1. INTRODUCTION

Salmonella spp. are one of the most important agents that leads to enteritis in the world. Additionally, it can lead to more critical health problems such as bacteremia and enteric fever[1]. This group of bacteria include more than 2600 serotypes and consists of two species called *S. enterica* and *S. bongori. S. enterica* spp. are responsible 99% of *Salmonella* infections and *S.* Enteritidis and *S.* Typhimurium are the most commonly isolated serotypes both in our country and in developed countries[2]. Virulence factors which responsible for invasion, extraintestinal spread and intracellular survival are encoded by genes located in *Salmonella* pathogenicity island (SPI). While some pathogenicity island are seem to be preserved in *Salmonella* genus, some others are special for certain serotypes. Based on presence of SPI and SPI features, *Salmonella* serotypes differ from each other in terms of adaptation in host cell, virulence factors and severity of infections[3].

In most cases, resultant infections do not necessitate antibiotic treatment due to the self limiting nature of disease. However, antibiotic treatment may be necessary for some situations like invasive infections, advanced age and immunosupression[4]. In such cases ampicillin is widely used to treat *Salmonella* infections[5]. Therefore resistance to ampicillin has emerged and beta-lactam enzymes are primarly responsible of ampicillin resistance[6].

The objectives of this study were to investigate beta-lactam resistance, the epidemiological relationship, serotype distribution and prevalence of the beta-lactamase genes namely bla_{TEM}, bla_{PER}, bla_{CTX-M}, bla_{SHV} and virulence genes of clinical ampicillin resistant *S. enterica.*

2. EXPERIMENTAL

Antimicrobial susceptibility

Salmonella enterica strains isolated in Bacteriology Laboratory between 2010-2012 years were used in our study. *In vitro* antibiotic susceptibilities of 117 *S. enterica* isolates were examined using modified Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standarts Institute (CLSI)[7]. Standart ampicillin(10µg), cefotaxime(30µg), ciprofloxacin(5µg) sulfamethoxazole/trimethoprim (23,75/ 1,25µg) (Oxoid, United Kingdom) discs were used to detect the resistance. The other part of the study was performed in ampicillin resistant strains. MIC values

of the isolates were also determined by broth microdilution in accordance with the recommendations of CLSI and *S. enterica* ATCC 04059 was used as control strain[8].

DNA isolation

DNA isolation was performed for use in PCR studies. For this purpose, isolates were suspended and homogenized at 200 µl of sterile ultrapure water. Then isolates were incubated in heat block at 95 °C for 10 minutes. Microtubes centrifuged at 13000 rpm for 5 minutes. The supernatants were transferred to sterile microtubes and stored at -20 ° C for use in PCR studies.

Serotypes and epidemiological relation

The strains were serotyped by Turkish Public Health Agency, National Microbiology Reference Laboratory. Epidemiological relations of the isolates was analyzed by PCR using Enterobacterial repetitive intergenic consensus (ERIC)-2 and ERIC-1R primer[9]. To evaluate similarity between these isolates, Jaccard coefficients were derived from the banding patterns. Dendrograms were constructed according to the unweighted pair group method (UPGMA) with arithmetic mean method, using Jaccard coefficients and MEGA software version 4.0.

Beta-lactamase genes

bla$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{PER}$ genes were determined using primers targeting the relevant regions[10-12] by convantional multiplex PCR method. PCR assays were run in 25 µl amplification mixture composed of 5µl bacterial DNA template, 2,5 µl taq buffer 1,5 mM $MgCl_2$, 200 µM dNTP, 30 pmol forward and reverse primers and 1.25 U taq polimerase.

Virulence genes

Six different virulence genes were analysed with two different multiplex PCR reaction, using primers targeting the relevant genes[13,14]. For this purpose, 5µl bacterial DNA tamplate, 2,5 µl Taq Buffer, 1,5 mM $MgCl_2$, 200 µM dNTP, 20 pmol forward and reverse primers, 1.25 U taq polimerase were prapared in 25 µl volume.

3. RESULTS

As a result of the disc diffusion test, ten (8,5%) out of 117 *S. enterica* isolates were detected resistant to ampicillin. These resistant strains were susceptible to ciprofloxacin, cefotaxime, sulfamethoxazole/trimethoprim. Ampicillin MIC range of the isolates were found as 512-128 µg/mL. The strains were divided into seven different clusters based on ERIC-PCR results. Detected serotypes were as follows; Five *S.* Enteritidis, 2 *S*. Infantis, 1 *S.* Typhimurium, 1 *S*. Corvallis. One isolate could not be serotyped. Five strains involved bla_{TEM} genes, two strains contained $bla_{CTX\text{-}M}$ genes. bla_{PER} and bla_{SHV} genes were not encountered. *InvA*, *pipD*, *sopB*, which are of virulence genes, were detected in all strains, *sifA* in seven, *pefA* in four and *sopE* in three strains.

4. DISCUSSION

Salmonella genus involves many members. Some *Salmonella* serotypes are known to be more commonly isolated. In the light of this information and existing data; most frequently isolated *Salmonella* sertoype is known to be *S.* Enteritidis[15]. In our study, five of ampicillin resistant *Salmonella* strains were determined as *S*. Enteritidis. In 2014, Maraki et al.[4] determined *S*. Enteritidis as the most commonly isolated serotype of *Salmonella* (37,3%). Another study carried out by Ozdemir and colleagues[16], *S.* Enteritidis was determined as the most commonly isolated serotype from *Salmonella* isolates collected from 4 different provinces of Turkey. Our results showed consistency with the literature.

Serotyping is the basic phenotypic method for epidemiological investigation of isolates. Nonetheless couldn't differentiate the strains of the same serotype. Genotypic methods like Pulsed field gel electhrophoresis (PFGE), ERIC-PCR, Repetitive element palindromic PCR (REP-PCR), can distinguish the strains more effectively. Although PFGE is the golden standard method for fingerprinting, due to the lack of equipment and to avoid protocols lasting four-five days, a simpler method of ERIC-PCR was preferred[17,18]. In our study strains were divided into 7 unrelated clusters by ERIC-PCR method with acceptable (≥0,90) discriminatory index (DI) value of 0,92. When literature and data obtained in this study are evaluated, the ERIC-PCR is considered to be useful and easily applicable method for genotyping of strains.

Recently, *Salmonella* strains show resistance against many antibiotic groups. Ampicillin, which is a member of beta-lactam antibiotics, is the fist line agent used in the treatment of *Salmonella* infections. *Salmonella* strains that resistant to ampicillin and other beta-lactams pose a risk for public health[5]. According to disc diffusion results ampicillin resistant strains were susceptible to other group of antibiotics such as ciprofloxacin, cefotaxime, sulfamethoxazole/trimethoprim.

According to data gained in abroad, rate of ampicillin resistance against *Salmonella* varies from a country to other. As for studies abroad; India, ampicillin resistance in *S. enterica* isolates was detected as 25% in 2011[19]. It was 33% in *Salmonella* isolated from children in Cambodia[20], 46% in Korea[21], 55% in Spain[22] and 8% in the United States[23]. In our country there were a few studies of clinical *Salmonella* strains which were isolated from children's hospital within 5 years. The rate of ampicillin resistance in *Salmonella* strains were determined 25,8% in 2012[24] , 19% in 2014[25]. However, resistance rates were higher than our study. At this point the stress of starting antibiotic treatment empirically in paediatric patients, before culture results, might be responsible of higher ampicillin resistance rates than our study.

In our study in five isolates having *bla*$_{TEM}$ genes and in two isolates having *bla*$_{CTX-M}$ genes were found. *bla*$_{CTX-M}$ positive isolates were in *S*. Infantis serotype. Four of five *bla*$_{TEM}$ gene positive isolates were in *S*. Enteritidis serotype while remaining one strain was in *S*. Typhimurium. While most common beta-lactamase genes of *Salmonella* isolates are the variants of *bla*$_{CTX-M}$ and *bla*$_{SHV}$[26] , no *bla*$_{SHV}$ gene was found in isolates used in our study. Different rates of beta-lactam resistance genes were reported in studies carried out abroad. Among ampicillin resistant 20 *Salmonella* isolates, beta-lactamase genes were found to be *bla*$_{SHV}$ 100%, *bla*$_{TEM}$ 85% *bla*$_{CTX-M}$ 5%[2]; In beta-lactam resistant 90 strains in Spain; *bla*$_{TEM}$ 22%, *bla*$_{CTX-M}$ 1% [22]. In Netherlands, a study with 34 *Salmonella* strains isolated from humans and environment, Hasman et al.[6] detected *bla*$_{TEM}$ gene in 19 (55%) strains, *bla*$_{CTX-M}$ gene in six (17%) and *bla*$_{SHV}$ gene in three (8%) strains and all 34 isolates were found resistant to penicillin. A study conducted on *S*. Typhimurium in 2011 in our country detected 23% *bla*$_{CTX-M}$ gene, 76% *bla*$_{TEM}$ gene and 100% *bla*$_{SHV}$. *bla*$_{PER}$ gene could not be detected in any isolates[27].

Salmonella bacteria carry many different and complex virulence factors. Investigating the presence of virulence factors coded as different pathogenicity island, will guide us in the matter of discovering *Salmonella* pathogenesis. Our study was thought as the

first study carried out in our country about the virulence factors of *Salmonella*, at least three virulence factors were detected in all strains. *invA* gene was determined in all isolates involved in this study and it was present independent from conditions like serotype and resistance genes. Dione et al.[26] detected *invA* gene in 99,5% of strains in a study. Another study conducted by Smith et al.[5] in 2010 encountered *invA* gene in all. Determination high level of *InvA* gene in different regions despite different serotypes and antimicrobial susceptibility profiles indicated to existence of a preserved region in this gene. Thus the idea of using this gene for rapid diagnosis of *Salmonella* with PCR as a target region has rised and this idea had led to studies with positive outcomes[28].

Generally *sopE* gene, which is shown the lowest prevalence, has associated with epidemical cases[26,29]. In our study, belonging of three strains that involve this gene to the same group according to ERIC-PCR and isolation of them in a short time indicated that they may have been isolated after a community onset epidemic.

Hughes et al.[14], detected *pipD* and *sopB* genes in all strains and these virulence factors has been associated with enteritis. Detection of these genes in all resistant isolates indicated a possible relationship between these virulence factors and resistance. A study conducted by Dione et al.[26] and data obtained from our study give similar results. Also, Sim et al.[30] detected alterations in beta-lactam resistance as a result a mutations created in these two genes. Although our study did not directly show the realtionship between resistance and virulence factors, it showed necessitation of further extensive studies about the relationship between these two factors.

pefA gene detected in four strains at this study, may be located on the same or different plasmid with different virulence (*spv*) genes. Only a fraction of *Salmonella* serotypes carry different sized plasmids which is named as serovar specific. However, its is known that not every plasmid carrying serotype includes *pefA* gene. Therefore, it is thought that *pefA* gene has a lower prevalence compared with the other virulence factors[31]. Hughes et al.[14] showed in a study that only three isolates out of 32 involved *pefA* gene. Skyberg et al.[13] performed a study on *Salmonella* of different serotypes, *pefA* gene was found 11 out of 152 strains, and it was only present in serotypes of *S*. Typhimurium and *S.* Enteritidis. Low prevalence of *pefA* gene in our study was compatible with the results of the other studies. Additionally,

three out of four *pefA* gene carrying strains belonged to *S*. Enteritidis serotype and this result was consistent with the literature data.

sifA gene, which enables to sustain vitality of *Salmonella* bacteria in macrophages was detected in seven strains in our study. Hur and colleagues[32] detected *sifA* gene in all of 42 strains. Skyberg et al.[13] determined the *sifA* gene 137 out of 158 isolates. Existence rate of *sifA* gene in this study was detected similar with the studies conducted abroad.

5. CONCLUSION

As a result, in the light of studies both in our country and in abroad, it is known that there are *Salmonella* strains that are resistant to antimicrobial agents some of them are beta lactamase producing. Further investigation on resistance and virulence profiles of *Salmonella* strains will enable us to better understand the pathogenesis of infections and to be able to take better measurements and give proper treatments.

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