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

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Article info
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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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 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, and 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. independent 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 aminoglycosides [8]. Enzymes aac(6’)-I, aac(3’)-II, aph(3’)-II, ant (3’)-I, and ant(2’)-I are responsible for

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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.

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(3)-Ib 23 (45.1%), aph(3)-Ia 23 (45.1%) isolates, rmtB 15 (29.4%), npmA 19 (37.3%), 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 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 | AGC GHT ACC TFG TTA CGA CTT | 877 | 56 | aac(3)-II | [16] |
| 3   | aac(3)-Ia-F | ATATEGGATGCATACGCCC | 623 | 50 | aph(3)-Ia | [17] |
| 4   | aac(3)-Ib-R | GACGCCTCTAACCCGAA | 635 | 58 | rmtA (3) | [18] |
| 5   | ampiR | CTG CGG CCG TAC ATC CTC CC | 173 | 55 | rmtB | [18] |
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 (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.

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CRediT authorship contribution statement

Omar B. Ahmed: Conceptualization, Methodology, Software, Data curation. Atif H. Asghar: Visualization. Faye 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.
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References

[1] L. Lansbury, B. Lim, V. Baskaran W Lim Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020, 81, 266–75.
[2] G.L. Dandagi, Nosocomial pneumonia in critically ill patients—J. Biomed. Sci. 15 (2008) 5–14.
[3] M.-P. Mingeot-Leclercq, Y. Bahar, M.E. Ahmed. Aminoglycoside usage and monitoring in a Saudi Arabian teaching hospital: a ten-year laboratory audit, J. Clin. Pharm. Ther. 25 (2000) 303–307.
[4] A.H. Asghar, O.B. Ahmed, Aminoglycoside resistance on Klebsiella pneumonia in a hospital in China, Int. J. Antimicrob. Agents 57 (2021) 106245.
[5] C.I. Liu, P. Du, N. Xiao, F. Ji, T.A. Russo, J. Guo. Hypervirulent Klebsiella pneumoniae is emerging as an increasingly prevalent K. pneumoniae pathotype responsible for nosocomial and healthcare-associated infections in Beijing. Virulence. 2020, 11, 1215–1225.
[6] G. Arcari, G. Raponi, F. Sacco, G. Bibbolino, F.M. Tulkens, Aminoglycoside-Modifying Enzymes in Escherichia coli and Klebsiella pneumoniae infections in COVID-19 patients: a 2-month retrospective analysis in an Italian hospital, Int. J. Antimicrob. Agents. 57 (2021) 106245.
[7] T. Hosoda, S. Harada, K. Okamoto, S. Ishino, M. Kaneko, M. Suzuki, M. Mrozugchi, COVID-19 and Fatal Sepsis Caused by Hypervirulent Klebsiella pneumoniae. Japan, 2020, Emerging Infectious Diseases. 27 (2) (2021) 556–559.
[8] B. Llano-Sotelo, E.F. Azucena, L.P. Kotra, S. Mobashery. Aminoglycoside Resistance Gene, aac(6’)-31, and Its Dissemination among Genetically Unrelated Clinical Isolates in a Brazilian Hospital, Antimicrob. Agents Chemother. 51 (2007) 2611–2614.
[9] H. Yigit, A.M. Queenan, C.J. Anderson, A. Domenne-Sanchez, J.W. Biddle, C.D. Steward, S. Aliberti, K. Bush, F.C. Tenover, Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of Klebsiella pneumoniae, Antimicrob. Agents Chemother. 45 (2001) 1151–1161.