Microbes, Infection and Chemotherapy

ORIGINAL RESEARCH

https://doi.org/10.54034/mic.e2160

Detection of virulence factors and antimicrobial susceptibility pattern of clinically significant *Klebsiella pneumoniae* isolates in a tertiary care hospital.

Author: Smrithi Surendranath[1,*], Amritha Sabitha-Kurup[1], Reena John[2]

Abstract

Introduction: *Klebsiella pneumoniae* is associated with a variety of infections across all age groups, both in community and hospital settings. Many strains of *K. pneumoniae* produce virulence factors such as a capsule, siderophores, biofilm, and hypermucoviscosity, which facilitate adhesion, invasion, and colonization. Additionally, the rising antibiotic resistance in *K. pneumoniae* strains poses a significant challenge, limiting treatment options. This study aims to identify the virulence factors and assess the antimicrobial susceptibility of clinically significant *K. pneumoniae* isolates. **Materials and Methods**: *K. pneumoniae* isolates were obtained from urine, sputum, blood, cerebrospinal fluid, pus swabs/aspirates, and tissue samples. Standard biochemical tests were performed for identification. Capsule formation, hypermucoviscosity, siderophore production, and biofilm formation were detected phenotypically. Antibiotic susceptibility testing was carried out using the disk diffusion method, and primary and confirmatory tests were conducted for extended-spectrum beta-lactamases (ESBL) and carbapenemase production. **Results**: Capsule formation, hypermucoviscosity, siderophore production, and biofilm formation were detected in 100%, 15%, 40%, and 15% of the isolates, respectively. Of the isolates, 40% were multidrug-resistant, 55% produced ESBL, 8% expressed AmpC beta-lactamase, and 10% produced carbapenemase. **Conclusion**: Capsule formation was the most prevalent virulence factor, followed by siderophore production. The majority of *K. pneumoniae* isolates demonstrated susceptibility to amikacin, piperacillin-tazobactam, and carbapenems. However, 40% of the isolates were multidrug-resistant, with ESBL production being the most common resistance mechanism.

Keywords: Biofilm, ESBL, *K.pneumoniae*, Virulence factors.

[1]Assistant Professor, KMCT Medical College, Kozhikode, Kerala, India.
[2]Professor and Head, Amala Institute of Medical Sciences, Thrissur, Kerala, India.

*https://orcid.org/0000-0002-6116-395X

Corresponding author:
Smrithi Surendranath
Address: Kozhikode, Kerala, India.
E-mail: snath4496@gmail.com

Submitted: april 30, 2024
Reviewed: june 28, 2024
Approved: october 03, 2024

How to cite: Surendranath S, Sabitha-Kurup A, John R. Detection of virulence factors and antimicrobial susceptibility pattern of clinically significant Klebsiella pneumoniae isolates in a tertiary care hospital.. Microbes Infect Chemother. 2024; 4: e2160. https://doi.org/10.54034/mic.e2160

Introduction

Klebsiella pneumoniae is a significant gram-negative pathogen responsible for 75%-86% of clinical Klebsiella infections. Its capacity to cause severe infections, coupled with the growing shortage of effective treatments, has made it increasingly notorious. One of its hallmark infections is primary pulmonary pneumonia, characterized by thick, brick-red, "currant jelly" sputum, resulting from extensive necrosis and hemorrhage. Carrier rates in the oropharynx of healthy individuals range from 1% to 6%, increasing to 20% in hospitalized patients (1). This colonization can serve as a source of lung infections, particularly in individuals with underlying conditions such as alcoholism, diabetes mellitus, and chronic obstructive pulmonary disease. Notably, *K. pneumoniae* has emerged as a leading cause of community-acquired liver abscess in Asia and among Asian migrants in Western countries (2).

K. pneumoniae exhibits various virulence factors that contribute to its pathogenicity, including capsule formation, fimbriae, hypermucoviscosity, siderophore production, and biofilm formation. These factors enable the bacterium to evade host immune defenses and establish chronic, multidrug-resistant infections. Hypervirulent strains of *K. pneumoniae* (HVKp) are particularly concerning, as they infect both healthy and immunocompromised individuals and are highly invasive (1).

The evolving antimicrobial resistance patterns of *K. pneumoniae* have underscored the need for a deeper understanding of its interaction with the host. Strains exhibiting hypervirulence or multidrug resistance have acquired additional genetic traits, further complicating treatment (2). Among these, extended-spectrum beta-lactamase (ESBL)-producing strains were once confined to nosocomial infections but are now increasingly isolated from

community settings. Infections caused by ESBL-producing bacteria are associated with more severe clinical outcomes, delayed therapeutic responses, prolonged hospital stays, higher healthcare costs, and increased mortality. Additionally, there has been a notable rise in infections caused by carbapenem-resistant *K. pneumoniae* (CR-Kp) since its initial detection in 1996. CR-Kp's resistance to penicillins, cephalosporins, and carbapenems presents a significant treatment challenge, with limited therapeutic options (3).

Despite *K. pneumoniae* being identified over a century ago, there remains a lack of comprehensive studies on many of its virulence factors, and aspects of its pathogenesis remain unclear. The increasing prevalence of *Klebsiella*-associated infections, the emergence of new clinical syndromes, and escalating antimicrobial resistance necessitate further research. This study was conducted over a one-year period at a tertiary care teaching hospital in Thrissur, Kerala, to investigate the virulence factors and antimicrobial susceptibility patterns of clinically significant *K. pneumoniae* isolates, contributing to the development of effective management strategies and antibiotic protocols for this region.

Methods

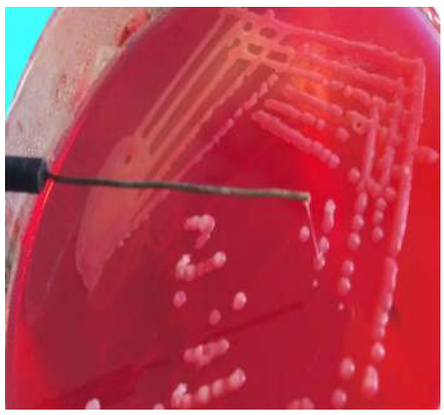
TA cross-sectional study was conducted over one year in the Department of Microbiology, Government Medical College, Thrissur, from March 2017 to February 2018. The sample size was calculated based on previous microbiology records from the institution, resulting in 100 samples. Institutional ethics committee approval was obtained, and informed consent was collected prior to isolate sampling. Clinically significant *Klebsiella pneumoniae* isolates were obtained from urine, sputum, blood, cerebrospinal fluid (CSF), pus swabs/aspirates, and tissue samples from inpatients, and were identified using standard microbiological methods. Isolates were considered "clinically significant" if they were obtained in pure culture or as part of mixed cultures with appropriate clinical history and symptoms. Repeat isolates from the same patient were excluded.

Upon receipt of samples, their macroscopic appearance was noted, followed by Gram staining. The samples were inoculated onto blood agar and MacConkey agar plates and incubated overnight at 37°C. The plates were examined at 24 and 48 hours, and identification was carried out using biochemical tests.

Capsule Detection: Capsule detection was performed using negative staining with India ink (4).

Hypermucoviscosity Test: Hypermucoviscosity was detected by using a standard inoculation loop to touch the surface of an isolated colony from an 18-24-hour incubation plate. A string of >5 mm indicated a hypermucoviscosity-positive phenotype (Figure 1) (5).

Figure 1: String test for hypermucoviscosity



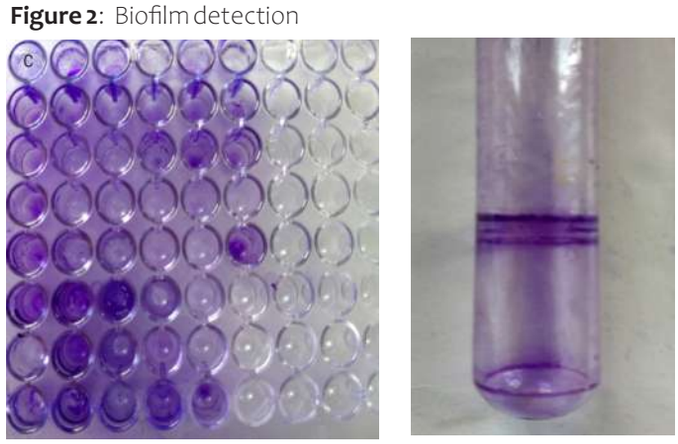
Siderophore Production: Siderophore production was detected by streaking bacterial isolates on iron-restricted agar medium, prepared by supplementing nutrient agar with 200 mM 2,2'-dipyridyl. Bacterial growth after incubation at 37°C for 24 hours was considered positive for siderophore production (4).

Biofilm Detection:

Tissue Culture Plate Method: Isolates were inoculated into brain heart infusion broth and incubated at 37°C for 18-24 hours. The cultures were diluted (1:100) with fresh broth and transferred into 96-well polystyrene tissue culture plates. The plates were incubated at 37°C for 24 hours, followed by washing with phosphate-buffered saline (pH 7.2). The adherent biofilm was fixed using 2% sodium acetate, stained with 0.1% crystal violet, and the excess stain was washed off with distilled water. The optical density (OD) of the stained biofilm was measured at 630 nm using an ELISA reader. A mean OD >0.240, 0.120-0.240, and <0.120 indicated strong, moderate, and weak biofilm production, respectively (6).

Tube Adherence Method: Isolates were inoculated into 5 mL of brain heart infusion broth (BHIB) in sterile test tubes and incubated at 37°C for 48 hours. After incubation, the supernatants were discarded, and the tubes were stained with 0.1% crystal violet. Tubes were then washed with distilled water and dried. The presence of a stained layer adhered to the inner wall indicated a positive biofilm result (Figure 2) (6).

Figure 2: Biofilm detection

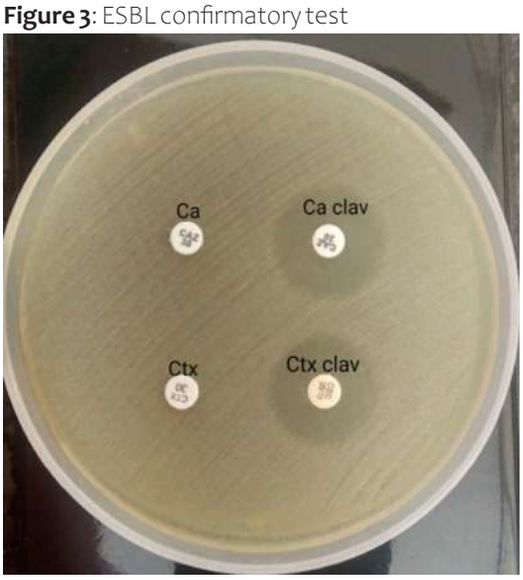


a. Microtitre plate method b. Tube method

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included ampicillin, cefazolin, ceftriaxone, cefepime, amikacin, gentamicin, ciprofloxacin, cotrimoxazole, piperacillin-tazobactam, imipenem, and nitrofurantoin (for urine isolates only).

ESBL Screening and Confirmation: ESBL screening was done using the Kirby-Bauer disc diffusion technique with cefpodoxime (10 µg), ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). Resistance was indicated by an inhibition zone ≤17 mm for cefpodoxime, ≤22 mm for ceftazidime, ≤27 mm for aztreonam, ≤27 mm for cefotaxime, and ≤25 mm for ceftriaxone. Confirmation was performed using the standard disk diffusion method with ceftazidime (30 µg) and cefotaxime (30 µg), each alone and in combination with clavulanate (10 µg). A ≥5 mm increase in the inhibition zone for the antibiotic when combined with clavulanate indicated ESBL production (Figure 3) (7).

Figure 3: ESBL confirmatory test



Ca-Ceftazidime, Ca clav – Ceftazidime clavulanate, Ctx – Cefotaxime, Ctx clav – Cefotaxime calvulanate

AmpC Beta-lactamase Detection: Screening for AmpC beta-lactamase was done using cefoxitin (30 µg). An inhibition zone ≤18 mm indicated AmpC beta-lactamase production. Confirmation was done using the AmpC disc test.

Carbapenemase Screening and Confirmation: Carbapenemase screening was performed using the imipenem disk diffusion method. A zone diameter ≤19 mm was considered positive for carbapenemase. Confirmation was done using the Modified Carbapenemase Inactivation Method (7).

After coding, the data were entered into a Microsoft Excel spreadsheet (version 10). Descriptive analysis was presented using frequencies and percentages.

Results

100 isolates of *K.pneumoniae* from inpatients with significant clinical history were taken out of which 34 were urine samples, 22 were from sputum, 16 were aspirates from wound infections , 15 were pus swabs, 7 were from blood culture, 5 from tissues of diabetic foot ulcer and 1 was from CSF.

Capsule was the most common virulence factor (100%) which was found in all isolates followed by siderophore production (40%).15% of the isolates showed hypermuco-viscosity and 15% of the isolates had biofilm.

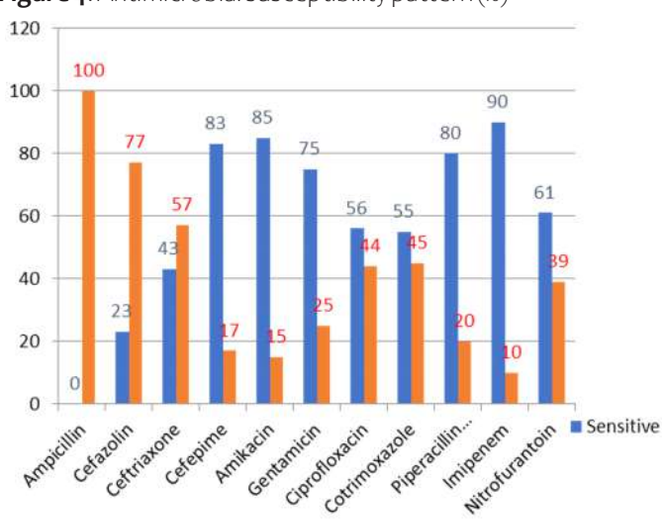
There were 15 isolates that produced biofilm out of which 10 were strong biofilm producers and 5 were moderate biofilm producers (Table 1). Out of the 6 isolates from urine which showed biofilm 5 were obtained from catheterized patients.

Table 1. Mean optical density values of biofilm producers

Sl no	Mean OD value	Strength of biofilm
1	0.473	Strong
2	0.688	Strong
3	0.622	Strong
4	0.308	Strong
5	0.459	Strong
6	0.428	Strong
7	0.645	Strong
8	0.284	Strong
9	0.291	Strong
10	0.257	Strong
11	0.222	Moderate
12	0.211	Moderate
13	0.137	Moderate
14	0.198	Moderate
15	0.189	Moderate

Antimicrobial susceptibility testing was done by disk diffusion method and the pattern was as follows (figure 4):

Figure 4. Antimicrobial susceptibility pattern (%)



K.pneumoniae possess many resistance mechanisms. ESBL, AmpC and Carbapenemase was tested phenotypically in this study. ESBL was the most common antibiotic resistance mechanism found in isolates (55%). 10% of the isolates showed carbapenemase production and AmpC was detected in 8% of the isolates. 40% of the isolates were multidrug-resistant.

Discussion

K. pneumoniae is responsible for numerous infections, including pneumonia, sepsis, urinary tract infection (UTI), bacteremia, meningitis, and pyogenic liver abscesses. In this study, the majority of isolates were obtained from urine samples, followed by pus swabs. According to a study conducted by Barwa R et al. at Mansoura University, out of 80 isolates, 50 were from urine and 30 from sputum (8). In a study conducted by Bora A et al. in Northeast India, 57.89% of isolates were from urine, 15.79% from sputum, 10.53% from blood, and 15.79% from pus (9). The increased number of isolates from urine samples may be due to *K. pneumoniae* being a major agent of UTI, second only to *Escherichia coli*.

The capsule is an elaborate polysaccharide matrix that mediates resistance to phagocytosis, prevents complement-mediated lysis and opsonization, and averts the activation of the immune response (10). Such capsulated strains are particularly evident in extraintestinal infections, such as septicemia, meningitis, and UTI. All the analyzed isolates showed capsule production. This is in concordance with studies conducted by Aljabany et al. and El Fertais-Aisani et al., who both observed 100% capsule production (4,11). This may be attributed to the fact that capsular polysaccharide is a nearly universal feature of environmental strains of Klebsiella.

The frequency of strains with the hypermucoviscosity phenotype was higher in infections causing liver abscess than in those from other sites of *K. pneumoniae* infection (12). El Fertais-Aisani R et al. observed 9.2% of hypermucoviscous phenotype (11). According to a study conducted by Chou A et al. in Houston, USA, 7.9% of *K. pneumoniae* isolates carried one or both hypervirulent *Klebsiella pneumoniae* (hvKP)-associated genes, *magA* and *rmpA*, at Ben Taub General Hospital, and 3.9% of isolates from the Veteran Affairs Medical Centre carried both hvKP-associated genes (13). Guo Y et al. reported a hypermucoviscous phenotype in 22.8% of *K. pneumoniae* isolates in a study conducted in China (14). In this study, 15% of the isolates were found to be hypermucoviscous, based on the string test.

K. pneumoniae secretes small molecules called siderophores, which help in iron acquisition from the host. This process causes the host cells to become stressed, inducing inflammation and cell signaling pathways. This allows the bacteria to escape from the lungs to the spleen, worsening the infection. *K. pneumoniae* has many iron uptake protein systems, such as enterobactin, aerobactin, ferrichrome, and coprogen (15). In this study, 40% of the isolates showed siderophore production on phenotypic detection. Aljabany et al. reported that 100% of isolates produced siderophores (4). In the study conducted by Ferreira RL et al, it was observed that the enterobactin (*ent*B) gene was found in 100%, the yersiniabactin (*ybt*S) gene in 60% and the aerobactin siderophore system (*iut*A) gene in 40% of the isolates (16).

A biofilm is a living ecosystem made of millions of adherent bacterial cells embedded within a self-produced matrix of extracellular polymeric substances. Since immune responses are significantly reduced in proximity to foreign bodies, medical implants and catheters are particularly susceptible to biofilm formation (17). The most clinically significant *K. pneumoniae* biofilms are those formed on the inner surfaces of catheters and other indwelling devices, thereby reducing the lifespan of many medical devices and leading to implant failure. Biofilms protect microorganisms from phagocytosis, opsonization, and the ciliary action of epithelial cells, helping them survive (18). Bacterial populations in biofilms are also more resistant to antibacterial agents than free-living cells (19), making treatment difficult once a biofilm is established.

Vuotto C et al. in Italy reported an increase in biofilm production in XDR strains compared to MDR and sensitive strains (20). In a study conducted by Nirwati H et al. in Tehran, 85.63% of the isolates were biofilm producers, in which 26.9% isolates were strong, 28.7% isolates were moderate, and 29.9% isolates were weak biofilm producers (21). A study in Coimbatore, India, showed that 26.7% of the isolates from urinary tract infections were biofilm producers (6). This study showed 15% biofilm production, and ten of these isolates were multidrug-resistant. This may be due to the biofilm restricting the entry of antibiotics into the bacterial cells.

Another concern is the decreasing susceptibility pattern of *K. pneumoniae* isolates to most antibiotics. According to a study conducted by Shilpa K et al. in Bangalore, Karnataka, India, the susceptibility pattern was as follows: amikacin (66%), ciprofloxacin (68%), gentamicin (62%), cefepime (60%), imipenem (56.66%), and co-trimoxazole (50%) (22). Resistance to ciprofloxacin may have been contributed by an increase in fluoroquinolone prescribing practices for uncomplicated UTIs in recent years.

Al Benwan et al. from Kuwait reported that a relatively high proportion of *K. pneumoniae* was associated with ESBL production in catheter-associated urinary tract infection (CA-UTI) (23). Some studies have shown more than 50% of *K. pneumoniae* isolates producing ESBL (4,24). In India, a high prevalence of ESBL-producing *Klebsiella pneumoniae* strains has been reported by various groups. Parveen RM et al. in Pondicherry showed that out of 39 isolates of *K. pneumoniae* isolated from blood cultures over three months, 97.2% were found to be ESBL positive by phenotypic testing (25). This is in concurrence with this study, which found 55% of isolates to be ESBL producers.

The incidence of carbapenem resistance in Klebsiella is rapidly increasing. Chiu SK et al. reported an average prevalence of carbapenem non-susceptible *K. pneumoniae* as

2.13% (26). Carbapenemase production was detected phenotypically in 32.07% of clinical isolates of Klebsiella spp. using the Modified Hodge test by Chauhan K et al. in a study in Uttar Pradesh (27). In this study, 10% of isolates were found to produce carbapenemase based on the modified carbapenemase inactivation method.

Conclusion

Klebsiella pneumoniae is a ubiquitous pathogen responsible for a wide range of infections. The capsule was the most common virulence factor, followed by siderophore production. The majority of *K. pneumoniae* isolates showed good sensitivity to amikacin, piperacillin-tazobactam, and carbapenems, while 40% of the isolates were multidrug-resistant. *K. pneumoniae* also exhibits various resistance mechanisms, with ESBL production being the most prevalent. Antibiotics should be prescribed in adequate therapeutic doses and for a sufficient duration to help reduce the emergence and spread of multidrug-resistant organisms in the community.

Author's contribution

Smrithi Surendranath: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. **Amritha Sabitha Kurup**: Writing – Review & Editing. **Reena John**: Supervision.

Ethics statement

The authors declare that the present study was conducted under the strictest ethical conditions after getting approval from Institutional Ethic Committee, Government medical college, Thrissur.

Financial support

None.

Conflict of interest

The authors declare no conflicts of interest.

Availability of data

The datasets generated and /or analyzed during current study available from the corresponding author on reasonable request.

Acknowledgment

The support provided by the teaching and non-teaching staff of Department of Microbiology, Government Medical college Thrissur, Kerala is gratefully acknowledged.

References

1. Choby JE, Howard-Anderson J, Weiss DS. Hypervirulent Klebsiella pneumoniae - clinical and molecular perspectives. J Intern Med. 2020;287(3):283-300.
2. Paczosa MK, Mecsas J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. Microbiol. Mol. Biol. Rev. 2016;80(3):33 629-61.
3. Karampatakis T, Tsergouli K, Behzadi P. Carbapenem-Resistant Klebsiella pneumoniae: Virulence Factors, Molecular Epidemiology and Latest Updates in Treatment Options. Antibiotics (Basel). 2023;12(2):234.
4. Aljabani AAJ, Alhasani . Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. Afr. J. Microbiol. Res. 2016;10(22):829-843.
5. Ballén V, Gabasa Y, Ratia C, Ortega R, Tejero M, Soto S. Antibiotic Resistance and Virulence Profiles of Klebsiella pneumoniae Strains Isolated From Different Clinical Sources. Front Cell Infect Microbiol. 2021;11:738223.
6. Palanisamy M. Antibacterial susceptibility and virulence factors of Klebsiella species isolated from urinary tract infections in Coimbatore, South India. South Indian j. biol. sci. 2015;1(3):145-150.
7. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Pennsylvania: CLSI, 2020
8. Barwa R, Shabaan M. Molecular Characterization of Klebsiella pneumoniae Clinical Isolates with Elevated Resistance to Carbapenems. Open Microbiol J. 2017;11:152–159.
9. Bora A, Ahmed G. Detection of NDM-1 in Clinical Isolates of Klebsiella Pneumoniae from Northeast India. J Clin of Diagn Res. 2012;6(5):794-800
10. Karampatakis T, Tsergouli K, Behzadi P. Carbapenem-Resistant Klebsiella pneumoniae: Virulence Factors, Molecular Epidemiology and Latest Updates in Treatment Options. Antibiotics. 2023; 12(2):234.
11. El-Fertas-Aissani, R,Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathol Biol. 2013;61(5):209-216.
12. A.M. Parrott, J. Shi, J. Aaron, D.A. Green, S. Whittier, F. Wu. Detection of multiple hypervirulent Klebsiella pneumoniae strains in a New York City hospital through screening of virulence genes. Clin Microbiol Infect. 2021;27(4): 583-9.
13. Chou A, Nuila RE, Franco LM, Stager CE, Atmar RL, Zechiedrich L. Prevalence of hypervirulent Klebsiella pneumoniae-associated genes rmpA and magA in two tertiary hospitals in Houston, TX, USA. J Med Microbiol. 2016;65(9):1047-48.
14. Guo Y, Wang S, Zhan L, Jin Y, Duan J et al. Microbiological and Clinical Characteristics of Hypermucoviscous Klebsiella pneumoniae Isolates Associated with Invasive Infections in China. Front Cell Infect Microbiol. 2017;7:24.
15. Zhu J, Wang T, Chen L, Du H. Virulence Factors in Hypervirulent Klebsiella pneumoniae. Front Microbiol. 2021;12:642484.
16. Ferreira RL, da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MCA, da Silva EML, Freire CCM, da Cunha AF, Pranchevicius MDS. High Prevalence of Multidrug-Resistant Klebsiella pneumoniae Harboring Several Virulence and β-Lactamase Encoding

Genes in a Brazilian Intensive Care Unit. Front Microbiol. 2019;9:3198.
17. Edminston CE, McBain AJ, Roberts C, Leaper D. Clinical and Microbiological Aspects of Biofilm Associated Surgical Site Infections. Adv Exp Med Biol. 2015;830:47-67.
18. Guerra MES, Destro G, Vieira B, Lima AS, Ferraz LFC, Hakansson AP, Darrieux M, Converso TR. Klebsiella pneumoniae Biofilms and Their Role in Disease Pathogenesis. Front Cell Infect Microbiol. 2022;12:877995.
19. Ghafourian S, Mohebi R, Rezaei M, Raftari M, Sekawi Z, Kazemian H et al. Comparative analysis of biofilm development among MRSA and MSSA strains. Roum Arch Microbiol Immunol. 2012;71(4):175-82.
20. Vuotto C, Longo F, Pascolini C, Donelli G, Balice MP et al. Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. J Appl Microbiol. 2017;123(4):1003-18.
21. Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, Hakim MS, Meliala A, Aman AT, Nuryastuti T. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proc. 2019;13(11):20.
22. Shilpa K, Ruby Thomas, Allavarapu Ramyashree. Isolation and Antimicrobial sensitivity pattern of Klebsiella pneumoniae from sputum samples in a tertiary care hospital. International Journal of Biomedical and Advance Research. 2016;7(2):053-057.
23. Al Benwan K, Al Sweih N, Rotimi VO. Etiology and antibiotic susceptibility patterns of community- and hospital-acquired urinary tract infections in a general hospital in Kuwait. Med Princ Pract. 2010;19(6):440-6.
24. El-kersh TA, Marie MA, Al-sheikh YA, Al-kahtani SA. Prevalence and risk factors of community-acquired urinary tract infections due to ESBL-producing Gram negative bacteria in an Armed Forces Hospital in Southern Saudi Arabia. J. Med. Sci. 2015;4(7):321-30.
25. Parveen RM, Khan MA, Menezes GA, Harish BN, Parija SC et al. Extended-spectrum β-lactamase producing Klebsiella pneumoniae from blood cultures in Puducherry, India. Indian J Med Res. 2011;134(3):392–395.
26. Chiu SK, Ma L, Chan MC, Lin YT, Fung CP et al. Carbapenem Nonsusceptible Klebsiella pneumoniae in Taiwan: Dissemination and Increasing Resistance of Carbapenemase Producers During 2012–2015. Scientific Reports. 2018;8:8468.
27. Chauhan K, Pandey A, Asthana AK, Madan M. Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallobetalactamase in clinical isolates of Escherichia coli and Klebsiella species. Indian J Pathol Microbiol. 2015;58(1):31-35