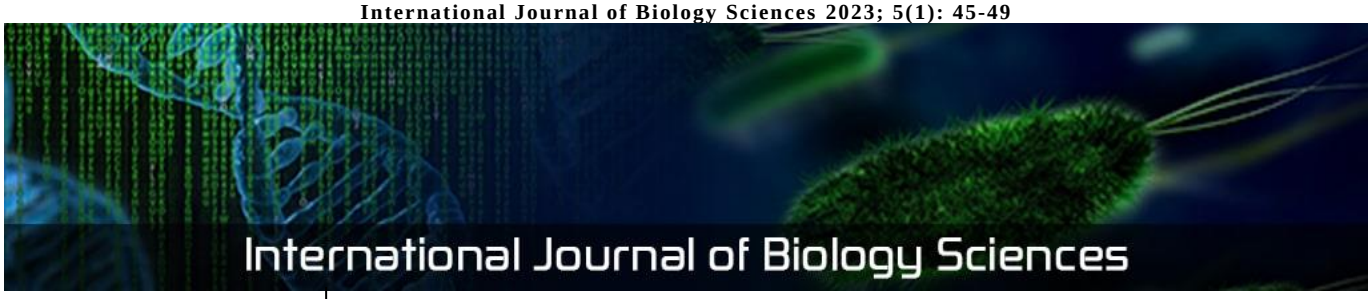

ISSN Print: 2664-9926
ISSN Online: 2664-9934
Impact Factor: RJIF 5.45
IJBS 2023; 5(1): 45-49
www.biologyjournal.net
Received: 11-11-2022
Accepted: 19-12-2022

Dr. E Subbalakshmi
Associate Professor,
Department of Microbiology,
Saveetha Institute of Medical and Technical Sciences,
Chennai, Tamil Nadu, India

Dr. P Banumadhi Radha
Post Graduate Student,
Department of Microbiology,
Saveetha Institute of Medical and Technical Sciences,
Chennai, Tamil Nadu, India

V Divya
Post Graduate Student,
Department of Microbiology,
Saveetha Institute of Medical and Technical Sciences,
Chennai, Tamil Nadu, India

Corresponding Author:
Dr. E Subbalakshmi
Associate Professor,
Department of Microbiology,
Saveetha Institute of Medical and Technical Sciences,
Chennai, Tamil Nadu, India

Evaluation of *in vitro* activity of ceftazidime/avibactam among carbapenamase producing *Klebsiella pneumoniae* from clinical isolates in tertiary care hospital

Dr. E Subbalakshmi, Dr. P Banumadhi Radha and V Divya

DOI: https://dx.doi.org/10.33545/26649926.2023.v5.i1a.146

Abstract
The emergence of multidrug resistant organisms pose a great challenge to global health. Carbapenem group of drugs have been used as treatment therapy for these multidrug resistant organisms. Ceftazidime-avibactam is a newer combination consisting of ceftazidime, a 3rd generation anti pseudomonal cephalosporin and avibactam, a beta lactamase inhibitor. Avibactam helps in restoring the antibacterial activity of ceftazidime against carbapenamase producing Enterobacterales. Ceftazidime-avibactam (CAZ-AVL) provides us a valuable strategy to combat carbapenem resistant infections especially in Enterobacterales. The objective of the study was to find out carbapenem resistant strains of *Klebsiella pneumoniae* from clinical isolates and to detect the carbapenamase activity by phenotypic and genotypic detection methods. Carbapenamase detection from the clinical isolates was done by meropenem hydrolysis test (mCIM). The resistant strains were evaluated for the activity of ceftazidime/avibactam by Estrip method. Molecular analysis of all the carbapenem resistant strains were done for bla_{OXA48} and bla_{KPC}. Among 2500 isolates which were subjected to study from January-June 2022, 306 isolates grew *Klebsiella pneumoniae* among which 34 (11%) isolates were found to be multidrug resistant organisms (MDRO). Modified carbapenem inactivation method (mCIM) was used for carbapenamase activity. Ceftazidime/avibactam susceptibility was done using Estrip. 28(82.4%) isolates were found susceptible (≤8/4ug/ml) and 6(17.6%) isolates were found resistant (≥16/4ug/ml). Among CAZ/AVL resistant strains bla_{KPC} was more prevalent than bla_{OXA48}. In our study it was found that prevalence of OXA-48 was more than KPC among the resistant strains of *Klebsiella pneumoniae* and the susceptibility of ceftazidime/avibactam was more in Enterobacterales.

Keywords: *Klebsiella pneumoniae*, multidrug resistance organisms, carbapenemase, ceftazidime/avibactam

Introduction
Resistance in Gram-negative organisms have been emerging these years. The resistance is probably due to indiscriminate use of antibiotics and prolonged hospital stay. Multidrug resistant Gram-negative organisms are treated with carbapenems especially in ICU (intensive care units) in most of the hospitals. Because of the rampant use, we are now facing problems with carbapenem resistance [1]. The resistance to carbapenem group of antibiotics are due to over expression of ESBL (extended spectrum of beta-lactamase), associated with porin loss, production of carbpenemhydrolysing enzymes like KPC (class A) AmpC beta-lactamase, MBL (class B metallo-beta-lactamase) and Carbapenamases like OXA-48 Class D) [2]. Among these carbapenamase enzymes prevalence of blaOXA-48 has been widely observed in Belgium, France and Spain [3]. The primary report of *Klebsiella pneumoniae* carbapenemase (KPC)-in *K. pneumoniae* was reported North Carolina in 2001 [4]. In 2005 it was noted that this KPC producing carbapenamase was found to be spreading worldwide [5]. In addition OXA-48 like and New Delhi metallo-beta-lactamases are the other carbapenemases of clinical significance in Enterobacterales. In India NDM-1 (New Delhi beta-lactamase) is the most frequently identified carbapenamase [3]. Besides KPC, it was observed that prevalence of OXA-48 was higher in parts of Southeast Asia and India [6]. Study showed that ceftazidime/avibactam showed wide range of *in vitro* activity against

KPC-producing Enterobacterales even in the presence of other hydrolyzing enzymes. Recent data from US, showed effectiveness of ceftazidime/avibactam against 120 KPC producers, even in the presence of other β-lactamases which has the ability to hydrolyze ceftazidime [7]. The activity of OXA-48 by ceftazidime/avibactam is complex due to its inability to hydrolyze ceftazidime. However, few studies have proved the coproduction of OXA-48-like enzymes with extended-spectrum β-lactamases (ESBLs) [8]. Hence, avibactam is necessary to restore the activity of ceftazidime when there is coproduction of OXA-48 and ESBL [5]. The mortality rates lies between 20% and 50% in critically ill patients with invasive infections due to carbapenem-resistant Enterobacterales (CRE) [9, 10].

Avibactam is a β-lactamase inhibitor which is active against class C enzymes, many of class a enzymes (including KPCs) and few class D (OXA) carbapenemases [11]. The object of this study was done to find out the *in vitro* activity of ceftazidime/avibactam among MDR Klebsiella pneumoniae from various clinical samples in a tertiary care centre.

Materials and Methods

The prospective study was done at Saveetha Institute of Medical and Technical Sciences, Chennai was done during the period from January to June 2022. Ethical clearance was obtained from the institution. Statistical analysis was done using SPSS software. Descriptive and inferential statistical analysis was done for the analysis. All the samples from in-patients of various departments were used for the study. All samples from outpatients were excluded in our study. The samples included urine, blood, exudate, body fluids CSF (cerebrospinal fluid), tissue samples etc. All the samples were processed after preliminary identification and were streaked on MAP (MacConkey agar), CAP (Chocolate agar) and BAP (blood agar), CLED (Cysteine lactose electrolyte deficient) agar depending on the samples and were processed in VITEK 2 system for organism identification and antibiotic susceptibility testing. Samples which were resistant to carbapenem group of drugs were taken for further study. Carbapenemase detection was done by mCIM as given by CLSI 2022 [12].

mCIM Method: 1ul loopful of bacteria from an overnight blood culture was emulsified into 2ml of TSB (Trypticase Soy Broth). The sample was vortexed for 10-15 seconds. 10ug meropenem disc was dispensed in the tube and the disc was completely immersed in broth and incubated at 37 deg C for 4 hours. On a MHA (Muller Hinton agar plate) ATCC (American type culture collection) strain *E. coli* 25922was streaked by lawn culture. The meropenem disc was removed making sure that the excess fluid was removed and kept at the centre of MHA. The plate was incubated overnight and looked for zone of inhibition. If the meropenem disc was hydrolysed there would have been no-zone of inhibition. Carbapenamase activity was detected by this method.

It is much more sensitive than modified Hodge test. If zone of inhibition was ≥19mm it was considered negative.

E strip by Ceftazidime and Ceftazidime + avibactam

E strip was done using ceftazidime + avibactam to assess the *in vitro* activity of these drugs on MHA plate. Susceptibility was observed when there was zone of inhibition of ≤ 8/4ug/ml and for resistance organisms it was ≥16/4 ug/ml.

PCR detection by gel electrophoresis.

PCR detection of the resistant strain was done by gel electrophoresis. The genes detected were bla OXA48 and bla KPC.

The primer pair used to detect the OXA-48 gene was OXA-48F (5′-GCTTGATCGCCCTCGATT-3′) and OXA-48R (3′-GATTTGCTCCGTGGCCGAAA-5′)

The primer for KPC blaKPC (forward primer 5′-ATGTCACTGTATCGCCGTCT-3′; reverse primer 5′-CCTAAATGTGACAGTGGTTGG).

The extraction procedure for both the genes were as follows

Plasmid DNA isolation:

The plasmid DNA from *Klebsiella pneumoniae* was isolated using the QIAprep Spin MiniPrep kit. The pellet was obtained by centrifuging about 5 ml of overnight grown bacterial culture at 8000 rpm for 3 minutes at room temperature. The pellet was resuspended in 250 microlitres of Buffer P1 and transferred to a micro-centrifuge tube. About 250 microlitres of buffer P2 was added to the suspension and mixed thoroughly by inverting the tubes 4-6 times until the solution becomes clear. To the lysate about 350 microlitres of buffer N3 was added and immediately mixed by inverting the tubes 4-6 times. The lysate was spun at 13000 rpm for 10 mins at room temperature. About 800 microlitres of cleared lysate was added to the QIAPrep 2.0 spin column by pipetting. The tubes were then spun for 30-60 seconds. The flow through was discarded.

The column was washed by adding 0.5 mL of Buffer PB. The tubes were spun for 30-60 seconds and the flow through was discarded. The column was spun for an additional time of 1 minute to remove any residual wash buffer.

The QIAPrep 2.0 column was placed in a sterile 1.5 mL tube.

About 50 microlitres of Buffer EB was added to the centre of the column. The tubes were incubated at RT for 1 minute and spun for 1 min at 12000 rpm.

About 5µl of eluted plasmid DNA was loaded on to 0.7% agarose gel.

The plasmid DNA was visualized using a UV trans-illuminator.

Results

During the period of 6 months (January 2022-June 2022) we received 2500 samples from various departments. The samples included sputum, CSF, urine, blood and sterile fluids. Among those 2500 isolates, 306 isolates grew *Klebsiella pneumoniae*. Out of the 306 isolates, 34 (11%) isolates were found to be multidrug resistant organisms (MDRO). These MDR *Klebsiella pneumoniae* were isolated from patients of different age group (11.8% were <40 years, 23.5% were between 40-50 years, 64.7% were >50 years of age). MDR *Klebsiella pneumoniae* were isolated more from men (70.6%) compared to women (29.4%). In our study, the clinical wards (61.8%) had more MDR *Klebsiella pneumoniae* compared to that of various Intensive Care Units {Medical ICU (32.8%), Surgical ICU (2.9%) and Respiratory ICU (2.9%)} (Table-1). Out of 34MDR *Klebsiella pneumoniae* strains, they were isolated more from exudate samples (41.2%) like wound swab, pus, tissue followed by urine samples (32.4%), respiratory samples (14.7%) and least in blood samples (11.8%).

Phenotypic and genotypic tests were performed amid the MDR *Klebsiella pneumoniae* isolated from various clinical samples to look for the carbapenemase activity. Modified

carbapenem inactivation method (mCIM) was carried out and all the 34(100%) isolates were mCIM positive. (Fig 1) Ceftazidime/avibactam susceptibility for the MDR strains were tested using Estrip, (Fig 2) 28(82.4%) isolates were found susceptible (≤8/4ug/ml) and 6(17.6%) isolates were found resistant (≥16/4ug/ml). The results of the molecular analysis of the resistant strains of MDR *Klebsiella pneumoniae* showed bla_{OXA-48} {positive 22(64.7%) and negative 12(35.3%)} and bla_{KPC} {positive 14(41.2%) and negative 20(58.8%)}.

Table 1: Frequency of distribution of patients studied

Ward/ICU	No. of Patients	%
WARD	21	61.8
MICU	11	32.4
SICU	1	2.9
RICU	1	2.9
Total	34	100.0

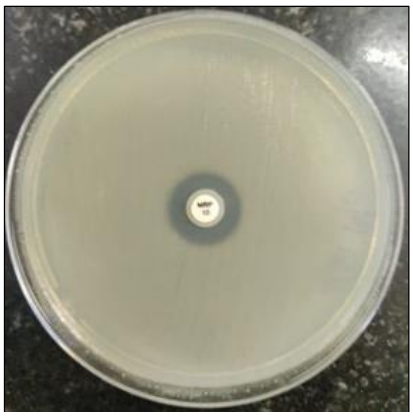

Fig 1: Positive mCIM method

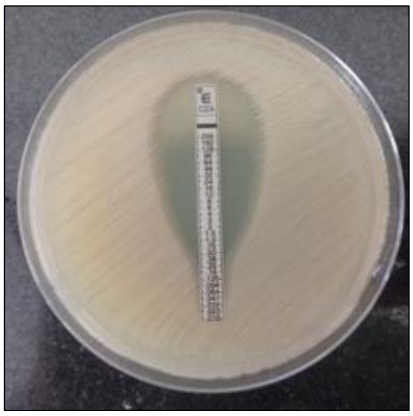

Fig 2: Ceftazidime-avibactam susceptibility in MDR *Klebsiella pneumoniae*

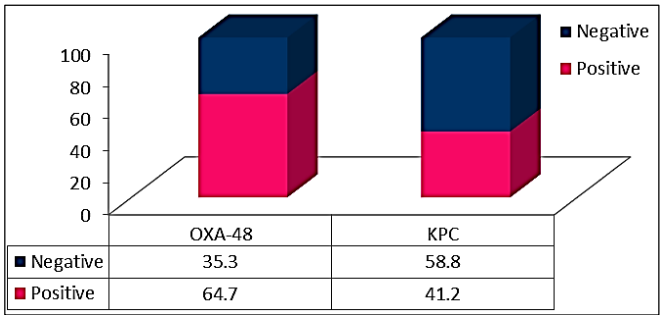

Fig 3: Distribution of OXA-48 and KPC genes among MDR Klebsiella Pneumoniae

Discussion

This study was focused to find out the *in vitro* activity of ceftazidime/avibactam in MDR *Klebsiella pneumoniae*. We encounter MDR organisms of *Klebsiella pneumoniae* in clinical wards compared to that of ICUs. Our study was focused to find out the *in vitro* activity of ceftazidime/avibactam among MDR strains of *Klebsiella pneumoniae*. In our study there were 34 multidrug resistant (11%) strains out of 306 strains of *Klebsiella pneumoniae*. These MDR strains which were beta carbapenem resistant were responsible for more mortality [13]. Emphasizing that in 2017 WHO (World health organization) declared carbapenem resistant Enterobacterales (CRE) as first critical pathogens. Ceftazidime-avibactam (CZA) is a newer combination of a third-generation cephalosporin with second generation beta-lactamase inhibitor [14]. The antibacterial activity against carbapenemase-producing Enterobacterales, is restored through the combination of ceftazidime-avibactam [11]. In our study out of 34 MDR strains 28 strains showed susceptibility to ceftazidime/avibactam. Only 6 strains were resistant to ceftazidime/avibactam. In Young *et al*. [14] study, he observed that 21 strains out of 386 isolates in *Klebsiella pneumoniae* were resistant to Ceftazidime/avibactam. It was observed that mortality rate was lower with patients on treatment with ceftazidime and avibactam when used in combination with ceftazidime. This was observed in Tumbarello *et al*. study where the mortality observed was 37% [15]. The mutations in the omega loop of the KPC enzyme leads to enhanced ceftazidime hydrolysis. This cannotbe inhibited by avibactam resulting in resistance to ceftazidime/avibactam. The resistance could be due to mutation in tyrosine for aspartic acid substitution at omega loop of the KPC enzyme at position 179 [16]. The resistance in *Klebsiella pneumoniae* to Ceftazidime/avibactam is due tomutation in $bla3_{KPC}$ Shields RK *et al*. study [17]. Besides carbapenemase activity, decreased outer membrane permeability and increased efflux pump mechanism can also lead to CZA resistance in *Pseudomonas aeruginosa* as discovered by Winkler and colleagues. Few other studies also described the overexpression of AmpC as a resistance mechanism for CZA, especially in *Pseudomonas aeruginosa* [18, 19, 20]. Molecular analysis was done for all34 strains of MDR *Klebsiella pneumoniae*. We looked for presence of bla_{KPC} and bla_{OXA-48} genes. In H.A. Hakeam, H. Alsahli, L. Albabtain *et al*. blaOXA-48 was observed in 31 isolates out of 50 isolates (66%).Our molecular analysis showed presence of bla_{OXA-48} more than bla_{KPC}. 22 (64.7%) strains were OXA-48 positive 14(41.2%) were KPC positive and 11 strains had both OXA and KPC positive. It is similar to Oteo *et al*. [21] study in Spanish hospitals which showed 90.5% of *Klebsiella pneumonia* harbouring OXA-48. Azimi *et al*. study [22], reported first case of OXA 48 in Iran. In Haekaem *et al*. study [23] $bla_{OXA\ 48}$ was observed in CRE Enterobacterales which is similar to our study. We analysed the presence of OXA and KPC in ceftazidime/avibactam resistant strains. In this study, among the 6 ceftazidime/avibactam resistant strains, 2(33.3%) strains harbored both OXA-48 and KPC; Only 3(50%) strains were KPC positive which were OXA-48 negative. In our study, among ceftazidime/avibactam resistant strains KPC was more prevalent than OXA 48.1(16.6%) strain had both OXA-48 and KPC negative. Here the mechanism could have been due to impermeability or porin loss. In Azimi *et*

al. study [22] also, out of 28 MDR strains of *Klebsiella pneumoniae* 27 strains showed presence of bla_{OXA48} and one strain was negative which is similar to our study. Interestingly, De la Calle *et al*. study observed that there was absence of resistance to ceftazidime-avibactam during the treatment of infections due to CRE, inhabiting the bla_{OXA-48} gene [24]. It has been observed that ceftazidime/avibactam is a promising drug especially in ICUs.

Conclusion

Though the sample size was small, it clearly indicates ceftazidime/avibactam is an ideal drug for Enterobacterales especially in intensive care units. Ceftazidime-avibactam, a beta-lactamse antibacterial agent is definitely an important drug for the management of MDR Gram-negative pathogens. Especially in Indian scenario, because of the cost factor limited data is currently available to support its clinical efficacy. Implementation of antimicrobial stewardship and strict infection control practices can help in preserving the susceptibility of ceftazidime-avibactam combination.

Conflict of interest: There is no conflict of interest in the study.

Funding: There was no funding for this study.

Ethical clearance: The ethical clearance was obtained from Saveetha Institute of Medical and Technical Sciences, Chennai. (Enclosed Ethical clearance).

Acknowledgement

We would like to acknowledge,

1. Statistician-Niharika S, Ashika R, Swathi Gupta for creation of Tables, Listing and Graphs (TLG) and Statistical Analysis Reporting (SAR) and Dr. K.P. Suresh, Ph.D. (Biostatistics) for reviewing methodology and results.
2. Dr. Vijayashree Priyadarshini, Associate Professor (clinical genetics), centre for cellular and Molecular Research, Saveetha dental college and hospitals, Chennai. For molecular analysis
3. Pfizer pharmaceuticals, India for providing E strips of Ceftazidime/avibactam.
4. Professors and technicians of Saveetha Institute of Medical and Technical Sciences, Chennai.

References

1. Taggar G, Attiq Rehman M, Boerlin P, Diarra MS. Molecular epidemiology of carbapenemases in enterobacteriales from humans, animals, food and the environment. Antibiotics. 2020;9:1-22.
2. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. Outbreak of OXA-48-positive carbapenem-resistant Klebsiella pneumoniae isolates in France. Antimicrob Agents Chemother. 2011 May;55(5):2420-3. DOI: 10.1128/AAC.01452-10.
3. Albiger B, Glasner C, Struelens M, Grundmann H, Monnet D. The European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts in 38 countries, Euro Surveill. 2015 May;20:30062.
4. Yigit H, Queenan AM, Anderson GJ, *et al*. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother. 2001;45:1151-61.
5. Munoz-Price LS, Poirel L, Bonomo RA, *et al*. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis. 2013;13:785-96.
6. Rahman M, Shukla SK, Prasad KN, *et al*. Prevalence and molecular characterisation of New Delhi metallo-β-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant Enterobacteriaceae from India. Int. J Antimicrob Agents. 2014;44:30-7.
7. Castanheira M, Mills JC, Costello SE, Jones RN, Sader HS. Ceftazidime avibactam activity tested against Enterobacteriaceae isolates from U.S. hospitals (2011-2013) and characterization of β-lactamase producing strains. Antimicrob Agents Chemother. 2015;59:3509-17.
8. Aktaş Z, Kayacan C, Oncul O. *In vitro* activity of avibactam (NXL104) in combination with β-lactams against gram-negative bacteria, including OXA48 β-lactamase-producing Klebsiella pneumoniae. Int. J Antimicrob Agents. 2012;39:86-9.
9. Morrill HJ, Pogue JM, Kaye KS, La Plante KL. Treatment options for carbapenem-resistant Enterobacteriaceae infections. Open Forum Infect Dis. 2015;2:ofv-050.
10. Satlin MJ, Chen L, Patel G, *et al*. Multicenter clinical and molecular epidemiological analysis of bacteremia due to carbapenem-resistant Enterobacteriaceae (CRE) in the CRE epicenter of the United States. Antimicrob Agents Chemother. 2017;61:p02349-16.
11. Van Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-Lactam/β-Lactamase Inhibitor Combinations. Clin Infect Dis. 2016 Jul;63(2):234-41.
12. CLSI 32nd edition performance standards for antimicrobial testing. Clinical and laboratory standard Institute, p. 122.
13. Chew KL, Lin RTP, Teo JWP. Klebsiella pneumoniae in Singapore: Hyper virulent infections and the Carbapenemase threat. Front Cell Infect Microbiol. 2017;7:515.
14. Neuner EA, Gallagher JC. Pharmacodynamic and pharmacokinetic considerations in the treatment of critically ill patients infected with carbapenem-resistant Enterobacteriaceae. Virulence. 2017;8(4):440-52.
15. Tumbarello M, Trecarichi EM, Corona A, De Rosa FG, Bassetti M, Mussini C, *et al*. Efficacy of ceftazidime-avibactam salvage therapy in patients with infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Clin Infect Dis. 2019;68(3):355-64.s
16. Livermore DM, Warner M, Jamrozy D, *et al*. *In vitro* selection of ceftazidime-avibactam resistance in Enterobacteriaceae with KPC-3 carbapenemase. Antimicrob Agents Chemother. 2015;59:5324-30.
17. Shields RK, Chen L, Cheng S, Chavda KD, Press EG, Snyder A, *et al*. Emergence of ceftazidime-avibactam resistance due to plasmid-borne blaKPC-3 mutations during treatment of carbapenem resistant Klebsiella pneumoniae infections. Antimicrob Agents Chemother. 2017;61:e02097-16.

18. Winkler ML, Papp-Wallace KM, Hujer AM, Domitrovic TN, Hujer KM, Hurless KN, *et al*. Unexpected challenges in treating multidrug-resistant Gram-negative bacteria: resistance to ceftazidime-avibactam in archived isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2015;59:1020-1029.
https://doi.org/10.1128/AAC.03681-14.24
https://doi.org/10.1128/AAC.04238-14.
19. Castanheira M, Mills JC, Farrell DJ, Jones RN. Mutation-driven beta-lactam resistance mechanisms among contemporary ceftazidime non-susceptible Pseudomonas aeruginosa isolates from U.S. hospitals. Antimicrob Agents Chemother. 2014;58:6844-6850.
20. Bowler LL, Zhanel GG, Ball TB, Saward LL. Mature Pseudomonas aeruginosa biofilms prevail compared to young biofilms in the presence of ceftazidime. Antimicrob Agents Chemother. 2012;56:4976-4979.
21. Oteo J, Hernández JM, Espasa M, Fleites A, Sáez D, Bautista V, *et al*. Emergence of OXA-48-producing Klebsiella pneumoniae and the novel carbapenemases OXA-244 and OXA-245 in Spain. J Antimicrob Chemother. 2013 Feb;68(2):317-21. DOI: 10.1093/jac/dks383.
22. Azimi L, Nordmann P, Lari AR, Bonnin RA. First report of OXA-48-producing Klebsiella pneumoniae strains in Iran. GMS Hyg. Infect Control. 2014 Mar;9(1):Doc-07. Doi: 10.3205/dgkh000227. PMID: 24653971; PMCID: PMC3960935.
23. Hakeam HA, Alsahli H, Albabtain L, Alassaf S, Al-Duhailib Z, Althawadi S. Effectiveness of ceftazidime-avibactam versus colistin in treating carbapenem-resistant Enterobacteriaceae bacteremia. Int. J Infect Dis. 2021 Aug;109:1-7.
Doi: 10.1016/j.ijid.2021.05.079. Epub 2021 Jun 4. PMID: 34091006.
24. De la Calle C, Rodríguez O, Morata L, Marco F, Cardozo C, García-Vidal C, *et al*. Clinical characteristics and prognosis of infections caused by OXA-48 carbapenemase-producing Enterobacteriaceae in patients treated with ceftazidime-avibactam. Int. J Antimicrob Agents. 2019 Apr;53(4):520-524. Doi: 10.1016/j.ijantimicag.2018.11.015. Epub 2018 Nov 22. PMID: 30471403.