Original Article

Detection of aacC1 and aacC2 Genes in Clinical Isolates of Klebsiella Pneumoniae

Fatemeh Bahrami Chegeni 1, Kourosh Cheraghipour1, Pegah Shakib1*.

1. Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

*correspondence: **Pegah Shakib,** Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran. Email: shakib.pegah@yahoo.com

Abstract:

Introduction: Klebsiella pneumoniae is one of the main agent of nosocomial infections. Reports around the world emphasize on the resistance to aminoglycoside antibiotics in recent years. The purpose of this study is to determine the frequency of aacC1 and aacC2 genes in clinical isolates of Klebsiella pneumoniae.

Methods: A total of 100 Klebsiella pneumoniae were collected from tertiary university hospitals, Khorramabad city, Iran, from February to August 2018. The obtained samples were identified by standard biochemical and microbiological tests. Susceptibility pattern of isolates were determined according to Clinical Laboratory Standards Institute (CLSI) advices using disk diffusion method. After DNA extraction, all Klebsiella pneumoniae isolates were evaluated for the presence of aacC1 and aacC2 genes using PCR assay.

Findings: Out Of 100 Klebsiella pneumonia isolates the highest resistance was related to kanamycin (35%), tobramycin (29%), and amikacin (23%). The aacC1 and aacC2 genes was detected in 22.8 and17.2 percent, respectively.

Conclusion: Our results indicate that the prevalence of the aacC1 and aacC2 genes was high and it is clear that we witness an increase in resistance to antibiotics in clinical isolates. Therefore, we expect an increase in the resistance to aminoglycoside antibiotics in the near future.

Keywords: Klebsiella pneumoniae, PCR assay, aacC1, aacC2.

Introduction:

Klebsiella pneumoniae (K. pneumoniae) is opportunistic gram-negative and aerobic bacilli from Enterobacteriaceae family with a polysaccharide capsule(1). K. pneumoniae is a main cause of human pathogen contains pneumonia, sepsis, urinary tract infections (UTI) and nosocomial infection in hospitalized patients(1, 2). K. pneumoniae infections mostly treated by antimicrobial agents such as third generating cephalosporins and fluoroquinolones(3, 4). Since 1984, emergence of antimicrobial resistant K. pneumoniae strains has significantly increased(5). Moreover, can cause treatment failure with diverse antimicrobials agents and a main threat to public health universally(6). Resistance to aminoglycosides can be carried out through different mechanisms include enzymatic modification of this drug, modification of the ribosomal target and decreased intracellular antibiotic accumulation by alterations of the outer membrane

[DOR: 20.1001.1.23222913.2020.9.1.4.1]

permeability, decreased inner membrane transport or active efflux (7-9). Aminoglycoside modifying enzymes (AMEs) are the most common mechanism of bacterial resistance to aminoglycoside such as aminoglycoside acetyl transferases (AACs)(2, 10). AACs modify aminoglycosides substrates and disrupt their binding to the ribosome by transferring the acetyl group from acetyl coenzyme A (AcCoA) onto amine moieties of the aminoglycosides frames. The family consists of four large classes formed based on the specific location of the transfer of the acetyl group on aminoglycoside including AAC (1), AAC (3), AAC (2'), AAC (6')(11, 12). Meanwhile, acetyltransferases AAC (3) are the most common enzymes in Enterobacteriaceae family. The enzyme substrate AAC (3) -II is gentamicin and faramicin, whereas, the three aac (3) -IIa and aac (3) -IIb and aac (3) -Ib and aac (3) -Ic genes are coding for resistance enzymes to this antibiotic (13). The transfer of antibiotic resistance genes is carried out through chromosomes, plasmids and transposes. Resistance to aminoglycosides is coded by aaaC1 genes (acetyl transferase, which causes resistance to gentamicin)(14).The aim of this study was determination of the frequency of aacC1 and aacC2 genes in clinical isolates of Klebsiella pneumoniae from tertiary university hospitals, Khorramabad, Lorestan province, Iran.

Methods:

Sample collection:

This descriptive study, was done in a period from February to August 2018 in tertiary university hospitals, located in Khoramabad, Lorestan province, Iran.

In this study 100 K. pneumoniae isolated from clinical samples included urine, blood, ulcers, respiratory secretions, and other body fluids. The samples were cultured on Blood agar and MacConkey agar (Oxoid, UK). After incubation for 18-24hour in 370C, colonies were identified phenotypically by differential biochemistry tests including gram stain, catalase, oxidase, Simmons Citrate agar, urea agar, SIM, TSI, MR and VP(15).

Antimicrobial susceptibility pattern:

The determination of antimicrobial susceptibility pattern of K.pneumoniae isolates was performed by Kirby-Bauer disc diffusion test using Mueller-Hinton agar (MHA) and amikacin (30µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30µg), and netilmicin (10 µg) accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines(16).

Genotypic Detection of aacC1 and aacC2 genes by polymerase chain reaction (PCR) assay:

DNA extraction was performed by Genomic DNA Extraction kit (SinaClon, Iran).detection of aacC1 and aacC2 genes from all K.pneumoniae isolates was performed using PCR method. PCRs doing based on the sequence of primers mentioned in table1(17). The PCR reaction was performed at a final reaction volume of 25 µl, contained 12.5 µl of master mix PCR,1 µl of each primer (10 pmol/µl), 3µl of

[DOR: 20.1001.1.23222913.2020.9.1.4.1]

extracted DNA, and 7.5 µl distilled water(DDW).

PCR amplification was carried out conditions: initial denaturation at 94◦C for 7 min, followed by 35 cycles each of 30 sec denaturation at94◦C, 30 sec annealing at 55◦C for aacC1 and45 sec annealing at 59◦C for aacC2 , 40s extension for 72◦C, and final extension at 72◦Cfor 5 min. The PCR products were electrophoresed on a 1.5% agarose gel containing Cyber Green in a TBE 0.5X buffer.

Findings:

Antimicrobial susceptibility testing:

K. pneumoniae strains showed variable degrees of resistance to aminoglycosides as evaluated by disk diffusion method according to the CLSI standard procedure (Tables 2). In clinically isolated strains the highest resistance related to Kanamycin (46%), and the lowest resistance related to Netilmicin (29%). Chi-square test revealed that there was no significant difference between disk diffusion method in K. pneumoniae strains in the resistant or the susceptible category (P>0.05).

Results of PCR assay for detection of aacC1 and aacC2 genes:

In this study, all isolates screened by PCR for the presence of aacC1 and aacC2 genes. Of the 63 isolates, 8 were positive for aacC1 (22.8%), and six for aacC2 (17.2%).

Discussion:

Recently the increasing of antimicrobial resistance isolates is one of the major problems in Health Centers in worldwide. Because they increase the cost of treatment and the duration of the treatment, and even increase the mortality rate(18, 19). K.pneumoniae is one of the most important pathogenic bacteria that found in clinical settings due to its epidemic tendency and antimicrobial resistance (20, 21). Aminoglycosides are commonly used antibiotics to treat infections caused by K.pneumoniae. Indeed, the emergence and prevalence of Aminoglycoside resistant K. pneumoniae isolates has been reported in previously studies in many countries (22-25). Nowroozi et al., in Iran showed79% resistance to kanamycin and 55% resistance to gentamicin. Therefore, investigation of resistance genes in clinical bacterial isolated with molecular methods have a serious role in controlling, and spreading of resistant pathogenic bacteria(26). The main mechanism of resistance against aminoglycosides in K. pneumoniae isolates is the production of modifying enzymes, such as Aminoglycoside Acetyltransferase (AAC)(26, 27). Our study aimed to determine the prevalence of aminoglycoside resistance genes (aacC1 and aacC2 genes) in K. pneumoniae isolates from different clinical samples of tertiary university hospitals, Khorramabad city, Iran. Al-Marzooq et al., in Malaysia described aacC2 gene with prevalence 67.7% was the most common aminoglycoside-resistance gene and aacC1 detected at 2.7% of in K. pneumoniae isolates which in contrast to the result of our study(28). The finding of aacC1 and aacC2 genes in this study is in agreement with Mohamed Abo-State in Egypt records frequency of aacC1and aac C2 genes 56% and 5%, respectively(29). Meanwhile, we found isolates that did not

[DOR: 20.1001.1.23222913.2020.9.1.4.1]

have any of the genes examined, probably strains without aacC1 and aacC2 genes involved had to other mechanisms of resistance to aminoglycoside include other Acetyltransferase, alterations of the outer membrane permeability, decreased inner membrane transport or active efflux.

Conclusion:

The present study reported the prevalence of the antimicrobial resistance to Aminoglycoside and the emergence of aacC1 and aacC2 genes by molecular methods in K. pneumoniae to minimize the spread of antibiotic resistance.

References:

1. Li B, Zhao Y, Liu C, Chen Z, Zhou D. Molecular pathogenesis of Klebsiella pneumoniae. Future microbiology. 2014;9(9):1071-81.
2. Pomakova D, Hsiao C, Beanan J, Olson R, MacDonald U, Keynan Y, et al. Clinical and phenotypic differences between classic and hypervirulent Klebsiella pneumonia: an emerging and under-recognized pathogenic variant. European journal of clinical microbiology & infectious diseases. 2012;31(6):981-9.
3. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infection Control & Hospital Epidemiology. 2008;29(12):1099-106.
4. Chen L, Todd R, Kiehlbauch J, Walters M, Kallen A. Notes from the Field: Pan-Resistant New Delhi Metallo-Beta-Lactamase-Producing Klebsiella pneumoniae-Washoe County, Nevada, 2016. MMWR Morbidity and mortality weekly report. 2017;66(1):33-.
5. Eisen D, Russell EG, Tymms M, Roper EJ, Grayson ML, Turnidge J. Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multiresistant Klebsiella pneumoniae. Journal of clinical microbiology. 1995;33(3):713-7.
6. Giannella M, Bartoletti M, Morelli M, Tedeschi S, Cristini F, Tumietto F, et al. Risk factors for infection with carbapenem-resistant Klebsiella pneumoniae after liver transplantation: the importance of pre-and posttransplant colonization. American Journal of Transplantation. 2015;15(6):1708-15.
7. Lindemann PC, Risberg K, Wiker HG, Mylvaganam H. Aminoglycoside resistance in clinical Escherichia coli and Klebsiella pneumoniae isolates from Western Norway. Apmis. 2012;120(6):495-502.
8. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007;128(6):1037-50.
9. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nature reviews microbiology. 2015;13(1):42.
10. El-Badawy MF, Tawakol WM, El-Far SW, Maghrabi IA, Al-Ghamdi SA, Mansy MS, et al. Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among Klebsiella pneumoniae clinical isolates recovered from Egyptian patients. International journal of microbiology. 2017;2017.

[DOR: 20.1001.1.23222913.2020.9.1.4.1]

11. Shakya T, Wright GD. Mechanisms of aminoglycoside antibiotic resistance. Aminoglycoside Antibiotics. 2007:119-40.
12. Ramirez MS, Nikolaidis N, Tolmasky M. Rise and dissemination of aminoglycoside resistance: the aac (6′)-Ib paradigm. Frontiers in microbiology. 2013;4:121.
13. Maynard C, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, et al. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal Escherichia coli isolates of animal and human origin. Journal of clinical microbiology. 2004;42(12):5444-52.
14. Garneau-Tsodikova S, Labby KJ. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. MedChemComm. 2016;7(1):11-27.
15. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology-e-book: Elsevier Health Sciences; 2014.
16. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011.
17. Sáenz Y, Brinas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, et al. Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origins. Antimicrobial agents and chemotherapy. 2004;48(10):3996-4001.
18. Woodford N, Tierno PM, Young K, Tysall L, Palepou M-FI, Ward E, et al. Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center. Antimicrobial agents and chemotherapy. 2004;48(12):4793-9.
19. Yao X, Doi Y, Zeng L, Lv L, Liu J-H. Carbapenem-resistant and colistin-resistant Escherichia coli co-producing NDM-9 and MCR-1. The Lancet infectious diseases. 2016;16(3):288-9.
20. Ben-David D, Kordevani R, Keller N, Tal I, Marzel A, Gal-Mor O, et al. Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. Clinical Microbiology and Infection. 2012;18(1):54-60.
21. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proceedings of the National Academy of Sciences. 2015;112(27):E3574-E81.
22. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. The Lancet infectious diseases. 2013;13(9):785-96.
23. Mehrgan H, Rahbar M, Arab-Halvaii Z. High prevalence of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a tertiary care hospital in Tehran, Iran. The Journal of infection in developing countries. 2010;4(03):132-8.
24. Waiwarawooth J, Jutiworakul K, Joraka W. The prevalence and susceptibility patterns of ESBL-producing Klebsiella pneumoniae and Escherichia coli in Chonburi hospital. Journal of Infectious Diseases and Antimicrobial Agents. 2006;23(2):57-65.
25. Chong Y, Yakushiji H, Ito Y, Kamimura T. Clinical and molecular

[DOR: 20.1001.1.23222913.2020.9.1.4.1]

epidemiology of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a long-term study from Japan. European journal of clinical microbiology & infectious diseases. 2011;30(1):83-7.
26. Liang C, Xing B, Yang X, Fu Y, Feng Y, Zhang Y. Molecular epidemiology of aminoglycosides resistance on Klebsiella pneumonia in a hospital in China. International journal of clinical and experimental medicine. 2015;8(1);1381.
27. Haldorsen BC, Simonsen GS, Sundsfjord A, Samuelsen Ø. Increased prevalence of aminoglycoside resistance in clinical isolates of Escherichia coli and Klebsiella spp. in Norway is associated with the acquisition of AAC (3)-II and AAC (6′)-Ib. Diagnostic microbiology and infectious disease. 2014;78(1):66-9.
28. Al-Marzooq F, Yusof MYM, Tay ST. Molecular analysis of antibiotic resistance determinants and plasmids in Malaysian isolates of multidrug resistant Klebsiella pneumoniae. PloS one 2015;10(7):e0133654.
29. Abo-State MAM, Saleh YE-S, Ghareeb HM. Prevalence and sequence of aminoglycosides modifying enzymes genes among E. coli and Klebsiella species isolated from Egyptian hospitals. Journal of radiation research and applied sciences.2018;11(4):408-15.

Tables and Charts:

Table 1: Sequence of primers using in this study.

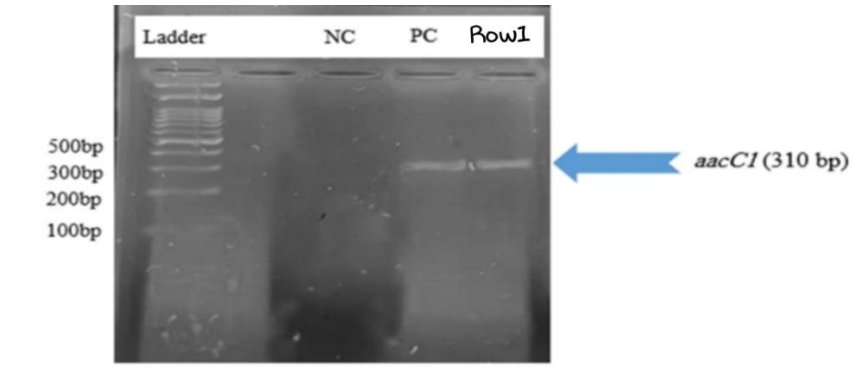
Target gene	Sequence of primers (5′ to 3′)	Amplicon size (bp)
aacC1	F :ACCTACTCCCAACATCAGCC R: TAGATCTCACTACGCGCCTG	310
aacC2	F: CTCTTGATGGTGCATGCCTC R: ATTGATTCAGCAGGCCGAAC	347

Table 2: Antimicrobial susceptibility test in K. pneumoniae isolates.

Antibiotic	Sensitive %	Resistance %	Intermediate %
Kanamycin	35	46	19
Amikacin	27	38	35
Tobramycin	36	40	24
Netilmicin	57	29	14
Gentamicin	40	35	25

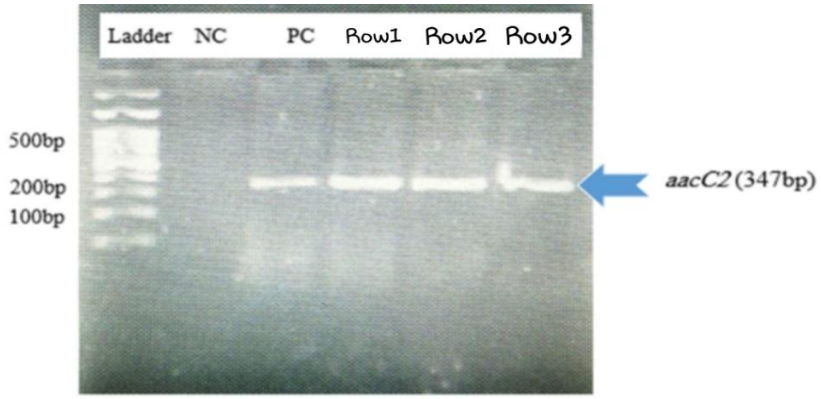
[DOR: 20.1001.1.23222913.2020.9.1.4.1]

Figure 1: PCR produces electrophoresis on agarose gel for detection of aacC1 gene.



Ladder: Molecular marker / PC: Positive Control / NC: Negative Control

Figure 2: PCR produces electrophoresis on agarose gel for detection of aacC2 gene.



Ladder: Molecular marker / PC: Positive Control / NC: Negative Control

[DOR: 20.1001.1.23222913.2020.9.1.4.1]