Detection of Multiresistant *Salmonella* Typhimurium DT104 by Multiplex PCR

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Abstract: Phage typing was almost the only way to confirm DT104, but it is so expensive and complicate to perform that it is available in just a few laboratories. Therefore, other rapid and accurate method becomes necessary. PCR has been programmed to detect DT104 and known to be very useful. There have been many different PCR programs to identify DT104, but in this study, *InvA*, *Mdh*, *Pse-1* and *CmlA/TetR* genes were used to amplify the specific regions of DT104 by multiplex PCR, which produce 393, 943, 468 and 602 bp PCR product, respectively. All DT104 were positive to these four genes, and ACSSuT type *S. Typhimurium*, other *Salmonella* spec. and other bacteria are negative to the specific genes.

Keywords: *Salmonella* Typhimurium DT104, Multiplex PCR, ACSSuT, GuSCN

Introduction: Antibiotic resistance in *Salmonella* has been on the rise. The major increased incidence of resistance can be attributed to *S. Typhimurium* definitive phage type 104(DT104) (Poppe et al., 1998). It has been revealed that the arrangement of antibiotic resistant genes is made up with at least two novel groups of mobile DNA elements, integrons (*Inc* and *Ind*), that are located in chromosome, but are considered to be originated from R-plasmid (Briggs et al., 1999).

In the present study, multiplex PCR was applied to detect *S. Typhimurium* DT104, and the condition for reaction was optimized.

Materials and Methods: The three strains of *S. Typhimurium* DT104 isolated from bovine were obtained from Cornell University. The eight strains of multidrug resistant *S. Typhimurium* strains, 15 strains of different *Salmonella* serotypes isolated from swine and other bacterial strains isolated in our laboratory during several years or stored as reference strains were used in this study. The ninety-five strains of *S. Typhimurium* isolated between 1996-1999 were tested to determine the resistant patterns. Antimicrobial susceptibility tests were preformed by a modified micro broth dilution method as described by Sahm and Washinton (Sahm et al., 1991). DNA was extracted according to the method based on the binding of DNA
to silicates in the presence of high concentration of GuSCN (Jones et al., 1993; Boom et al., 1999). The reaction mixture (50 µl total volume) consists of 20 µl of sterile water, 1 µl of each primer (20 pmole), 5 µl of 10 µl PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl), 8 µl of dNTP (2.5 mM each dATP, dTTP, dGTP and dCTP), 3.5 mM MgCl₂, 1 µl of Taq DNA polymerase and 1 µl of template DNA. The mixture was subjected to following conditions: pre-denaturation at 95 °C for 3 min and 30 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 45 s and extension at 72 °C for 1 min and final extension at 72 °C for 5 min and then at 4 °C. The PCR product was visualized by gel electrophoresis in 2 % agarose gel.

**Result:** The ninety-five strains of *S. Typhimurium* were tested for antimicrobial resistance (Figure 1). All strains were phage typed and were reported not to be DT104. Multiplex PCR was programmed using extracted DNA from DT104 and performed for all strains. But, there was no strain that was positive to all four genes except Cornell strains. All four genes were positive to all DT104, and *InvA* and *Mdh* were also positive to R type ACSSuT. *S. Typhimurium* and other *Salmonella* serotypes. Other bacterial species failed to produce any band, but *Shigella* and *Klebsiella* were positive to *InvA* and *Mdh*. *Yersinia* was weakly positive to *InvA* gene (Figure 2).

Figure 1. Resistant types of *S. Typhimurium*

![Graph showing resistant types of *S. Typhimurium*](image)

Figure 2. Multiplex PCR detection specificity for DT104, resistant and non-resistant *S. Typhimurium*, different *Salmonella* serotypes and other bacterial strains. Lane 1-3, DT104; lane 4-11, R type ACSSuT *S. Typhimurium*; lane 12, *S. Typhimurium*; lane 13-27, Other *Salmonella* serotypes; lane 28-38 Other bacterial species; lane 39, Negative control; M, standard size maker (φX 174-Hinc)
Discussion and Conclusions: In this study, \textit{InvA}, \textit{Mdh}, \textit{Pse-1} and \textit{ClmA/TetR} genes were chosen. \textit{InvA} gene was positive to all \textit{S. Typhimurium} and 15 strains of different \textit{Salmonella} serotypes, showing high specificity for \textit{Salmonella}. But, \textit{Shigella} and \textit{Klebsiella} were also positive and \textit{Yersinia} showed weak positive band. Therefore, it was possibly thought that there be more bacterial strains, which produce unexpected positive band. Also, \textit{Mdh} gene was positive to \textit{Shigella} and \textit{Klebsiella}. \textit{ClmA/TetR} and \textit{Pse-1} were very specific and positive only to DT104 among all 124 bacterial strains. GuSCN has been known as a powerful agent in the purification and detection of both DNA and RNA. Using the multiplex PCR program set up in the present study, it took about 5 hour to confirm DT104 from sample treatment to electrophoresis to visualize the result.

References

