TEM GENE INVESTIGATION IN ISOLATED *KLEBSIELLA PNEUMONIAE* FROM COCKROACHES OF HOSPITALS

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Abstract: *Klebsiella pneumoniae* is a known cause of community-acquired bacterial pneumonia and is an important hospital-acquired pathogen that causes severe morbidity and mortality. This bacterium can be spread among different creatures in nature and finally transmitted to humans. 250 cockroaches were collected from different hospitals in Chaharmahal Va Bakhtiari province located in southwest Iran. The samples were examined for the presence of *K. pneumoniae* by plating onto a combination of culture media. Genomic bacterial DNA was extracted and was amplified sequence-specific target (*TEM* gene) by PCR assay. Out of 250 cockroach samples, 179 samples (71.60%) were positive for *K. pneumoniae*. In addition, out of all specimens that amplified by PCR in this research, 32 samples (17.87%) were positive for *TEM* gene. In present study unfortunately observed a high prevalence of *K. pneumoniae* which is very disturbing, but prevalence of *TEM* gene in isolated *K. pneumoniae* was low. Therefore, in our opinion detection of *TEM* gene by molecular methods can provide useful information about its epidemiology and risk factors associated with *K. pneumoniae* infection.

Keywords: *Klebsiella pneumoniae*, *TEM* gene, Cockroaches, Hospitals.

Introduction: The studies of done in the nineteenth century, proved that insects have important role in transmission disease to man that one of them are cockroaches [1]. Most cockroaches infected with pathogenic bacteria causing leprosy, dysentery, bubonic plague, pimples, abscesses and food poisoning. Cockroaches also have a symbiotic life with over 100 species and 60 species of bacteria, yeast, and 90 species and 45 species of protozoa [2]. Important carriers of bacteria can be noted German cockroaches (*Blattella germanica*) and American cockroach (*Periplaneta americana*). German cockroaches are one of the common-place pests that carry pathogenic bacteria have been reported since many years ago [3]. The bacteria contaminant could be from air, water, food or contact with the vectors harboring the pathogens. Cockroaches stay in filthy environments in the house, shops and even hospitals where both clinical and environmental samples coincide [4], [5].

The medical importance of cockroaches is much greater and they are among the medically important pests in urban environments that cause serious public health problems and especially they have been associated with an outbreak of dysentery [6]. Recently most pathogenic bacteria have been isolated from cockroaches, such as *Salmonella spp*, *Campylobacter spp*, *Shigella spp*, *Pseudomonas aeruginosa* and *Kelebsiella pneumoniae* [7], [8].

Klebsiella was first discovered by Karl Friedlander at 1882 [9]. *Klebsiella* consist of 7 types that *Klebsiella oxytoca* and *Klebsiella rhinoscleromatis* have also been demonstrated in human clinical specimens. *Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative, rod shaped, lactose fermenting, non-motile and encapsulated bacillus of the *Enterobacteriaceae* family [10], [11]. It is an opportunistic pathogen that is found in water, soil, and plants as well as existing as a normal flora in mucosal surfaces such as the intestines, pharynx, mouth, and skin in mammalian also this bacteria was recognized as a community-acquired pulmonary pathogen, mainly between patients with a history of chronic alcoholism [10], [12], [13]. In human, *K. pneumoniae* can colonize the gastrointestinal tract, bowel, bladder, skin or pharynx, which may cause various clinical outcomes, including pneumonia, thrombophlebitis, bacteremia and urinary tract infection [13]. The class A enzymes are mainly plasmid encoded, of the first to be described at amino acid sequence level were the enzymes TEM-1 and TEM-2 [14]. Because presence of the *TEM* gene with virulence of bacteria has a direct relationship, the purpose of present research was study of the presence of *TEM* gene in *K. pneumoniae* isolated from cockroaches from Chaharmahal Va Bakhtiari province hospitals.

Material and Methods:

Sampling:Cockroaches were randomly collected for six months (June to September 2014), from six hospitals located in Chaharmahal Va Bakhtiari province (Kashani Shahrekord, Imam Ali Farrokhshahr, Shohada Farsan, Imam Reza Lordegan and Valiasr Boroujen). Total 250 cockroaches were collected with hands covering sterile-gloves from different parts of the hospitals. Live cockroach specimens were immediately carried to Biotechnology Research Center of Islamic Azad University and killed using chloroform soaked cotton. The tubes containing samples were filled with 70% ethanol for 5 minutes to decontaminate their external

surface and were allowed to air dry. Then cockroaches were washed with sterile normal saline to remove residue ethanol. Finally their viscera were removed with sterile forceps under a dissecting microscope and instruments were sterilized after every dissection. Cockroaches gut was then kept in 2 ml sterile normal saline for 5 minutes to produce a homogenate specimen.

Bacterial Isolation: After the isolation and put in test tube containing of normal saline the gut homogenate kept in buffered peptone water and samples were inoculated on blood agar and Mac-Conkey Agar (MCA) then incubated for 24h at 37ºC. For the growth of Gram-negative bacteria such as *K. pneumoniae* BPW used that was inoculated in seven primary media (Sheep Blood Agar, Chocolate agar, Mac-Conkey, Desoxycholate Citrate Agar (DCA), SS agar, Mannitol Salt Agar (MSA) and Xylose Lysine Deoxycholate (XLD). In addition identification of gram-negative bacteria was attaining by use of standard methods (API System, bioMerieux, France). The biochemical reagents and test used for identification *K. pneumoniae* included: triple sugar iron agar, simmons citrate, indole, urease, motility and H2S. The biochemical characters of *K. pneumoniae* identified were positive citrate utilization test, negative methyl red test, negative indole test, positive urease test, positive voges-proskauer test, sucrose, acid and abundant gas production from glucose, lactose, mannitol sugar fermentation tests and maltose.

DNA extraction and PCR: *K. pneumoniae* genomic DNA was extracted using DNA extraction kit (DNP[TM] Kit; CinnaGen, Iran) according to the manufacturer's recommendation. In order to detection of *TEM* gene, PCR reaction were performed in a total volume of 25 µL containing 2 µL of DNA sample, 1 µM of each primers (TEM-F: 5'-TCCGCTCATGAGACAATAACC -3' and TEM-R: 3'-ATAATACCGCACCACATAGCAG -5'), 2 mM MgCl2, 5 µL of 10X PCR buffer AMS, 200 µM dNTPs and 1 unit of Taq DNA polymerase (CinnaGen Co, Iran). The PCR assay was performed at 95°C for 5 min and then for 32 cycles of 94°C for 1 min, 58°C for 40 sec, 72°C for 40 sec, and a final extension at 72°C for 5 min, with a final hold at 10°C in a thermal cycler (Mastercycler gradient, Eppendrof, Germany).

The amplified products were run on 1% agarose gel and staining with ethidium bromide (0.5 mg/ml) in a dark room. A 100 bp ladder molecular weight marker (Roche, USA) was used to measure the molecular weights of amplified products. Aliquots of 14 µl of PCR products were applied to the gel .Constant voltage of 84 for 20 min was used for products separation. The images of ethidium bromide stained DNA bands were digitized using an UVItec documentation system (UK).

Statistical analysis: All data were analyzed by using MS Excel 2007 and SPSS software (Version 17.SPSS Inc, USA) and p value was calculated using Chi-square and Fisher's exact tests to find any significant relationship. P value less than 0.05 was considered statistically significant.

Results: Out of 250 samples cockroaches collected from hospitals in this study, 179 samples (71.60%) were infected with *K. pneumoniae*. The *TEM* gene was successfully amplified TEM-F and TEM-R primers. Agarose gel electrophoresis of the PCR amplified products for *TEM* gene was shown in Fig. 1. From samples that assayed by PCR in this research, only 32 samples (17.87%) were positive to *TEM* gene (Table 1).

Table 1. Frequency of *K. pneumoniae* and *TEM* gene in different hospital

Hospitals	Number of Samples	positive klebsiella	positive TEM gene
Imam Reza Lordegan	60	39	9
Valiasr Boroujen	60	52	11
Shohada Farsan	40	19	0
Imam Ali Farokhshahr	30	21	3
Kashani Shahrekord	60	48	9
Total	250	179	32

Fig. 1. Agarose gel electrophoresis of the products amplified with PCR using the specific primers for *TEM* gene of *K. pneumoniae*. Lane M; 100 bp DNA ladder (Fermentase, Germany), Lane 1, 2 and 5; PCR products of the positive samples, Lane 3 and 4; negative samples.

Discussion: *Klebsiella* consist of 7 types that include *Klebsiella pneumoniae, Klebsiella planticola, Klebsiella terrigena, Klebsiella rhinoscleromatis, Klebsiella ozaenae, Klebsiella ornithinolytica* and *Klebsiella oxytoca* that two species *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens [10], [11]. *K. pneumoniae* is one of a most common cause of gram- negative sepsis which usually inhabits the human and animal intestinal tract [10], [15]. Some gram-negative bacilli such as *K. pneumoniae* and *Escherichia coli* strains enzymes particular type of produce β-lactamases. Family *Enterobacteriaceae*, produce β-lactamases are encoded by plasmids. Among the most important are β-lactamases include *TEM* and *SHV*. For the first time called *TEM-1* from a blood culture (Temonera) in Greece was isolated from E. coli [16], [17]. One of the first β-lactamases is *TEM-1* that by plasmid of the coded, but other bacteria such as *Vibrio cholerae* and *Haemophilus* strains of *Pseudomonas aeruginosa* and *Neisseria* also has the ability to produce it. Today, reports indicate that the prevalence of beta-lactamase *TEM-1* beta-lactamase, especially in parts of the world suggests that this type of β-lactamases is a global problem [18], [19]. According to a study in 2011 by Shebani et al. out of 70 samples of *E. coli,* 27 samples (38.58 %) were ESBL positive and 43 samples (61.42%) were ESBL negative that only 10 samples (37.04%) of *TEM-1* beta-lactamase gene and 17 samples (62.96%) were free of *TEM-1* beta-lactamase gene [19]. *TEM* has been responsible for several unrelated outbreaks in the United States [20] and recently reported from Europe with the same frequency [21]. A study from Korea revealed that the *SHV* is the most common ESBLs found in Korea [22]. *SHV* (especially *SHV-5*), is commonly encountered and reported worldwide [22], [23]. In another study in India, reported frequency of ESBL producing *Klebsiella spp*. reported between 6-87% [24]- [26]. Our study and other studies in all over the world suggest that high prevalence of the *K. pneumoniae* that is very disturbing, and also relatively low prevalence of *TEM* gene in cockroaches. Since the abundance of cockroaches in hospitals is very much and pathogenic *K. pneumoniae* strains with *TEM* gene can transmitted to patients and staff of hospitals, we suggest cockroaches eradication program and constant monitoring on these programs.

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