The rationale behind antibiotic resistance pattern in
*Klebsiella pneumoniae*

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Abstract. Presently, there is an increase in antibiotic resistance in bacteria, due to relax prescription of antibiotics, especially in Iran. Undoubtedly, in toxin antitoxin (TA) system, a toxin neutralized by antitoxin, which known as a potent antimicrobial target; but there is no extensive survey on the prevalence of TA loci in large scale of *Klebsiella pneumoniae*. Therefore, this study aims to determine the prevalence of different TA loci in clinical and environmental *K. pneumoniae* isolates. For this reason, 48 *K. pneumoniae* clinical isolates and 49 *K. pneumoniae* environmental isolates were subjected for evaluation of different TA loci. The results of current study indicated that there is no association between antibiotic resistances and presence of TA loci in clinical and environmental *K. pneumoniae*. The role of TA loci as a potent target in antibiotic resistant *K. pneumoniae* has been complicated. Therefore, more studies should be performed to explain why TA loci are presented in *K. pneumoniae* and what is the rationale behind antibiotic resistant *K. pneumoniae*?

INTRODUCTION

The main concern in antibiotic resistance is because of antibiotic abuse (Laxminarayan *et al.*, 2014). The bacteria acquired resistance to antibiotics via different mechanisms including the enzymatic inactivation of antibiotics, target alteration, efflux pumps (Bari *et al.*, 2008; Giedraitiene *et al.*, 2011), antibiotic resistance genes (Hassan *et al.*, 2012), and importantly existence of toxin antitoxin (TA) systems (Ghafourian *et al.*, 2013; Soo *et al.*, 2014) that causes an alteration of bacterial physiology. Toxin-antitoxin (TA) system is defined as a sophisticated system, which based on the function of unstable antitoxin towards the stable toxin. Post segregation killing (PSK) implied the vertical inheritance TA system, which located on plasmid. According to this inclusive definition eventual fate of cell depends on plasmid inheritance and obviously, follow-up TA system. Inevitability of death will be occurred in daughter cell groups, which did not inherit plasmid due to the unstable antitoxin degradation and clearly, the stable toxin has inherent ability for killing cell (Sherro *et al.*, 2013; Yamaguchi *et al.*, 2011).

The functions of TA system are: persister cell formation (Maisonuneuve *et al.*, 2011), stress resistance (Aizenman *et al.*, 1996), protection from bacteriophages (phages) (Fineran *et al.*, 2009), regulation of biofilm formation (Wang & Wood, 2012), Program Cell Death (PCD) and gene regulation.
In 2007, a study of TA systems in clinical isolates of vancomycin resistant entrococcus revealed that all the isolates were positive for mazEF TA loci (100%). Many of these TA systems were presented on plasmids carrying the vanA gene cassette (Schmidt et al., 2007).

The prevalence and functionality of the mazEF TA system in clinical isolates of MRSA have been evaluated in a single study (Williams et al., 2011), which demonstrated that all MRSA strains harbor the mazEF TA system and that mazEF is transcribed in all MRSA isolates. Also, it be demonstrated that mazEF TA loci are unique TA loci in clinical isolates of Enterococcus faecalis and E. faceium (Soheili et al., 2015). They showed by disruption of toxin the pathogenicity of bacteria were fallen.

Amitai and colleagues demonstrated that 5% of bacterial cells were viable and 95% were killed after toxin activation because the increased toxin could not be neutralized by the antitoxin. However, when co-expressing mazE (antitoxin) and neutralizing mazF (toxin), 85% of the cells were viable because the toxin was neutralized and inhibited by the antitoxin (Amitai et al., 2004).

Hence, artificial disruption of antitoxin can lead to bacterial cell killing. However, the most important step for potency of TA system, as an antibacterial target, is to identify a TA system that is prevalent in all resistant strains. There are no reports about Prevalence of TA loci in clinical and environmental isolates of K. pneumoniae. Therefore, the current study aims to identify the rationale behind antibiotic resistant K. pneumoniae clinical and environmental isolates by evaluation the prevalence of TA loci in K. pneumoniae clinical and environmental isolates.

METHODS

Bacterial Isolates

97 K. pneumoniae clinical (n=48) and environmental (n=49) isolates were collected during period November 2013 to February 2014. Clinical and environmental isolates were collected from hospital and sewage, respectively.

Antimicrobial susceptibility testing

All K. pneumoniae were subjected to antibiotic susceptibility assay by disk diffusion method as CLSI guideline with the following set of antibiotics: gentamicin (10µg), ciprofloxacin (5µg), cefoxitin (30µg), pipracilin/tazobactem (100/10µg), aztreonam (30µg), chloramphenicol (30µg), nitrofurantion (300µg), cephalothin (30µg), amikacin (30µg), cefuroxime (30µg), tetracycline (30µg), cefazolin (30µg), and amoxicillin (30µg).

Presence of different Toxin antitoxin loci

PCR was performed to identify mazEF, relEB, and mqsRA (type II TA system) with specific primers.

Statistical analysis

SPSS version 16 with chi-square program was applied to evaluate the association between antibiotic resistance pattern and the prevalence of TA systems in both K. pneumoniae clinical and environmental isolates. \( P_{value} \leq 0.05 \) considered as a significant.

RESULTS

Our results demonstrated that among all 97 K. pneumoniae clinical and environmental isolates, the highest antibiotic resistance observed for amoxicillin (97.9%), while the lowest antibiotic resistance was seen for amikacin (5.2%) (Figure 1). Expectedly, our data showed that the antibiotic resistance pattern in clinical isolates were high, when it compare with environmental isolates. Surprisingly, exception was seen for cephalotin and cefoxitin. Note, in the competition for resistance to amoxicillin, no winner observed in both clinical and environmental K. pneumoniae.

Unexpectedly, mazEF and relBE TA loci were not presented in all antibiotic resistant K. pneumoniae. Our results demonstrated that only 8.2% (n=8) and 34% (n=33) of
**DISCUSSION**

As literature, type II TA system is more common among other types of TA systems in bacteria (Sberro et al., 2013). *mazEF* (Ghafourian et al., 2013), *relBE* (Yamaguchi et al., 2011), *mqsRA* (Soo et al., 2014) belong to the type II TA system, which could have a role in antibiotic resistance (Ghafourian et al., 2013). Poor knowledge about the exact mechanism for antibiotic resistance based TA loci, led us to face with surprising data in the current study. In one hand, presents of *mazEF* and *relBE* TA loci in both resistance and sensitive isolates observed; on the other hand, unlike the other study (Ghafourian et al., 2013) there is no meaningful difference between antibiotic resistant *K. pneumoniae* and presence of TA loci. This complication says that there is only one way to understand the exact role of TA loci about antibiotic resistance and bacterial strategy for their resistance, which it is more and more study. Therefore, it is suggested that the research should not focus only in one type of TA system; also the other types of TA systems shall be included. Despite, Williams et al. (Williams et al., 2011) demonstrated that *mazEF* TA system presented in all MRSA clinical isolates and *relBE* TA loci were positive in all *P. aeruginosa* clinical isolates or Schmidt et al. (Schmidt et al., 2007) demonstrated that *vanA* harbored by plasmid that contain *mazEF* TA loci; our results showed non of studied TA loci were observed in antibiotic resistance pattern in *K. pneumoniae*. 

*K. pneumoniae* were possess *mazEF* and *relBE* TA loci, respectively. The *mazEF* were dominant to environmental *K. pneumoniae* (n=6), whereas the opposite results were seen in positive *relBE K. pneumoniae* (18 clinical isolates contained *relBE*). In addition, *mqsRA* TA loci were introduced as ghost in all *K. pneumoniae*. 

The statistical analysis emphasized that there is no significant difference between antibiotic resistant *K. pneumoniae* and presence of TA loci in both clinical and environmental isolates.
resistant K. pneumoniae, hence, the role of TA loci in resistance to antibiotic and as a potent target in antibiotic resistant K. pneumoniae are complicated.

REFERENCES


