

Full Length Research Paper

A study of the detection of Salmonella heidelberg in some Egyptian foods

Sedki Hegazy

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Egypt (Now, on Secondment to College of Applied Medical Science, Majmaah University, Al-majmaah 11952, P.O.Box 1816, KSA). E-mail: sedki_hegazy@yahoo.com.

Accepted 09 July, 2015

A total of two hundred samples of Egyptian foods including chicken, beef, milk, *Kushary* and *Sogok* (sausage) were obtained from local markets and super markets. The foods were examined to detect *Salmonella heidelberg*. As a result, *S. heidelberg* were detected in 6 of 40 (15%) chicken, 2 of 40 (5%) beef, 1 of 40 (2.5%) milk, 1 of 40 (2.5%) *Kushary*, and 4 of 40 (10%) *Sogok*. The effects of temperature, pH, and antibiotics on growth of *S. heidelberg* were studied. The results indicated that 35°C was the optimum temperature and 6.8 was the optimum pH for *S. heidelberg* growth. Antibiotic susceptibility patterns indicated that 4 out of 14 (28.5%) of *S. heidelberg* isolates (E5, E7, E12 and E13) were resistant to cefazolin, bacitracin, ceftazidime and sulphamethoxazole/ trimethoprim 19:1 respectively. This study emphasized that the presence of *S. heidelberg* in some Egyptian foods under investigation, such as chicken, beef, milk, *Kushary* and *Sogok* (sausage), is of concern due to the fact that they have the potential to cause human infections, and are resistant to some antibiotics. So, the current control measures on animal origin foods should be improved.

Key words: Detection, isolation, characterization, Salmonella heidelberg, Egyptian foods.

INTRODUCTION

Salmonellosis is a significant health problem (Kaldhone et al., 2008). In 1999, there were an estimated 1.4 million cases of *Salmonella* infections in the United States, which resulted in 17,000 hospitalizations and 585 deaths (Mead et al., 1999). Since then, the overall rate of salmonellosis in the United States has decreased by approximately 9%; however, the rate of *Salmonella enterica* serovar Heidelberg infections increased by 25% over the same period (CDC. 2006). *S. enteric* serovar Heidelberg ranks fourth among serotypes causing human salmonellosis (CDC, 2005, 2008; FDA, 2010) but is often the most commonly detected serotype among turkey and chicken *Salmonella* isolates submitted to the U.S.

Department of Agriculture's National Veterinary Services

Laboratory (NVSL) (Ferris et al., 2002; Foley et al., 2008). Annually, human infections with *S. enterica* serovar Heidelberg lead to approximately 84,000 cases of salmonellosis and contribute to approximately 7% of the *Salmonella*-related deaths in the United States, the second highest percentage after *S. enteric* serovar Typhimurium (Hennessy et al., 2004; Kennedy et al., 2004; Vugia et al., 2004).

In addition to that, *Salmonella enterica* serovar Heidelberg ranks among the top 3 serovars isolated from persons infected with *Salmonella* in Canada (EDPNML, PHAC, CSCHAH, 2009), and is more frequently reported in North America than in other regions of the world (WHO, 2009). Although many *Salmonella* Heidelberg infections result in mild to moderate illness, the bacterium also causes severe illness with complications such as septicemia, myocarditis, extraintestinal infections, and death (Burt et al., 1990; Vugia et al., 2004).

The most common source of *S. enterica* serovar Heidelberg infections is likely the consumption of undercooked or mishandled poultry products, such as turkey, chicken, and eggs (Hennessy et al., 2004). *Salmonella* surveillance data collected in poultry processing plants as part of U.S. Department of Agriculture's Pathogen Reduction-Hazard Analysis Critical Control Point Program showed that 11.4% of broiler chickens and 7.1% of turkeys carried *Salmonella* infections in 2006 (U.S. Department of Agriculture, 2007). These findings, coupled with the fact that there has been a nearly fourfold increase in the per capita consumption of poultry products in the United States over the past half century (Buzby and Farah 2006), indicate that the contamination of poultry products with *S. enterica* serovar Heidelberg is a major public health concern. Surveillance data show that antimicrobial resistance among *S. enterica* serovar Heidelberg isolates has been increasing.

In addition to that, sources of human Salmonella heidelberg infection include consumption of poultry or eggs and egg-containing products (Hennessy et al., 2004; Bucher et al., 2007). In Canada, S. heidelberg is commonly isolated from healthy chickens from farm, abattoir, and retail sources (Diarrassouba et al., 2007; PHAC, 2006). It has also been isolated, although less frequently, from ground beef, pork, and turkey meat (Escartín et al., 2000; Zhao et al., 2008) and from clinical samples from various animal species (PHAC, 2006). Moreover, a variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of food-borne illness (Beuchat, 1995; D'Aoust, 1997; Doyle et al., 1997).

The incidence of human infections by *S*. heidelberg increased by 20% from 1996 to 2005, even though the overall number of cases of salmonellosis decreased by 9% (CDCP, 2006). However, since 2005, the incidence of *S*. heidelberg infections has decreased such that in 2009, the overall incidence had decreased by 33% as compared to 1996 baseline data (CDCP, 2010). *S*. heidelberg infections are likely caused by the consumption of contaminated meat, poultry, eggs, or egg containing products (Bucher et al., 2007; Chittick et al., 2006; Hennessy et al., 2004). The FoodNet data indicated that the principal risk factor for *S*. heidelberg infections is the consumption of eggs prepared outside the home (Hennessy et al., 2004).

S. heidelberg appears more invasive than other gastroenteritis- causing serovars; ≈9% of isolates of this serovar received through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) during 2003 to 2005 were recovered from (PHAC, 2005). blood samples Treatment with antimicrobial agents may be life-saving in the case of invasive infections (Dutil et al., 2010). However, data from the National Antimicrobial Resistance Monitoring System (NARMS) indicates that the percentage of S. enteric serovar Heidelberg isolates from humans and poultry (chicken and turkey) that were resistant to cephalosporins increased overall from 1997 to 2003. For example, in 1997, none of the isolates from humans and 1.6% of poultry isolates were resistant to ceftiofur (Tio); by 2003, the numbers increased to 5.2 and 7.4%,

respectively (FDA, 2006). This increase in cephalosporin resistance is likely associated with the spread of the AmpC _-lactamase, which is encoded by *bla*CMY (Winokur et al., 2000; Zhao et al., 2003; Aarestrup et al., 2004).

In addition to that, the spread of multidrug resistance among *S. heidelberg* isolates is a risk to the management of salmonellosis in both veterinary and human clinical practice. Therefore, an increased understanding of pathogen distribution and mechanisms of antimicrobial resistance transmission is important for development of strategies to limit salmonellosis due to multidrug-resistant strains (Kaldhone et al., 2008). The main objective of this study is to detect *S. heidelberg* in some Egyptian food. As such, the isolates were characterized by studying the relation of pH and temperature to growth of the isolates and their susceptibility to different antimicrobial agents.

MATERIALS AND METHODS

Sample collection and analysis

A total of two hundred samples of Egyptian foods including (quantity of samples are given in brackets): chicken (40), beef (40), milk (40), Kushary (40), and sausage (Sogok) (40) were used in this study. All samples under investigation were collected from local markets and super markets (solid in Cairo and Assuit city, Egypt) in sterile plastic bags and stored at -4°C until they were analyzed.

Enrichment and isolation

Pre-enrichments and isolation were performed as a standard method for isolation of *Salmonella* from foods. Food sample (25 g) were added to 225 ml buffer peptone water (BPW) (Oxoid) incubated for 24 h at 37°C. A total of 214 bacterial isolates were obtained in the form of highly pure culture from food samples under investigation (200 samples); these bacterial isolates were screened and the suspected similar ones were placed together for the purpose of selection and identification processes. 93 isolates were collected and morphologically identified as gram negative, and subjected for dropping of 0.1 ml (three drops) of pre-enrichment on to modified semi-solid reppaport vassilliadis agar (MSRV) (Oxoid) and incubated for 24 h at 42°C.

Identification

Presumptive Salmonella colonies were sub-cultured on tryptic soya agar (TSA) (Difco) plates for 24 to 48 h at 37°C in order to obtain pure culture according to the study of Stefanovicova et al. (1998). Sub-culturing of positive samples was done on modified brilliant green agar (MBGA) (Oxoid) and xylose-lysine-desoxycholate

agar (XLDA) (Oxoid), and incubated for 24 h at 37°C. 38 out of 93 G-ve isolates were pre-identified as *Salmonella*. Biochemical tests were carried out by BBL[®] Entrotube miniaturized diagnostic kit (Bacton Dikinson), and *Salmonella* serological confirmation was investigated by slid agglutinations O, H antiserum (Behring), according to the study of Hendriksen (2003).

Effect of temperature on Salmonella heidelberg growth

All *S. heidelberg* (14) isolates were inoculated into conical flasks containing nutrient broth and incubated for 24 h at different temperatures namely 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C.

Effect of pH values on Salmonella heidelberg growth

The pH values of nutrient broth media were adjusted by a model Jenway 3020 pH meter using 1N Na₂OH and 1N HCl at different values (1.2, 2.3, 3.1, 4.3, 5.4, 6.8, 7.0, 8.2, 9.1, and 10.2). Confirmation of measurements was carried out using pH meter. All *S. heidelberg* (14) isolates were inoculated into conical flasks containing nutrient broth and incubated at 35°C for 24 h.

Antimicrobial susceptibility test

Antimicrobial susceptibility of the *S. heidelberg* isolates (14) was studied by the disk diffusion technique on Isosensitest medium by using the zone size criteria recommended by the disk manufacturer and on the basis of the breakpoints established by Swedish reference group for antibiotics (SRGA, 1990). The following antibiotics and antimicrobial agents (Oxoid) were used: Cefazolin, ceftazidime, polymxin B, tetracycline, cefonicid, chloramphenicol, amoxycillin clavulanic acid (augmentin) 2:1, bacitracin, ampicillin, gentamycin, kanamycin, penicillin G, sulphamethoxazole/ trimethoprim 19:1 and nitrofurantion.

RESULTS AND DISCUSSION

Detection of Salmonella heidelberg

S. heidelberg is among the most commonly detected bacteria from retail meats and food animals and ranks fourth among serovars associated with human infections, causing an estimated 84,000 illnesses in the United States annually (Hennessy et al., 2004; CDCP, 2008, FDA, 2009). While most *Salmonella* infections are selflimiting and resolve within a few days, *S. heidelberg* tends to cause a disproportionately high percentage of invasive infections (Vugia et al., 2004) for which antimicrobial therapy is often warranted, making antimicrobial resistance a significant concern (Han et al., 2011).

The examination of food samples of animal origin for their presence has become routine all over the world. However, *Salmonella enterica* infections are a significant public health concern worldwide, with an estimated 1.028 million cases, 19,000 hospitalizations, and 400 deaths in the United States each year (Scallan et al., 2011). Human salmonellosis is typically associated with the consumption of contaminated foods, such as fresh and processed meat and poultry, eggs, and fresh produce (Benenson and Chin, 1995; Mead et al., 1999; Tauxe, 1991).

Moreover, sources of human *S. heidelberg* infection include consumption of poultry or eggs and egg-containing products (Hennessy et al., 2004; Bucher et al., 2007). In addition to that, *Salmonella* surveillance data collected in poultry processing plants as part of U.S.

Department of Agriculture's Pathogen Reduction-Hazard Analysis Critical Control Point Program showed that 11.4% of broiler chickens and 7.1% of turkeys carried *Salmonella* infections in 2006 (U.S. Department of Agriculture, 2007). It is confirmed that, poultry, meat, milk, dairy products, person-to-person spread and pet-toperson exposures have caused many outbreaks (Gangarosa, 1978; Todd, 1985).

In the current study, 200 food samples including, chicken (40), beef (40), milk (40), Kushary (40), and sausage (Sogok) (40) were collected from different supermarkets and local market solid in Cairo and Assuit city, Egypt. Total viable counts (TVCs) found in the individual food samples were recorded (data not shown). 238 bacterial isolates were obtained in the form of highly pure culture, these bacterial isolates were screened and the suspected similar ones were placed together. Only 93 G-ve isolates were selected and subjected for identification processes.

The isolates were identified according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984). Only 38 out of 93 of these G-ve bacterial isolates were identified from different food samples. According to the preidentification (Gram's stain, cell shape and cell arrangement), the bacterial isolates (38) were identified as Salmonella sp. Only 14 of Salmonella isolates out of 93 G-ve bacterial isolates (15%) were suggestive of being belonging to S. heidelberg; these isolates were identified as S. heidelberg according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984) and Bergey's Manual of Determinative Bacteriology (Hensyl, 1994). All isolates were grown as well on modified semi-solid reppaport vassilliadis agar (MSRV) (Oxoid), modified brilliant green agar (Oxoid) and xylose-lysinedesoxycholate agar (XLDA) (Oxoid). Biochemical tests carried out by BBL[®] Entrotube TM miniaturized diagnostic kit (Bacton Dikinson) and the S. heidelberg serological confirmation were investigated by slid agglutinations O, H antiserum (Behring).

| Destarial inclute | Foods* | | | | | | | | | | |
|---------------------------|----------|----------|----------|----------|-----------------|--|--|--|--|--|--|
| Bacterial isolate | Chicken | Beef | Milk | Kushary | Sausage (Sogok) | | | | | | |
| Salmonella heidelberg E1 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E2 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E3 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E4 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E5 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E6 | 1 (2.5%) | - | - | - | - | | | | | | |
| Salmonella heidelberg E7 | - | 1 (2.5%) | - | - | - | | | | | | |
| Salmonella heidelberg E8 | - | 1 (2.5%) | - | - | - | | | | | | |
| Salmonella heidelberg E9 | - | - | 1 (2.5%) | - | - | | | | | | |
| Salmonella heidelberg E10 | - | - | - | 1 (2.5%) | - | | | | | | |
| Salmonella heidelberg E11 | - | - | - | - | 1 (2.5%) | | | | | | |
| Salmonella heidelberg E12 | - | - | - | - | 1 (2.5%) | | | | | | |
| Salmonella heidelberg E13 | - | - | - | - | 1 (2.5%) | | | | | | |
| Salmonella heidelberg E14 | - | - | - | - | 1 (2.5%) | | | | | | |
| Total (%) | 15 | 5 | 2.5 | 2.5 | 10 | | | | | | |

Table 1. Detection of *S. heidelberg* in some Egyptian foods.

* 40 samples from each food.

Only 14 isolates (E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13, and E14) were identified as S. heidelberg by both biochemical and serological testing as mentioned before (Table 1). S. heidelberg were detected in 6 of 40 (15%) chicken, 2 of 40 (5%) beef, 1 of 40 (2.5%) milk, 1 of 40 (2.5%) Kushary, and 4 of 40 (10%) Sogok as shown in Table 1. Same isolates of S. heidelberg, by several investigators, were isolated from chickens (PHAC, 2006; Bucher et al., 2007; Diarrassouba et al., 2007), beef (Escartín et al., 2000; Sørensen et al., 2002; Zhao et al., 2006; Van et al., 2007; Anonymous, 2008; Zhao et al., 2008), turkey (Nayak and Kenney, 2002; Fakhr et al., 2006; Kaldhone et al., 2008); poultry (Chambers et al., 1998; Demczuk et al., 2000; Mammina et al., 2003; Van et al., 2007); eggs (Chambers et al., 1998; Demczuk et al., 2000).

In addition to that, presence of Salmonella spp. was detected in meat, poultry and shellfish. Thus, it highlights the considerably high prevalence of Salmonella spp. in raw meat and poultry, in which 32/50 (64%) of pork samples, 31/50 (62%) of beef samples, and 16/30 (53.3%) of chicken samples were contaminated with Salmonella spp. However, the rate of Salmonella contamination in shellfish (18.0%) was much lower than that in meat and poultry (60.8%) (Van et al., 2007). Moreover, the prevalence of Salmonella in beef cattle was 4.6% (11/240), and the rate was significantly higher in fasted cattle (7.46%) than in non-fasted cattle (0.94%) (Abouzeed et al., 2000). S. heidelberg strains were isolated from humans, retail meats, and food animals (Kaldhone et al., 2008; Lynne et al., 2009; Nayak et al., 2004; Oloya et al., 2009; Zhao et al., 2008).

A total of 298 Salmonella serovar Heidelberg isolates were recovered, representing 21.6% of all Salmonella serovars from retail meats, of which 178 (59.7%) were from ground turkey, 110 (36.9%) were from chicken breast, and 10 (3.4%) were from pork chops; moreover, none was found in ground beef (Zhao et al., 2008). In addition to that, Bryan and Doyle (1995) reported that turkey and chicken meat can be vehicles of food borne salmonellosis because the raw product is initially contaminated with Salmonella cells when it is delivered to the consumer or due to subsequent undercooking, cross-contamination, or improper thawing. All bacterial isolates were selected to study the effect of temperature, pH and antimicrobial susceptibility on bacterial growth.

Effect of temperature and pH on growth of *Salmonella heidelberg* isolates

Earlier studies of predictive models describing the effects of environmental factors such as temperature, pH, water activity, and preservative agents on the growth, survival, or inactivation of *Salmonella* spp. are still limited (Park et al., 2007). The effect of temperature on growth of *S. heidelberg* isolates were studied at different temperatures including: 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C respectively. The results indicated that the optimal temperature for growth of all *S. heidelberg* isolates was 35°C, as such the increase or decrease of temperature indicates weak growth (Table 2). Survival populations of *S. heidelberg* at low-temperature (4, 7 and 10°C) showed a maximum growth of 2 logs in brain heart infusion broth (BHI) at 10°C among all the treatments (Morey and

| Bacterial isolate | Temperature (°C) | | | | | | | | | | | | | |
|---------------------------|------------------|----|----|----|----|-----|-----|----|----|----|--|--|--|--|
| Bacterial isolate | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | | | | |
| Salmonella heidelberg E1 | - | w | + | + | + | + | +++ | + | - | - | | | | |
| Salmonella heidelberg E2 | - | w | + | + | + | + | ++ | + | - | - | | | | |
| Salmonella heidelberg E3 | - | VW | w | + | + | ++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E4 | - | - | w | + | + | ++ | +++ | ++ | vw | - | | | | |
| Salmonella heidelberg E5 | - | VW | w | + | + | ++ | +++ | ++ | - | - | | | | |
| Salmonella heidelberg E6 | - | - | w | + | ++ | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E7 | - | VW | w | + | + | ++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E8 | - | VW | w | + | ++ | ++ | +++ | + | vw | - | | | | |
| Salmonella heidelberg E9 | - | - | w | + | ++ | ++ | +++ | + | - | - | | | | |
| Salmonella heidelberg E10 | - | VW | w | + | + | ++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E11 | - | - | w | + | ++ | ++ | +++ | ++ | - | - | | | | |
| Salmonella heidelberg E12 | - | VW | w | + | + | ++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E13 | - | - | w | + | ++ | ++ | +++ | + | - | - | | | | |
| Salmonella heidelberg E14 | - | vw | w | + | + | ++ | ++ | + | - | - | | | | |

Table 2. Growth of S. heidelberg isolates at different temperatures.

-, No growth; vw, very weak growth; w, weak growth; +, moderate growth; ++, good growth; +++, abundant growth; ++++, excellent growth.

Singh, 2012).

On the other hand, the effect of pH values on growth of *S. heidelberg* isolates were studied at different pH values including: 1.3, 2.2, 3.2, 4.4, 5.2, 6.8, 7.1, 8.3, 9.2, and 10.0 respectively. The results indicated that the optimal pH values for growth of all *S. heidelberg* isolates was 6.8, as such the increase or decrease of temperature indicates decreased growth (Table 3). However, there is little information about the relation of temperature, pH and growth of *S. heidelberg* as mentioned before.

Antimicrobial susceptibility

Antibiotic resistance of fourteen S. heidelberg isolates recovered from food samples were tested for antibiotic susceptibility against 15 antibiotics. The antibiotic resistance rates for each source and for the whole set of isolates are shown in Table 4. The isolates were studied by the disk diffusion technique on Iso-sensitest medium by using the zone size criteria recommended by the disk manufacturer and based on the breakpoints established by Swedish reference group for antibiotics (SRGA, 1990). Fifteen antibiotics and antimicrobial agents were applied during this study against the growth of the fourteen S. heidelberg isolates (Table 4). The following antibiotics and antimicrobial agents (Oxoid) were used: cefazolin, polymxin B, tetracycline, Amoxycillin clavulanic acid (augmentin) 2:1, chloramphenicol, bacitracin, ampicillin, Gentamicin, cefonicid, ceftazidime, kanamycin, penicillin sulphamethoxazole/ trimethoprim 19:1 G. and nitrofurantion and streptomycin, respectively.

Results of the antimicrobial susceptibility patterns

indicated that S. heidelberg isolate (E5) was resistant to cefazolin and S. heidelberg isolate (E7) was resistant to bacitracin. S. heidelberg (E12) was resistant to ceftazidime and S. heidelberg isolate (E13) was resistant to Sulphamethoxozole/Trimethoprim 19:1 as shown in Table 4. Briefly, in the current study, the results indicated that of the 4 out of 14 isolates, 28.5% were resistant to four antimicrobial agents tested and of the 10 out of 14 isolates, 71.5% were susceptible to other eleven antimicrobial agents under investigation (Table 4). In the same way, 42% of the isolates were resistant to at least 1 of the 15 antimicrobial agents tested, and 4% of the isolates were resistant to 8 or more antimicrobial agents. Resistances to streptomycin (32%), tetracycline (30%), and kanamycin (24%) were most commonly detected (Kaldhone et al., 2008).

Conversely, the resistance rates were lower than those observed for NARMS isolates from turkey and ground turkey, where typically more than 50% of isolates in 199 of 298 isolates (67%) were resistant to at least one antimicrobial agent, with 16.4% (n _ 49) of the isolates being resistant to at least five antimicrobials. Six isolates (3.0%), all recovered from ground turkey, were resistant to at least nine antimicrobials, including beta-lactams, aminoglycosides, chloramphenicol, sulfamethoxazole, and tetracycline. For chicken and turkey isolates combined, resistance to tetracycline was most common (39.9%), followed streptomycin by (37.8%),(27.7%), gentamicin (25.7%),sulfamethoxazole kanamycin (21.5%), ampicillin (19.8%), amoxicillinclavulanic acid (10.4%), cefoxitin (9.0%), and ceftiofur (9.0%). Rare isolates were resistant to chloramphenicol

| Pastarial isolate | pH values | | | | | | | | | | | | | |
|---------------------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|------|--|--|--|--|
| Bacterial isolate | 1.3 | 2.2 | 3.2 | 4.4 | 5.2 | 6.8 | 7.1 | 8.3 | 9.2 | 10.0 | | | | |
| Salmonella heidelberg E1 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E2 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E3 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E4 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E5 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E6 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E7 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E8 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E9 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E10 | | - | - | + | + | +++ | +++ | + | - | - | | | | |
| Salmonella heidelberg E11 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E12 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E13 | | - | - | + | + | +++ | ++ | + | - | - | | | | |
| Salmonella heidelberg E14 | | - | - | + | + | +++ | +++ | + | - | - | | | | |

Table 3. Growth of S. heidelberg isolates in relation to different pH values.

-, No growth; +, moderate growth; ++, good growth; +++, abundant growth; ++++, excellent growth.

 Table 4. Susceptibility of some antibiotics against S. heidelberg isolates.

| | | Antibiotics | | | | | | | | | | | | | |
|---------------------------|----------------|-------------|--------------|--|-----------------|------------|------------|------------|-----------|-------------|-----------|--------------|--|----------------|--------------|
| Bacterial isolate | Cefazolin | Polymxin B | Tetracycline | A moxycillin clavulanicacid(augmentin)2:1 | Chloramphenicol | Bacitracin | Ampicillin | Gentamicin | Cefonicid | Ceftazidime | Kanamycin | Penicillin G | Sulphamethoxozole/Trimethoprim1 9:1 | Nitrofurantion | Streptomycin |
| Salmonella heidelberg E1 | s ¹ | s | s | s | s | s | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E2 | S | S | s | S | S | S | S | s | s | S | s | S | S | S | S |
| Salmonella heidelberg E3 | S | S | S | S | S | S | S | s | s | s | s | S | S | S | S |
| Salmonella heidelberg E4 | S | s | S | S | S | s | s | s | s | S | s | s | S | S | S |
| Salmonella heidelberg E5 | s | s | s | s | s | s | s | s | s | s | s | s | r ² | s | S |
| Salmonella heidelberg E6 | s | s | s | s | s | s | s | s | s | s | s | s | S | s | S |
| Salmonella heidelberg E7 | r | s | s | s | S | s | s | s | s | s | s | s | s | s | S |
| Salmonella heidelberg E8 | s | s | s | S | s | s | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E9 | s | s | s | S | s | s | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E10 | s | s | s | S | s | s | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E11 | s | s | s | s | s | s | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E12 | s | s | s | s | s | s | s | s | s | r | s | s | s | s | s |
| Salmonella heidelberg E13 | s | s | s | s | s | r | s | s | s | s | s | s | s | s | s |
| Salmonella heidelberg E14 | s | s | s | s | s | s | s | s | s | s | s | s | s | s | s |

 s^1 : sensitive; r^2 : resistant.

(1%), nalidixic acid (1.0%), and trimethoprimsulfamethoxazole (0.7%). All isolates were susceptible to

amikacin, ceftriaxone, and ciprofloxacin (Zhao et al., 2008).

Fifty-eight Salmonella enterica serovar Heidelberg isolates isolated from food animals were tested for antimicrobial susceptibilities and further characterized for selecting antimicrobial resistance genes, plasmid carriage, class 1 integrons, and genetic relatedness using pulsed-field gel electrophoresis (PFGE). Seventy-two percent of isolates displayed resistance to at least one of the antimicrobial agents tested, while 24% exhibited resistance to eight or more antimicrobial agents. Resistance was most commonly observed to tetracycline (71%), streptomycin (62%), and kanamycin (52%). Isolates obtained from cattle and swine displayed the highest rates of resistance while isolates from chickens more often displayed susceptibility to the tested antimicrobials (Lynne et al., 2009). In addition to that, Patchanee et al. (2008) reported that all S. heidelberg isolates from swine were resistant to one or more of the antimicrobials tested and the majority (73.3%) showed multidrug resistance to streptomycin, tetracycline, and kanamycin (R-type: StTeKm). About 80% of the S. heidelberg isolates of human origin were pan-susceptible, however, one isolate showed multidrug resistance to 10 of the 12 antimicrobials tested.

On the other hand, some researchers were of the opinion that S. heidelberg was resistant to many antimicrobial agents including, cephalosporin (Folster et al., 2010), quinolone (Boscán-Duque et al., 2007), ceftiofur and nalidixic acid (Donado-Godoy et al., 2012), gentamicin, sulfamethoxazole, tetracycline, kanamycin, and streptomycin (Fakhr et al., 2006), gentamicin, spectinomycin, streptomycin, tetracycline (Nayak and 2002), gentamicin, streptomycin Kenney, and sulfisoxazole (Abouzeed et al., 2000). This study emphasized that the presence of S. heidelberg in some Egyptian foods under investigation, such as chicken. beef, milk, Kushary and Sogok (sausage), is of concern due to the fact that they have the potential to cause human infections, and are resistant to some antibiotics. As such, the current control measures on animal origin foods should be improved.

REFERENCES

- Aarestrup FM, Hasman H, Olsen I, Sorensen G (2004). International spread of *bla*CMY-2-mediated cephalosporin resistance in a multiresistant *Salmonella enterica* serovar Heidelberg isolate stemming from the importation of a boar by Denmark from Canada. Antimicrob. Agents Chemother., 48: 1916–1917.
- Abouzeed YM, Hariharan H, Poppe C, Kibenge FS (2000). Characterization of *Salmonella* isolates from beef cattle, broiler chickens and human sources on Prince Edward Island. Comp. Immunol. Microbiol. Infect. Dis., 23(4) :253-266.
- Anonymous (2008). NARMS retail meat annual report, 2005. U.S. Food and Drug Administration, Washington,

- DC. <u>http://www.fda.gov/cvm /2005NARMSAnnualRpt.htm.</u> <u>Accessed 5 November 2008</u>.
- Benenson AS, Chin J (1995). Control of communicable diseases manual. American Public Health Association, Washington, DC.
- Beuchat LR (1995). Pathogenic microorganisms associated with fresh produce. J. Food Prot., 59: 204-216.
- Boscán-Duque LA, Arzálluz-Fisher AM, Ugarte C, Sánchez D, Wittum TE, Hoet AE (2007). Reduced susceptibility to quinolones among *Salmonella* serotypes isolated from poultry at slaughter in Venezuela. J. Food Prot., 70(9): 2030-1035.
- Bucher O, Holley RA, Ahmed R, Tabor H, Nadon C, Ng LK (2007). Occurrence and characterization of *Salmonella* from chicken nuggets, strips, and pelleted broiler feed. J. Food Prot., 70: 2251–2258.
- Burt CR, Proudfoot JC, Roberts M, Horowitz RH (1990). Fatal myocarditis secondary to *Salmonella* septicaemia in a young adult. J. Emerg. Med., 8: 295–297.
- Buzby JC, Farah HA (2006). Chicken consumption continues longrun rise. Amber Waves, 4: 5.
- CDC (2005). *Salmonella* surveillance: annual summary, 2004. Centers for Disease Control and Prevention, Atlanta, GA.
- CDC (2006). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. MMWR Morb. Mortal. Wkly. Rep., 55: 392–395.
- CDCP (Centers for Disease Control and Prevention), (2008). Salmonella surveillance: annual summary, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA.
- CDCP (Centers for Disease Control and Prevention), (2008). Salmonella surveillance: annual summary. 2006. Centers for Disease Control and Prevention, Atlanta, GA.
- CDCP (Centers for Disease Control and Prevention), (2006). FoodNet surveillance report, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA.
- CDCP (Centers for Disease Control and Prevention), (2010). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 States, 2009. U.S. Department of Health and Human Services, CDC, Atlanta, GA.
- Chambers JR, Bisaillon JR, Labbe Y, Poppe C, Langford CF (1998). *Salmonella* prevalence in crops of Ontario and Quebec broiler chickens at slaughter. Poult. Sci., 77: 1497–1501.
- Chittick P, Sulka A, Tauxe RV, Fry AM (2006). A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: clues for disease prevention. J. Food Prot., 69: 1150–1153.
- D'Aoust J (1997). Salmonella species, p. 135-137. In MP.

Doyle, LR. Beuchat,T J. Montville (ed.), Food microbiology: fundamentals and frontiers. American Society for Microbiology, Washington, D.C.

- Demczuk W, Ahmed R, Woodward D, Clark C, Rodgers F (2000). Laboratory surveillance for enteric pathogens in Canada, 2000 annual summary. National Laboratory for Enteric Pathogens, National Microbiology Laboratory, Health Canada. The Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada.
- Diarrassouba F, Diarra MS, Bach S, Delaquis P, Pritchard J, Topp E, (2007). Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler chicken farms. J. Food Prot., 70: 1316–1327.
- Donado-Godoy P, Gardner I, Byrne BA, Leon M, Perez- Gutierrez E, Ovalle MV, Tafur MA, Miller W (2012). Prevalence, risk factors, and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. J. Food Prot., 75(5): 874-883.
- Doyle MP, Zhao T, Meng J, Zhao S, (1997). *E. coli* O157:H7, p. 175-178. In MP. doyle, LR. Beuchat, TJ. Montville (ed.), Food Microbiology: fundamentals and frontiers. American Society for Microbiology, Washington, D.C.
- Dutil L, Rebecca Irwin, Rita Finley, Lai King Ng, Brent Avery, Patrick Boerlin, Anne-Marie Bourgault, Linda Cole, Danielle Daignault, Andrea Desruisseau, Walter Demczuk, Linda Hoang, Greg B. Horsman, Johanne Ismail, Frances Jamieson AM, Pacagnella A, Pillai DR (2010). Ceftiofur Resistance in *Salmonella enterica* Serovar Heidelberg from Chicken Meat and Humans, Canada. Emerg. Infect. Dis., 16(1): 48–54.
- Escartín EF, Lozano JS, García OR (2000). Quantitative survival of native *Salmonella* serovars during storage of frozen raw pork. Int. J. Food Microbiol., 54: 19–25.
- Fakhr MK, Sherwood JS, Thorsness J, Logue CM (2006). Molecular characterization and antibiotic resistance profiling of *Salmonella* isolated from retail Turkey meat products. Foodborne Pathol. Dis., 3(4): 366-374.
- FDA (Food and Drug Administration) (2009). National antimicrobial resistance monitoring system—enteric bacteria (NARMS): 2006 executive report. U.S. Department of Health and Human Services, U.S. FDA, Silver Spring, MD.
- FDA (Food and Drug Administration) (2010). National Antimicrobial Resistance Monitoring System—enteric

bacteria (NARMS): 2007 executive report. U.S. Department of Health and Human Services, FDA, Rockville, MD.

- FDA (2006). National Antimicrobial Resistance Monitoring System—Enteric Bacteria (NARMS): 2003 executive report. Department of Health and Human Services, U.S. Food and Drug Administration, Washington, DC. Available from <u>http://www.who.int/salmsurv/links/GSSProgressReport2</u> 005. pdf
- Ferris KE, Timm JM, Aalsburg AM, Munoz M (2002). Salmonella serotypes from animals and related sources reported during July 2001–June 2002. Proc. Annu. Meet. U.S. Anim. Health Assoc., 106: 467–497.
- Foley SL, Lynne AM, Nayak R (2008). Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. J. Anim. Sci. 86: E149–E162.
- Folster JP, Pecic G, Bolcen S, Theobald L, Hise K, Carattoli A, Zhao S, McDermott PF, Whichard JM (2010). Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from humans in the United States. Foodborne Pathol. Dis., 7(2): 181-187.
- Gangarosa EJ (1978). What have we learned from 15 years of *Salmonella* surveillance ? In: Proceedings of the National Salmonellosis Seminar. Washington, 1978.
- Han Jing, Donna E. David, Joanna Deck, Aaron M. Lynne, Pravin Kaldhone, Rajesh N, Stefanova R, Foley SL (2011). Comparison of *Salmonella enterica* Serovar Heidelberg Isolates from Human Patients with Those from Animal and Food Sources. J. Clin. Microbiol., 49(3): 1130–1133
- Hendriksen RS (2003). A global *Salmonella* surveillance and laboratory support project of the World Health Organization. Laboratory Protocols Level 1 Training Course Identification of *Salmonella* 4th Ed. April 2003.
- Hennessy TW, Cheng LH, Kassenborg H, Ahuja SD, Mohle-Boetani J, Marcus R, (2004). Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: a case-control study in FoodNet sites. Clin. Infect. Dis., 38: S237–243.
- Hensyl WR (1994). Bergey's Manual Of Determinative Bacteriology 9th ed. Williams & Wilkins, Baltimore. Philadeiphia, Hong Kong, London, Munich, Sydney, Tokyo. Page 155 – 156 and group 5, page 179 - 209. higher organisms in drinking water: a review. Canada J. Microbiol., 54(7): 509-524.
- Kaldhone Pravin, Rajesh Nayak, Aaron M. Lynne, Donna E. David, Patrick F. McDermott, Catherine M. Logue, Steven L. Foley (2008). Characterization of Salmonella enterica serovar Heidelberg from Turkey-Associated Sources. Appl. Environ. Microbiol., 74(16): 5038–5046. Kennedy M, R. Villar, DJ. Vugia, T. Rabatsky-Ehr, MM

- . Farley, M. Pass, K. Smith, P. Smith, PR. Cieslak, B. Imhoff, PM. Griffin. (2004). Hospitalizations and deaths due to *Salmonella* infections, FoodNet, 1996–1999. Clin. Infect. Dis., 38(3): S142–S148.
- Krieg NK (Ed) (1984). Bergey's Manual Of Systematic Bacteriology Vol. I, Baltimore, Hang Kong, London and Sydney. (Volume 1), section 4, page 172 – 173 and section 5, page 414.
- Lynne AM, Kaldhone P, David D, White DG, Foley SL (2009). Characterization of antimicrobial resistance in *Salmonella enterica* serotype Heidelberg isolated from food animals. Foodborne Pathol. Dis., 6(2): 207-215.
- Mammina C, Talini M, Pontello M, Di Noto AM, Nastasi A (2003). Clonal circulation of *Salmonella enterica* serotype Heidelberg in Italy?. Euro. Surveill., 8(11): 222-225.
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999). Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607–625.
- Morey A, Singh M (2012). Low-temperature survival of *Salmonella* spp. in a model food system with natural microflora. Foodborne Pathol. Dis., 9(3):218-223.
- Nayak R, Kenney PB (2002). Screening of *Salmonella* isolates from a turkey production facility for antibiotic resistance. Poult. Sci., 81(10): 1496-1500.
- Nayak R, Stewart T, Wang RF, Lin J, Cerniglia CE, Kenney PB (2004). Genetic diversity and virulence gene determinants of antibiotic-resistant *Salmonella* isolated from preharvest turkey production sources. Int. J. Food Microbiol., 91(1): 51–62.
- Oloya J, Doetkott D, Khaitsa ML (2009). Antimicrobial drug resistance and molecular characterization of *Salmonella* isolated from domestic animals, humans, and meat products. Foodborne Pathol. Dis., 6: 273–284.
- Park SY, Seo KY, Ha SD (2007). A response surface model based on absorbance data for the growth rates of *Salmonella enterica* serovar typhimurium as a function of temperature, NaCl, and pH. J. Microbiol. Biotechnol., 17(4): 644-649.
- Patchanee P, Zewde BM, Tadesse DA, Hoet A, Gebreyes WA (2008). Characterization of multidrugresistant *Salmonella enterica* serovar Heidelberg isolated from humans and animals. Foodborne Pathol. Dis., 5(6): 839-851.
- PHAC (Public Health Agency of Canada) (2005). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph (Ontario, Canada): Public Health Agency of Canada, 2007.
- PHAC (Public Health Agency of Canada) (2006). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2006 [cited 2009 May 13]. Available from <u>http://www.phac-aspc.gc.ca/cipars-picra/</u> index_e.html

- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011). Foodborne illness acquired in the United Statesmajor pathogens. Emerg. Infect. Dis., 17: 7–15.
- Sørensen O, van Donkersgoed J, McFall M, Manninen K, Gensler G, Ollis G (2002). *Salmonella* spp. shedding by Alberta beef cattle and the detection of *Salmonella* spp. in ground beef. J. Food Prot., 65: 484–491.
- SRGA (Swedish Reference Group for Antibiotics) (1990). Antimicrobial Susceptibility Testing Of Bacteria-A Reference And Methodology Manual. The swedish Medical Society and statens Bacteriologiska Laboratorium, Stockholm, sweden.
- Stefanovicova A, Rehakova H, Skarkova A, rijpens N, Kuchta T (1998). Confirmation of presumptive *Salmonella* colonies by the polymearase chain reaction. J. food prot., 61(10): 1381-1383.
- Tauxe RV (1991). *Salmonella*: a postmodern pathogen. J. Food Prot., 54: 563–568.
- Threlfall EJ, Hall ML, Rowe B, (1992). Salmonella bacteraemia in England and Wales, 1981-1990. J. Clin. Pathol., 45: 34-36.
- Tietjen M, DYC. Fung, (1995). Salmonellae and food safety. Crit. Rev. Microbiol., 21(1): 53-83.
- Todd ECD (1985). Foodborne and waterborne disease in Canada ± 1978 annual summary. J. Food Prot., 48: 990±6.
- U.S. Department of Agriculture (2007). Progress report on *Salmonella* testing of raw meat and poultry products, 1998–2006. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, DC.
- Van TTH, Moutafis G, Istivan T, Tran LT, Peter J Coloe (2007). Detection of *Salmonella* spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance. Appl. Environ. Microbiol., 73(21): 6885–6890
- Vugia DJ, Samuel M, Farley MM, Marcus R, Shiferaw B, Shallow S (2004). Invasive *Salmonella* infections in the United States, Food- Net, 1996–1999: incidence, serotype distribution, and outcome. Clin. Infect. Dis., 38: S149–156.
- WHO (World Health Organization) (2009). WHO Global Salm-Surv. Progress report(2000–2005). Geneva: The Organization; 2006 [cited 2009 May
- Winokur PL, Brueggemann A, DeSalvo DL, Hoffmann L, Apley MD, Uhlenhopp EK, Pfaller MA, Doern GV (2000). Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC _-lactamase. Antimicrob. Agents Chemother., 44: 2777–2783.
- Zhao S, White DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, Abbott JW, Hall-Robinson E, McDermott PF (2008). Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl. Environ. Microbiol.,

74: 6656-6662.

- Zhao S, McDermott PF, Friedman S, Abbott J, Ayers S, Glenn A, Hall-Robinson E, Hubert SK, Harbottle H, Walke RD, Chiller TM, White DG (2006). Antimicrobial resistance and genetic relatedness among *Salmonella* from retail foods of animal origin: NARMS retail meat surveillance. Foodborne Pathol. Dis., 3: 106–117.
- Zhao S, Qaiyumi S, Friedman S, Singh R, Foley SL, White DG, McDermott PF, Donkar T, Bolin C, Munro S, Baron EJ, Walker RD (2003). Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol., 41: 5366– 5371.