Multiclonal spread of Klebsiella pneumoniae across hospitals in Khartoum, Sudan


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Multiclonal spread of *Klebsiella pneumoniae* across hospitals in Khartoum, Sudan

Einas A. Osman\(^a,b\), Nagwa E. El-Amin\(^c\), Leena L. Al-Hassan\(^d,x,1\), Maowia Mukhtar\(^b,e,1\)

\(^a\) Faculty of Medical Laboratories, Microbiology Department, Ibn Sina University, Algerief West, Khartoum, Sudan
\(^b\) Bioscience Research Institute, Ibn Sina University, Khartoum, Sudan
\(^c\) Department of Pathology, College of Medicine, Qassim University, Saudi Arabia
\(^d\) Department of Global Health and Infection, Brighton and Sussex Medical School, BN1 9PX, UK
\(^e\) Institute of Endemic Diseases, University of Khartoum, Sudan

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**A B S T R A C T**

**Objectives:** Multidrug-resistant (MDR) *Klebsiella pneumoniae* is increasing worldwide with poorly characterised epidemiology in many parts of the world, particularly in Africa. This study aimed to investigate the molecular epidemiology of *K. pneumoniae*, to identify the diversity of sequence types (ST), and to detect carbapenem resistance genes in major regional hospitals in Khartoum, Sudan.

**Methods:** *Klebsiella pneumoniae* isolates (n = 117) were cultured from four hospitals in Khartoum, from April 2015 to October 2016. The isolates were characterised by sequencing of 16S-23S rDNA internal transcribed spacer (ITS) region. Molecular epidemiology was determined by multilocus sequence typing (MLST), and analysed by maximum likelihood phylogeny (PhyML). Antimicrobial susceptibility was determined by disk diffusion. Isolates phenotypically resistant to carbapenem were screened for carbapenemase genes: *blaNDM, blaOXA48, blaOXA58, blaVIM* and *blaGES* by PCR.

**Results:** ITS sequencing confirmed the 117 isolates as *K. pneumoniae*. MLST revealed 52 different STs grouped in four distinct clusters by PhyML. All isolates were MDR, and carbapenem-producing *K. pneumoniae* (CP-KP) isolates accounted for 44(37.6%) mostly harbouring *blaNDM* (28/44) and *blaOXA48* (7/44), with several isolates harbouring multiple genes.

**Conclusion:** MDR and CP-KP *K. pneumoniae* is widespread in Khartoum hospitals, with a diverse population of 52 STs clustering in four major lineages. There is an urgent need for systematic epidemiological studies of drug-resistant infections across all healthcare institutions in Sudan to inform local infection prevention and control strategies.

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1. **Introduction**

The incidence of multidrug-resistant (MDR) and carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) infections has increased during the last decade throughout the world, assigning *K. pneumoniae* as one of the global priority pathogens by the World Health Organization (WHO) [1,2] with great medical significance implicated in urinary tract infections, pneumonia, bacteremia, meningitis, and abscesses [3]. Therefore, MDR *K. pneumoniae* is considered a significant health problem associated with significant morbidity and mortality because of limited antibiotic treatment options [4]. Carbapenems are usually the last-resort antibiotic by virtue of their broad spectrum of activity; however, CP-KP has created significant clinical challenges for clinicians globally, resulting in ineffective treatments and high rates of clinical failure. Furthermore, this problem is aggravated by the localisation of resistance genes on mobile elements facilitating gene transfer to susceptible isolates [5].

The burden of MDR is underestimated in many low- and middle-income countries (LMICs), because of limitations in diagnostic facilities [6]. In Sudan, epidemiology of MDR and CP-KP is not well described despite being a major public health threat, with fragmented data on the prevalence and distribution [7–9]. Quantification of CP-KP in healthcare facilities and understanding of the evolving epidemiology of CP-KP in Sudan is critical to informing national and regional infection prevention and control (IPC) efforts.

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Corresponding author at: G.19 Medical Research Building, Brighton and Sussex Medical School, BN1 9PX, UK.

E-mail address: lal-hassan@bsms.ac.uk (LL. Al-Hassan).

1 These authors contributed equally to the work.
We have previously reported a high rate of misidentification of *K. pneumoniae* across Khartoum hospitals [10]. In this study, we aimed to characterise the local epidemiology by multilocus sequence typing (MLST), and the prevalence of carbapenemase genes among *K. pneumoniae* isolates across Khartoum State hospitals, thereby determining the level of inter/intra-hospital transmission.

2. Material and methods

2.1. Bacterial isolates and antimicrobial susceptibility testing

A total of 117 isolates of *K. pneumoniae* from patient samples were cultured in clinical microbiology laboratories (CML) of four major teaching hospitals in Khartoum: Ribat National Hospital (RNH), Omdurman Teaching Hospital (OTH), Soba University Hospital (SUH), and Khartoum Bahri Teaching Hospital (KBTH) between April 2015 and October 2016. CML identify clinical specimens to the genus levels by conventional phenotypic and biochemical methods. No specific selection criteria were implemented, as the study aimed to collect and characterise all *K. pneumoniae* isolates identified in the hospitals’ CML. All acquired isolates were then confirmed genotypically by amplification of 16S-23S rDNA internal transcribed spacer (ITS) of *K. pneumoniae* as described by our preceding study [11]. All primer sequences and expected amplicon sizes are listed in Table S1. Clinical data associated with the isolates were collected which include age, gender, location of patient, type of culture, and antibiotic therapy before and during the infection.

Susceptibility tests were done by disk diffusion for the following 10 antibiotics: amoxicillin/ampicillin (AMC/AMP) (30 μg); piperacillin-tazobactam (TZP) (110 μg); cefoxitin (FOX) (30 μg); ciprofloxacin (CIP) (5 μg); gentamicin (CEN) (10 μg); amikacin (AK) (30 μg); trimethoprim-sulfamethoxazole (SXT) (30 μg); meropenem (MEM) (10 μg); imipenem (IMP) (10 μg). The procedure was performed and results interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

2.2. Molecular typing of *K. pneumoniae* isolates

MLST was done to identify the STs of all the *K. pneumoniae* isolates by amplification and sequencing of the seven housekeeping genes [13]. The results of sequences were analysed in the PubMLST database (https://bigd.ebi.ac.uk/pubmlst/klebsiella/klebsiella.html) and BLAST (https://blast.ncbi.nlm.nih.gov/), then assigned an allele number. The allele numbers were combined to yield a specific ST.

A concatenated alignment with maximum likelihood phylogeny (PhyML) was constructed using Seaview to determine relatedness of isolates [14], and the PhyML was analysed with metadata using Phandango [15].

2.3. Molecular detection of carbapenemase genes

All isolates phenotypically resistant to carbapenems were screened for the presence of carbapenemase genes: *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>OXAM</sub>, and *bla*<sub>GES</sub> by multiplex-PCR using specific primers listed in Table S1 [16,17]. PCR reaction conditions were prepared using ready master mix (APSLABS, India), 0.5 μL of each primer (25 nmol concentration), and 1 μL of template DNA (10 ng) in a total volume of 25 μL. PCR thermal profile for NDM comprised initial denaturation at 94 °C for 10 min followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C, and a final extension step of 10 min at 72 °C. The PCR amplification for multiplex (VIM, IMP and KPC) was carried out as follows: 94 °C for 10 min; 30 cycles of 94 °C for 40 s, 55 °C for 40 s and 72 °C for 1 min; and 72 °C for 7 min. The same conditions were used for multiplex GES and OXA-48 PCR, but with an annealing temperature at 57 °C instead of 55 °C. The PCR products were analysed by gel electrophoresis.

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th><em>K. pneumoniae</em> (n = 117)</th>
<th>CP-KP (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>28 (23.9)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>1–15</td>
<td>21 (17.9)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>16–30</td>
<td>17 (14.5)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>31–45</td>
<td>16 (13.7)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>46–60</td>
<td>19 (16.3)</td>
<td>6 (31.6)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>16 (13.7)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44 (37.6)</td>
<td>16 (35.8)</td>
</tr>
<tr>
<td>Female</td>
<td>73 (62.4)</td>
<td>28 (64.2)</td>
</tr>
<tr>
<td>Hospitals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribat National Hospital (RNH)</td>
<td>41 (35.0)</td>
<td>16 (36.4)</td>
</tr>
<tr>
<td>Omdurman Teaching Hospital (OTH)</td>
<td>34 (29.1)</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>Soba University Hospital (SUH)</td>
<td>27 (23.1)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Khartoum Bahri Teaching Hospital (KBTH)</td>
<td>15 (12.8)</td>
<td>7 (15.9)</td>
</tr>
<tr>
<td>Hospital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>103 (88.0)</td>
<td>36 (81.8)</td>
</tr>
<tr>
<td>Outpatient</td>
<td>14 (12.0)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Source of specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>28 (23.9)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Urine</td>
<td>27 (23.1)</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>Wound swab</td>
<td>27 (23.1)</td>
<td>13 (29.5)</td>
</tr>
<tr>
<td>Umbilical swab</td>
<td>11 (9.4)</td>
<td>9 (11.4)</td>
</tr>
<tr>
<td>Sputum</td>
<td>9 (7.7)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Pus</td>
<td>8 (6.83)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>4 (3.42)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>CSF</td>
<td>2 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Eye swab</td>
<td>1 (0.85)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid.
3. Results

A total of 117 MDR *K. pneumoniae* isolates were cultured from different clinical samples collected from in- and outpatients across four hospitals in Khartoum, Sudan. Table 1 summarises the distribution pattern of *K. pneumoniae* by age group, gender, hospital, and source of specimens. The highest number of *K. pneumoniae* isolates was from Ribat National Hospital (RNH; *n* = 41; 35.0%), followed by Omdurman Teaching Hospital (OTH; *n* = 34; 29.1%), Soba University Hospital (SUH; *n* = 27; 23.1%), and Khartoum Bahri Teaching Hospital (KBTH; *n* = 15; 12.8%). Isolates were obtained from patients with a range of ages; however, the largest number of isolates were from infants (<1 year, 23.9%). More than 80% of isolates were from inpatients, from a variety of samples, but more commonly blood and urine samples or wound swabs (70.1% collectively).

As listed in Table 2, all isolates were MDR, with particularly high resistance rates (>50%) to amoxicillin/ampicillin, piperacillin/tazobactam, cefpirome, and gentamicin. The majority of isolates were sensitive (>60%) to amikacin and trimethoprim/sulfamethoxazole. Carbapenem resistance was observed in 50 isolates (42.8%), with total resistance at 13.7% and 29.1% for imipenem and meropenem, respectively. Antibiotic use prior to the first positive culture was common (61.4%) in patients with a carbapenem-resistant *K. pneumoniae*; only 17/44 (38.6%) had no antibiotic therapy in the preceding 14 days (Table S2).

MLST revealed 52 different STs, 15 of which are novel and assigned to ST3460–3474. ST41, 383, and 649 were present in different hospitals in Khartoum as seen in Table 3. The STs of the 44 CP-KP revealed that recurrent STs were ST383 (*n* = 8, in SUH, RNH, OTH), followed by ST 101 (*n* = 5, in OTH, KBTH, RNH), ST 48 (*n* = 3 in KBTH), 649 (*n* = 3, SUH, RNH), ST 846 (*n* = 3, RNH) and ST 3229 (*n* = 2, KBTH). All other STs were identified in individual isolates (Table 3).

As seen in Fig. 1, there is a large diversity of STs among the isolates, with several lineages present. Results indicate that despite distinct STs present in each hospital, with only a few recurrent STs listed above, all STs fall into four major clusters that are present across hospitals. Notably, ST2461 and ST2674 are highly similar (part of cluster 2 in Fig. 1); however, isolates come from RNH and SUH, indicating inter-hospital spread. Cluster 4 appears to contain the largest diversity, with three sub-clusters across the four hospitals of isolates that are both CP-KP and non-CP-KP. Some hospitals appear to have clonally related strains, such as in OTH: ST-654, -1289, -657, -2870, -219, -2260, -524, -2461, -2674, belonging to cluster 2 (Fig. 1). On the other hand, SUH appears to have a pool of diverse CP-KP and non-CP-KP. Notably, the novel STs 3460–3474 are all carbapenem-sensitive with the exception of ST 3467.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Antimicrobial susceptibility profiles of <em>K. pneumoniae</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic</td>
<td><em>K. pneumoniae</em> (n = 117)</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
</tr>
<tr>
<td><strong>AMC/AMP</strong></td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td><strong>TZP</strong></td>
<td>7 (5.9%)</td>
</tr>
<tr>
<td><strong>Cefpirome</strong></td>
<td>7 (5.9%)</td>
</tr>
<tr>
<td><strong>Cefpirome</strong></td>
<td>45 (38.5%)</td>
</tr>
<tr>
<td><strong>Impenem</strong></td>
<td>89 (76.1%)</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td>79 (67.5%)</td>
</tr>
<tr>
<td><strong>Gentamicin</strong></td>
<td>41 (35.0%)</td>
</tr>
<tr>
<td><strong>Amikacin</strong></td>
<td>78 (66.5%)</td>
</tr>
<tr>
<td><strong>SXT</strong></td>
<td>79 (67.5%)</td>
</tr>
</tbody>
</table>

AMC/AMP = amoxicillin/ampicillin; SXT = trimethoprim-sulfamethoxazole; TZP = piperacillin-tazobactam.

All isolates exhibiting phenotypic resistance to carbapenems were screened for acquired carbapenemases as outlined in the Methods. Forty-four isolates (37.6% of the total 117 isolates) harboured NDM (*n* = 32), OXA-48 (*n* = 10), GES (*n* = 6), and VIM (3), of which several isolates co-harboured several genes: two with NDM and VIM, two with OXA-48 and GES, one NDM and GES, and one with NDM, VIM, and OXA-48 (Fig. 54). NDM is present across a range of STs in multiple hospitals.

4. Discussion

Khartoum city is the capital of Sudan and includes three localities: Khartoum, Bahri, and Omdurman. Hospitals in the three localities are under the umbrella of Khartoum State Ministry of Health and they provide service to the residence of the State as well as those who are referred from other states. This study aimed to characterise the molecular epidemiology of a total of 117 MDR *K. pneumoniae* across four major teaching hospitals that serve the majority of the residence of the city: RNH and SUH are located in Khartoum locality, OTH in Omdurman locality, and KBTH located in Bahri locality. The majority of isolates were from RNH, and as detailed in Table 1, the isolates were most commonly from infants (<1 year old). Carbapenem resistance was unexpectedly high (>40%), while >60% of isolates were sensitive to amikacin. Our data (Table S2) revealed that antibiotic usage (14 days before and during hospital admission) was a risk factor for CP-KP, mostly in patients taking combined antibiotic therapy.

During the 19-month surveillance period 37.6% (*n* = 44/117) of *K. pneumoniae* isolates harboured a carbapenemase, namely NDM, VIM, OXA-48, and GES. Patients who were colonised or infected with CP-KP had high rates of certain comorbidities including renal disease, diabetic wounds, and solid malignancy (data not shown). Notably, the majority of CP-KP isolates were isolated from urine and wound, followed by blood, sputum and pus samples (Table 1), similar to the results of other studies in Sudan, Uganda, and Nigeria [18–20].

The emergence of CP-KP limits the use of carbapenems in patients with severe infections, leading to increased mortality rates. In the last decade, infections caused by carbapenem-resistant *K. pneumoniae* increased significantly in many countries in the region (Africa and the Middle East) [18,21–26]. Currently in Sudan, carbapenems are the only available choice for the treatment of MDR *K. pneumoniae* and other ESBL and AmpC-producing Gram-negatives. However, in most cases, antibiotic prescription of carbapenems is not based on AST, but rather based on previous treatment failures or to be used as prophylaxis. There is therefore an urgent need to address improving CML diagnostics, which in turn would have a direct impact on limiting the misuse of antibiotics, particularly carbapenems. It is important to note that the limited diagnostic capacities in CML across hospitals in Khartoum has led to frequent outsourcing of cultures and general laboratory diagnostics where patients are in many instances directed to private laboratories. Subsequently, the local data on epidemiology and resistance are fragmented.

In this study, the prevalence of MDR was 51.2% among the *K. pneumoniae* isolates, with >40% of isolates being carbapenem-resistant. Our results indicate that isolates remain >60% sensitive to amikacin and trimethoprim-sulfamethoxazole (SXT); however, gentamicin resistance reached 64%. Most antibiotics in Sudan are obtainable over the counter, which may have contributed to the high MDR rates noted in the study. Antibiotic usage in the community and its role in the transmission of resistance is an important issue to study in the future.

As seen in Table 1, CP-KP was isolated from 36/44 (81.8%) inpatients, and 8/44 (18.2%) outpatients, indicating the possible dissemination of CP-KP in community-acquired infections.
Carbapenem resistance mediated by NDM was present across a diversity of STs and lineages, suggesting the successful dissemination and maintenance among diverse strains.

The molecular epidemiological characterisation of *K. pneumoniae* in Khartoum, Sudan revealed a high diversity of 52 different STs, including 15 novel STs. However, the PhyML analysis revealed that despite this diversity in STs, they fall within four main clusters circulating in different hospitals. The largest diversity of STs was observed in SUH and RNH (Fig. 1), where it is apparent that multiple lineages are present simultaneously in the hospital. OTH, on the other hand, has the least diversity and appears to have one major CP-KP clone of closely related STs (645, 1289, 657, 2870, 219, and 2260) in addition to other sporadic STs. The diversity observed in this study may also represent isolates brought to the hospitals from diverse geographical settings, as these hospitals are tertiary hospitals serving most of the country.

Successful global clones ST-383 and -101 were identified in our study and are present in multiple hospitals indicating the introduction and maintenance of international clones in Sudanese hospitals, thereby confirming the role of travel and immigration in transmission of resistance worldwide [27,28]. It is also important to note that despite the global spread of ST-258-KPC-producer, in our study we did not detect the KPC gene, and the global ST258 was not identified [4].

In conclusion, our study provides an overview of the molecular epidemiology of *K. pneumoniae* in Khartoum thereby collecting samples from four hospitals representing the three regions, revealing a heterogeneous population of MDR *K. pneumoniae*.

![Fig. 1. Maximum likelihood phylogeny (PhyML) of concatenated STs identified in the study. The STs fall into four major clusters that are heterogeneous with multiple STs from different hospitals, indicating unique diverse STs to each hospital, but intra-hospital circulating clones. The largest heterogeneity is observed in cluster 4, which appears to have three sub-clusters. Two singletons: ST48 and ST2923 are also identified that do not fall within the clusters. Resistance genes are spread across different STs and clusters (also detailed in Table 3).](image-url)
isolates and the presence of four major clusters highlighting a large inter-hospital spread of diverse clones in Khartoum, Sudan. The data highlighted an alarming rate of ~40% carbapenem resistance.

**Funding**

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**Competing interests**

None declared.

**Ethical approval**

This work contains no human or animal data. Institutional approval was obtained from The Sudanese Ministry of Health.

**Author contributions**

All authors contributed equally and participated in design and implementation, analysis, interpretation of the study, and the development of the manuscript. EA collected the strains and conducted the laboratory work and data analysis. LAF performed part of the data analysis. EA and LAF drafted the manuscript. NAE facilitated the isolate collection and reviewed the manuscript. MM supervised the laboratory work and revised the manuscript. All authors had full access to the data and approved the final manuscript.

**Appendix A. Supplementary data**


**References**


