University of Thi-Qar Journal of Science (UTJsci)
E-ISSN:2709-0256, P-ISSN: 1991-8690 Vol. (10), No.1(Special Issue: ISCAMET), April 2023

Molecular characterization of Klebsiella pneumoniae isolated from COVID-19 patients as a secondary infection

Nathum Jabber Habeeb
Department of Biology, College of Science, University of Thi-Qar,
Iraq
nazmjbarhbyb@gmail.com

Khwam Reissan Hussein
Al-Nasiriyah Technical Institute, Southern Technical University,
Iraq
krhussein@stu.edu.iq

Abstract- Viral respiratory infection such as severe acute coronavirus-2 disease (SARS-CoV-2) and bacterial co-infections commonly are identified and a major cause of morbidity, mortality requiring prompt and antibacterial diagnosis. Sputum samples were collected from 100 patients including 68 males and 32 females with age ranged between 16 -85 years from the beginning of October 2021 to the end of March 2022. Specimens have been collected from isolation wards at Al-Hussein Teaching Hospital in Thi-Qar province, south of Iraq from patients that were diagnosed with COVID-19 and taking preventive treatment for bacterial infections. The results have shown the most common isolates were *Klebsiella pneumoniae* as the pathogen registered in 20 (32.2%) of the total 62 bacterial positive cases, followed by the *Candida* spp. 18 (29%). The current study also has demonstrated that the bacterial isolates were varied in their resistance and sensitivity to the antibiotics. A DNA fragment encoding the open reading frame (903 bp) of Cation-efflux pump FieF was successfully shown a positive PCR band and isolated from only 10 (47.6%) isolates of *K. pneumoniae* isolates were named Thi-Qar1-Thi-Qar11 by conventional PCR using Cation-efflux pump FieF gene specific primer. The obtained nucleotide sequences of the Cation-efflux pump FieF from the above-mentioned Thi-Qar isolates were deposited in the GenBank under accession numbers MZ069095-MZ069104.

Keywords— *Klebsiella pneumoniae*, Molecular, COVID-19

I. INTRODUCTION

COVID-19 virus is mainly spread in close contact with an infected person. The virus can also spread via contaminated surfaces (Marquès & Domingo, 2021). Persons remain infectious in moderate cases for up to 10 days and in serious cases for two weeks. Different methods for the diagnosis of the disease have been developed. The standard approach is using a nasopharyngeal swab (rRT-PCR) in real-time, reverse transcription-polymerase chain reaction(Tahamtan & Ardebili, 2020). In viral respiratory infection such as influenza, bacterial co-infections commonly are identified and are a major cause of morbidity and mortality requiring prompt and antibacterial diagnosis (Esper *et al.*, 2011). The bacterial joint infection was shown to be as high in patients with serious influenza (Esper *et al.*, 2011; Shah *et al.*, 2016) and is linked to greater disease severity, increased use of health resources, and increased risk of death (Martín-Loeches *et al.*, 2011) . In patients infected with serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the prevalence, incidence, and characteristics of bacterial infection are unclear and have been raised as a significant gap of understanding (Cox *et al.*, 2020). Although COVID-19 treatment with antibiotics is ineffective, it is prescribed for several reasons in patients with suspected or documented COVID-19. This means not excluding bacterial co-infection when presenting but also bacterial possibilities(Langford *et al.*, 2020). During the secondary infection may be occurred. In particular, the various guidelines advocate the use of empirical antibiotics for severe COVID-19 patients in the treatment of extra-excessive death in patients with bacterial superinfection during influenza pandemics (Alhazzani *et al.*, 2020). However, this assumption gives cause for concern regarding the overuse of antibiotics and the damage resulting from bacterial resistance. To ensure sustainable use of antibiotics and to minimize adverse effects of overuse, an understanding of the percentage of COVID-19 patients with acute airborne bacterial co-infection with the culprit pathogen is vital. In the refining of Empirical Antibiotic Management Guidelines, this knowledge could also have significant impacts on patients with COVID-19(Langford *et al.*, 2020). A swift review to determine the prevalence of Bacterial Infection in COVID-19 patients and to identify those people's most prevalent respiratory co-infecting (Uyeki *et al.*, 2020). The secondary bacterial infection was reported to 15 percent in Chinese hospitals. Their admission was reported. Of the secondary bacterial diseases, 27 of the 28 patients died (Huttner *et al.*, 2020). It was reported that 29 of 69 sputum-growing patients to identify respiratory bacteria/psycho-coinfection upon hospital admission. 5 out of 69 (7 percent), including *C. Albicans* (2/25:40%), *Enterobacter cloacae* (2/5:5:55), and *Acinetobacter baumanni* (1/5:20%) had

positive microbiology. Among all studies reported in COVID-19 for bacterial and fungal coinfection, very few atypical organisms have been identified in one obstetric patient admitted with COVID-19 in China (Haramoto *et al.*, 2020). Because of the widespread of the coronavirus and association with severe bacterial infections in Thi-Qar province south of Iraq was spread. This study is aimed to isolate and to detect molecular profile of the secondary bacterial infections caused by *Klebsiella pneumoniae* associated with COVID-19 infections.

II. MATERIALS AND METHODS

The sputum samples were collected randomly from 100 patients which have included 68 male and 32 female in age ranged between (16 -85) years from the beginning of October 2021 to the end of March 2022. Specimens were collected from isolation wards at Al-Hussein Teaching Hospital in Al-Nasiriyah for patients diagnosed with COVID-19 and taking preventive treatment for bacterial infection in patients infected with COVID-19. Good sputum samples depend on thorough health care worker education and patient understanding throughout all phases of the collection process. Food should not be ingested 1 to 2 hours before take sputum, with the mouth just before sputum or saline rinsing. A deep coughed specimen should be instructed in patients. In order to decrease saliva contamination, the substantial should be excluded into a sterile container. Specimen transportation to the laboratory must be done immediately because even a moderate amount of time at room temperature can result in loss of some infectious agents, thus all the samples were transported to the laboratory to be inoculated on blood agar, MacConkey agar, and chocolate agar to be incubated at 37 ˚C for 24 to 48 hours.

Klebsiella pneumoniae isolates were performed using blood and MacConkey agar as the method that illustrated (Mahon & Manuselis 2000) . The primary detection of *K. pneumoniae* was assesses via culturing, microscopical, motility, and biochemical tests (catalase, ndole, oxidase, citrate utilization, urease and glucose) (Mahon & Manuselis 2000). Muller-Hiton agar media were used for sensitivity test. The confirmatory diagnosis of this bacterium was done using Analytical Profile Index (API) 20E system (Bio-Merieux, France). Antimicrobial susceptibility test was performed by following Minimum Inhibitory Concentration (MIC) on Vitek2 compact system according to the CLSI (2020) and World Health Organization. Genomic DNA was extracted from bacterial growth. Specific primer set for cepA-f gene cepA-F 5`-CAACTCCTTCGCCTATCCCG-3` cepA-R 5`-TCAGGTCAGACC AAACGGCG-3` (Fang *et al.*, 2002) was used by polymerase chain reaction (PCR) technique using specific primers with using PCR thermo cycler system (Eppendrof, UAS). The method was preceded according to instructions of the company. After PCR amplification, the reaction product was separated by electrophoresis on agarose gel (1 %) and then the PCR product image was taken (Sambrook & Rusell 2001). PCR products were sent by Macrogen Corporation – Korea, using ABI3730XL, an automated DNA sequences. The results were then emailed and analyzed with the appropriate genetic software https://www.ncbi. nlm.nih.gov/ .

III. RESULTS:

Klebsiella pneumonaie isolates which have been identified from sputum COVID-19 patients,was twenty one, included: 17 (80.9%) isolates from male patients and 4 (19%) isolates from females. An antimicrobial susceptibility test was applied on the identified 21 out of 62 (33.8%) isolates of *K. pneumonaie* towards 15 different antibiotics that belongs to different generic classes of antimicrobial antibiotics. All isolates were resistant to ampicillin (100%), followed by cefazolin (71.4%). All isolates were high sensitive to amikacin, ciprofloxacine and tigecycline approximately 100%, followed by 95.2% for levofloxacin. According to the results of antibiotics susceptibility test, this study has identified 11(52.3%) isolates that were confirmed ESPL positive by utilizing Vitek-2 system.

Among the Cephalosporins class, all the positive ESPL isolates resist to all the antimicrobial agents in this group, surprisingly. However, Cefoxitin was still effective in fighting this microbe as recorded in 19 out of 21 sputum samples. On the other hand, isolates where negative to ESPL test were found susceptible to the several agents of antimicrobial classes, except isolates (K12 and K13) that unexpectedly have shown an extensive drug resistance as both were resist to 9 antimicrobial agents. While the rest recorded less than 2 antimicrobial agent resistances. Most notably, isolated *Klebsiella* spp. found susceptible to the antibiotics Amikacin, Ciprofloxacin, and Tigecycline in all the studied samples, suggests recommending these antimicrobial agents in fighting *Klebsiella* associated with Covid-19 infections.

A DNA fragment encoding the open reading frame (1051 bp) of Cation-efflux pump FieF. It has shown successfully positive PCR band and was isolated from 10 (47.6%) strains of *K. pneumoniae* (Figure 1). Samples were then sent for sequencing, and the obtained results have shown the nucleotide acid sequences of the Cation-efflux pump FieF from the above-mentioned Thi-Qar isolates were deposited in the GenBank under the accession numbers MZ069095- MZ069104.

Multiple sequence alignment and phylogenetic neighbor-joining trees for these fragments are shown in Figure 2. Similarly, translated sequence variants have also been examined against the reference (WP-008807873.1).

IV. DISCUSSION:

The effects of sex-differences on the results from the bacteriology shows that the 100 hospitalized confirmed infected patients with Covid-19. Collected sputum samples have shown a positive bacterial growth in 44 out of 62 (70.9%) in males, whereas in females were only 18 out of 62 (29.1%), suggesting the respiratory system of men are more susceptible to secondary bacterial growth and bacterial pneumonia than women (Barbagelata *et al.*, 2020; Sharifipour *et al.*, 2020). Most importantly of the chest nosocomial infections which have been reported by this study, were found among non-elder groups in contrast to other studies highlighted advancement in age as one of the most deterministic factors in increasing the risk of death post Covid19 infections(Velavan & Meyer, 2020).

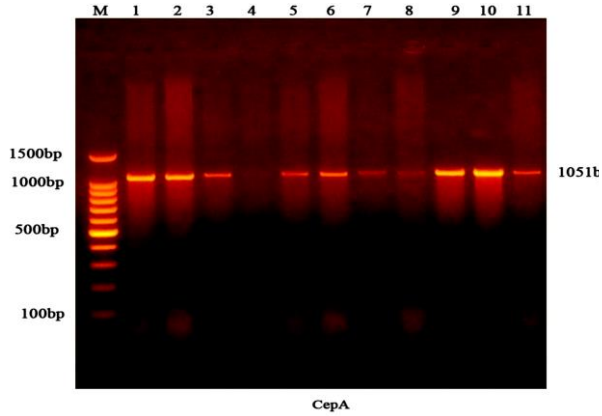

Figure 1: The amplification of CepA gene of *K. pneumoniae* (N=11) Samples were fractionated on agarose gel (1.5%) electrophoresis M: 100bp ladder marker. Lanes 1-9 resemble 1051bp PCR products.

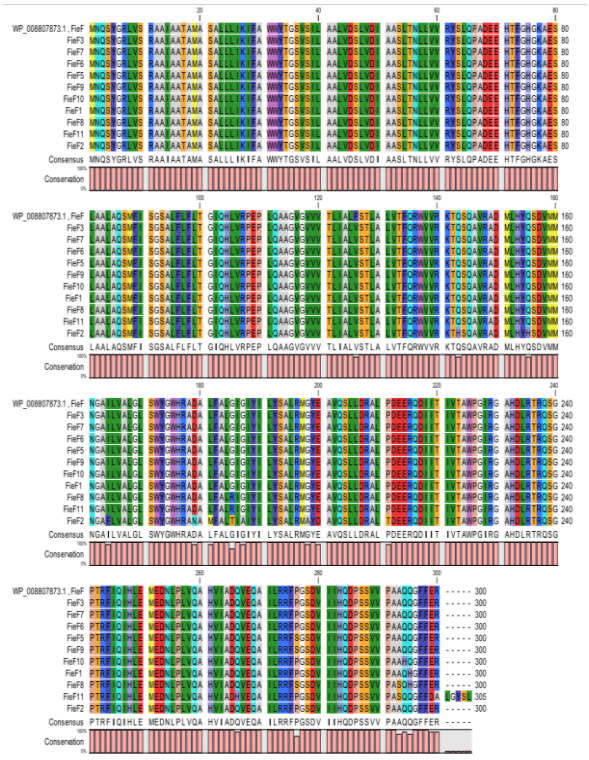

Figure 2: Multiple sequence alignment of ten Fief amino acid sequences from ten clinical isolates of *K. Pneumoniae*

Secondary bacterial infections (pneumonia) of hospitalized patients that are usually resistant to most of the antimicrobial classes are considered as serious health issue faces that are still challenges to the entire medical field so far. One of these superbugs is *K. pneumonaie*. The results have come to confirm that, 11 out of 21 samples were tested by the Vitek2 were positive ESPL. This is due to production of plasmid-borne extended-spectrum β-lactamases that can be detected in this system (Garcia-Menino *et al.*, 2021). It is worth mentioning that in most of the cases have reported that the prevalence of multidrug resistant *K. pneumoniae* in hospitals and healthcare centers post the Covid-19 pandemic were substantially higher than the pre-pandemic time in Wuhan-China and many other nations. These studies have shown a significant contribution from the MDR *K. pneumoniae* in the severity of the disease and the increased mortality rates among intubated patients (Li *et al.*, 2020; Ramadan *et al.*, 2020) .

Since 11 isolates were strongly resistant to most of the Cephalosporins agents, it is therefore essential to study the gene that is responsible to the pathogen susceptibility to antiseptics, particularly chlorhexidine, as the sequences that render *Klebsiella* spp. vulnerable to chlorhexidine were found compatible with a cation efflux pump Fief (cepA) that is associated with the locus of Cephalosporins gene resistance (Fang *et al.*, 2002). Genetic analysis on Cation-efflux pump FieF gene extracted from 11 *Klebsiella* isolates revealed multiple single nucleotide polymorphisms (SNP) that have showed significant effects on protein transcription. The PCR findings were sequenced and submitted to the Genbank as the first *K. pneumoniae* cepA (FieF) segment attributed to multidrug resistance. In details, the translated nucleotide sequences of the ten FieF revealed a complete coding sequence (cds) with 300 amino acids. The multiple sequence alignment showed the translated amino acid sequence of the ten FieF sequences along with the reference FieF amino acid sequence (WP_001262961.1). It has conferred the presence of genetic variations in the form of single nucleotide polymorphism (SNP) among the whole length of FieF protein sequences (Wand *et al.*, 2015). The phylogenetic tree based on the nucleotide sequences of FieF sequences which that recorded in this study conferred four clades. These clades were different from the four clades derived upon using the amino acid protein sequences. Finally, it is more informative to deduce the sequence variations according to the difference in amino acid sequences rather than the nucleotide sequences (Fang *et al.*, 2002).

V. CONCLUSION

The results have indicated i the need for an urgent attention to secondary infections caused by multidrug resistant *K. pneumonia*e that is attributed by COVID-19 infections.

ACKNOWLEDGMENTS

The authors thank and gratitude the medical staff of Molecular and Laboratory staff of Thi-Qar Health Department for their cooperation.

REFERENCES

Alhazzani, W., Møller, M. H., Arabi, Y. M., Loeb, M., Gong, M. N., Fan, E. (2020). Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive Care Med, 46(5), 854-887.

Barbagelata, E., Cilloniz, C., Dominedo, C., Torres, A., Nicolini, A., & Solidoro, P. (2020). Gender differences in community-acquired pneumonia. Minerva Med, 111(2), 153-165. doi:10.23736/S0026-4806.20.06448-4

Cox, M. J., Loman, N., Bogaert, D., & O'Grady, J. (2020). Co-infections: potentially lethal and unexplored in COVID-19. The Lancet Microbe, 1(1), e11.

Esper, F. P., Spahlinger, T., & Zhou, L. (2011). Rate and influence of respiratory virus co-infection on pandemic (H1N1) influenza disease. J Infect., 63(4), 260-266.

Fang, C. T., Chen, H. C., Chuang, Y. P., Chang, S. C., & Wang, J. T. (2002). Cloning of a cation efflux pump gene associated with chlorhexidine resistance in *K. pneumoniae*. Antimicrob Agents Chemother, 46(6), 2024-2028. doi:10.1128/AAC.46.6.2024-2028.2002

Garcia-Menino, I., Forcelledo, L., Rosete, Y., Garcia-Prieto, E., Escudero, D., & Fernandez, J. (2021). Spread of OXA-48-producing K. pneumoniae among COVID-19-infected patients: The storm after the storm. J Infect Public Health, 14(1), 50-52. doi:10.1016/j.jiph.2020.11.001

Haramoto, E., Malla, B., Thakali, O., & Kitajima, M. (2020). First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Science of Total Environment, 737, 140405.

Huttner, B., Catho, G., Pano-Pardo, J. R., Pulcini, C., & Schouten, J. (2020). COVID-19: do not neglect antimicrobial stewardship principles! Clin Microbiol Infect, 26(7), 808-810.

Langford, B. J., So, M., Raybardhan, S., Leung, V., Westwood, D., MacFadden, D. R. (2020). Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect.

Li, T., Li, Z., Deans, E. E., Mittler, E., Liu, M., Chandran, K. (2021). The shape of pleomorphic virions determines resistance to cell-entry pressure. Nat Microbiol, 6(5), 617-629. doi:10.1038/s41564-021-00877-0

Mahon CR, Manuselis G, (2000) editors. Textbook of diagnostic microbiology. WB

Marquès, M., & Domingo, J. L. (2021). Contamination of inert surfaces by SARS-CoV-2: Persistence, stability and infectivity. A review. Environmental Research, 193, 110559. doi:10.1016/j.envres.2020.110559.

Martín-Loeches, I., Sanchez-Corral, A., Diaz, E., Granada, R. M., Zaragoza, R., Villavicencio, C. (2011). Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A (H1N1) virus. Chest, 139(3), 555-562.

Ramadan, H. K., Mahmoud, M. A., Aburahma, M. Z., Elkhawaga, A. A., El-Mokhtar, M. A., Sayed, I. M. (2020). Predictors of Severity and Co-Infection Resistance Profile in COVID-19 Patients: First Report from Upper Egypt. Infect Drug Resist, 13, 3409-3422. doi:10.2147/IDR.S272605

Sambrook J, Rusell DW (2001). Vectors and strains. Molecular cloning. 3:A3.

Shah, N. S., Greenberg, J. A., McNulty, M. C., Gregg, K. S., Riddell IV, J., Mangino, J. E., et al. (2016). Bacterial and viral co-infections complicating severe influenza: Incidence and impact among 507 US patients, 2013–14. J Clin Virol, 80, 12-19.

Sharifipour, E., Shams, S., Esmkhani, M., Khodadadi, J., Fotouhi-Ardakani, R., Koohpaei, A. (2020). Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis, 20(1), 646. doi:10.1186/s12879-020-05374-z

Tahamtan, A., & Ardebili, A. (2020). Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Molecul Diagn, 20(5), 453-454. doi:10.1080/14737159.2020.1757437

Uyeki, T. M., Bundesmann, M., & Alhazzani, W. (2020). Clinical management of critically ill adults with coronavirus disease 2019 (COVID-19).

Velavan, T. P., & Meyer, C. G. (2020). The COVID-19 epidemic. Trop Med Int Health, 25(3), 278-280. doi:10.1111/tmi.13383

Wand, M. E., Baker, K. S., Benthall, G., McGregor, H., McCowen, J. W., Deheer-Graham, A. (2015). Characterization of pre-antibiotic era *Klebsiella pneumoniae* isolates with respect to antibiotic/disinfectant susceptibility and virulence in Galleria mellonella. Antimicrob Agents Chemother, 59(7), 3966-3972. doi:10.1128/AAC.05009-14