مجلة دجلة • المجلد ٦ ،العدد ٤ (٢٠٢٣)، ص: ١٠ - ١٧
ISSN: 2222-6583

Antibiotic Susceptibility and Biofilm Formation of *Klebsiella pneumoniae*

Ahad A. Al-Majeed Al-Dulaymi[1], Hasan A. Aal Owaif [2]
[1] College of Biotechnology, Al-Nahrain University, Baghdad, Iraq
Ahad.Abdulmajeed21@ced.nahrainuniv.edu.iq
[2] Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq
hasan.abdulhadi@nahrainuniv.edu.iq

Abstract
Klebsiella pneumoniae is considered the second cause pathogenic in Enterobacteriaceae after *E. coli*. Also, medically recognized as one of the important opportunistic pathogens that causing worldwide Healthcare-associated infections. In this study 25 samples of *Klebsiella pneumoniae* collected from different clinical cases was provided by CAC center in Baghdad, Iraq. The isolates were identified by VITEK2 system. The results showed that all 25 sample (100%) were *K. pneumoniae*. The present study shows that (100%) of the *K. pneumoniae* isolates have the ability to form biofilms and they were divided into three groups: strong biofilm-producer (11 isolate, 44%), moderate biofilm-producer (11 isolate, 44%) and weak biofilm-producer (3 isolate, 12%). Also this study shows that 25 sample of *K. pneumoniae* highly resistant against Ampicillin, Amoxicillin-Clavulanic acid, Aztreonam, Doxycycline, Cefotaxime, Tetracycline, Erythromycin, Ceftazidime in the rate of (n=25, 100%) except Ceftriaxone (n=23, 92%), while it shows high Sensitivity against Levofloxacin (n=22, 88%), Piperacillin-Tazobactam (n=23, 92%), Ciprofloxacin (n=24, 96%), Amikacin, Gentamicin and Imipenem shows sensitivity in rate (n=25, 100%) for all.

Key words: *K. pneumoniae*, Biofilm, Antibiotic Resistance.

1.Introduction

Klebsiella pneumoniae is a significant cause of severe morbidity and mortality, particularly in immunocompromised patients, kids, and newborns (Attia *et al*., 2023). Their weakened immune systems, frequent use of medications, and intrusive equipment all add to their susceptibility to infections. Healthcare workers' hands act as reservoirs for disease transmission (Gupta, 2002). *K. pneumoniae* infections that are resistant to various antibiotics have become a severe public health issue in recent decades, making these infections particularly difficult to treat. The continuous horizontal transmission of antimicrobial resistance genes via mobile elements is essential for *K. pneumoniae* to survive in the hospital environment (Navon-Venezia *et al*., 2017). *K. pneumoniae's* ability to form biofilms on the surfaces of medical devices and diseased tissues is thought to be one of the virulence factors required for pathogenesis, allowing the bacteria to persist for long periods despite immune system responses and antimicrobial therapy. A biofilm is a kind of polymerization in which bacteria adhere to inert or active surfaces through extracellular polymeric substances (EPS) (Li and Ni, 2023; Ashwath *et al*., 2022). Polysaccharides, proteins, nucleic acids, lipids, and extracellular DNA (eDNA) constitute

the majority of the EPS (Bertoglio *et al*., 2018). *K. pneumoniae* biofilm polysaccharides are made up of mannose, glucose and amines. Due to the variability of the environment, the expression of proteins in biofilms differs. Although the kinetics of biofilm development varies amongst strains, the process of biofilm formation remains consistent, including initial adhesion, micro-colony production, maturation, and dispersion (Al-Bayati and Samara-Singhe, 2022). Many bacterial activities are required for each phase, including exercise, adhesion, transport, stress response, metabolic pathway activation, and extracellular matrix formation (Domka *et al*., 2007).The biofilm matrix provides biofilms with overall characteristics that can protect resident cells from desiccation, chemical interference, and other bacterial invasions. Furthermore, biofilms help bacterial cells in avoiding death by the human phagocytic system and ensuring that the biofilm community adheres to the medium surface (Yan and Bassler, 2019). It is expected that bacterial biofilms are 10-1,000 times more resistant to antimicrobial drugs than planktonic bacteria (De la Fuente-Núñez *et al*., 2016). Furthermore, the bacteria in these stable communities are frequently in close proximity to one another, increasing the possibility of chemical signal transduction and gene transfer between bacterial cells of the same or different species (Koraimann and Wanger, 2014). This increases the conditions needed for the propagation of drug resistance genes. According to a previous study, biofilm production was involved in 65-80% of bacterial infections (Al-Bayati and Samara-Singhe, 2022). Most serial isolates obtained from individuals with recurrent infections were shown to be strong biofilm producers in vitro (Sanchez *et al*., 2013). Antibiotic resistance manifests itself in biofilm-producing bacteria via a number of strategies, including limits antibiotic penetration into the complex biofilm structure, slower bacterial growth inside the biofilm, and resistance gene exchange. Bacterial biofilms additionally present a substantial danger of spreading between patients and across the hospital environment (Percival *et al.*, 2015).

2.3 Antibiotic Susceptibility test

Kirby-Bauer method was used perform the antibiotic susceptibility test for 15 different antibiotics including: (Amikacin (30µg), Ampicillin (10µg), Amoxicillin-Clavulanic acid (30µg), Aztreonam (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Doxycycline (30µg), Erythromycin (15µg), Gentamicin (10µg), Imipenem (10µg), Levofloxacin (5µg), Piperacillin-Tazobactam (100/10 µg) and Tetracycline (30µg). the suspension of bacteria was prepared by taking a few single colonies of each isolate into 5ml of normal saline to make turbidity equals to 0.5Mcfarland standards ($1.5x10^8$ CFU/ml). A sterile cotton swab was merged in this bacterial suspension and used to spread on nutrient agar medium then it was left for 10 min to dry. The antimicrobial discs were placed on the agar with sterile forceps pushed firmly to confirm contact with the agar. Then, the plates were incubated for 24h at 37°C. After incubation, inhibition zone around each disc was measured in millimeter (mm)

using a metric ruler and the results were interpreted according to Clinical Laboratories Standards Institute (CLSI, 2022).

3. Results

3.1 Identification of *K. pneumoniae*

The results of the conformation identification by VITEK2 system of the 25 isolate shows that (25 isolate, 100%) are related to *K. pneumoniae.*

3.2 Biofilm formation of *K. pneumoniae*

The 96-well microtiter plate was used to detect the ability of *K. pneumoniae* to form biofilm. The method was considered a quantitative biofilm assay based on the production of exopolysaccharide. The results revealed that (25 isolate, 100%) have the ability to form the biofilm in different quantities under the same experimental conditions as shown in figure1.

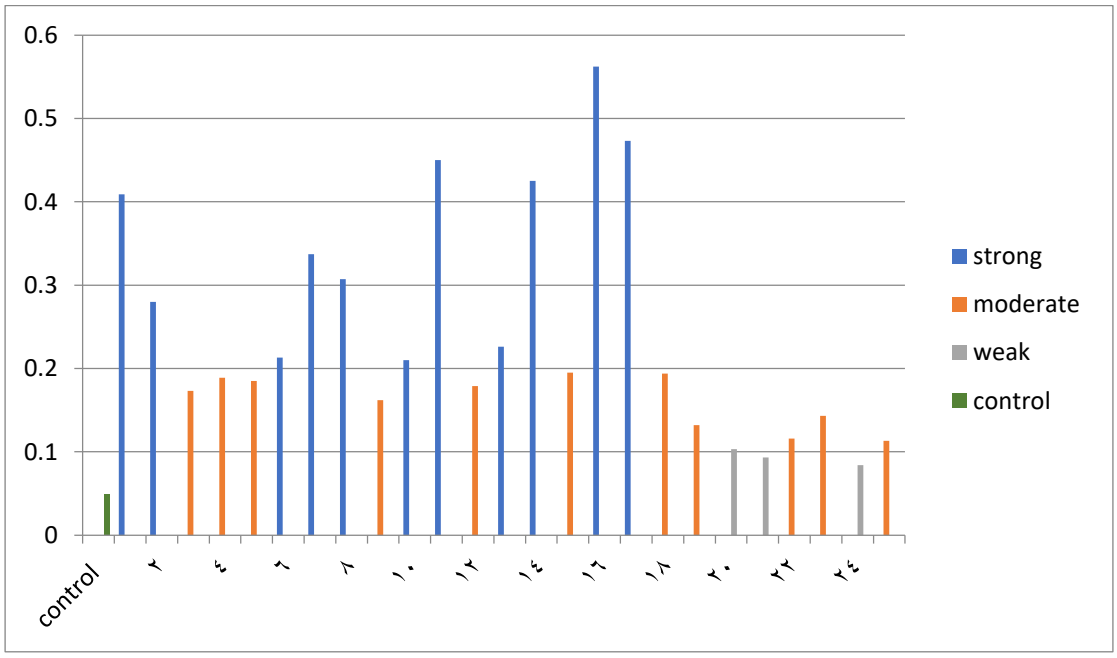

Figure 1: Biofilm formation O.D values of *K.pneumoniae*.

These 25 isolate were divided into three groups, strong biofilm-producers (11 isolate, 44%), moderate biofilm-producers (11 isolate, 44%) and weak biofilm producers (3 isolates, 12%) as shown in table 1.

Table 1: Biofilm formation by *K. pneumoniae* isolates.

Biofilm	No. of isolates	The percentage (%)
Strong biofilm	11	44%
Moderate biofilm	11	44%
Weak biofilm	3	12%

3.3 Antibiotic sensitivity test

An antibiotic susceptibility test was performed for *K. pneumoniae* the results showed that *Klebsiella pneumoniae* (n=25) had (25 isolate, 100%) resistant to Ampicillin, Amoxicillin-

Clavulanic acid, Aztreonam, Doxycycline, Cefotaxime, Tetracycline, Erythromycin and Ceftazidime, while (23 isolate, 92%) were resistant to Ceftriaxone as shown in figure 2.

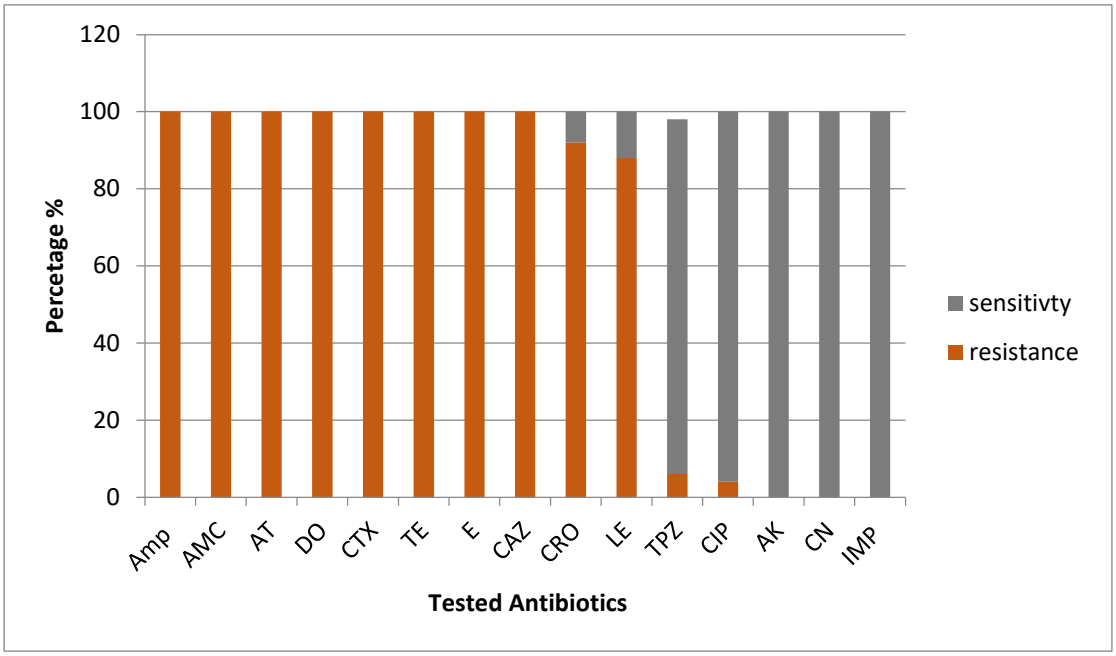

Figure 2: percentage of antibiotic resistance and sensitivity patterns of *K. pneumoniae.*

Whilst these 25 sample of *K. pneumoniae* shows high sensitivity against Levofloxacin (22 isolate, 88%), piperacillin-tazobactam (23 isolate, 92%), Ciprofloxacin (24 isolate, 96%) and Amikacin, Gentamicin and Imipenem shows sensitivity in the rate of (25 isolate, 100%) as shown in figure 3.

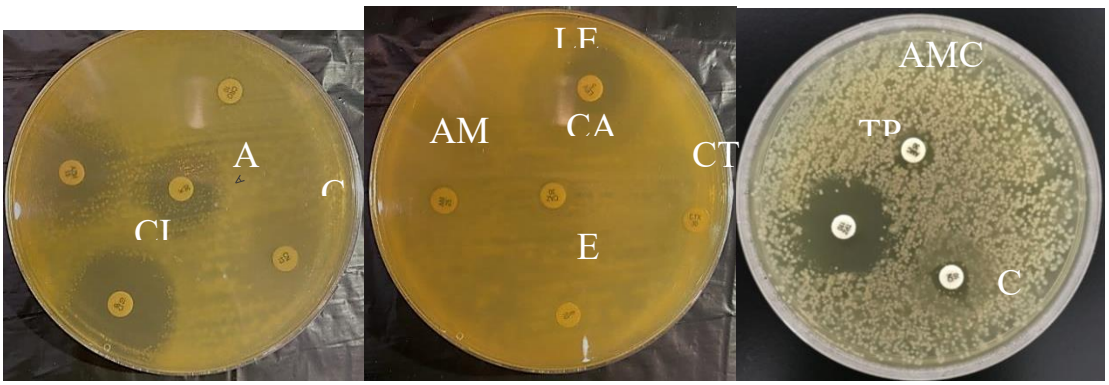

Figure 3: shows the antibiotic resistance and sensitivity of *K. pneumoniae.*

Discussion

In this study the results of biofilm formation by *K. pneumoniae* isolates were agreed with the findings of another studies for example these results were submitted by Haghighifar *et al*., (2021) and Nirwati *et al*., (2019) that reported that the biofilm- producing isolates were (100%) and (85.63%) respectively ; while another study in Iraq by Abbas *et al*., (2020) showed that the ratio of biofilm- producing isolates was (6.6%). This study found considerably of phenotypic antibiotic-resistant *K. pneumoniae*, which is cause for concern. Antibiotic resistance is an outcome of unregulated and excessive antibiotic use (Lateef, 2004). B-lactams are the primary type of treatment for *K. pneumoniae* infections. The 100% resistance rate to Ampicillin and Amoxicillin-Clavulanic acid, a beta-lactam, matches up with previous research that revealed

increased resistance to Ampicillin. (Penes *et al*., 2017; Lagha *et al*., 2021; Ranjbar and Chehelgerdi, 2019 and Cepas *et al*., 2019). Whereas Monobactam antibiotics for example Aztreonam (which is a subgroup of beta-lactam antibiotics, wherein the beta-lactam ring alone and not fused to another ring which works only against aerobic gram-negative bacteria) shows high rate of resistance in *K. pneumoniae* isolates reach to (100%) in this study and this results agreed with another study by Wilhelm *et al*., (2023) who tested 22 isolate of *K. pneumoniae* and discovered that 19 isolate (86%) were resistant to Aztreonam (Wilhelm *et al*., 2023). Aminoglycosides inhibit protein synthesis by attaching to the bacterial ribosome. Since its introduction, aminoglycoside intake has been regulated due to long-term usage being linked to kidney and auditory nerve damage, leading to hearing impairment (Goñi-Urriza *et al*., 2000). In agreement with these findings, *K. pneumoniae* exhibited weak resistance to gentamicin and amikacin when compared to earlier research (Fernández-Martinez *et al*., 2018; Yang *et al*., 2020). Because of its infrequent use, aminoglycoside resistance may be low in this study. In this study, *K. pneumoniae* isolates don't show any resistance against carbapenems. Similar to a study in Saudi Arabia resistance to Imipenem (3.4%) was the weakest, while Imipenem was found to be the most effective antibiotic against *K. pneumoniae* at a ratio of (100%) in another Iraqi study by Raouf (2022) (Ngoi *et al*., 2021; Raouf *et al*., 2022). Meanwhile, cephalosporin resistance was discovered 100% of the isolates were resist to this type, including Ceftazidime, Cefotaxime and Ceftriaxone. This agreed with another studies for example Raouf, 2022 found that 97% of *K. pneumoniae* resistant to cefotaxime, while shalash and Tuwajj, 2023 found that 100% of *K. pneumoniae* isolates were resistant against ceftriaxone (Shlash and Tuwaij, 2023) , and the results of Ceftazidime was matched with the results of Meng *et al*., 2019 who found 94% of *K. pneumoniae* isolates were resistant to this one (Meng *et al*., 2019; Hasan *et al*., 2020). High resistance to tetracycline and doxycycline (100%) for both were observed in this study. The increased resistance rate of these antibiotics classes in this study could be attributed to common drug abuse in health care. The susceptibility rate against fluoroquinolones for example ciprofloxacin 96% and Levofloxacin 88% is similar to the findings of another reports, Ghanem *et al*., 2017 found that 3.9% of *K. pneumoniae* isolates were resistant against ciprofloxacin, while Akter *et al*., 2019 found that 84% of isolates were sensitive against Levofloxacin. Macrolide antibiotics which effects on the protein synthesis of the bacterial cell in this study shows high resistance rate (100%) in *K. pneumonaie* isolates which agrees with another studies Bokaeian *et al*., 2014 found that (70%) of *K. pneumoniae* isolates were resistant against this antibiotic while, Safika *et al*., 2022 found that the resistance rate against these isolates were (100%). The widespread usage of antimicrobial agents has resulted in a high incidence of MDR *K. pneumoniae* strains (Ciccozzi *et al*., 2019). The present research reveals multidrug resistance to various antimicrobials more than three antibiotic classes.

Refrences

Abbas, O. N.; Mhawesh, A. A. and Al-shaibani, A. B. (2020). Molecular identification of pathogenic Klebsiella pneumoniae strains producing biofilm. Medico-Legal update, 20(3): 1068-1074.

Akter, T., Khan, S., Nahar, S., Fatema, K., Shaha, M., Hossain, M. J., Sultana, H. and Karim, S.U. (2019). Determination of prevalence and antimicrobial sensitivity patterns of Klebsiella

pneumoniae from sputum sample of a tertiary care hospital. International Medical Journal, 24(3): 29-35.

Al-Bayati, M. and Samara-singhe, S. (2022). Biofilm and gene expression characteristics of the Carbapenem-resistant Enterobacterales, Escherichia coli IMP, and Klebsiella pneumoniae NDM-1 associated with common bacterial infections. Int. J. Environ. Res. Public Health 19:4788. Doi: 10.3390/ijerph19084788.

Ashwath, P., Deekshit, V. K., Rohit, A., Dhinakaran, I., Karunasagar, I., Karunasagar, I., *et al*. (2022). Biofilm formation and associated gene expression in multidrug-resistant Klebsiella pneumoniae isolated from clinical specimens. Curr. Microbiol. 79:73. Doi: 10.1007/s00284-022-02766-z.

Attia, N., Al-Ghazzawi, E., El-Khwsky, F., Metwally, D. and Ramadan, A. (2023). *Klebsiella pneumoniae* isolated from an Egyptian pediatric hospital: Prevalence, antibiotic resistance, biofilm formation, and genotyping. Microbes and Infectious Diseases, 4(3). Doi: 10.21608/mid.2023.209787.1520.

Bertoglio, F., Bloise, N., Oriano, M., Petrini, P., Sprio, S., Imbriani, M., *et al*. (2018).Treatment of biofilm communities: an update on new tools from the Nano sized world. Appl. Sci. 8:845. Doi: 10.3390/app8060845.

Bokaeian, M., Saeidi, S., Shahi, Z. and Kadaei, V. (2014). tetA and tetB genes in Klebsiella pneumoniae isolated from clinical samples. Brief lands, 1(2), e18152.

Cepas, V., Lopez, Y., Munoz, E., Rolo, D., Ardanuy, C., Marti, S., Xercavins, M., Horcajada, J. P., Bosch, J. and Soto, S. M. (2019). Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. Microb. Drug. Resist. 25(1):72-9.

Ciccozzi, M.; Cella, E.; Lai, A.; De Florio, L.; Antonelli, F.; Fogolari, M.; Di Matteo, F.M.; Pizzicannella, M.; Colombo, B.; Dicuonzo,G.; *et al*. (2019). Phylogenetic Analysis of Multi-Drug Resistant Klebsiella pneumoniae Strains From Duodenoscope Biofilm: Microbiological Surveillance and Reprocessing Improvements for Infection Prevention. Front. Public Health, 7, 219.

CLSI. (2022). Performance Standards for Antimicrobial Susceptibility Testing, M100 (34 Edition). Clinical and Laboratory Institute.

Domka, J., Lee, J., Bansal, T., and Wood, T. K. (2007). Temporal gene-expression in Escherichia coli K-12 biofilms. Environ. Microbiol. 9, 332–346.

de la Fuente-Núñez, C., Cardoso, M. H., de Souza Cândido, E., Franco, O. L., and Hancock, R. E. W. (2016). Synthetic antibiofilm peptides. Biochim. Biophys. Acta 1858, 1061–1069.

Fernández-Martínez, M.; Ruiz Del Castillo, B.; Lecea-Cuello, M.J.; Rodríguez-Baño, J.; Pascual, Á.; Martínez-Martínez, L. (2018). Prevalence of Aminoglycoside-Modifying Enzymes in Escherichia coli and Klebsiella pneumoniae Producing Extended Spectrumβ-Lactamases Collected in Two Multicenter Studies in Spain. Microb. Drug Resist, 24, 367–376.

Ghanem, S., El-Shafey, H. M., El-Kelani, A. T. and Manzoor, N. (2017). Antimicrobial resistance patterns of Klebsiella isolates from clinical samples in a Saudi hospital. African Journal of Microbiology Research, 11(23), 965-971.

Goñi-Urriza, M.; Capdepuy, M.; Arpin, C.; Raymond, N.; Pierre Caumette, C.Q. (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp. Appl. Environ. Microbiol, 66, 125–132.

Gupta, A. (2002). Editor Hospital-acquired infections in the neonatal intensive care unit-*Klebsiella pneumoniae*. Seminars in perinatology, Elsevier.

Haghighifar, E., Norouzi, F. and Kamali, R. (2021). Molecular detection of extended- spectrum β-lactamase (ESBLs) and biofilm formation in uropathogens Klebsiella pneumoniae in Iran. Medical Journal of the Islamic Republic of Iran, 35, 72.

Hasan, M.; Hossain, M.K.; Rumi, N.A.; Rahman, M.S.; Hosen, M.A. (2020). Isolation and characterization of multiple drug-resistant bacteria from the waste of hospital and non-hospital environment. Asian J. Med. Biol. Res, 6, 460–468.

Kirmusaoğlu, S. (2019). The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents, Antimicrobials, Antibiotic Resistance, Antibiofilm strategies and activity methods. Intech Open, UK.

Koraimann, G. and Wagner, M. A. (2014). Social behavior and decision making in bacterial conjugation. Front. Cell. Infect. Microbiol. 4:54.

Lagha, R.; Ben Abdallah, F.; ALKhammash, A.A.H.; Amor, N.; Hassan, M.M.; Mabrouk, I.; Alhomrani, M.; Gaber, A. (2021). Molecular characterization of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from King Abdulaziz Specialist Hospital at Taif City, Saudi Arabia. J. Infect. Public Health, 14, 143–151.

Lateef, A. (2004). The microbiology of a pharmaceutical effluent and its public health implications. World J. Microbiol. Biotechnol, 20, 167–171.

Li, Y. and Ni, M. (2023). Regulation of biofilm formation in Klebsiella pneumoniae. Frontiers in Microbiology, 14. Doi: 10.3389/fmicb.2023.1238482.

Meng, X., Yang, J., Duan, J., Liu, S., Huang, X., Wen, X., Huang, X., Fu, C., Li, J., Dou, Q., Liu, Y., Wang, J., Yan, Q., Zou, M., Liu, W., Peng, Z., Chen, L., Li, C. and Wu, A. (2019). Assessing Molecular Epidemiology of Carbapenem-resistant Klebsiella pneumoniae CRKP with MLST and MALDI-TOF in central china. Scientific Reports, 9(1): 2271.

Navon-Venezia, S., Kondratyeva, K. and Carattoli, A. (2017). *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS microbiology reviews, 41(3):252-75.

Ngoi, S.T.; Teh, C.S.J.; Chong, C.W.; Abdul Jabar, K.; Tan, S.C.; Yu, L.H.; Leong, K.C.; Tee, L.H.; AbuBakar, S. (2021). In vitro efficacy of flomoxef against extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae associated with urinary tract infections in Malaysia. Antibiotics, 10, 181.

Nirwati, H., Sinanjung, K., Fahrunissa, F., Wijaya, F., Napitupulu, S., Hati, V.P., Hakim, M.S., Meliala, A., Aman, A.T. and Nuryastuti, T. (2019). Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klatan, Indonesia. BMC proceedings, 13(11):20.

Penes, N.O.; Muntean, A.A.; Moisoiu, A.; Muntean, M.M.; Chirca, A.; Bogdan, M.A.; Popa, M.I. (2017). An overview of resistance profiles ESKAPE pathogens from 2010–2015 in a tertiary respiratory center in Romania. Rom. J. Morphol. Embryol, 58, 909–922.

Percival, S.L., Suleman, L., Vuotto, C., Donelli, G. (2015). Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of medical microbiology, 64(4):323-34.

Ranjbar, R. and Chehelgerdi, A.F.K.M. (2019). Molecular characterization, serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the Klebsiella pneumoniae strains isolated from different types of hospital-acquired infections. Infect. Drug Resist, 12, 603–611.

Raouf, F.E.A., Benyagoub, E., Al-Khudhairy, M.K., Akrami, S., and Saki, M. (2022). Extended-spectrum beta-lactamases among Klebsiella pneumoniae from Iraqi patients with community-acquired pneumonia. Rev. Assoc. Med. Bras., 68, 833-837.

Sanchez, C. J., Mende, K., Beckius, M. L., Akers, K. S., Romano, D. R., Wenke, J. C.,et al. (2013). Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infect. Dis. 13:47.

Shlash, A.A.A.A. and Tuwaij, N.S.S. (2023). Molecular investigation of Ambler class A and C β-lactamase among Ceftriaxone resistant Klebsiella pneumoniae infections in Najaf city, Iraq. Biochem. Cell. Arch, 18(2): 2511-2522.

Wilhelm, C. M., Inamine, E., Martins, A. F. and Barth, A. L. (2023). Evaluation of Aztreonam and Ceftazidime/ Avibactam synergism against Klebsiella pneumoniae by MALDI-TOFMS. Antibiotics, 12(6): 1063.

Yan, J., and Bassler, B. L. (2019). Surviving as a community: antibiotic tolerance and persistence in bacterial biofilms. Cell Host Microbe 26, 15–21.

Yang, Y.; Peng, Y.; Jiang, J.; Gong, Z.; Zhu, H.; Wang, K.; Zhou, Q.; Tian, Y.; Qin, A.; Yang, Z.; et al. Isolation and characterization of multidrug-resistant *Klebsiella pneumoniae* from raw cow milk in Jiangsu and Shandong provinces, China. Trans bound. Emerg.Dis. 2020, 68, 1033–1039.