A Comparative Multiplex PCR Methods for the Identification of Five Major Virulence Genes in Three Important Serotypes of Shigella Isolated From Patients with Diarrhea in Iran

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Background & Objectives: Shigella infection is one of the prevalent causes of diarrhoeal disease in most developing countries. Conventional microbiological diagnostic tests for Identification of Shigella species are time-consuming and requires a lot of work and cost. The object of the present research was to isolate and identify of three important serotypes of Shigella species including Shigella flexneri 2a, Shigella sonnei and Shigella dysenteriae type 1 from patients with bacillary dysentery and to detect their five major virulence genes, ShET1, ShET2, virG, virF and ipaBCD by using a rapid and novel comparative multiplex PCR. Analyses of the genetic markers related to the she pathogenicity island and shiga toxin gene allowed us to develop and validate stimulant diagnosis among three important Shigella´s serotypes.

Methods: At first phase, a total of 524 stool specimens of hospitalized patients with diarrhea were cultured for the isolation of Shigella Spp. in Tehran, Iran from February 2008 to September 2010. Among a total of 59 strains of Shigella isolated during this period, we identify Shigella flexneri 2a (3.38%) 2, Shigella flexneri-non 2a (27.12%) 16, Shigella sonnei (57.62%) 34, Shigella dysenteriae type 1 (1.7%) 1and otherserotypes (10.16%) 6, respectively, was discreted by using the differential mPCR approach. Serological assay confirmed the results of mPCR. In next step, the resulting five bands for ShET1, ShET2, virG, virF and ipaBCD were even and discrete with product sizes of 553, 968, 738, 607 and 330 bp, respectively. Our results revealed only (32.2%) 19 of the Shigella flexneri isolates was positive for ShET1 and the prevalence of ShET-2 was (93.22%) 55 in all the Shigella Spp studied. The all of the isolates possessed the ipaH and virG, while (96.61%) 57 and (55.93%) 33 harboured the virFand ipaBCD genes, respectively. Finally, in order to determination sensitivity of our comparative mPCR Methods, we used a purified DNA with a minimum concentration 20 ng/µl up to2 pg/µl.

Conclusion: We conclude that this comparative mPCR procedure is more reliable, sensitive, easier, reproducible and specific which is significantly faster than current conventional detection assays.

Keywords: Identification; MPCR; Shigella; Diarrhea; Virulence Genes, Iran