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# Increasing frequency of Aminoglycoside-Resistant *Klebsiella pneumoniae* during the era of pandemic COVID-19

Omar B. Ahmed<sup>a,\*</sup>, Atif H. Asghar<sup>a</sup>, Fayez S. Bahwerth<sup>b</sup>

<sup>a</sup> Department of Environmental and Health Research, The Custodian of the Two Holy Mosques Institute for Hajj and Umrah Research, Umm Al-Qura University, Saudi Arabia  
<sup>b</sup> King Faisal Hospital Makkah, Ministry of Health, Makkah, Saudi Arabia

## ARTICLE INFO

## Article history:

Received 13 May 2021  
 Accepted 18 May 2021  
 Available online xxxx

## Keywords:

*K. pneumoniae*  
 Aminoglycosides  
 COVID-19  
 Resistant genes

## ABSTRACT

The emergence of multidrug resistance to aminoglycosides in *K. pneumoniae* isolates is a growing concern, especially during pandemic Coronavirus disease 2019 (COVID-19). The study identifies antibiotic resistance in *K. pneumoniae* isolated from tertiary hospitals during pandemic COVID-19. Among 220 clinical isolates, the total rate of *K. pneumoniae* was found to be 89 (40.5%). Phenotyping results confirmed the resistance of aminoglycoside antibiotics in 51 (23.2%) of *K. pneumoniae* isolates. PCR results confirmed the existence of one or more aminoglycoside genes in 82.3% of the 51 isolates. The *rmtD* gene was the highest-detected gene (66.7%), followed by *aac(6′)-Ib* (45.1%), *aph(3′)-Ia* (45.1%), *rmtB* (29.4%), *armA* (21.6%), *aac(3)-II* (7.8%), and *rmtA* (3) (11.8%). Significantly, higher resistance strains showed a higher prevalence (61.5%) of aminoglycoside genes ( $p < 0.05$ ). During COVID-19, there is a higher risk of acquiring MDR bacterial infections, so the monitoring of multidrug resistant bacteria must be continuously undertaken to implement effective measures in infection control and prevention.

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Selection and peer-review under responsibility of the scientific committee of the Emerging Trends in Materials Science, Technology and Engineering.

## 1. Introduction

The high number of the admitted pandemic Coronavirus disease 2019 (COVID-19) patients to hospital wards and intensive care units (ICU) may be accompanied by secondary bacterial infections, thus patients may be given an additional empirical broad-spectrum antibiotic (e.g. aminoglycosides). It was reported that co-infection was found in 7–14% of patients admitted to the hospitals [1]. Nosocomial infections are mostly caused by *Staphylococcus*, *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas* spp., and *Klebsiella pneumoniae* [2]. *Klebsiella* is a Gram-negative bacterium that can cause nosocomial infections. These isolates can carry virulence plasmids that harbor resistant genes (such as aminoglycoside resistant genes) with higher frequency which may result in disseminated infections (e.g. liver, lungs, and eyes) [3,4]. Sometimes viral agents can be associated with secondary *K. pneumoniae* infection (pneumonia) as a part of nosocomial infections which may lead to high mortality as a result of co-existence with respiratory diseases [5]. As a result, *K. pneumoniae* bacteria may

develop into the so called multiple drug resistant (MDR) *K. pneumoniae* and hence the aminoglycosides are antibiotics that have good antibacterial activity. They act by inhibiting the synthesis of proteins inside bacteria by attaching to the amino group site of 16 S RNAs inside subunits of the 30S ribosome, which may make being coded by the genes difficult, making them unread and inhibiting translocation [6,7]. Inactivating enzyme production in these bacteria is the most known resistance mechanism to aminoglycosides [7]. Enzymes that are responsible for modifying aminoglycosides (AMEs) include O-adenyltransferases (ANT), (N-acetyltransferases (AAC), and O-phosphotransferases (APH)), and they are encoded through DNA molecules known as plasmids. Known AME-encoding genes are *aac(3)-II*, *aac(6′)-I*, *ant(3′)-I*, *aph(3′)-II*, and *ant(2′)-I* in *K. pneumoniae*. Other mechanisms include uptake reduction or decreasing cell permeability besides methylating 16S RNA in ribosomes. Such a reaction may be enhanced and regulated by the *rmtA* gene. The second mechanism is decreased drug quantity inside cells. Aminoglycoside resistance is independent (i.e. inactivating enzymes), which can also be seen in *K. pneumoniae*. This resistance is characterized by involving all other types of aminoglycosides because of an efflux system that reduces the quantity of amino-glycosides [8]. Enzymes *aac(6′)-I*, *aac(3)-II*, *aph(3′)-II*, *ant(3)-I*, and *ant(2)-I* are responsible for

\* Corresponding author.

E-mail address: [obmohamed@uqu.edu.sa](mailto:obmohamed@uqu.edu.sa) (O.B. Ahmed).

resistance to aminoglycosides with variable degrees [7]. The outbreak of MDR *K. pneumoniae* during pandemic COVID-19 has been reported in many parts of the world [9–11], but very few laboratory-based reports have been published [12–16]. The present study was carried out to identify antibiotic resistance aminoglycoside in *K. pneumoniae* isolated from tertiary care hospitals during current pandemic COVID-19.

## 2. Materials and methods

### 2.1. Sample identification

A stock of 220 Gram-negative isolates were collected from 5 tertiary hospitals in Makkah, Saudi Arabia in the period of January 2020 to January 2021. Bacterial isolates were identified with a Vitek-2 Compact System using AST-GNI cards (Biomerieux). DensiCHEK Plus was established within a 0.5 McFarland standard in a 0.48% sterile sodium chloride solution [17–23]. Bacterial suspension was manually loaded using cards of a VITEK 2 system. The system automatically filled each test card, sealed it, and incubated it for a period of about 3 h. The system then analyzed the data after repeated kinetic fluorescence measurement before automatic result reporting.

### 2.2. Antibiotic-Susceptibility tests

The Standards Institute (CLSI) [12]. The turbidity of each inoculum was adjusted to a 0.5 McFarland standard. For phenotypic screening, antimicrobial susceptibility was done by using antimicrobial discs such as AK (30 µg), GN (10 µg), TOB (10 µg), amoxicillin clavulanic acid (AMC) ((20/10 µg), ciprofloxacin (CIP) (5 µg), cefepime (CPM) (30 µg), imipenem (IMP; 10 µg), cefotaxime (CTX; 30 µg), ampicillin (AMP; 10 µg), aztreonam (ATM; 30 µg), and cefuroxime (CXM; 30 µg) (Table 1). The isolates were classified as susceptible, intermediate, or resistant to each antibiotic according to CLSI guidelines [12].

### 2.3. PCR analysis

The cooled in ice and then centrifuged at 3000 g for 10 s. The upper layer containing DNA was used for PCR. at 94 °C for 4 min, 30 amplification (shown in Table 2) for 30 s, 72 °C for 1 min (extension), and 72 °C.

### 2.4. Statistical analysis

We using SPSS software (v.25, IBM, United States). The chi-squared test was used to compare between different variables,

**Table 1**  
The primers used in PCR reaction in results.

| No. | Primer       | Sequence                    | Size | A.T. | Gene       | Refs. |
|-----|--------------|-----------------------------|------|------|------------|-------|
| 1   | 16SrDNA-F    | AGA GTT TGA TCM TGG CTC AG  | 1500 | 55   | 16S rDNA   | [14]  |
| 2   | 16SrDNA-R    | ACG GHT ACC TTG TTA CGA CTT |      |      |            |       |
| 3   | aac(3)-II-F  | ATATCGGATGCATACGCGG         | 877  | 56   | aac(3)-II  | [16]  |
| 4   | aac(3)-II-R  | GACGGCCTCTAACCGGAAGG        |      |      |            |       |
| 5   | aph(3')-Ia-F | CGAGCATCAAATGAACTGC         | 623  | 50   | aph(3')-Ia | [17]  |
| 6   | aph(3')-Ia-R | GCGTTGCCAATGATGTTACAG       |      |      |            |       |
| 7   | rmtA-f       | CTA GCG TCC ATC CTT TCC TC  | 635  | 58   | rmtA (3)   | [18]  |
| 8   | rmtA-r       | TTT GCT TCC ATG CCC TTG CC  |      |      |            |       |
| 9   | rmtB-F       | GCT TTC TGC GGG CGA TGT AA  | 173  | 55   | rmtB       | [18]  |

**Table 2**  
Frequency of the isolates used in the study.

| Species                  | Frequency (%) |
|--------------------------|---------------|
| <i>K. pneumoniae</i>     | 89 (40.5%)    |
| <i>E. coli</i>           | 58 (26.4%)    |
| <i>K. oxytoca</i>        | 8 (3.6%)      |
| <i>A. baumannii</i>      | 19 (8.6%)     |
| <i>Chr. indologenes</i>  | 6 (2.7%)      |
| <i>P. aeruginosa</i>     | 23 (10.5%)    |
| <i>Pr. mirabilis</i>     | 9 (4.1%)      |
| <i>Enterobacter spp.</i> | 8 (3.6%)      |
|                          | 220 (100%)    |

## 3. Results

A total number of 220 of clinical specimens (Gram-negative bacteria) were collected from 5 tertiary hospitals in Makkah, Saudi Arabia. They were obtained from patients of various body sites. The majority of isolated strains were *K. pneumoniae* 89 (40.5%), followed by *E. coli* 58 (26.4%) and *P. aeruginosa* 23 (10.5%) (Table 2). The antimicrobial susceptibilities of the isolates are shown in Table 3. Phenotyping results confirmed the resistance of two or more aminoglycoside antibiotics in 51 (57.3%) out of the 89 *K. pneumoniae* isolates (Table 4). All the 51 isolates showed multidrug resistance phenotypes. Resistance rates for amoxicillin clavulanic acid, ciprofloxacin, cefotaxime, ampicillin, aztreonam, and cefuroxime antibiotics were 100%, while resistance rates for cefepime and imipenem antibiotics were 98% and 74.5%, (Table 3). while single genes were detected in 7 (13.8%), as shown in Table 3. The rmtD gene was the highest-detected gene 34 (66.7%), followed by aac(6')-Ib 23 (45.1%), aph(3')-Ia 23 (45.1%) isolates, rmtB 15 (29.4%), npmA 19 (27.3%), armA 11 (21.6%), aac(3)-II 4 (7.8%), and rmtA (3) 6 (11.8%) isolates, as shown in Figs. 1 and 2. No rmtC gene detected in the studied isolates. Significantly, higher-resistance

## 4. Discussion

The COVID-19 pandemic may lead to emergence to another pandemic, that of MDR bacteria. The emergence of *K. pneumoniae* strains resistant to aminoglycoside has widely in hospitals may lead to difficulties in treatment, *pneumoniae* infections during the COVID-19 pandemic. The total rate of *K. pneumoniae* in our study was 89 (40.5%). Phenotyping results confirmed the resistance of two or more aminoglycoside antibiotics in 51 (57.3%) out of the 89 *K. pneumoniae* isolates. Worldwide, aminoglycoside-resistant rate of *K. pneumoniae* has increased reported during the pandemic, and since then is known to break out in many countries [19,20].

The isolates of the present Almost 100% of the isolates were resistant to amoxicillin clavulanic acid, ciprofloxacin, cefotaxime, ampicillin, aztreonam, and cefuroxime. In Saudi Arabia, amikacin with gentamycin and tobramycin are common aminoglycosides for enterobacteria treatments [21–33]. In a study conducted at

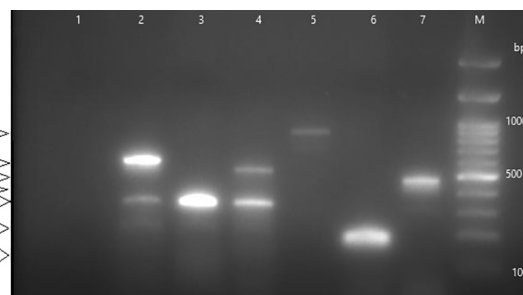
**Table 3**  
Antimicrobial-susceptibility patterns of aminoglycoside-resistant *K. pneumoniae*.

| Antibiotic    | Sensitive  | Intermediate | Resistant  |
|---------------|------------|--------------|------------|
| Amikacin      | 0 (0%)     | 0 (0%)       | 51(100%)   |
| Gentamicin    | 1 (2%)     | 0 (0%)       | 50 (98%)   |
| Tobramycin    | 0 (0%)     | 1 (2%)       | 50 (98%)   |
| Ciprofloxacin | 0 (0%)     | 0 (0%)       | 51 (100%)  |
| Cefepime      | 0 (0%)     | 2 (4%)       | 49 (96%)   |
| Imipenem      | 11 (21.5%) | 2 (4%)       | 38 (74.5%) |
| Cefotaxime    | 0 (0%)     | 0 (0%)       | 51 (100%)  |
| Ampicillin    | 0 (0%)     | 0 (0%)       | 51 (100%)  |
| Aztreonam     | 0 (0%)     | 0 (0%)       | 51 (100%)  |
| Cefuroxime    | 0 (0%)     | 0 (0%)       | 51(100%)   |

**Table 4**  
Frequency of phenotypic and genotypic aminoglycoside-resistant *K. pneumoniae*.

| Species              | Phenotypic | Genotypic |            |            |
|----------------------|------------|-----------|------------|------------|
|                      |            | Single    | Combined   | Total      |
| <i>K. pneumoniae</i> | 51 (57.5%) | 7 (13.7%) | 35 (68.6%) | 42 (82.3%) |

Makkah, amikacin was the most effective against the common Gram negative bacteria, while high antimicrobial resistance was observed to routine antibiotics [22]. The emergence of Aminoglycoside-Resistant *K. pneumoniae* strains during pandemic COVID-19 has been described in many studies, whereas MDR *Klebsiella pneumoniae* may be associated with both COVID-19 ICU and non-COVID-19 ICU patients [9–11]. may be the reason for elevated resistance rates [23–33]. Arteaga-Livias et al.2021 suggested that cross contamination via the hands of the staff and the limitation of an inappropriate use of PPE might facilitate the spread of MDR bacteria amid pandemic COVID-19 [24]. Our results showed that mainly seven different kinds of PCR patterns were revealed in 42 (82.3%) of the *K. pneumoniae* isolates. Resistance against aminoglycosides may be associated with the broad prevalence of plasmids among *K. pneumoniae*, which become sources of resistance acquisition through lateral gene transfer. Modifications made by AME genes decrease the binding affinity of drugs for their targets and hence lead to a loss in antibacterial effects [25]. Among these, rmtD is the most predominant type, This may be explained by antibiotic stress in Makkah hospital settings specially during mass gatherings



**Fig. 2.** Detection of aminoglycoside resistant genes.

(pilgrimage) that may facilitate the prevalence and coexistence of resistance genes within *K. pneumoniae* isolates. In conclusion, the frequency aminoglycoside-resistant *K. pneumoniae* in Makkah hospitals was highly resistant to amikacin, gentamicin, and tobramycin, and multiple-resistant to amoxicillin clavulanic acid, ciprofloxacin, cefotaxime, ampicillin, aztreonam, and cefuroxime. so the monitoring of Multidrug resistant bacteria must be continuously undertaken to implement effective measures in infection control and prevention.

#### Funding

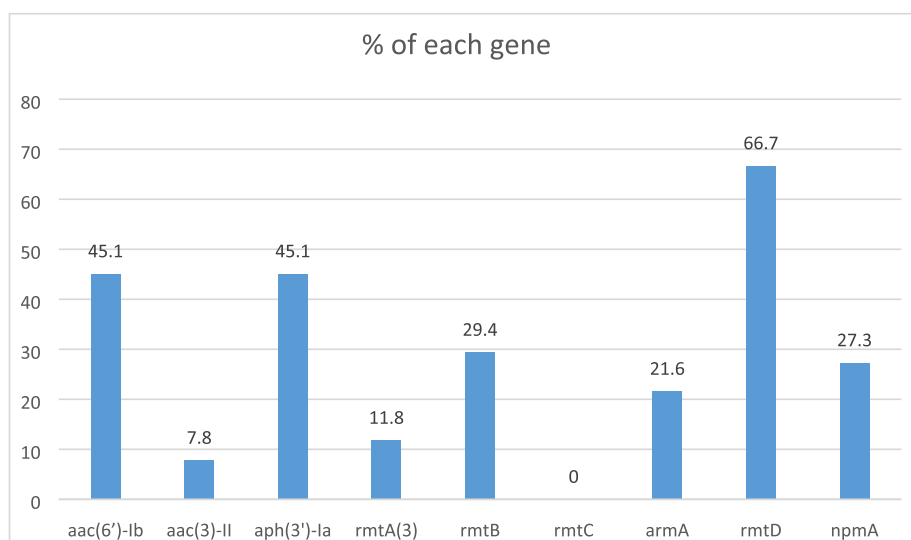
The research work was funded by the Deanship of Scientific Research—Umm Al-Qura University, Makkah, Saudi Arabia, by grant number No. 18-MED-1-01-0027.

#### CRedit authorship contribution statement

**Omar B. Ahmed:** Conceptualization, Methodology, Software, Data curation. **Atif H. Asghar:** Visualization. **Fayez S. Bahwerth:** Investigation, Supervision, Software, Validation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



**Fig. 1.** Distribution of aminoglycoside resistant genes.

## Acknowledgments

The authors would like to acknowledge the Deanship of Scientific Research at Umm Al-Qura University for supporting this work (grant number. 18-MED-1-01-0027).

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