Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i2.4866

RESISTANCE PHENOTYPE AND MOLECULAR EPIDEMIOLOGY OF CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE ISOLATES IN KING ABDUALLAH MEDICAL COMPLEX, JEDDAH

Suhailah Khalil Abdulhalim[1*], Ohoud suliman mannaa[2], Almontaserbellah Abdulhadi Suror[3], Ghazwah Rehim Aljadany[4], Noha khalid mohammed Nouh[5], Ala helyyel alharbi[6], Dr. Abdulaziz Mohammed Felemban[7], Bayan Sagga[8]

[1*]Senior Specialist Genetics at King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia
[2]King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia
[3]King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia
[4]King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia
[5]King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia
[6]Maternity & Children's Hospital in the north, Ministry of Health, Jeddah Saudi Arabia
[7]King abdullah medical complex – Jeddah
[8]Ministry of health, Jeddah Saudi Arabia

***Corresponding Author:** suhailah Khalil Abdulhalim
*Senior Specialist Genetics at King Abdullah Medical Complex, Ministry of Health, Jeddah Saudi Arabia

Abstract:

Although amikacin, meropenem, and colistin are options that still can be used, but the spread of CRKP has become a big problem all over the world, especially because this type of bacteria is resistant to most wide-spread antibiotics being used. Several studies have shown that the CRKP strains have been identified with these technology-based designs that are applied retrospectively. While the function of the molecular epidemiology and the influence of CRKP on outcomes were precisely outlined, a relevant paragraph explaining this phenomenon is still not available. Moreover, the existing options for therapy of CRKP are not efficient and this area still awaits a breakthrough. Consequently, the objective of the research study is to perform the molecular analysis of CRKP and the resistance patterns in the King Abdullah Medical Complex (KAMCJ), Jeddah. In this study the strain was subjected to growing in colonies during January 2022- August 2023 for a total of 260 CRKP isolates. An antimicrobial susceptibility test has been performed through these advanced techniques with VITEK-2 automated microbiology analyzer platform as well as broth microdilution approach. Then, to detect the carbapenemase and drug resistance genes, the XpertCarba-R assay and modified carbenem acid inactivation method have been conducted. The finding of this study indicated that the blaOXA-48 and blaNDM genes resulted in the transmission of K. pneumonia in patients' admissions at the intensive care unit. In the study, it was found out that patients who had CRKP were statistically related to their gender and chronic obstructive pulmonary disease. Besides that, CRKP dispersal would be limited and the performance of infection control measures supervised through CRKP strains surveillance.

Keywords: Molecular Epidemiology, Resistance Phenotype, CRKP, Antibiotic resistance, pert Carba-R assay, blaNDM, blaOXA-48.

1. INTRODUCTION

In the past thirty years, several multidrug resistant organisms have been discovered, which presents new challenges to the field of pharmacology as well the implementation of infection control practices. In the category of Enterobacteriaceae, Klebsiella pneumonia is a type of Gram-negative bacteria that lives abnormally and colonizes to the maximum population of the.
the gastrointestinal tract (Lynch et al., 2021; Clark and Zhanel, 2019; Su et al., 2019; Shaidullina et al., 2020; Zhao et al., 2020; Mirzaie and Ranjbar, 2021; Fursova et al., 20 Klebsiella pneumoniae diffusion is stimulated and induces the evolution of the high efficiency of resistant strain selection (Fursova et al., 2022; Elbrolosy et al., 2021; Kuzina et al., 2023). Klebsiella pneumonia is a major, gram-negative, and opportunistic pathogen that could lead to surgical site infections, urinary tract, bloodstream, respiratory, and ventilator-associated pneumonia.In clinical settings, the antibiotics' bactericidal effects on K. pneumoniae are fading gradually as a result of the ongoing rise of carbapenem-resistant K. pneumoniae (CRKP), which makes the choice of clinical treatment more challenging (Guo et al., 2023; Shelenkov et al., 2020; Hazirolan and Karagoz, 2020, Lalaoui et al., 2018; Mouftah et al., 2021; Zhou et al., 2020; Guo et al., 2023; Zhang et al., 2021). CRKP is dominated by high diversity of plasmid carrying carbapenemase enzymes which can include K. pneumonia carbapenemases (KPCs), MBLs (metallo-ß-lactamases) and extended-spectrum beta-lactamases (ESBLs) (Gong et al., 2022; Al-Zahrani & Alasiri During the last two decades, CRKP has caused a massive number of deaths and is endemic in many countries. Contrary to the carbapenem-susceptible K. pneumoniae, CRKP mortality is associated with higher patient mortality (Cienfuegos-gallet et al., 2022; Xu et al., 2022; Ahmed et al., 2021). The World Health Organization stated that coming up with fresh and efficient antibiotic drugs for the treatment of CRKP is a priority (Puente et al., 2019; Wang et al., 2022; Lin et al., 2024; Huang et al., 2022; Tsui et al., 2023). Here, in Fig. 1, the surge of Klebsiella pneumoniae is shown.

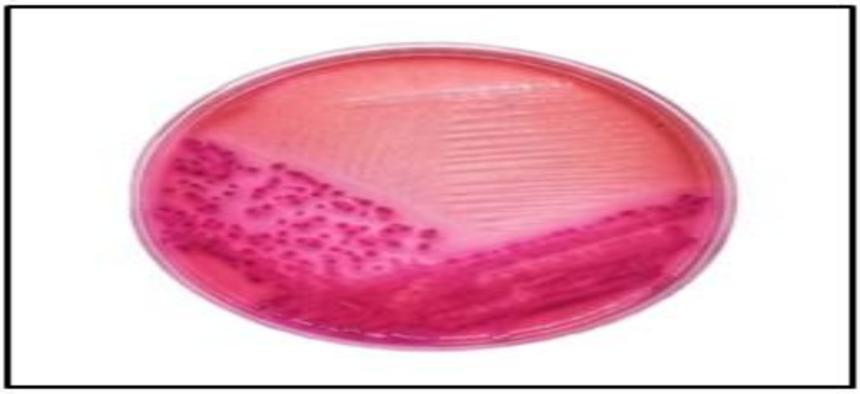


Figure 1: Klebsiella Pneumoniae

It is only recently that there have been a series of reports on maticillin-resistant Klebsiella pneumoniae isolates. While awareness of the molecular epidemiology of CRKP in the whole Middle East and Gulf Countries is, unfortunately, general insufficient. In the KSA, studys found that there were mostly OXA-48 and NDM strains of carbapenemases. However, the majority of the research work encountered has been small-scale and based on retrospective design. A few latest research outcomes demonstrated that CRKP infection, which can result from comorbidities, has high fatality. Accordingly, it would be useful to have a deep inception into CRKP molecular epidemiology in this zone and its implication to healthcare as well as to develop local guidelines from them. Consequently, to overcome this research gap, the current research work, in particular, was designed to investigate drug resistance, molecular epidemiology, and genetic relationship of CRKP isolates from patients in the King Abdullah Medical Complex in Jeddah (KAMCJ). The aim of this work is to obtain isolates of MDR bacteria and understand the resistance profile as measured against K. pneumoniae.

The remainder of the paper is structured as follows: Chapter 2 ethnography and literature review review, Chapter 3 the essence of materials and methods of the research, and Chapter 4 the most significant results and a discussion of the analysis. Moreover, chapter 5 points out to the end of the study and further areas of focus.

2. RELATED LITERATURE REVIEW

Alraddadi et al., (2022) are determined to finding the molecular epidemiology of carbapenem-resistant Enterobacterales from swabs of their institute members. In this study, an inclusive sample size of a total 189 patients older than 14 years of age who tested CRE-postive were included. In subsequent step, data trend involving the outcome variable ("mortality") and other categorical covariates were applied the use of both uni-variate and multi-variate logistics models. Through the evaluation, it was observed the the CRE bacteremic still being the only significant predictor of 30-day all cause except death with its 95% CI of 2.8. In addition, the univariate analysis showed nosocomial pneumonia, high Pitt bacteremia score, and high comorbidity index indicated by Charlson comorbidity index to be the significant factors associated with mortality. Although this specific protocol for a CRE failed to emerge due the high heterogeneity of treatment, the common principles of the treatment could still be pointed out

Alshahrani and co. (2022) studied the laboratory characteristics of carbapenem-resistant k.pneumoniae clinical isolates from adult patients with comorbidities using the method of molecular examination. A type of hospital has been considered throughout the year of April 2021 to December 2021, named as the King Abdullah Hospital, from Bisha province, Saudi Arabia. According to the descriptive statistics and a chi-square test, the nature of the association between gene-carrying isolates and patients' demographics and other underlying medical conditions were examined. Among the study populations, the presence of a carbapenem-gene carrying K. pneumoniae was statistically significantly associated with gender, COPD, as well as admission to the intensive care unit (correctness - $p = 0.015$, $p = 0.028$, and $p = 0.002$, respectively). Thus, this study involved just a minimal number of predicted factors and producers of carbapenemase alone.

In their study, Alhazmi et al., (2022), reported on the molecular identification of CRKP strains that were also providing the blaNDM and blaOXA-48 genes using the allele-specific PCR assays Jeddah. A subject sample has collected been from 191 CRKP-isolated patients in the clinical specimens of KFAFH in Saudi Arabia. The results of a study were analyzed, and the data was examined by statistical methods. Study findings revealed that all the isolates were either intermediate or high resistant to one or both of the carbapenems test drug. The fact that the CRKP strains showed moderate (37.7%) and low (14%) tenacity against colistin and tigecycline, concurring the effect on the growth inhibition was indowed as well. Nevertheless, the DNA profiling technique just done on the utmost common clones of CRKP was undertaken in very particular hospital and so the other hospitals in the Kingdom of Saudi Arabia were not considered.

The authors Zaman et al., (2018) conducted a study of the genetic and clonal profiling diversity of multidrug resistance among multidrug/CRKPs. Through the use of PCR and sequencing, amino acid sequence of exactly 71 non-streptococcal isolates was evaluated, they were all isolates from a tertiary care hospital in Saudi Arabia. To follow, by using Splits Tree4 a phlogeny was performed. The study showed that OXA-48 polyclonal gene was present in 8/71 (67.6%) and total 9/71 (12.7%) isolates contained NDM-1. Consequently, the gene encoding the CTX-M-15 most predominant extended spectrum antibiotic β-lactamase was found in 47/71 (66.2%) isolates. On the other hand it was found that very little information was known about the different gene components implicated in the NDM-1 gene.

By utilizing various epidemiological and clinical factors the relationship with the ket. pneumoniae bloodstream infection has been studied by hafez et al. In this case, the study of 152 k Pneumoniae BSI cases, from Saudi Arabia was considered. Application of statistics techniques on tests the records of the patients has be done. Consequently, the research has demonstrated that women have a very little probability of getting it but men are more infected than them. Moreover, at all the age categories neurology conditions were the most seen causes for getting K.pneumoniae BSI, which is reflected by children, adults, and the elderly.

CRKP genotypic profiles and HG were reported from Egyptian University Hospital isolation by Taha et al., (2023). The DNA extraction successfully from 500 different clinical samples of Tanta University Hospitals, Egypt suddenly shrank to 160 K. pneumoniae specimens. Afterwards, this polymerase chain reaction was used to determine the capsular serotypes and carbapenemase genes produced by the bacteria. Further, an antimicrobial susceptibility test was also conducted. Finally, the statistical tools have been reached to take analysis of the control variables. The statistical analysis revealed that the most common form of scapular type was seen quite prevalent in K1; hence, the corresponding value was 30.6%. Lastly, the molecular CR gene the 31.3% (50/160) phenotypic carbapenem resistance. Yet the design of a study could be cross-sectional and thus limiting the external validity of these outcomes.

Grame, DeDDiiC-LjoBoVCi and SaLiMOViC-Besic obtained the phenotypic and molecular characterization of carbapenemase-producing Enterobacterial K. pneumonia clinical isolates (Grame, DeDDiiC-LjoBoVCi and SaLiMOViC-Besic, 2020). Three different types of carbepenemase OXA-type 48 and one carbapenemase type OXA-143 were identified in the Clinical Center Sarajevo. Specimens such as bronchoalveolar lavage, wounds swabs, and anal swab have given so many samples like Sputum. The existing samples were isolated. Culture, microscopic viewing, and biochemical testing were applied for the determination of the molecular makeup of K. pneumoniae isolates using the VITEK 2 compact system. The result of the examination indicated that clone spreader direction from the colonised patients could be possible, and cross transmission of the bacteria in the hospital environment might also happen. While they have not yet found any similarity of the two different genetic patterns, it is highly probable.

The study by Gandor and his colleagues was on the isolated characterization of the CRKP group from the intensive care units at Zagazig University Hospitals (Gandor et al, 2022). Through this, we have chosen a sample of 119 patients with infections confirmed by K. pneumoniae. For screening the carbapenemase as a detection method, the modified hodge test (MHT) and modified carbapenem inactivation method (MCIM) were used as phenotypic tests. Finally, the results of the research has shown that about the fragment that was 56.2% enriched with blaNDM, 41.0% with blaOXA-48, and 32.4% with blaKPC. However, there were some drawbacks in this work having of that a mere one gene type was missing to be considered. The other is finance is that comprehensive exothe not too considered because of limited sources of funds.

Lau et al., (2021) conducted an investigation of CRKP epidemiology in a university hospital in Malaysia regarding the clinical and culture based genotyping of the isolates. Carbapenem referring (CRKP) strains found in a tertiary hospital which was a total of 63 and these were isolated within the period from January 2016 to August 2017. Subsequently, the study outline the NDM carbapenemase gene and the reasons that the patient uses invasive devices such as catheters and artificial lungs through logistic regression. A study assessed the association of NDM producers, colonization or infection by CRKP with central venous catheters using and in-hospital mortality. The investigation resulted in a finding that these three factors were significantly associated with in-hospital death. The survival analysis also showed that on the other hand the Cox proportional hazard had a greater hazard ratio for patients with stoma while for non-diffusing mucoid-producing CRKP patients it had a lower hazard ratio for the time to next visit. Unlike other studies on CRKP strains, this study was conducted in stricter timespan; therefore, it was confined to vice versa strains that were generated in state hospitals under consideration.

Morningstar and the colleagues, Brek, et al., (2023) investigated the occurrence of carbapenemase-producing Klebsiella pneumoniae in Saudi Arabia and identified the involved molecular factors. During the months of March 2020 - April 2021, sampling from each single one out of 3 tertiary hospitals constituting Jazan region, a total number of 86 new isolates free of duplication was obtained and the species K. pneumoniae was the primary focus of the study. Such as fully automated systems, automated detection of susceptibilities of microorganisms against antibiotics and identification can be carried out. Among the PCR-multiplex method applied, the test and analysis of carbapenemase genes were identified. The result of the study is that 64 (74.4%) of the 86 carbapenemase-producing CRKP strains isolated from the patients were identified. The most prevalent carbapenemases gene identified was blaOXA-48 with a number of 65.1% isolates positively identified. The study I have done has had three limitations: the fund of this Women's General Studies Tradition was not enough; second, time; and third, some students' attitudes and interrogations. The other aspect which is also addressed by the study is that not all K. pneumoniae isolates were susceptible to the carbapenem.

On the same year, the research) of Indrajith et al., was focusing on the molecular characterization of CRKP isolates including multidrug resistance in clinical samples in India. From rainforest central south India, we altogether got one hundred forty-five total suspected that were pus and blood, urine, sputum, and biopsy samples. Identification was carried by a standard and an antibiotic sensitivity test biochemically and molecularly. Isolate identification showed 61% were K. pneumonia and of all ESBL resistant antibiotics 97%, of carbarenem antibiotics 58%, and 75% Multidrug Resistence(MDR) of isolates were confirmed. Also, the result of the research was positive for the MDR Kemp isolates with a regard to blaCTXM-1, blaSHVgetse, blaTEM gene, blaNDM-1 gene, blaGES gene, and blaIMP-1 gene. A limitation of such studies is that the samples might be collected only from certain hospitals; as a result, the validity of the study results might be relevant only to some other hospitals.

Pattolath et al.(2023), in their study wanted to explore the CRKP clinical and or molecular phenotype infections in a tertiary care hospital. With about 786 K. pneumoniae isolates collected from the tertiary care hospital in Mangalore as those that responded to the health care problem, this study was carried out in the tertiary care hospital located in Mangalore. In the isolates, the PCR method was used for assessing the descent while the CR isolates were screened by carba NP test. The multivariate analysis of the CRKP infection was coming to be studied in detail. A study found that!: CRKP accounted for more than half of all the isolates (68%). Moreover, the research results suggested that the CRKP patients presented with a higher probability of death rate and a greater incidence of septic shock. Although the study conflicted with other reports, the result could be the same in otherplaces. In the case at hand the antibiotic resistance is demonstrated to be only due to carbaepenemase production.

In the work of Samadi et al., (2024) a consideration of the molecular and phenotypic detection of carbapenemase-producing Klebsiellapneumoniae was conducted. Moreover, this research delved into the expression dimensons and prevalence of genes encoding carbapenemase and Cr-KPN isolates. For this purpose 671 cases from two referral hospitals in Shiraz have been considered. Confirmation or identification of the isolates was done by conventional bacterial assays, through molecular biology like PCR, and also biochemical assays depending on the case in question. The PCR assays with the multiplex technology for carbapenemase-producing Klebsiellapneumoniae were performed, and genotype and phenotype were finally known through modified carbapenem inactivation methods. The general rate of isolates for K. pneumoniae is 14.9%, the majority of which consist of blaIMI/IMP gene found in 91% of the subjects. In addition, blaIMI/NMC, blaNDM, blaOXA-48 and blaKPC were also verified by real-time PCR analysis so they could be seen enriched in different genomic sites or plasmids.

Lee et al, (2022) has also investigated the profile of non-carbapenemase-producing CRKP identification using molecular and clinical marks that were used as indicators. In this study, a number of 54 NC-CRKP-infected/colonized isolated patients were included in the study, taken from the tertiary teaching hospital in Malaysia, from January 2013 to Oct maine 2019. The accuracy of information we get from the findings has been put through statistical analysis test. The investigation ended that the incidence rate of 46.2% was detected in the hospital patients of NC-CRKP bloodstream infections. Then, resistance gene blaSHV, blaOXA, blaTEM, blaDHA, and blaCTX-M was found and 46.3% of the strains (25/54) showed porin loss as well. Yet, because of the substantial small number of individuals which were included in this analysis, statistics showed no significant correlations between the risk factor and the analysis of the multivariate binary logistic regression.

Wang et al. (2022) handled the CRKP outbreak, traced the transmission, and studied the drug resistance mechanisms in a tertiary hospital. Overall, sampling produced allogroup CRKP 45 strains in the teaching hospital from March to June 2018, in Northern China. Through the microbroth dilution method and the applied VITEK2 compact system the drug susceptibility testing is in practiced. This led to identification of known plasmid lineages, drug resistance determinants, their multilocus sequence typing (MLST), and PCR-based screening and sequence. To sum up, total 45 isolates were resistant to different antibiotics. Additionally it also proved that the most predominated plasmids were of IncFIB and IncFII type and all 45 CRKP isolates contained PMQR genes and ESBL genes along with carbapenemase genes. Nevertheless, the study is based on CRKP isolates; and it presents the scoping and confirming study at one large tertiary hospital in Northern China.

In their study on the CRKP isolates,(the molecular and clinical characteristics at a tertiary hospital in China as shown by Hu et al., 2023) Hu et al., (2023) demonstrated the CRKP isolates' molecular and clinical characteristics at a tertiary hospital in China. Validation of role of drug resistance mechanisms and their significance; this study examined drug resistance mechanisms. The data were generated when breadth carriage rate was examined in hospitalized patients from 45 CRKP strains that were identified in Zhongnan Hospital between August 2018 and December 2020. Implementing such automation DITEK 2 made it possible to perform both the antimicrobial susceptibility tests and the strain itself. The study pointed out that the CRKP strains carried many genes resistant to different antibiotics and also had a piece or parts considered to be part of its "viral qualities". Resulting ST11 and ST1049 were both shown to be the two sequence types of MLST from the analysis. Also, in three samples of CRKP isolates the resistance pattern was multidrug-resistance strain. Although the study was single-centered and perhaps with a small number of cases, it has been shown to be effective by patients' improvements.

The article by Kang et al., (2020) concentrated on the epidemiological aspects of CRKP at the molecular level and the patients-based disease burden in a tertiary hospital from Northern Jiangsu Province in China. Ultimately, 252 CRKP infection patients were gathered to complete epidemiological and clinical survey forms through the period of January to December 2016, which were carried out using survey forms. MLST was used to find the sequencing of genes and to explore the clonal characteristics and resistance-associated genes with the conventional DNA methods and the PCR techniques. In the end, KPC-2 (120/128, 93.8%) was the most important carbapenemase and ST11 (98/128, 76.5%) was the common cluster type, which was followed by 14.64 incident cases per 100,000 patient days of the hospital in 2016. But the details about the clinical data were not available all the time so it was not easy to know the whole infection picture on infection. It can also be the role of the number of observations taken is not large enough to conclude the study.

Liao et al., in the year 2023, surveyed the microbial and drug-resistance gene epidemiological characteristics of CRKP. A bacterial strain gathered from the Carbapenem-resistant Klebsiella pneumoniae (CRKP) sample in the First Affiliated Hospital of Gannan Medical University in China, between January 2018 and February 2021, was used for the study. The drug resistance gene expressions have been identified by applying both multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), then the resistance to the drug was captured using the VitekII compact system. The study came to the conclusion that the existence of blaNDM-1 was found within all 12 isolates and the presence of blaKPC-2 was found in all 35 isolates, while a detection rate of blaOXA-48 and blaIMP-4 accounted for 2.4%. In addition to the above groupings, 42 other isolates had blaSHV and blaCTX-M-1 which is followed by 12 other isolates carrying blaCTX-M-9 and 27 other isolates carrying blaTEM.

Wei et al., 2023, who focused their study on antimicrobial resistance gene profiling and the molecular characteristics of CRKP isolates at a major metropolitan hospital is the subject matter of this research. In this study, non-duplicate CRKP strains one hundred three strains gathered from the Guangxi Medical University in China for the span of time June 2018 to October 2020. Researchers mainly confirmed that CCAR isolates were positive for the metallo-beta-lactamase mainly NDM-1 gene. Meanwhile, antibacterial drugs can only be selected within the range that suits the weakened immunity as found in the present study.
Shen and her colleagues investigated the STs , MAMPR and CRNG among CRKP isolates collected from the patients in China. In total, 94 CRKP strains were collected from First People Hospital in Zungi City from the beginning year 2018 until the end year of 2020. With a VITEK analyzer it was determined the antibiogram which is carbapenemase production action method according modified carbapenem inactivation method. The study findings showed that the isolates were very active carbapenemase producers, with 72.3% of them being new Delhi metallo--β-lactamase and Klebsiella pneumoniae carbapenemase, which was 24.5% of the gene assets. Despite this, the subtypes of carbapenemase genes among VIM, NDM and KPC were not studied, and the number of isolates in this experiment was also small.

3. MATERIALS AND METHODS

3.1. Study Design

This study underscored the investigation of the molecular epidemiology and resistant characteristics of CR-Kp. Here, we evaluated the suitability of King Abdullah Medical Complex (KAMCJ), located in Jeddah, Saudi Arabia, as a study site. By the end of the given period, in particular from January 2022 until August 2023, we had collected and recorded in total 260 K. pneumoniae isolates. The study was a retrospective study oriented on the recent evolution. So, the criteria for inclusion is going to cover all those isolates of K. pneumoniae, whose phenotypic resistance or tolerance is detected and then sent to a microbiological laboratory. Even though this study picked out Carbapenem-susceptible K. pneumoniae and redundant samples here, the authors still need to analyze and define the target population and study setting first.

3.2. Data collecting and processing.

During this research work, a bacterium isolate from various specimens was brought to our microbiology lab, which include Urine Samples, blood samples/sputum/wounds and pus samples in King Abdullah Medical Complex, Jeddah A total number of 260 people have been measured among which 150 men participated and for the female, 110 have taken part. So, the new specimens of the medical samples came from the intensive care unit (ICU), the emergency department, the dialysis department, the cardio and neuro department, the female medical ward, the avadi holding of the emergency, the isolation ward, and the CCU of King Abdullah Medical Complex (KAMCJ). All strains used to enlarge were plated on the sheep blood agar and MacConkey agar plates. Afterwards, the plates were incubated with a 37 ◦C incubator overnight. For selected colonies growth of one particular bacteria, carried away isolates of bacteria originally mixed and grown on agar culture was inoculated into MacConkey media. In order to dig further, the pure colonies of all the bacterial isolates were inoculated in thioglycollate broth media supplemented with 20% (V/V) glycerol that were finally stored at -80 °C for further analysis.

3.3. Sample processing

Urine sample

By using the proper medium plates of blood blood sheep maker W/CLED agar, the area where it was streaked was cleaned off and was it placed into Class II type b2 biological safety cabinets under the barcode with the patient’s information. Next is a downstroke which is the central stroke that connects the top to bottom of the almost hand/scroll-like structure. In the case of the 1st result, we have a disposable loop and the latter has named "acquired late streaking" test. At 37o C, the plates will be dispensed for 48 hours starting now.

Blood samples

The blood was scanned with the BACT/ALERT® 3D machine (BioMérieux, France) and loaded further for 1 to 5 days How to use present continuous tense in the essays The machine will then transport the specimen to the cabin for safekeeping. In the research, we have used 3 media such as Blood medium, Chocolate medium, and MacConkey mediums that enable us to figure out a blood sample. Then, a syringe was thoroughly degassed, so that it was devoid of air and was filled with a substance for inoculating all the plates which has been labeled with the patent info. The fabric of the blood using an alcohol swab is thereafter subjected to a dry or wet screen to draw 1-3 ml of the blood. A piece of disposable loop is used for this. Afterward, the lecturer ran us through the gram staining procedure. This was by incubating the microplates at 37oC.

Sputum Samples

Sputum samples were cultured on the oxidation resistant mediums, blood medium, chocolate medium, and the MacConkey medium. Moreover, cotton swab, a throw away tube or reagent lasseled sample were applied as well. The collection of sputum in the sample container can be done by using a cotton swab which is dipped into the sample tube, and checked whether it sticks to the interior of the container or not. In the next step, the medium was smeared into a thin layer in three slopes by direction going in a circle. Next, the plates were left at 37 oC for 24–78 hours after the incubation.

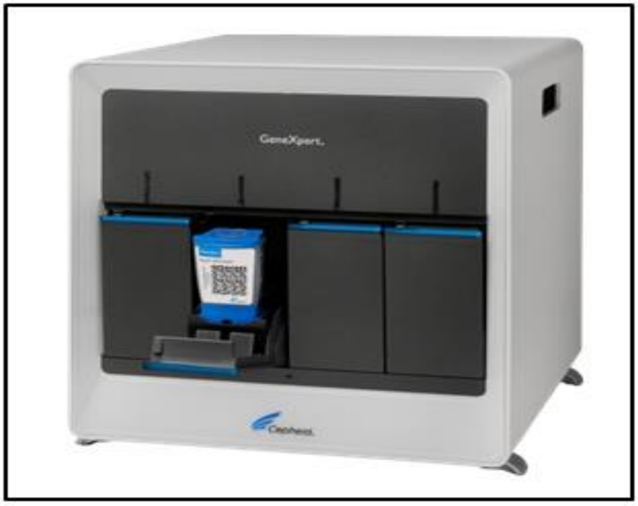
3.4. Identification of isolates

Due to the use of oxidase, gram stain, colony morphology, and standard biochemical tests, the isolates were unofficially named. Next, different specimens of blood, CSF, urine, wounds, and sputum are directly plated, or cultured, into the lab's Microbiology department. Immediate processing is needed for rapid studies and diagnosis output. After this the definitive species identification was carried out by the VITEK II (bioMérieux, France) automatic system using a special card for Gram-negative strains (ID-GNB) in conformity with the instructions provided by the manufacturer.

3.5. Antimicrobial susceptibility testing

Through VITEK®2 COMPACT (BioMérieux, France), MicroScan system and the Genexpert, AST of the isolates was done. These antimicrobial agents are tested for their resistance or activity to broad antimicrobial class-carbapenems (imipenem, meropænum, and ertapæneum) and additional eleven compounds, i.e., cefepime (CPE), gentamicin (GEN), amikacin (AMK), amoxicillin-clavulanate (With the each pure culture isolated and the transported to 1.5ml tubes containing Luria-Bertani (LB) broth and 20% (v/v) glycerol we stored it in -80 °C environment. Subsequently, the recommended management discs of (GN or GP) antibiotic were placed with the plate which were kept at 37oC for 24 or 48hrs. This is visibalized in the figure 2 where the vitek2 and the genexpert machines are presented.

(a)



(b)

Figure 2: (a) Vitek 2 compact (b) Genexpert

3.7. Statistical Analysis

The statistical tools we used, were IBM SPSS version 20, to ensure that all the data that was being, entered was understood. In our data and variable description infographic, we portray the data and the variables as percentages and frequencies. Pearson Chi-Square test was carried out for the significance of the frequency distribution with 95% confidence interval using a $\alpha=0.05$. The continuous variables were displayed in median range of the highest and the lowest value. Categorical variables are expressed as percents of such categories as a patient or an isolate.

4. RESULT AND DISCUSSION

In this section we have gone deeper into comparing the demographic features of the patients with CRKPinfection , results of antimicrobial susceptibility testing , and the epidemiology of the molecular detection of carbapenemase genes of CRKP. Thereafter, the association between the carbapenemase genes and age-groups, gender and specimens would be investigated which would later be discussed with acquired GeneXpert and XpertCarba-R results.

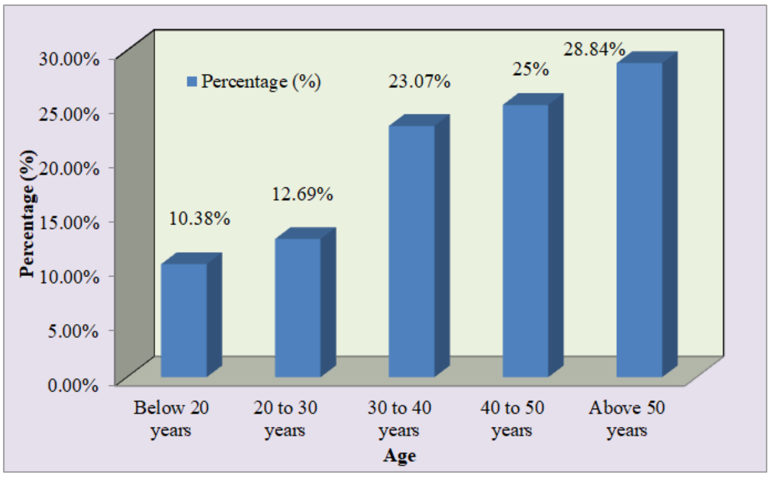
4.1. The demographic profile of cohorts of patients that harbor infections of CRKP

In this case, Tab1. provides data on the demographic profile of those patients with the CRKP infection according to the different age categories and gender.

Table 1: Patients' demographic profile

Patients	**Number of isolates (N)**	**Percentage (%)**
	Age	
Below 20 years	27	10.38%
20 to 30 years	33	12.69%
30 to 40 years	60	23.07%
40 to 50 years	65	25%
Above 50 years	75	28.84%
	Gender	
Male	150	57.69%
Female	110	42.30%

Based on the records, there were a total of 260 K. pneumoniae isolates that were recovered from the hospital and sent to the microbiology laboratory of King Abdullah Medical Complex (KAMCJ) in Jeddah. As far as the number of the respondents showing their categories, male respondents numbered more; this resulted in male specimens being 57.69% and those came from female individuals being 42.30%. Next was the age categories, which covered less than 20 years in ages, 20 to 30 years old, 30 to 40 years old, 40 to 50 years old, and above 50 years. A comparison of the numbers of isolates was conducted for all age categories and it was found that the numbers were high in the age category above 50 years (28.84%), while the numbers were low in the age category beneath the age of 20 years (10.38%). A demographic story plot was done through the graph form of Figure 3 below, on the left side.



(a)

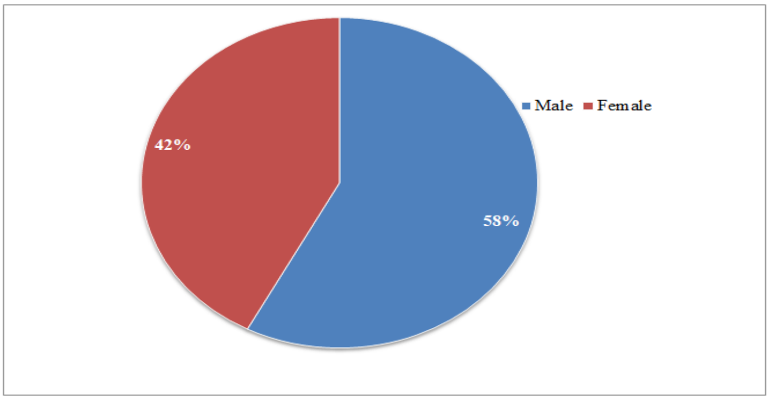


Figure 3: Demographic profile of respondents (a) Age (b) Gender

4.2. Analysis of antimicrobial susceptibility testing

The AST is used to identify the antimicrobial regimen, which is specifically effective for individual patients. And, this test is based on laboratory procedures, and it is performed by medical technologists. Here, the analysis of antimicrobial susceptibility testing is shown in below Table 2.

Table 2: Testing of AST

Isolates	Susceptible	Intermediate	Resistant
CPE	29	20	211
GEN	19	53	188
AMK	10	55	195
AMC	42	41	167
CAZ	12	35	207
TGC	33	67	160
CIP	35	50	175

Hence, among the clinical isolates, cefepime showed the greatest resistance which was 211 isolates, then other isolates of ceftazidime (207 isolates), amikacin (195 isolates), ciprofloxacin (188 isolates), gentamicin (175 isolates), and tigecycline (160 isolates Furthermore, to mid; Therefor position of the tigecycline is 67. However, the last drug got to the second highest spot (55) and the third one ended up in the third position 53. Then, scanning the following graph, amoxicillin-clavulanate is ranked by a 42 susceptible score as the first most effective drug while amikacin with 10 gets the lowest score. Figure of the testing of the AYES is shown in figure 4.

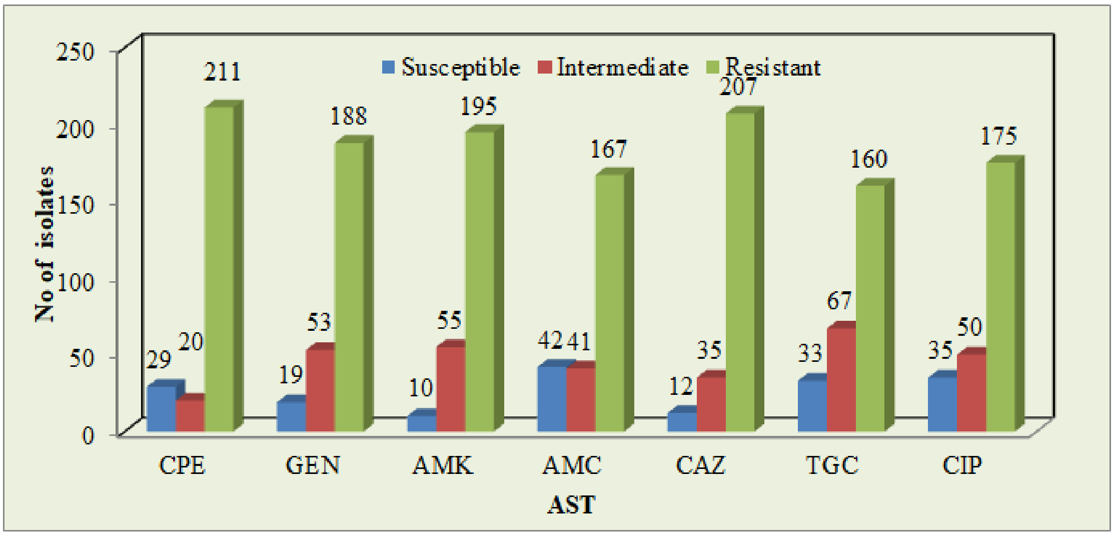


Figure 4: Analysis of AST

4.3. Epidemiology Molecular Detection of Carbapenemase Genes of CRKP Isolates

In this study, we selected five genes which are blaKPC, blaIMP, blaOXA-48, blaVIM, and blaNDM for the analysis as shown in the below table 3. This table demonstrate MICs of K. pneumoniae isolate.

Table 3: Descriptive statistics of molecular detection

Gene	Positive (%)	Negative (%)
blaKPC gene	6%	94%
blaIMP gene	1%	99%
blaOXA-48 gene	85%	15%
blaVIM gene	7%	93%
blaNDM gene	20%	80%

Table 4: Analysis of MIC of K. pneumoniae isolates with carbapenemase genes

K. pneumoniae Isolate CRKP- KAMCJ	**Imipenem**		**Meropenem**		**Ertapenem**	
	MIC *	**Interpretation**	**MIC ***	**Interpretation**	**MIC ***	**Interpretation**
CRKP- KAMCJ-10	≥20	R	≥20	R	-	-
CRKP- KAMCJ-15	≥20	R	≥20	R	≥10	R
CRKP- KAMCJ-25	≥20	R	≥20	R	≥10	R

Descriptive statistics method was used for the diagnosis of descriptive frequencies of genes. Among the five studied genes blaKPC, blaIMP, blaVIM, and blaNDM, gene blaOXA-48 is more often detected in molecular test than the other gene, i.e. 85%. Moreover, a BLD gene and other genes linked to the MDR phenotype were seen in 20% of isolates. Furthermore, in the same study, blaVIM gene, blaKPC gene, and blaIMP gene had the lowest percentage of positive molecular detection that totaled to 7%, 6%, and 1% respectively. Also, there were limited interpretations and minimum inhibitory concentration among CRKP isolates groups but a notable absence of KAMCJ-10.

4.4. Association between Carbapenemase genes and patients age groups, gender and type of specimen.

The Pearson correlation coefficient is a procedure that is adopted in order to explore whether two variables are in a statistical relationship or not as well as checking the degree of the relationship (which is termed as the “significant relationship”). Hence, this below table 5 illustrates result of correlation analysis.

Table 5: Pearson correlation analysis

Carbapenemase Genes	**Age**	**Gender**	**Specimen**	**ward**
blaKPC gene	Correlation Coefficient	-0.004	-0.073	0.063
	Sig.	0.983	0.341	0.490
blaIMP gene	Correlation Coefficient	-	-	-
	Sig.			
blaOXA-48 gene	Correlation Coefficient	0.098	0.099	-0.113*
	Sig.	0.197	0.191	0.005
blaVIM gene	Correlation Coefficient	-0.115	-0.071	-0.093
	Sig.	0.161	0.482	0.390
blaNDM gene	Correlation Coefficient	-0.088	0.86	0.105*
	Sig.	0.193	0.181	0.067

Based on the finding we observed that Age and Gender (0.098 and 0.099) respectively had weak relationship with blaOXA-48 genotype. The corresponding coefficients were low and statistically non-significant: age (−0.088); gender (−0.067) at 0.05 alpha level. But, surprisingly, the correlation between the blaNDM gene and ward status was positive and significant; so, the value obtained is 0.105*. Nevertheless, the blaOXA-48 had the weakly negative correlation coefficients with the ward; therefore eight positive coefficients were obtained, which result in the value of 0.113*.

4.5) A tool to guide the surge use of GeneXpert, Xpert Carba-R Assay

[In here, the results of analyte naming of IMP1, VIM, NDM, KPC, and OXA48 are in extension to Table 6]

Table 6: Findings from GeneXpert, Xpert Carba-R Assay

Analyte Name	**Analyte Result**	**Probe Check Result**
IMP1	Not detected	Pass
VIM	Not detected	Pass
NDM	Not detected	Pass
KPC	Not detected	Pass
OXA48	Detected	Pass

The results shows that the OxA 48 target DNAs is presented and the IMP, VIM, NDM and KPC target DNA sequences are not detected. The IMP, VIM, KPC, and ZND genes had calling failure or the call was below the detection limit of the assay. The IMP1, VIM, NDM, KPC, and OXA48 target DNA sets reached passing state in the probe check analysis.

5. CONCLUSION

The study focused on antibiotic resistance, molecular epidemiology and genetic relationship of (CRKP) isolates from patients with (KAMCJ) King Abdullah Medical Complex, Jeddah. Besides, we investigated the isolation and detection of multi-drug resistance pathogens and K. Pneumoniae susceptibility to certain antibiotics. As many as 260 CRP K isolates were collected at King Abdullah Medical Complex. Through VITEK-2 automated microbiology analyzer brands, bioMérieux (France), and broth microdilution method, the antimicrobial susceptibility was tested. This was followed by the discovery of the drug-resistant genes which was through the specimen sent to the Xpert Carba-R assay. This study showed that K. pneumoniae strains resistant to 3-rd generation cephalosporines were the most frequent in ICU patients — blaOXA-48 and blaNDM genes producers. Moreover, the course of the investigation presented the most significant occurrence of the positive molecular detections in the case of the gene, blaOXA-48, which was as high as 85%. Secondly, the gene of blaOXA-48 and blaNDM gave a coefficient of negative correlation between the independend variable (-0.113*, -0.105*). Yet the research examined only one hospital clinical setting, making out of the general data base. The study could be conducted further in the future by analyzing a bigger sample proportion and possibly involving geographical contexts or areas.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

I would like to thank the MOH, particularly the Research and Studies Department in Jeddah, Saudi Arabia, for the support and the approval for conducting this research.

ETHICAL APPROVAL

Permission to conduct this study was granted by the Research and Studies Committee of Ministry of Health under the research number of (A01474) and registered number with KACST (KSA: H-02-J-002).

AUTHOR CONTRIBUTIONS

SAk and AMF designed, supervised the project and reviewed the manuscript. NKN, BS, AHA, GRJ, OSM and AAS collected the samples and performed experiments and data analysis. All authors read and approved the final version.

REFERENCES

1. Ahmed, M.A.E.-G.E.-S. *et al.* (2021) ‘Emergence of Hypervirulent Carbapenem-Resistant Klebsiella pneumoniae Coharboring a bla NDM-1 -Carrying Virulent Plasmid and a bla KPC-2 -Carrying Plasmid in an Egyptian Hospital’, *mSphere*, 6(3), pp. 1–6. Available at: https://doi.org/10.1128/msphere.00088-21.
2. Al-Zahrani, I.A. and Alasiri, B.A. (2018) ‘The emergence of carbapenem-resistant Klebsiella pneumoniae isolates producing OXA-48 and NDM in the Southern (Asir) Province, Saudi Arabia’, *Saudi Medical Journal*, 39(1), pp. 23–30. Available at: https://doi.org/10.15537/smj.2018.1.21094.
3. Alhazmi, W., Al-Jabri, A. and Al-Zahrani, I. (2022) ‘The Molecular Characterization of Nosocomial Carbapenem-Resistant Klebsiella pneumoniae Co-Harboring blaNDM and blaOXA-48 in Jeddah’, *Microbiology Research*, 13(4), pp. 753–764. Available at:

https://doi.org/10.3390/microbiolres13040054.

4. Alraddadi, B.M. *et al.* (2022) ‘Molecular epidemiology and outcome of carbapenem-resistant Enterobacterales in Saudi Arabia’, *BMC Infectious Diseases*, 22(1), pp. 1–8. Available at: https://doi.org/10.1186/s12879-022-07507-y.
5. Alshahrani, A.M. *et al.* (2022) ‘Molecular Epidemiology of Carbapenem-Resistant K. pneumoniae Clinical Isolates from the Adult Patients with Comorbidities in a Tertiary Hospital, Southern Saudi Arabia’, *Antibiotics*, 11(12), pp. 1–13. Available at: https://doi.org /10.3390/antibiotics11121697.
6. Brek, T. *et al.* (2023) ‘Prevalence and molecular determinants of carbapenemase-producing Klebsiella pneumoniae isolated from Jazan, Saudi Arabia’, *Journal of Infection in Developing Countries*, 17(10), pp. 1420–1429. Available at: https://doi.org/10.3855/jidc.17662.
7. Cienfuegos-gallet, A. V *et al.* (2022) ‘Multicenter Genomic Analysis of Carbapenem-Resistant Klebsiella pneumoniae from Bacteremia in China’, *Microbiology Spectrum*, 10(2), pp. 1–12. Available at: https://doi.org/10.1128/spectrum.02290-21.
8. Elbrolosy, A.M. *et al.* (2021) ‘Characterization of virulence genetic profile and resistance patterns of clinical Klebsiella pneumoniae isolates: Classic versus hypermucoviscous phenotypes’, *Microbes and Infectious Diseases*, 2(3), pp. 516–528. Available at: https://doi.org/10.21608/MID.2021.74461.1147.
9. Fursova, A.D. *et al.* (2022) ‘Early response of antimicrobial resistance and virulence genes expression in classical, hypervirulent, and hybrid hvkp-mdr klebsiella pneumoniae on antimicrobial stress’, *Antibiotics*, 11(1), pp. 1–17. Available at: https://doi.org/10. 3390/antibiotics11010007.
10. Fursova, N.K. *et al.* (2020) ‘Emergence of Five Genetic Lines ST395NDM-1, ST13OXA-48, ST3346OXA-48, ST39CTX-M-14, and Novel ST3551OXA-48of Multidrug-Resistant Clinical Klebsiella pneumoniae in Russia’, *Microbial Drug Resistance*, 26(8), pp. 924–933. Available at: https://doi.org/10.1089/mdr.2019.0289.
11. Gandor, N.H.M. *et al.* (2022) ‘Characterization of Carbapenem-Resistant K. Pneumoniae Isolated from Intensive Care Units of Zagazig University Hospitals’, *Antibiotics*, 11(8), pp. 1–14. Available at: https://doi.org/10.3390/antibiotics11081108.
12. Gong, Y. *et al.* (2022) ‘Emergence of a Carbapenem-Resistant Klebsiella pneumoniae Isolate Co-harbouring Dual blaNDM– 6-Carrying Plasmids in China’, *Frontiers in Microbiology*, 13, pp. 1–8. Available at: https://doi.org/10.3389/fmicb.2022.900831.
13. Granov, D., Dedeic-Ljubovic, A. and Salimovic-Besic, I. (2020) ‘Characterization of Carbapenemase-Producing Klebsiella pneumoniae in Clinical Center University of Sarajevo, Bosnia and Herzegovina’, *Microbial Drug Resistance*, 26(9), pp. 1038–1045. Available at: https://doi.org/10.1089/mdr.2019.0188.
14. Guo, H. *et al.* (2023) ‘Global emergence of carbapenem-resistant Klebsiella pneumoniae co-carrying multiple carbapenemases’, *Computational and Structural Biotechnology Journal*, 21, pp. 3557–3563. Available at: https://doi.org/10.1016/j.csbj.2023.07.013.
15. Guo, Y. *et al.* (2023) ‘Virulence, antimicrobial resistance, and molecular characteristics of carbapenem-resistant Klebsiella pneumoniae in a hospital in Shijiazhuang City from China’, *International Microbiology*, 26(4), pp. 1073–1085. Available at: https://doi.org/10.1007/s10123-023-00357-x.
16. Hafiz, T.A. *et al.* (2023) ‘Klebsiella pneumoniae bacteraemia epidemiology: resistance profiles and clinical outcome of King Fahad Medical City isolates, Riyadh, Saudi Arabia’, *BMC Infectious Diseases*, 23(1), pp. 1–9. Available at: https://doi.org/10.1186/s12879-023-08563-8.
17. Hazirolan, G. and Karagoz, A. (2020) ‘Emergence of carbapenemase-producing and colistin resistant Klebsiella pneumoniae ST101 high-risk clone in Turkey’, *Acta Microbiologica et Immunologica Hungarica*, 67(4), pp. 216–221. Available at: https://doi.org/10.1 556/030.2020.01275.
18. Hu, F. *et al.* (2023) ‘Molecular and Clinical Characteristics of Carbapenem-Resistant Klebsiella

pneumoniae Isolates at a Tertiary Hospital in Wuhan, China', *Infection and Drug Resistance,* 16, pp. 65–76. Available at: https://doi.org/10.2147/IDR.S397975.

19. Huang, W. *et al.* (2022) 'Carbapenemase Production and Epidemiological Characteristics of Carbapenem-Resistant Klebsiella pneumoniae in Western Chongqing, China', *Frontiers in Cellular and Infection Microbiology,* 11, pp. 1–11. Available at: https://doi.org/10.3389/fcimb.2021.775740.
20. Indrajith, S. *et al.* (2021) 'Molecular insights of Carbapenem resistance Klebsiella pneumoniae isolates with focus on multidrug resistance from clinical samples', *Journal of Infection and Public Health,* 14(1), pp. 131–138. Available at: https://doi.org/10.1016/j.jiph.2020.09.018.
21. Kang, H. *et al.* (2020) 'Disease burden and molecular epidemiology of carbapenem- resistant Klebsiella pneumonia infection in a tertiary hospital in China', *Annals of Translational Medicine,* 8(9), pp. 1–10. Available at: https://doi.org/10.21037/atm.2020.03.122.
22. Kuzina, E.S. *et al.* (2023) 'High-Molecular-Weight Plasmids Carrying Carbapenemase Genes blaNDM-1, blaKPC-2, and blaOXA-48 Coexisting in Clinical Klebsiella pneumoniae Strains of ST39', *Microorganisms,* 11(2), pp. 1–16. Available at: https://doi.org/10.3390/microorganisms11020459.
23. Lalaoui, R. *et al.* (2018) 'Spread of Carbapenem and Colistin-Resistant Klebsiella pneumoniae ST512 Clinical Isolates in Israel: A Cause for Vigilance', *Microbial Drug Resistance,* 25(1), pp. 63–71. Available at: https://doi.org/10.1089/mdr.2018.0014.
24. Lau, M.Y. *et al.* (2021) 'Molecular characterization of carbapenem resistant klebsiella pneumoniae in Malaysia hospital', *Pathogens,* 10(3), pp. 1–15. Available at: https://doi.org/10.3390/pathogens10030279.
25. Lee, Y.Q. *et al.* (2022) 'Characterisation of Non-Carbapenemase-Producing Carbapenem-Resistant Klebsiella pneumoniae Based on Their Clinical and Molecular Profile in Malaysia', *Antibiotics,* 11(11), pp. 1–20. Available at: https://doi.org/10.3390/antibiotics11111670.
26. Li, Z. *et al.* (2021) 'Carbapenem-Resistant Klebsiella pneumoniae in southwest China: Molecular characteristics and risk factors caused by KPC and NDM producers', *Infection and Drug Resistance,* 14, pp. 3145–3158. Available at: https://doi.org/10.2147/IDR.S324244.
27. Liao, Y. *et al.* (2023) 'Drug Resistance Genes and Molecular Epidemiological Characteristics of Carbapenem-Resistant Klebsiella pneumonia', *Infection and Drug Resistance,* 16, pp. 1511–1519. Available at: https://doi.org/10.2147/IDR.S399142.
28. Lin, X.C. *et al.* (2024) 'The Global and Regional Prevalence of Hospital-Acquired Carbapenem-Resistant Klebsiella pneumoniae Infection: A Systematic Review and Meta-analysis', *Open Forum Infectious Diseases,* 11(2), pp. 1–15. Available at: https://doi.org/10.1093/ofid/ofad649.
29. Liu, S. *et al.* (2023) 'Retracted: Analysis of Carbapenemase-Resistant Genotypes of Highly Virulent Klebsiella pneumoniae and Clinical Infection Characteristics of Different MLST Types', *Evidence-Based Complementary and Alternative Medicine*, 2021, pp. 1–9. Available at: https://doi.org/10.1155/2023/9838103.
30. Lynch, J.P., Clark, N.M. and Zhanel, G.G. (2021) 'Escalating antimicrobial resistance among Enterobacteriaceae: focus on carbapenemases', *Expert Opinion on Pharmacotherapy*, 22(11), pp. 1455–1473. Available at: https://doi.org/10.1080/14656566.2021.1904891.
31. Mirzaie, A. and Ranjbar, R. (2021) 'Antibiotic resistance, virulence-associated genes analysis and molecular typing of Klebsiella pneumoniae strains recovered from clinical samples', *AMB Express*, 11(1), pp. 1–11. Available at: https://doi.org/10.1186/s13568-021-01282-w.
32. Mouftah, S.F. *et al.* (2021) 'Diversity of carbapenem-resistant Klebsiella pneumoniae ST14 and emergence of a subgroup with KL64 capsular locus in the Arabian Peninsula', *European Journal of Clinical Microbiology and Infectious Diseases,* pp. 1–9. Available at: https://doi.org/10.1007/s10096-021-04384-2.
33. Pattolath, A., Adhikari, P. and Pai, V. (2023) 'Clinical and Molecular Profile of Carbapenem Resistant Klebsiella pneumoniae Infections in a Tertiary Care Hospital –Mangalore', *Infection and Drug Resistance*, 16, pp. 4335–4348. Available at: https://doi.org/10.2147/IDR.S411056.

34. Puente, F. Del *et al.* (2019) ‘Epidemiology and outcome of Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae (KPC-KP) infections in cardiac surgery patients: a brief narrative review’, *Journal of Chemotherapy*, 31(7–8), pp. 359–366. Available at: https://doi.org/10.1080/1120009X.2019.1685794.
35. Samadi, N. *et al.* (2024) ‘Phenotypic and Molecular Detection of Carbapenemase-Producing Klebsiella pneumoniae Isolated from Patients Admitted to Teaching Hospitals in Shiraz, Iran’, *Jundishapur Journal of Microbiology*, 16(12), pp. 1–9. Available at: https://doi.org/10.5812/jjm-142449.
36. Shaidullina, E. *et al.* (2020) ‘Antimicrobial resistance and genomic characterization of OXA-48- and CTX-M-15-co-producing hypervirulent Klebsiella pneumoniae st23 recovered from nosocomial outbreak’, *Antibiotics*, 9(12), pp. 1–9. Available at: https://doi.org/10.3390/antibiotics9120862.
37. Shelenkov, A. *et al.* (2020) ‘Molecular typing, characterization of antimicrobial resistance, virulence profiling and analysis of whole-genome sequence of clinical klebsiella pneumoniae isolates’, *Antibiotics*, 9(5), pp. 1–15. Available at: https://doi.org/10.3390/antibiotics9050261.
38. Shen, M. *et al.* (2023) ‘Antimicrobial Resistance Patterns, Sequence Types, Virulence and Carbapenemase Genes of Carbapenem-Resistant Klebsiella pneumoniae Clinical Isolates from a Tertiary Care Teaching Hospital in Zunyi, China’, *Infection and Drug Resistance*, 16, pp. 637–649. Available at: https://doi.org/10.2147/IDR.S398304.
39. Su, J. *et al.* (2019) ‘In vitro bactericidal activity of trimethoprim-sulfamethoxazole/colistin combination against carbapenem-resistant klebsiella pneumoniae clinical isolates’, *Microbial Drug Resistance*, 25(2), pp. 152–156. Available at: https://doi.org/10.1089/mdr.2018.0085.
40. Taha, M.S. *et al.* (2023) ‘Genotypic Characterization of Carbapenem-Resistant Klebsiella pneumoniae Isolated from an Egyptian University Hospital’, *Pathogens*, 12(1), pp. 1–14. Available at: https://doi.org/10.3390/pathogens12010121.
41. Tsui, C.K.M. *et al.* (2023) ‘Genomic Epidemiology of Carbapenem-Resistant Klebsiella in Qatar: Emergence and Dissemination of Hypervirulent Klebsiella pneumoniae Sequence Type 383 Strains’, *Antimicrobial Agents and Chemotherapy*, 67(7), pp. 1–16. Available at: https://doi.org/10.1128/aac.00030-23.
42. Wang, N. *et al.* (2022) ‘Prevalence of Carbapenem-Resistant Klebsiella pneumoniae Infection in a Northern Province in China: Clinical Characteristics, Drug Resistance, and Geographic Distribution’, *Infection and Drug Resistance*, 15, pp. 569–579. Available at: https://doi.org/10.2147/IDR.S347343.
43. Wang, S. *et al.* (2022) ‘Molecular Epidemiology of Carbapenem-Resistant Klebsiella pneumoniae in a Tertiary Hospital in Northern China’, *Canadian Journal of Infectious Diseases and Medical Microbiology*, 2022, pp. 1–11. Available at: https://doi.org/10.1155/2022/2615753.
44. Wei, X. *et al.* (2023) ‘Molecular characteristics and antimicrobial resistance profiles of Carbapenem-Resistant Klebsiella pneumoniae isolates at a tertiary hospital in Nanning, China’, *BMC Microbiology*, 23(1), pp. 1–6. Available at: https://doi.org/10.1186/s12866-023-03038-x.
45. Xu, T. *et al.* (2022) ‘Clinical and resistance characterization of carbapenem-resistant Klebsiella pneumoniae isolated from intensive care units in China’, *Annals of Translational Medicine*, 10(20), pp. 1109–1109. Available at: https://doi.org/10.21037/atm-22-4323.
46. Yu, X. *et al.* (2019) ‘Molecular characterization of carbapenem-resistant Klebsiella pneumoniae isolates with focus on antimicrobial resistance’, *BMC Genomics*, 20(1), pp. 1–10. Available at: https://doi.org/10.1186/s12864-019-6225-9.
47. Zaman, T. uz *et al.* (2018) ‘Clonal diversity and genetic profiling of antibiotic resistance among multidrug/carbapenem-resistant Klebsiella pneumoniae isolates from a tertiary care hospital in Saudi Arabia’, *BMC Infectious Diseases*, 18(1), pp. 1–11. Available at: https://doi.org/10.1186/s12879-018-3114-9.
48. Zhang, W.X. *et al.* (2021) ‘Resistance Phenotype and Molecular Epidemiology of Carbapenem-Resistant Klebsiella pneumoniae Isolates in Shanghai’, *Microbial Drug Resistance*, 27(10), pp.

1312–1318. Available at: https://doi.org/10.1089/mdr.2020.0390.

49. Zhao, Y. *et al.* (2020) ‘Dynamic epidemiology and virulence characteristics of carbapenem-resistant klebsiella pneumoniae in wenzhou, China from 2003 to 2016’, *Infection and Drug Resistance*, 13, pp. 931–940. Available at: https://doi.org/10.2147/IDR.S243032.
50. Zhou, K. *et al.* (2020) ‘Novel Subclone of Carbapenem-Resistant Klebsiella pneumoniae Sequence Type 11 with Enhanced Virulence and Transmissibility, China’, *Emerging infectious diseases*, 26(2), pp. 289–297. Available at: https://doi.org/10.3201/eid2602.190594.