Prevalence of Enterotoxins, Sea, Sec and Seq Genes of Staphylococcus Aureus from Clinical Isolate in Isfahan

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ABSTRACT

Background and Objective:
Staphylococcus aureus produce wide variety of enterotoxins. There are different methods for detection of toxins produced by this bacterium. Among these methods, PCR is a highly sensitive test. This method has a high specificity and needs a short time to accomplish. The aim of this study was to investigate the prevalence of enterotoxin genes in Staphylococcus aureus isolates using the PCR method.

Materials and Methods:
200 strains of Staphylococcus aureus isolated from clinical cases. Biochemical tests were performed for definitive diagnosis of these strains. Multiplex PCR method was used to identify genes of staphylococcal enterotoxin A, B and Q.

Results:
DNA amplification fragments for sea, sec and seq genes were 552, 271 and 122 bp, respectively. Through the 200 tested isolates, 60 isolates were found sea, 31 isolates sec, and 22 isolates were seq. 56.5 percent of the isolates were found of other types of enterotoxin.

Conclusion:
Abundance of type A enterotoxin of this bacterium, cause the most researchers focus on this enterotoxin. Due to Staphylococcus aureus isolated from clinical cases, PCR method can be useful to identify strains of enterotoxin A, C and Q gene in this isolates.

1. Introduction

Staphylococcus aureus is a significant cause of disease in humans. The bacterium contains several toxins. Different enterotoxin produced by these bacteria. About 18 enterotoxin of these bacteria have been discovered. (1) These toxins contain proteins with molecular weights of 26 to 29 kDa. The most important feature of these toxins are capable of causing vomiting in primates, resistance to heat, pepsin digestion, being super antigens. (2) 5% of human food poisoning caused by enterotoxins. Reports of the enterotoxin poisoning are rising. (3) Bergdoll reports, 95 percent of poisoning caused by Staphylococcus aureus enterotoxin are due to the type of A, B, C, D, E. The 5% remaining was due to the other type. (4, 5) Enterotoxin A is the most important enterotoxin produced by Staphylococcus aureus. This toxin is the major cause of food poisoning in the world, and most studies have been performed on this toxin. (6) However, there is little information about the type C and Q enterotoxin. There are Different methods to identify the bacterial toxins such as latex agglutination, ELISA, immunochromatography. All these methods require conditions for expression of gene in order to identify the toxins. (7) In PCR method to identify the gene encoding of toxin is done, it can be taken to identify Staphylococcus aureus gene encoding this toxin. In this method, the strain producing low-toxin will be detected. (8, 9) The aim of this study was to investigate the prevalence of enterotoxin A, C and Q among clinical isolates of Staphylococcus aureus.
Methods

In this study, 200 isolates of Staphylococcus aureus were examined. After transferring a single colony on blood agar and preparation, the phenotypic tests were performed.

DNA extracting:

Extraction of genomic DNA was performed by the DNA extraction kit (k0512). The quality of extracted DNA was controlled on 1 percent agarose gel. Measuring the concentration of DNA, the spectrophotometer device was used. DNA extracted at 4 °C was maintained.(8, 10)

PCR reaction:

To perform PCR reaction following mix was used: One microliter of template DNA, 0.5 microliter of Taq Polymerase DNA, 0.5 of each primer, 2 ml of DNTP, 2.5 ml PCR buffer, 1.5 ml MgCl₂(8) Adding distilled water the volume reached 25 ml finally. The reaction was performed in 32 cycles. The thermocycler machine program has started with a step of 5 min at 94° C for initial melting stage, then 35 cycles consisting of 15 seconds at 94° C for melting stage, 15 seconds at 58° to stage annealing and 20 seconds at 72° C for an extension period continued. A final extension step of 5 min and a temperature of 72° C was also considered. To investigate the product of PCR reaction, 5 ml of product was used for electrophoresis on 1% agarose gel.(11, 12)

Results:

In this study, 200 clinical isolates were studied. Phenotypic testing was performed on isolates. The genomic DNA of samples was extracted through DNA extraction kit, and PCR reaction was performed to detect enterotoxins. To detect extracted DNA quality electrophoresis method was used on 1% agarose gel. After transferring the samples on the gel, the result after 60 minutes was studied. To detect genes of A, C, Q enterotoxin, we used primer set forth in Table 1. PCR reaction was performed on isolates of 200 patients. Phenotypic tests for the detection of Staphylococcus aureus isolates were carried out. After PCR, 60 isolates had the sea gene. 31 isolates with the sec gen, 11 isolates, the seq gene, 87 strains were also contains other types of enterotoxin.

Discussion

Expression of enterotoxin A to Q among different strains of Staphylococcus aureus has been observed.(13) Various methods are used to detect these enterotoxins. In this study 113 strains tested by one of the A, C or Q enterotoxin. The most isolated staphylococcal enterotoxin was A (30%), and staphylococcal enterotoxin type Q had the lowest prevalence (11%). Adwan and colleagues findings in 2005 showed that 37% of Staphylococcus aureus containing enterotoxin genes. The prevalence of A and Q enterotoxin was equal to 10.8 percent.(14) Kwon and colleagues in 2004 by multiplex PCR method showed that 19/14% of the isolates containing the enterotoxin. The most types isolated was sei enterotoxin.(15) These findings are consistent with the findings of Chiang and colleagues in 2006 which said 74/1% of strains of Staphylococcus aureus had enterotoxin gene. The most isolated enterotoxin was A type (28/6%) and 8/2% isolated strains was C type.(16) In this study the sea, sec and seq genes were used to identify strains that are capable of producing A, C, Q enterotoxin. The attention of Iranian researchers, focused on type A of staphylococcal enterotoxins. Because between serotypes of Staphylococcus aureus, the most gastroenteritis have been reported by this type. However, the few studies have been done on other types of bacteria, including C and Q types.

Conclusion

The presence of enterotoxin A gene in clinical strains may represent widespread dissemination of these isolates in the environment. Transmission of these strains in food and healthy individuals can increase gastroenteritis the community. The PCR test can be important to identify strains of A, C and Q enterotoxin gene in these people. The importance of the issue in people who are carriers of the bacteria, and are constantly dealing with food becomes more marked. These people may transmit infections to food. Abundance of staphylococcal enterotoxin type A, which has led the most studies and attention paid to this enterotoxin. However, the incidence of poisoning staphylococcal enterotoxin A, is more than the other Staphylococcal enterotoxins. So that it identifies the importance of this type of staphylococcal enterotoxin more than others.

References
