Gene Cell Tissue. 2014 July; 1(2): e18152. DOI: 10.17795/gct-18152

Published online 2014 May 22. **Research Article**

tetA and *tetB* Genes in *Klebsiella Pneumoniae* Isolated From Clinical Samples

Mohammad Bokaeian [1,*]; Saeide Saeidi [2,*]; Zahra Shahi [2]; Vahideh Kadaei [3]

[1]Department of Laboratory Sciences, Infectious Disease and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran
[2]Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, Kerman, IR Iran
[3]Department of Biology, Zabol University of Medical Sciences, Zabol, IR Iran

*Corresponding authors: Mohammad Bokaeian, Department of Laboratory Sciences, Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Khalij Fars Boulevar, Postal Code: 9816743463, Zahedan, IR Iran. Tel: +98-5413425723, Fax: +98-5413425732, E-mail: bokaeian.m@gmail.com; Saeide Saeidi, Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, Kerman, IR Iran. Tel: +98-9153495398, Fax: +98-5422240696, E-mail: s.saeedi12@yahoo.com

Received: February 10, 2014; **Revised:** April 24, 2014; **Accepted:** May 5, 2014

Background: The emergence of antibiotic resistance among clinical and nonclinical bacteria is a global public health problem. *Klebsiella Pneumoniae* is one of the most pathogens that contains a variety of gens and shows resistance to many antibiotics. Perpetual monitoring of the resistant bacteria is an important in order to limit the development of resistance among these pathogens.
Objectives: The current study aimed to monitor the prevalence of *tetA* and *tetB* resistance genes in *Klebsiella Pneumoniae* species isolated from the patients with urinary tract infection who hospitalized in Mir Hospital of Zabol, Iran from 2011 to 2012.
Materials and Methods: In the present cross-sectional study, a total of 30 strains of *K. pneumoniae* were isolated from urine cultures of hospitalized patients in Mir Hospital (Zabol, south-east of Iran) who had urinary tract infections from 2011 to 2012. Antibiotic susceptibility of isolates was evaluated for four antibiotics including ceftazidime, cefixime, tetracycline and erythromycin using standard Kirby-Bauer disk diffusion method. The *K. pneumoniae* genome was extracted by simple boiling method, and polymerase chain reaction (PCR) method also was used to detect *tetA* and *tetB* genes by specific pair of primers.
Results: The K. pneumonia isolates were resistant to erythromycin (70%), cefixime (53.3%), tetracycline (50%) and ceftazidime (36.6%). The amplification of *tetA* and *tetB* genes of K. pneumonia revealed that all of the isolates harbored these genes.
Conclusions: Resistance to tetracycline and other antibiotics, and the presence of various resistance genes in K. pneumonia strains are alarming signs in Zabol area. The current study strongly recommends limiting the consumption of antibiotics including tetracycline. Further studies should be conducted in order to find out the extent of the problem in other areas.

Keywords:Klebsiella Pneumoniae; Tetracycline; Drug Resistance, Bacteria

1. Background

The emergence of antibiotic resistance is a global public health problem. Gram-negative bacterial resistance is of particular importance, since there is a dearth of novel antibiotics directed against these organisms. The clinical utility of carbapenems and the agents of the last resort against multi-drug resistant Enterobacteriaceae are threatened with the growing incidence of pan resistant isolates (1, 2). Different species of *Klebsiella* genus are responsible for a wide variety of diseases in humans. *Klebsiella pneumoniae* has been associated with various ailments such as urinary tract infections, septicemia, diarrhea, and other diseases (3). In addition to being the primary cause of respiratory tract infections, K. pneumonia is commonly involved in acute pyelonephritis in pregnant women with urinary tract abnormalities such as urolithiasis, hydronephrosis or congenital deformities. Several reports, especially from the Asia Pacific region and the United States, have also shown that this pathogen has become the predominant cause of liver abscess (4, 5). Tetracycline and other antibiotics have been frequently used to treat diseases, but unfortunately repeated use of these compounds has resulted in the development of resistant strains. Tetracycline resistance in bacteria is mediated by four mechanisms: efflux, ribosomal protection, enzymatic inactivation, and target modification (6). At present, 23 genes encoding efflux pumps and 11 genes encoding ribosomal protection proteins, not including the recently described mosaic tetracycline resistance genes have been reported (7). In prior clinical surveys, *tetB* gene was identified as the most prevalent tetracycline resistance determinant with a wide host range due to the fact that it resides on highly mobile genetic elements that readily transfer between different bacterial genera (8). The gene *tetA* is located on conjugative plasmids of different incompatibility groups.

2. Objectives

The current study aimed to detect *tetA* and *tetB* genes in *Klebsiella Pneumoniae* isolated from clinical samples.

3. Materials and Methods

In the present cross-sectional study, a total of 30 *K. pneu-*

Implication for health policy makers/ practice/ research/ medical education:
In order to reduce the prevalence of bacterial resistance, more investigations on resistance monitoring is highly recommended.

moniae strains were isolated from urine cultures of hospitalized patients in Mir Hospital (Zabol, south east of Iran) who had urinary tract infections from 2011to 2012. All samples were collected aseptically from the patients and plated right after the collection. Identification of all causative microorganisms was performed by standard microbiologic methods (9).

3.1. Agar Disk Diffusion Assay

The susceptibility of all applied antibiotics was carried out using disc diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (CLSI) (10). Briefly, *K. pneumoniae* isolates were grown overnight on blood agar, and colony suspension was prepared equal to the turbidity of a 0.5 McFarland standard. The suspension (100 μL) was spread over a Mueller-Hinton agar plate and discs of the selected antibiotics were placed aseptically on the surface of inoculated media. Antibiotics concentrations were as follow: cefixime (30 μg), tetracycline (30 μg), erythromycin (15 μg) and ceftazidime (30 μg).

3.2. Amplification of Genes

The colonies of *K. pneumoniae* were suspended in TE (Tris + EDTA) buffer and their DNA was extracted by simple boiling method (11). The polymerase chain reaction (PCR) method was performed to detect *tetA* and *tetB* genes as described previously with minor modifications, using specific pair of primers (Table 1).

The PCR mixture consisted of 10 pmol of each primers, 10 ng DNA sample, 1.5 mM MgCl2, 0.2 mM each dNTP, and 5 U Taq DNA polymerase (Cinagen, Iran) in a total volume of 50 μL of PCR reaction.

4. Results

Overall, *K. pneumoniae* isolates were resistant to the agents including erythromycin (70%), cefixime (53.3%), tetracyclin (50%) and ceftazidime (36.6%) (Table 2). The amplification of *tetA* and *tetB* of *K. pneumoniae* revealed that all of the isolates harbored these genes (Figure 1).

5. Discussion

The *K. pneumoniae* isolates in the study were resistant to erythromycin (70%), cefixime (53.3%), tetracyclin (50%) and ceftazidime (36.6%). In the study conducted by Derakhsan et al. more than half (17/31) of *K. pneumoniae* isolates showed high resistance to different antibiotics including amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, aztreonam, and ceftazidime (12). All of the isolates in the current study harbored *tetA* and *tetB* genes. In the study by Tuckman et al. a total of 452 tetracycline-resistant and nonduplicate isolates were positive by PCR for at least one of the six examined tetracycline resistant determinants (13). In the latter study, 32% and 26% of isolates were positive for *tetB* and *tetA* respectively, whereas

Table 1. The PCR Primers Applied to Detect *tetA* and *tetB* Genes [a]

Primer	Sequence	Annealing Temperature
tetA	-	-
F	GTGAAACCCAACATACCCC	58
R	GAAGGCAAGCAGGATGTAG	60
tetB	-	-
F	CCTTATCATGCCAGTCTTGC	60
R	ACTGCCGTTTTTTCGCC	52

[a] Abbreviations: F, forward; R, reverse

Table 2. Antimicrobial Susceptibility of *Klebsiella Pneumonia* Isolates [a]

	Ceftazidime	Cefixime	Erythromycin	Tetracycline
Sensitive	16 (53.3)	10 (33.3)	2 (6.6)	13 (43.3)
Intermediate	1 (3.3)	2 (6.6)	5 (16.6)	1 (3.3)
Resistant	11 (36.6)	16 (53.3)	21 (70)	15 (50)

[a] Data are presented as No. (%).

Figure 1. PCR Products for *tetA* and *tetB* Genes

Lanes 1-8 show the fragment of *tetA* , and demonstrate the 888 base pare DNA size marker, lanes 9-16 show the fragment of *tetB* gene with 774 bp size marker.

tetC, tetD, tetE, and *tetM*, were collectively found in 4% of isolates (13). The tetracycline resistance is a common and developing aspect of resistance among the other bacteria. In the study by Skockova et al. for example, 102 *Escherichia coli* isolates were examined and about half (49.0%) of these isolates showed resistance to tetracycline. Antibiotic resistance and the corresponding gene(s) show time and geographical dependency. The most common gene detected in tetracycline-resistant isolates from 2010 to 2011 was *tetA* (81.3%), while *tetB* was most often (86.5%) found in isolates from 2005 to 2006 (14). The tetracycline resistance genes (including *tetA* and *tetB*) have been detected from clinical and non-clinical samples. Koo and Woo have identified *tetA* (52.4%) followed by *tetB* (41.3%) as the most frequent genes in tetracycline-resistant E. coli isolates from meat and meat products (15). In the study by Menggen et al. antimicrobial resistance genes, carried by 30 Salmonella isolates, were detected and common genes included *tetC* (60%, 18/30), cat1 (43.3%, 13/30) and *tetA* (40%, 12/30) (16). These studies provide a picture of the

tetracycline resistance genes burden among clinical and nonclinical *K. pneumoniae* isolates, as well as the utility of the novel broad-spectrum agent, and tigecycline against these pathogens. Moreover, these results support the general approach of reengineering the existing antimicrobial agents with acceptable safety profiles to evade the resistance mechanisms posed by bacterial pathogens.

In conclusion, resistance to tetracycline and other antibiotics and the presence of various resistance determinants in K. pneumonia strains is an alarming sign in Zabol area. Wide and inappropriate use of antibiotics may play an important role in resistance development. The current study strongly recommends limiting the consumption of antibiotics including tetracycline. Further studies should be conducted in order to find out the extent of the problem in other areas.

Acknowledgements

This study was supported by Institute of Plant Biotechnology University of Zabol, Zabol, Iran.

Authors' Contributions

Mohammad Bokaeian, data analysis and final approval of the manuscript; Saeide Saeidi, study design, data analysis and manuscript preparation; Zahra Shahi, data collection and final approval of the manuscript; Vahideh Kadaei, experimental studies and final approval of the manuscript.

Funding/Support

This study was funded and supported by Institute of Plant Biotechnology, University of Zabol.

References

1. Overturf GD. Carbapenemases: a brief review for pediatric infectious disease specialists. *Pediatr Infect Dis J.* 2010;**29**(1):68–70.
2. Aubron C, Poirel L, Ash RJ, Nordmann P. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. *Emerg Infect Dis.* 2005;**11**(2):260–4.
3. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998;**11**(4):589–603.
4. Chung DR, Lee SS, Lee HR, Kim HB, Choi HJ, Eom JS, et al. Emerging invasive liver abscess caused by K1 serotype Klebsiella pneumoniae in Korea. *J Infect.* 2007;**54**(6):578–83.
5. Ohmori S. Septic endophthalmitis and meningitis associated with Klebsiella pneumoniae liver abscess. *Hepatology Research.* 2002;**22**(4):307–12.
6. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev.* 2001;**65**(2):232–60.
7. Patterson AJ, Rincon MT, Flint HJ, Scott KP. Mosaic tetracycline resistance genes are widespread in human and animal fecal samples. *Antimicrob Agents Chemother.* 2007;**51**(3):1115–8.
8. Ross JI, Eady EA, Cove JH, Cunliffe WJ. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. *Antimicrob Agents Chemother.* 1998;**42**(7):1702–5.
9. Ganesan S, Taylor R, Rabheru K, Forbes I, Dumontet J, Procyshyn RM. Antipsychotic polypharmacy does not increase the risk for side effects. *Schizophr Res.* 2008;**98**(1-3):323–4.
10. Clinical and Laboratory Standards Institute . *Performance standards for antimicrobial disk susceptibility testing.*USA: Wayne, Pa; 2007.
11. Varaiya AY, Dogra JD, Kulkarni MH, Bhalekar PN. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infections. *Indian J Pathol Microbiol.* 2008;**51**(3):370–2.
12. Derakhshan S, Najar Peerayeh S, Fallah F, Bakhshi B, Rahbar M, Ashrafi A. Detection of Class 1, 2, and 3 Integrons Among Klebsiella pneumoniae Isolated from Children in Tehran Hospitals. *Archives of Pediatric Infectious Diseases.* 2013;**1**(4):164–8.
13. Tuckman M, Petersen PJ, Howe AY, Orlowski M, Mullen S, Chan K, et al. Occurrence of tetracycline resistance genes among Escherichia coli isolates from the phase 3 clinical trials for tigecycline. *Antimicrob Agents Chemother.* 2007;**51**(9):3205–11.
14. Skočková, A. , Cupáková, S. , Karpíšková, R. , Janštová, B. . Detection of tetracyclin resistance genes in Escherichia coli from cows milk. *Journal of Microbiology, Biotechnology and Food Sciences .* 2012;**1**:777–84.
15. Koo HJ, Woo GJ. Distribution and transferability of tetracycline resistance determinants in Escherichia coli isolated from meat and meat products. *Int J Food Microbiol.* 2011;**145**(2-3):407–13.
16. Menggen M, Wang H, Yu Y, Zhang D, Liu S. Detection of Antimicrobial Resistance Genes of Pathogenic Salmonella from Swine with DNA Microarray. *Journal of Veterinary Diagnostic Investigation.* 2007;**19**(2):161–7.