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Prevalence of *Cep* Gene of *Brucella* Isolated from Clinical Specimens in Hospitals from Kermanshah of Iran

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Brucellosis is primarily a disease of animals that can be transmitted to humans (zoonosis). *Brucella* is lack of classic virulence factors and tend to invade and chronic infection in host. Brucellosis is an occupational disease which infects more ranchers, farmer, slaughter, veterinarians and laboratory personnel. The aim of this study was to evaluate the prevalence of cep gene of *Brucella isolated* from clinical specimens in hospitals from Kermanshah of Iran. Of 100 serum specimens from patients referred to hospitals of Kermanshah in 2014 year were collected. These specimens were positive for Wright test (Ã1/80) and as well as they were verified by culture and biochemical tests. The cep sequence was attained from gene bank and the sequence was assessed to characterize and verifying the complete similarity (Alignment). For extraction of DNA from specimens, the kit from Cinnagen Company was applied. Then PCR test was performed and results analyzed by SPSS software. PCR reaction was performed for survey the presence of the pertinent gene in 100 specimens isolated from hospitals of Kermanshah, Iran. Among 100 clinical isolates of *Brucella*, 54 (54%) had cep gene. 75 cases (75%) of patients were males and females, respectively. According to the first time in this study was revealed the cep gene for diagnosis of brucellosis in the serum. So, cep gene can be used as a diagnostic component with other factors; however; this finding should be studied in animal models and diagnostic and therapeutic strategies linked with this gene will be also investigated.

Keywords: *Brucella*, cep gene, Prevalence.

Brucellosis is primarily a disease of animals that can be transmitted to humans (zoonosis)[1]. *Brucella* is lack of classic virulence factors and tend to invade and chronic infection in host[2]. Brucellosis is an occupational disease which infects more ranchers, farmer, slaughter, veterinarians and laboratory personnel[3]. The annually number of brucellosis in America is 100-200 cases while in developing and underdeveloped countries is about 500 000 cases[4]. Totally, A study review in 2010 showed that the distribution and epidemiology of brucellosis in the world is much varied and several factors such as; occupational, demographic, and socioeconomic characteristics are attributed[5]. This disease have heterogeneous distributed in different regions of Iran, its occurrence in different regions is not equal, as in some areas of south is low and in some parts of our country including Esfahan[6] and Arak[7] have highest prevalence. Recently, *Brucella* has caused disease in new parts of the worlds and as well as has considered as a re-emerging pathogen in some areas which caused changes in the epidemiology of this disease and result in raised the role of

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Brucella in travel-related diseases[8]. *Brucella* virulence factors are including lipopolysaccharide (LPS), secretory system type IV, cyclic1.2, glycan, superoxide dismutase, outer membrane proteins, membrane purine, phospholipid and lysine[9]. Microcapsule as well as is another *Brucella* virulence factor that plays an important role in preventing *phagolysozyme* integration. This structure prevents entrance of harmful drugs and enzymes and has been shown that these bacterial cells can prevent dehydration. *Brucella* species not previously thought to exist in the capsule, but recent studies have shown that the production of microcapsules has been observed in some species of *Brucella*[10, 11]. Rapid detection by PCR, using laboratory sensitive animal, Brucellin test and use of serological tests on serum, milk, vaginal mucus, semen and meat extract can helping[12, 13]. So, the aim of this study was to evaluate the prevalence of cep gene of *Brucella isolated* from clinical specimens in hospitals from Kermanshah of Iran.

MATERIALS AND METHODS

Bacterial strains and verifying of clinical samples

Of 100 serum specimens from patients referred to hospitals of Kermanshah province in 2014 year were collected. These specimens were positive for Wright test (Ã1/80) and as well as were confirmed by culture and biochemical tests. The average ages of patients were 70-18 years. Then clinical specimens were transferred to the laboratory and stored at -20 ° C until use.

Performance of PCR test

Firstly, cep sequence was attained from gene bank and the sequence was assessed to characterize and verifying the complete similarity (Alignment). The primers design for cep gene was carried out by proper softwares. Primers sequences are: F cep: CACGAGGATAGCGGGAATTA, R cep: TTTCGTCAGTGCCCTTCTCT. The condition for PCR in our study include: initial denaturation: 95 o C for 5 min, denaturation at 90 o C for 1 min, annealing temperature; 58 o C for 1 min, extension step: 72 o C for 1 min, 35 cycles and final extension: 72 o C in 5 min. For extraction of DNA from specimens, the kit from Cinnagen Company was applied. After that, the electrophoresis was done for assessing PCR results, then results analyzed by SPSS software.

Table 1. Frequency of cep gene in clinical specimens

Variable	Positive cases	%	Total
cep gene	54	54	100

RESULTS

The results of cep gene from clinical isolates of *Brucella*

PCR reaction was performed for survey the presence of the pertinent gene in 100 specimens isolated from hospitals of Kermanshah, Iran. Among 100 clinical isolates of *Brucella*, 54 (54%) had cep gene (Table 1, Figure1). 25 (25%) and 75 cases (75%) of patients were males and females, respectively.

DISCUSSION

In our study, it was found that 75% of patients were males and only 25 % of them were females, which can be due to considering this disease as an occupational disease. Because more men have jobs such as livestock, butchering, agriculture and veterinary medicine are more infected with brucellosis and nearby 90% of veterinary surgeons in the Middle West of the United States obtain this disease(14). *Brucella* infects phagocyte cells such as macrophages, neutrophils and epithelial cells. *Brucella* after enter

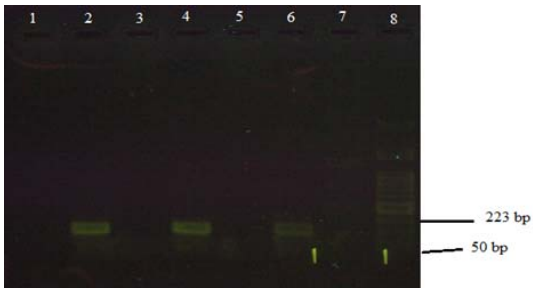

Fig. 1. Results of products electrophoresis with cep primers. Wells 3, 5,7. Negative control, wells 2, 4, 6. Examples of positive samples for cep gene and well 8 is related to DNA marker

to the cell; locate in vacuole-like structures. These vacuoles are then merging with lysosomes, due to the merge and acidification of the vacuole environment, bacterium excrete some virulence factors. Next, the vacuole membrane will connect to the endoplasmic reticulum, and provides the possibility of intracellular growth of *Brucella* and cause chronic infection[15]. Capsule is one of the most important factors in this bacterium. Capsule has several roles, including anti-phagocytosis, preventing the arrival of antibiotics, preventing dehydration, and antigen binding properties. But the most important role of capsule is anti-phagocytic property[16]. In the past it was believed that *Brucella* didn't have capsule, but recent studies have shown that some species of this bacterium have capsule which containing polysaccharides and proteins. The gene encoding the capsule is named cep and also in *Brucella* present antigen L which called antigen Vi in *Salmonella typhi* that has a similar role[17].

The innovation of this study is that the cep gene frequency in serum specimens examined that is very important because presence of cep gene in serum specimens can be indicating of bacteremia and septicemia. Diagnostic value of cep gene from other factors[18] that used for detection by PCR is not less and have a sensitivity approximately them, and because there is not in all strains of *Brucella* the factors and mechanisms of its pathogenesis is different from other factors, can be used as a complementary diagnostic factor in companying with other factors. Microcapsule also plays an important role in the escape of bacterium from *phagolysozyme* system. And because the study about distribution of capsule and cep gene was not available, this study has provided the first report on the *Brucella* status in Kermanshah province. *Brucella* strains which have eligible microcapsules are more likely to pathogenesis. The microcapsules also play a role in the process of anti-phagocytic and escape from the *phagolysozyme* system, so the presence of capsule can increase the pathogenicity of *Brucella*, Accordingly, in incidence of chronic infection is more effective and detecting of chronic infection in the laboratory is difficult(16). Hence can introduce the cep gene as a good candidate for vaccine design also as a promising candidate in detection of this bacterium. So, development and production of drugs which capable of inhibiting cep gene is most importance. In this study, frequency of cep gene was about 54% that shows about 50% of the strains studied in this investigation have high virulence.

CONCLUSION

According to the first time in this study was revealed the cep gene for diagnosis of brucellosis in the serum. So, cep gene can be used as a diagnostic component with other factors; however; this finding should be studied in animal models and diagnostic and therapeutic strategies linked with this gene will be also investigated.

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