA R. GCGTGTGGGCGCTAGATTAG | 340 | 4 [39] |
|           |                     | entB  | F. GTCAACTGCGGCTTGAGCCGTC R. TATGGCGTAAACACGGGTTGAT | 400 |          |
|           |                     | rmpA  | F. CATAAGATATGTTGACAG R. CTTGACTGAGGCACTTTCA | 461 |          |
|           |                     | K2    | F. CAACCATGGTGTGCGATTAG R. TGGTAGCCATATCCCTTTGG | 531 |          |
|           |                     | kfu   | F. GGCCTTTGTCGAGGCATAG R. GGGTCTGGGCAGAGATGTC | 638 |          |
|           |                     | magA  | F. GGTGCTTCTTACATCATGTC R. GCAATGCGGCGTTGCTTAG | 1283 |          |
| 3rd       | 35 cycles at 94 °C for 20 s, 56 °C for 10 s, 72 °C for 20 s | NDM   | F. GGTGGTGGCGACAGTTGTTTC R. CGGAATGCGTACATCAGCT | 521 | [39, 40] (Mushi et al. 2014) |
|           |                     | IMP   | F. TTGACACTCTGCATTTACAG R. GATTAGAAATGCAACGCCTCT | 232 | [17, 37] |
|           |                     | KPC   | F. CATCAGGGGTTTCTGTGCG R. ACAGCGCGATAGCTTTTCC | 498 |          |
|           |                     | OXA-48| F. GCTTGTAGCGGCTGCGATT R. GATTGTGCTCGGCGGAAGA | 281 |          |

s second, F Forward, R Reverse, bp base pair

* Annealing time changed from 90 s to 45 s
Capsule stain was used to detect capsule [14]. String test was used to differentiate between hvKp and cKp strains: if the grown colonies of *K. pneumoniae* form a string > 5 mm in length using a sterile loop, this demonstrates the hypermucoviscosity phenotype [15].

**DNA extraction and detection of virulent and resistant genes**

DNA was extracted using the guanidine chloride method [16]. The DNA samples were stored at −80 °C until used for PCR.

A primer sets targeting virulence, and carbapenem-resistant genes of *K. pneumoniae* are shown in Table 1. The primers were dissolved according to manufacturer guidelines to prepare 10 pmol/μl in all PCR reactions.

**PCR conditions**

PCR was carried out in a 20 μl volume using the Maxime PCR PreMix kit (iNtRON Biotechnology, Seongnam, Korea), 1 μl of each forward and reverse primer (10 pmol/μL), 2 μl of DNA, and then the volume was completed to 20 μl by distilled water. Four multiplex and single reaction PCR protocols were used for amplification of 16S rRNA, resistant and virulence genes, the initial melting temperature for all was 95 °C for 5 min, and a final extension was at 72 °C for 10 min. Details of annealing temperatures are listed in Table 1.

**Statistical analysis**

Data of research was analyzed using SPSS. Frequencies and Chi square test was used for comparison of different correlations and associations between variables (*p* value ≤ 0.05).

### Results

**Demographic data**

Sixty *K. pneumoniae* isolates were obtained from different hospitals in Khartoum State, 27 (45%) were from females, and 33 (55%) from males, 37 (61.7%) were from urine, 14 (23.3%) were from the blood of neonatal and adult sepsis, 5 (8.3%) were from wound swab, and 4 (6.7%) were from sputum.

**String test**

Out of sixty *K. pneumoniae* isolates, 10 (16.7%) were hypermucoviscous, and 50 (83.3%) isolates were classic.

**Susceptibility test results**

Most strains, 42 (70%), were resistant to ceftazidime, 18 (30%) to ciprofloxacin, 23 (38.3%) to chloramphenicol, 24 (40%) to gentamicin and only 5 (8%) resistant to imipenem. Multidrug resistant isolates were detected in 12 of urine isolates, 7 of blood, and 2 of wound swab isolates. Three neonatal blood isolates and one adult wound swab were showed extensively drug-resistant, more results are shown in Table 2.

**PCR results**

Detection of *K. pneumoniae* carbapenem-resistant and virulence genes

At least one of carbapenem-resistant genes were detected in 76.7% (46/60) of isolates; 68.3% (41/60) were positive for *bla*OXA-48 gene, 10% (6/60) were positive for *bla*NDM gene, 8.4% (5/60) were positive for *bla*KPC gene, and 3.3% (2/60) were positive for *bla*IMP gene. One neonatal blood isolate possesses three carbapenem-resistant genes (*bla*KPC, *bla*OXA-48, and *bla*IMP), six isolates possess two genes (four possess *bla*OXA-48 and *bla*NDM, two possess

### Table 2: Susceptibility testing profile of *K. pneumoniae*-strains among different clinical specimens and age groups

| Sex          | Ciprofloxacin Sensitive | Ciprofloxacin Resistant | Chloramphenicol Sensitive | Chloramphenicol Resistant | Gentamicin Sensitive | Gentamicin Resistant | Imipenem Sensitive | Imipenem Resistant | Ceftazidime Sensitive | Ceftazidime Resistant |
|--------------|--------------------------|-------------------------|----------------------------|---------------------------|---------------------|---------------------|-------------------|-------------------|---------------------|-----------------------|
| Male         | 20 (48%)                 | 13 (72%)                | 17 (46%)                   | 16 (70%)                  | 17 (47%)            | 16 (67%)            | 31 (56%)          | 2 (40%)           | 10 (56%)            | 23 (55%)              |
| Female       | 22 (52%)                 | 5 (28%)                 | 20 (54%)                   | 7 (30%)                   | 19 (53%)            | 8 (33%)             | 24 (44%)          | 3 (60%)           | 8 (44%)             | 19 (45%)              |
| p            | 0.082                    | 0.076                   | 0.143                      | 0.95                      | 0.049               | 0.38                |                   |                   |                     |                       |
| Sample N = 60|                          |                         |                            |                           |                     |                     |                   |                   |                     |                       |
| Urine        | 27 (64%)                 | 10 (56%)                | 24 (65%)                   | 13 (57%)                  | 24 (67%)            | 13 (54%)            | 37 (67%)          | 0 (0%)            | 15 (83%)            | 22 (52%)              |
| Blood        | 8 (19%)                  | 6 (33%)                 | 7 (19%)                    | 7 (30%)                   | 6 (17%)             | 8 (33%)             | 10 (18%)          | 4 (80%)           | 0 (0%)              | 14 (33%)              |
| Wound swab   | 3 (7%)                   | 2 (11%)                 | 3 (8%)                     | 2 (9%)                    | 3 (8%)              | 2 (8%)              | 4 (7%)            | 1 (20%)           | 1 (6%)              | 4 (10%)               |
| Sputum       | 4 (10%)                  | 0 (0%)                  | 3 (8%)                     | 1 (4%)                    | 3 (8%)              | 1 (4%)              | 4 (7%)            | 0 (0%)            | 2 (11%)             | 1 (5%)                |
| p            | 0.80                     | 0.95                    | 0.83                       | 0.23                      | 0.38                |                   |                   |                   |                     |                       |
| Total        | 42                       | 18                      | 37                         | 23                        | 36                  | 24                  | 55                | 5                 | 18                  | 42                    |

*p* = *p*-value, *N* = number.
and thirty-nine isolates possess one gene (34 \(\text{bla}_{\text{OXA-48}}\), two \(\text{bla}_{\text{NDM}}\), two \(\text{bla}_{\text{KPC}}\) and one \(\text{bla}_{\text{IMP}}\) and the remaining (14) were negative for all carbapenem-resistant genes. Eight hyper-virulent strains were harboring \(\text{bla}_{\text{OXA-48}}\) and two harboring \(\text{bla}_{\text{NDM}}\) genes.

For virulence genes \(\text{mrkD}\) detected in 47 (78.3%) isolates, \(\text{entB}\) in 56 (93.3%), \(\text{rmpA}\) in 3 (5%), \(\text{K2}\) in 31 (51.7%), \(\text{kfu}\) in 36 (60%) and \(\text{magA}\) in 8 (13.3%) isolates.

There was no significant statistical association between the presence of virulence genes and carbapenem resistant genes except between \(\text{entB}\) and NDM (\(p\)-value=0.005) (Table 3). A total of 92% (43/47) of \(\text{mrkD}\) gene-positive isolates were positive for one or more carbapenem-resistant genes. There was a strongly significant association between the presence of \(\text{mrkD}\) and \(\text{entB}\) genes (\(p\)-value=0.0005), they were co-existed in 46 isolates.

**Discussion**

In this study, eight hyper-virulent strains of \(K.\) pneumoniae were reported positive for carbapenem resistant genes (\(\text{OXA-48}\) and NDM). The presence of these strains in the clinical setting will complicate clinical practice and will cause fatal nosocomial infections [6]. Although antimicrobial-resistant hvKP strains are rarely reported worldwide [17–19], here in Sudan, they appear to be more prevalent.

Eight neonatal blood isolates were multidrug-resistant, and three of them were extensively resistant to all antibiotics that were used. Consequently, the emergence of MDR pathogens would increase the mortality and morbidity and prolong hospitalization and cost of treatment [20].

All neonatal blood isolates (12) were resistant to cefazidime. Cefazidime-resistant \(K.\) pneumoniae (CRKP) in the pediatric oncology units of some Sudanese hospitals may be the cause of recent reports of high mortality rate associated with \(K.\) pneumoniae infections among this group in different Sudanese hospitals [21]. According to Schiappa [22], high resistance rates to cefazidime could be due to the presence of a predominant enzyme (TEM-10) responsible for cefazidime resistance in bloodstream isolates.

The isolates showed varying degrees of resistance to the other antibiotics; ciprofloxacin 30%, gentamicin 40%,

| Table 3 The association between \(K.\) pneumoniae virulence and carbapenem resistant genes production |
|-------------------------------|-------------------------------|---------------------------------|-------------------------------|
| \(\text{mrkD}\) | \(\text{OXA-48}\) | \(\text{KPC}\) | \(\text{NDM}\) |
| Positive | Negative | Positive | Negative | Positive | Negative | Positive | Negative |
|---|---|---|---|---|---|---|---|
| \(\text{IMP}\) | \(\text{Positive}\) | \(\text{Negative}\) | \(\text{Positive}\) | \(\text{Negative}\) | \(\text{Positive}\) | \(\text{Negative}\) | \(\text{Positive}\) | \(\text{Negative}\) |
| \(\text{mrkD}\) | Positive | 1 (2) | 46 (98%) | 32 (68%) | 15 (32%) | 4 (9%) | 43 (91%) | 4 (9%) | 43 (91%) |
| | Negative | 1 (8%) | 12 (92%) | 9 (69%) | 4 (31%) | 1 (8%) | 12 (92%) | 2 (15%) | 11 (85%) |
| \(p\) | 0.33 | 0.93 | 0.92 | 0.47 |
| \(\text{entB}\) | Positive | 2 (4%) | 54 (96%) | 39 (70%) | 17 (30%) | 5 (9%) | 51 (91%) | 4 (7%) | 52 (93%) |
| | Negative | 0 (0%) | 4 (100%) | 2 (50%) | 2 (50%) | 0 (0%) | 4 (100%) | 2 (50%) | 2 (50%) |
| \(p\) | 0.70 | 0.42 | 0.51 | 0.005 |
| \(\text{rmpA}\) | Positive | 0 (0%) | 3 (100%) | 1 (33%) | 2 (67%) | 0 (0%) | 3 (100%) | 0 (0%) | 3 (100%) |
| | Negative | 2 (4%) | 55 (96%) | 40 (70%) | 17 (30%) | 5 (9%) | 52 (91%) | 6 (11%) | 51 (89%) |
| \(p\) | 0.74 | 0.18 | 0.59 | 0.51 |
| \(\text{k2}\) | Positive | 1 (3%) | 30 (97%) | 21 (68%) | 10 (32%) | 1 (3%) | 30 (97%) | 3 (10%) | 28 (90%) |
| | Negative | 1 (3%) | 28 (97%) | 20 (69%) | 9 (31%) | 4 (14%) | 25 (86%) | 3 (10%) | 26 (90%) |
| \(p\) | 0.94 | 0.92 | 0.14 | 0.93 |
| \(\text{kfu}\) | Positive | 0 (0%) | 36 (100%) | 26 (72%) | 10 (28%) | 1 (3%) | 35 (97%) | 3 (8%) | 33 (92%) |
| | Negative | 2 (8%) | 22 (92%) | 15 (63%) | 9 (38%) | 4 (17%) | 20 (83%) | 3 (13%) | 21 (88%) |
| \(p\) | 0.08 | 0.43 | 0.05 | 0.60 |
| \(\text{magA}\) | Positive | 1 (13%) | 7 (88%) | 7 (88%) | 1 (13%) | 1 (13%) | 7 (88%) | 1 (13%) | 7 (88%) |
| | Negative | 1 (2%) | 51 (98%) | 34 (65%) | 18 (35%) | 4 (8%) | 48 (92%) | 5 (10%) | 47 (90%) |
| \(p\) | 0.12 | 0.21 | 0.65 | 0.80 |
and ceftazidime (70%). Resistance to these antibiotics may also be due to the presence of Extended-Spectrum Beta-lactamases (ESBLs) and other mechanisms like efflux pumps and porin mutations [23], which were not covered in this study.

Although chloramphenicol is used as a treatment of choice for MDR gram-negative bacilli bacteria [24], 38% of our isolates were resistant to it, which may be caused by transferable enzymatic resistance to aminoglycosides, that is common in some hospitals [25].

In the current study, 94% (51/54) of the isolates harboring carbapenem-resistant genes were phenotypically susceptible to imipenem. This confirms what Walsh [26] said that this gene is not stable and relies upon other synergistic mechanisms to mediate resistance against carbapenems. In addition to imipenem, other antibiotics were analyzed in this study. Although five strains of *K. pneumoniae* in this study were resistant to imipenem, only three of them were positive for carbapenem-resistant genes (*OXA*-48), the rest two strains may possess other carbapenem-resistant genes not covered in this study or possessed another mechanism of resistance [27].

Of 46 *K. pneumoniae* isolates detected of having carbapenem-resistant genes, 10 had multiple genes co-occurring. This finding agrees with Ali & Omer [28] and Satir [29], which showed a multiplicity of genes in their isolates.

A total of 80% (4/5) of *KPC* and 100% (2/2) of *IMP* genes were positive among infant blood samples, and this may be due to organisms harboring these genes having a high ability to cause systemic infections, particularly in immunocompromised patients [30].

In this study, we found the essential gene for *K. pneumoniae* siderophores system *entB* gene is positive in 93.3% of all *K. pneumoniae* isolates, the rest (6.7%) of isolates that do not possess *entB* may contain other entero-bactin (*entA*, C, D, E or F), or other siderophores systems like yersiniabactin or aerobactin as reported by Lawlor [20]. Furthermore, *mrkD* gene is presented in 78.3% of the isolate. This gene has been found to be important in adhesion, as reported by Chen et al. (2012) [30]. The *rmpA* gene was detected in 5% of isolates, in contrast with Aljanaby and Alhasani [20], who found the *rmpA* gene present in 62.5% of *K. pneumoniae* isolates. This difference may be attributed to its mode of inheritance as plasmid-mediated, as mentioned by [20], indicating the limited spread of this gene in our local strains in Sudan.

The capsular serotype gene *K2* was present in 51.7% of isolates; the rest of isolates may contain other capsular serotypes, as mentioned by Ho [31]. This study showed that *K2* is present in 80% of hypermucoviscous strains, indicating that there is a relationship between the presence of *K2* gene and hypermucoviscous strains of *K. pneumoniae*, which is in agreement with the study by Guo [32] which found that *K2* is the most common capsular serotype in hypermucoviscous strain. In contrast to other studies [20, 33–35], which found *K1* was the most prevalent capsular serotype among hypermucoviscous *K. pneumoniae*.

The *kfu* gene (which codes for an iron uptake system) was present in 60% of isolates. The study showed no association between the presence of *kfu* gene and hypermucoviscosity. This finding disagrees with previous studies [20, 36, 37], which showed that *kfu* gene is associated with hypermucoviscosity phenotype, which may be attributed to diversity in geographical locations of studies.

The *magA* gene was found in 13.3% of isolates. The study showed no association between the presence of *magA* gene and hypermucoviscous strains. Although this gene is highly essential for *K. pneumoniae*, which confirms bacterial mucoviscosity, its prevalence among local isolates is not high, suggesting that other genes play a role in the formation of mucoviscosity [20].

**Conclusion**

The study reported for the first time in Sudan the following findings:

1. Presence of carbapenems resistant genes in hyper-virulent strains of *K. pneumoniae* isolated from hospitalized patients.
2. Presence of MDR and XDR strains of *K. pneumoniae* in neonatal ward in some Sudanese hospitals.

**Limitations**

- Low sample size.
- DNA sequencing not done due to financial issues.

**Abbreviation**

| Abbreviation | Description |
|--------------|-------------|
| *Bio* | β-lactamase; *cKp*: Classic *K. pneumoniae*; *CLSI*: Clinical and Laboratory Standards Institute; *CPS*: Capsular polysaccharide; *entB*: Entero-bactin B; *ESBL*: Extended-spectrum β-lactamase; *hvKp*: Hyper-virulent *Klebsiella pneumoniae*; *IPM*: Imipenem; *Kfu*: Klebsiella Ferric Uptake; *KPC*: Klebsiella pneumoniae carbapenemase; *OXA*-48: Oxacillinase 48; *magA*: Mucoviscosity-Associated Gene A; *MDR*: Multi Drug Resistant; *mxd*: Mannose Resistant Klebsiella like hemoagglutinin D; *NDM*: New Delhi metallo; *PCR*: Polymerase chain reaction; *rmpA*: Regulatory of Mucoid Phenotype A; *SPSS*: Statistical Package for the Social Sciences; *XDR*: Extensively drug-resistant. |

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Authors’ contributions
AMA, HNA, SAA, EFA and EHO designed the study, AMA, SAA, EFA and EHO performed the experiments, HNA, AMA, and SAA analyzed the data, HNA, AMA and LAH wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available at https://doi.org/10.6084/m9.figshare.12401684.

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The research was approved by the institutional ethics committee of the deanship of scientific research, Sudan University of Science and Technology. Written consent was waived by the ethical committee of Sudan University of Science and Technology, meeting No (SUST/DSR/1EC/EA2/2017) Date (07/01/2017) because we are using a previously collected human bio-specimens with limited data.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Greenwood D, Slack RC, Barer MR, Irving WL. Medical microbiology. E-Book: a guide to microbial infections: pathogenism, immunity, laboratory diagnosis and control. Amsterdam: With STUDENT CONSULT Online Access: Elsevier Health Sciences; 2012.
2. Khaertnyov KS, Anokhin VA, Rizvanov AA, Davydkov YN, Semyenova DR, Lubin SA, Skvortsova NN. Virulence factors and antibiotic resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis. Frontiers in medicine 2018; 5.
3. Huyhn DTN, Kim A-Y, Kim Y-R. Identification of Pathogenic Factors in Klebsiella pneumoniae Using Impedimetric Sensor Equipped with Biomimetic Surfaces. Sensors. 2017;17(6):1406.
4. Lee C-R, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016;7:895.
5. Al-Zahrani IA, Alasni BA. The emergence of carbenemase-resistant Klebsiella pneumoniae isolates producing OXA-48 and NDM in the Southern (Asir) province, Saudi Arabia. Saudi Med J. 2018;39(1):23.
6. Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Li H, Zhang F, Li S, Wang R, Wang H. High prevalence of hypervirulent Klebsiella pneumoniae infection in China: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob Agents Chemother. 2016;60(10):6115–20.
7. Adam MA, Elhayag W. Prevalence of metallo-β-lactamase acquired genes among carbenemases susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC Infect Dis. 2018;18(1):668.
8. Dahab R, Ibrahim AM, Altyab HN. Phenotypic and genotypic detection of carbenemase enzymes producing gram-negative bacilli isolated from patients in Khartoum State. F1000Research. 2017;6:1656.
9. Copur Cicek A, Saral A, Ozad Duzgun A, Yasar E, Cizmeci Z, Ozdem Balci P, Sari F, Firat M, Ahhtop YA, Ak S, et al. Nationwide study of Escherichia coli producing extended-spectrum beta-lactamases TEM, SHV and CTX-M in Turkey. J Antimicrob. 2013;56(11):647–50.
10. Borner A, Saaider-Odes L, Riesenberk K, Eskira S, Peled N, Nativ R, Schlaffer F, Sherf M. Attributable mortality rate for carbenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol. 2009;30(10):972–6.
11. Chesbrough M. District laboratory practice in tropical countries. Cambridge: Cambridge University Press; 2006.
12. Leboffe MJ, Pierce BE. A photographic atlas for the microbiology laboratory. Englewood: Morton Publishing Company; 2012.
13. CLSI C. Performance standards for antimicrobial susceptibility testing. Clinical Lab Standards Institute 2016.
14. McKinney RE. Staining bacterial polysaccharides. J Bacteriol. 1953;66(4):453.
15. Aljanaby AAJ, Alhasani AHA. Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. Afr J Microbiol Res. 2016;10(22):829–43.
16. Sabeel S, Salih MA, Ali M, El-Zaki S-E, Abozeid N, Eldagi ZAM, Altayb HN, Elegali A, Ibrahim NY, Alamri BK. Phenotypic and genotypic analysis of multidrug-resistant Mycobacterium tuberculosis isolates from Sudanese patients. Tuberc Res Treat. 2017;2017:8340746.
17. Su S-C, Liu M, Li D-Y, Yeh J-Y, Lin J-C, Chang F-Y. Community-acquired liver abscess caused by serotype K1 Klebsiella pneumoniae with CTX-M-15-type extended-spectrum β-lactamase. Antimicrob Agents Chemother. 2008;52(2):804–5.
18. Cheng N-C, Yu Y-C, Tai H-C, Hshue P-R, Chang S-C, Lai S-Y, YW C-F, Fang C-T. Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial Klebsiella pneumoniae necrotizing fasciitis. Clin Infect Dis. 2015;5(5):930–9.
19. LI W, Sun G, Yu Y, Li N, Chen M, Jin R, Jiao Y, Wu H. Increasing occurrence of antimicrobial-resistant hyperpyrexial (hypermucoviscous) Klebsiella pneumoniae isolates in China. Clin Infect Dis. 2013;58(2):225–32.
20. Behera B, Das A, Mathur P, Kapil A, Gadepalli R, Dhawan B. Tigecycline susceptibility report from an Indian tertiary care hospital. Indian J Med Res. 2009;129(4):446.
21. Abdelaziz M, Hamadalin Y, Hashim O, Bashir T, Mahjoub E. Microbiological profile of neonatal sepsis at a maternity hospital in omdurman, Sudan. Sudan J Med Sci. 2019;14:451–5.
22. Schiappa DA, Hayden MK, Matushek MG, Hashemi FN, Sullivan J, Smith KY, Miyashiro D, Quinn JP, Weinstein RA, Trenholme GM. Cefazidime-resistant Klebsiella pneumoniae and Escherichia coli bloodstream infection: a case-control and molecular epidemiologic investigation. J Infect Dis. 1996;174(3):529–36.
23. Singh-Moodley A, Perovic O. Antimicrobial susceptibility testing in predicting the presence of carbenemase genes in Enterobacteriaceae in South Africa. BMC Infect Dis. 2016;16(1):536.
24. Yu W-L, Ko W-C, Cheng K-C, Lee H-C, Ke D-S, Lee C-C, Fung C-P, Chuang Y-C. Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. Clin Infect Dis. 2006;42(10):1351–8.
25. Melki AH, Hassan AN, Elsayed DEM. Extended spectrum beta lactamases among multi drug resistant Escherichia coli and Klebsiella species causing urinary tract infections in Khartoum. Afr J Bacteriol Res. 2010;3(3):18–21.
26. Walsh TR. Emerging carbenemases: a global perspective. Int J Antimicrob Agents. 2010;36:58–14.
27. Meletis G. Carbenem resistance: overview of the problem and future perspectives. Therapeutic Adv Infect Dis. 2016;3(1):15–21.
28. Ali AH, Al Fahril AO. J Clin Rev Case Rep. 2019;6:3.
29. Sair S, Elkhalifa A, Ali M, El Hussein A, Elkhidir I. Detection of Carbapenem resistance genes among selected Gram Negative bacteria isolated from patients in Khartoum State, Sudan. Clin Microbiol J. 2016;5:266. https://doi.org/10.4172/2327-5073.1000266 Page 2 of 4. Clin Microbiol, an open access journal ISSN: 2327-5073 Volume 5• Issue 6• 1000266.
30. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of Klebsiella pneumoniae carbenemases (KPC) resistance. Infect Drug Resist. 2012;5:133.
31. Ho J-Y, Lin T-L, Li C-Y, Lee A, Cheng A-N, Chen M-C, Wu S-H, Wang J-T, Li T-L, Tsai M-D. Functions of some capsular polysaccharide biosynthetic genes in Klebsiella pneumoniae NTUH K-2044. PLoS ONE. 2011;6(7):e21664.
32. Guo Y, Wang S, Zhan L, Jin Y, Duan J, Hao Z, Lv J, Qi X, Chen L, Kreiswirth BN. Microbiological and clinical characteristics of hypermucoviscous Klebsiella pneumoniae isolates associated with invasive infections in China. Front Cellular Infect Microbiol. 2017;7:24.
33. Liu YM, Li BB, Zhang YY, Zhang W, Shen H, Li H, Cao B. Clinical and molecular characteristics of emerging hypervirulent Klebsiella pneumoniae bloodstream infections in mainland China. Antimicrob Agents Chemother. 2014;58(9):5379–85.
34. Qu T-t, Zhou J-c, Jiang Y, Shi K-r, Li B, Shen P, Wei Z-q, Yu Y-s. Clinical and microbiological characteristics of Klebsiella pneumoniae liver abscess in East China. BMC Infect Dis. 2015;15(1):161.
35. Yan Q, Zhou M, Zou M. Liu W-e: hypervirulent Klebsiella pneumoniae induced ventilator-associated pneumonia in mechanically ventilated patients in China. Eur J Clin Microbiol Infect Dis. 2016;35(3):387–96.
36. Hsieh P-F, Lin T-L, Lee C-Z, Tsai S-F, Wang J-T. Serum-induced iron-acquisition systems and TonB contribute to virulence in Klebsiella pneumoniae causing primary pyogenic liver abscess. J Infect Dis. 2008;197(12):1717–27.
37. Ma L-C, Fang C-T, Lee C-Z, Shun C-T, Wang J-T. Genomic heterogeneity in Klebsiella pneumoniae strains is associated with primary pyogenic liver abscess and metastatic infection. J Infect Dis. 2005;192(1):117–28.
38. Mahmudunnabi G, Montaz F, Foyeal MJ, Rahman MM, Islam K. Molecular detection and PCR-RFLP analysis using PstI and AluI of multidrug-resistant Klebsiella pneumoniae causing urinary tract infection in women in the eastern part of Bangladesh. J Genetic Eng Biotechnol. 2018;16(1):77–82.
39. Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailouf F, Kassis-Chikhani N, Arlet G, Decré D. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of Klebsiella pneumoniae. J Clin Microbiol. 2014;52(12):4377–80.
40. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed Res Int. 2014;2014:303104.

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