1. INTRODUCTION

Salmonella is a leading cause of severe economic losses in poultry and foodborne illness in humans worldwide (Lin *et al*., 2014; Sallam *et al*., 2014). Though there are more than two thousand different subspecies of Salmonella, few of them are able to cause serious conditions in humans and chickens (Rodpai *et al*., 2013). *Salmonella enteritidis* (*S. enteritidis*) is invasive in laying hens, and the potential for its vertical transmission has been documented (Cooper *et al*., 1989). Some *S. enteritidis* strains have been found to cause anorexia, diarrhea, and reduced egg production in experimentally infected laying hens (Gast and Beard, 1992). *S. enteritidis* is also invasive in broiler chickens (Lister, 1988) and often being isolated from the muscles of raw chicken carcasses purchased from retail outlets (Humphrey, 1991). The disease which caused by any salmonella rather than *S. pullorum-gallinarum* (typhoid) is known as paratyphoid (PT), although signs of severe PT infection in young poultry are generally similar to those produced by *S. pullorum-gallinarum*.

The presence of Salmonella in the intestinal tract, skin and among the feathers of chickens may cause carcasses contamination during slaughtering and processing and possibly it is responsible by the introduction of this microorganism in the slaughterhouses (Paiao *et al*., 2013).

Human infections with *S. enteritidis* are a major problem in many countries worldwide. There have been 419 confirmed cases of salmonella

illness linked to raw chicken including frozen raw breaded chicken products in Canada during the year 2018 (BCCDC, 2018). Cases of *S. enteritidis* acquired in the EU have increased in humans by 3% since 2014. In laying hens, the prevalence increased from 0.7% to 1.21% over the same period. There were 94530 human cases of salmonellosis reported in the EU in 2016. *S. enteritidis* the most widespread type of salmonella, accounted for 59% of all salmonellosis cases originating in the EU and is mostly associated with the consumption of eggs, egg products and poultry meat (ECDC, 2018). In 2014, 88175 confirmed cases of human salmonellosis causing 9830 hospitalizations and 65 fatalities were reported across the European Union (EU). Among these, 16000 cases of human salmonellosis were reported from Germany. As in previous years, *S. enteritidis* was the predominant serovar (44.4% of all isolates) (EFSA and ECDC, 2014).

Antimicrobial resistance in *S. enteritidis* and other *Salmonella spp* is an increasing problem leading to serious health hazards in the world (Singh *et al*., 2013). The reason of this problem could be due to overuse and misuse of antibiotics in developing countries (Ikwap *et al*., 2014).

In Libya, there were few researchers studied the prevalence of salmonella in chicken slaughterhouses as well as in humans. Abubaker (1993) collected 120 samples from chickens carcass, 60 samples from liver and 60 samples from cecal contents at a chicken slaughterhouse. He found prevalence of 25.83%, 1.66% and 5%, respectively. Serotyping of salmonella isolated

from children with diarrhea in Zliten City resulted in 21 being *S. heidelberg* and 3 *S. enteritidis* (Ali *et al*., 2005). Therefore, this study is planned with the following objectives:

1. To determine the prevalence of *S. enteritidis* in some chicken slaughterhouses in Tripoli – Libya.
2. To investigate the susceptibility of salmonella isolates to antibiotics.

2. REVIEW OF LITURATURE

2.1. General characterization of salmonella

The genus salmonella is a member of the bacterial family *Enterobacteriaceae* and can be divided into five biochemically distinct subgenera (Krieg and Holt, 1984; Holt *et al*., 1994). However, the degree of genetic relatedness among the salmonellae is so great that some scholars have suggested that the genus actually consists of only two species (Grimont *et al*., 2000)

Phenotypic as well as genotypic methods are used to discriminate salmonella serotypes. The phenotypic methods such as bio-typing, phage typing and resistance to antibiotics are considered to be the first steps in discrimination. Among the genotypic methods, plasmid profile analysis, the analysis of restriction patterns of chromosomal and plasmid DNA and ribotyping are mostly used (D'Aoust, 1989).

2.2. Morphology and staining

Salmonellae are straight, non-spore-forming rods, measuring about 0.7—1.5 & 2.0—5.0 µm (Holt *et al*., 1994; Lightfoot, 2004) . Salmonellae are gram negative, but cells can be stained readily with common dyes, such as methylene blue or carbolfuchsin. Paratyphoid salmonellae are usually peritrichously flagellated and motile, although naturally occurring non-motile mutants occasionally are encountered. Typical salmonella are colonies on agar

media about 2 to 4 mm in diameter, round with smooth edges, slightly raised and glistening (Holt *et al*., 1994) .

2.3. Growth requirement

Salmonellae are facultatively anaerobic and can grow well under both aerobic and anaerobic conditions. The optimum temperature to support the growth of salmonella is 37°C, but some growth is observed generally over a range of about 5 to 45°C. Salmonella can grow within a pH range of approximately 4.0 to 9.0, with an optimum pH around 7.0, although some cellular characteristics such as flagella and fimbria may not be expressed under extreme pH conditions (Holt *et al*., 1994; Lightfoot, 2004) . The nutritional requirements of salmonella are relatively simple, and most culture media that supply sources of carbon and nitrogen can support their growth. The viability of Salmonella cultures can be maintained for many years in simple media, such as peptone agar or nutrient agar, which have been stab-inoculated, sealed, and held at room temperature (Krieg and Holt, 1984).

2.4. Biochemical properties

Typical PT salmonellae ferment glucose (to produce both acid and gas), dulcitol, mannitol, maltose, and mucate but do not ferment lactose, sucrose, malonate, or salicin. They can produce hydrogen sulfide on many types of media, decarboxylate ornithine and lysine, use citrate as a sole source of

carbon, and reduce nitrates to nitrites. PT salmonellae do not hydrolyze urea or gelatin and do not produce indole (Holt *et al*., 1994).

2.5. Susceptibility to Chemical and Physical Agents

2.5.1. Physical Agents: Heat and Irradiation

With the exception of a few distinctively thermoresistant strains (such as *S. senftenberg* 775W), salmonella generally are quite susceptible to destruction by heat. For example, cooking to an internal temperature of 79°C in conventional or convection ovens always eliminated inoculated *S. typhimurium* from roasting chickens (Schnepf and Barbeau, 1989). The heat resistance of *S. enteritidis* can be increased by prior exposure to alkaline conditions (Humphrey *et al*., 1991), and decreased by prior refrigeration (Saeed and Koons, 1993). Isolates of *S. enteritidis* with enhanced heat tolerance have been reported to also be more tolerant of acid and hydrogen peroxide and survived longer on surfaces (Humphrey *et al*., 1995). Salmonella strains of several serotypes have been able to survive cooking methods for eggs that allow some of the yolk to remain liquid (Humphery *et al*., 1989). Liquid whole egg is pasteurized in the United States according to USDA specifications that require a minimum treatment time of 3.5 minutes at 60°C (Baker, 1990). A combined treatment of heating for 25 minutes in a 57°C water bath and then in hot air for 60 minutes at 55°C achieved a 7 log reduction in *S. enteritidis* numbers inside intact shell eggs (Hou *et al*., 1996).

Steam pelleting treatment of poultry feed under precisely defined conditions has been reported to eliminate salmonellae in a manner dependent on temperature, time, and moisture (Himathongkham *et al*., 1996). The addition of propionic acid was found to improve *S. enteritidis* destruction in poultry feed by heating. (Matlho *et al*., 1997).

Irradiation has received considerable attention as a potential method for eliminating salmonellae from foods and feedstuffs. Most salmonella strains appear to be highly susceptible to the lethal effects of irradiation (Thayer *et al*., 1990). Gamma radiation has been applied successfully to reducing the levels of Salmonella contamination in poultry meat (Nassar *et al*., 1997), egg products (Matic *et al*., 1990), shell eggs (Serrano *et al*., 1997), and poultry feeds (Leesson and marcotte, 1993). Rradiation doses are expressed in kiloGrays (kGy) or Grays (Gy) (Torgby-Tetteh *et al*., 2014). The doses used for radiation are typically between 2 and 8 kGy (IAEA, 2002).

Combined heat and radiation treatments have been shown to be more effective in eliminating salmonellae than either treatment alone (Thayer *et al*., 1991). Ultraviolet radiation has been found effective for reducing salmonella contamination of poultry carcasses (Wallner-pendleton *et al*., 1994), hatching eggs (Baily *et al*., 1996), shell eggs and egg belts. (Gao *et al*., 1997).

2.5.2. Chemical Disinfection

Diverse chemical treatments have been shown to reduce, but not eliminate, contaminating salmonellae on carcasses and hatching eggs (Nassar *et al*., 1997). The application of hydrogen peroxide (Mulder *et al*., 1987), acetic acid (Dickens and Whittemore, 1994; Davies and Breslin, 2003) lactic acid (Izat *et al*., 1990 and Dvorak, 2005), potassium sorbate (Morrison and Fleet., 1985 and Dvorak, 2005), chlorine, or trisodium phosphate (Kim *et al*., 1991; Quinn and Markey, 2001) have all been reported to lower the incidence or level of salmonella contamination on broiler carcasses. Fumigating with formaldehyde (Willams, 1970; Gradel *et al*., 2004) , hydrogen peroxide, or ozone (Baily et al., 1996; Ramesh *et al* ., 2002), spraying with polyhexamethylene biguanide hydrochloride (Cox *et al*., 1994; Gradel *et al*., 2004), or dipping in a peroxidase catalyzed compound (Kuo *et al*., 1996; Davies and Breslin, 2003) have been found to be useful for controlling salmonellae on hatching eggs. Prior application of a vacuum increased salmonella elimination from egg shells by disinfectants (Cox *et al*., 1999). However, recontamination of surfaces after disinfection often can diminish any potential gains from using chemical treatments (Berrag *et al*., 1997).

Studies of the efficacy of chemical treatment of poultry feeds to inhibit salmonellae have produced variable results. Inclusion of an organic acid mixture in feed was reported to reduce significantly the eventual level of salmonellae in feed contaminated with mouse droppings containing *S.*

typhimurium (Larsen *et al*., 1993; Van *et al*.,2002). Treatment with ethyl alcohol likewise reduced Salmonella populations in feed (Ha *et al*., 1997). Smyser and Snoeyenbos (1979), however, studied 12 compounds as potential antagonists of salmonellae in poultry feed (including organic acids) and found that only formalin was consistently effective. The application of chemical disinfectants in poultry housing facilities, although prominent in many Salmonella control programs, is also of somewhat uncertain efficacy (Van *et al*., 2002).

Phenolic and quaternary ammonium compounds are often used for this purpose, but cleaning and disinfection has not always been successful in eliminating salmonellae from contaminated houses (Mason, 1994; Gradel *et al*., 2004). The presence of chick fluff, feces, feed, or wood shavings can interfere with the activity of many chemical disinfectants (Berchieri and Barrow, 1996). Moreover, some chemical disinfectants appear to have reduced potency when used with field (well, stream, or pond) sources of water (Davison and Benson, 1996; Wales *et al*., 2010) . Chemical disinfection of poultry facilities can also be compromised by the improper performance of cleaning and disinfection protocols and by the recontamination of the environment by infected mice (Davies and wary, 1996). Formaldehyde fumigation has been found to be highly effective for decontaminating poultry houses and hatcheries (Whistler and Sheldon, 1989; Gradel *et al*., 2004), but

safety considerations have limited its availability and use. Ozone fumigation has also been applied successfully as a hatchery disinfectant (Lin and Tsen, 1999). In a large field trial in Pennsylvania, cleaning and disinfection was only 50% effective in eliminating *S. enteritidis* from laying houses (Schlosser *et al*., 1999).

2.6. Environmental Factors

The environmental persistence of salmonellae creates continuous opportunities for horizontal transmission of infection within and between flocks. Williams and Benson (Williams and Benson, 1978; Humphrey, 2000) observed the survival of *S. typhimurium* for 16 months in feed and 18 months in litter stored at 25°C. However, used litter has also sometimes been reported to exert an inhibitory effect on Salmonella growth or survival (Tucker, 1967), perhaps because dissolved ammonia leads to a gradual increase in pH over time (Turnbull and Senoeyenbos, 1973). Water activity has been identified as an important supporting factor in allowing the persistence of salmonellae (Oppara *et al*., 1992). Overall water activity levels in poultry houses correlate with the probability of isolating Salmonella from environmental drag swabs of floor litter (Carr *et al*., 1995), although the unequal distribution of salmonellae throughout houses does not seem to depend on any corresponding pattern of water activity levels (Hayes *et al*., 2000). Both *S. enteritidis* and *S. typhimurium* survived in large numbers in aerosols for several hours

(McDermid and Lever, 1996). Nevertheless, *S. enteritidis* has been shown able to survive (and produce filaments) at low water activity. (Mattick *et al*., 2000).

2.7. Virulence Factors

Three general categories of toxins have been reported to play roles in the pathogenicity of PT salmonellae. Endotoxin is associated with the lipid A portion of Salmonella cell wall lipopolysaccharide (LPS). If released into the bloodstream of an infected animal when bacterial cells are lysed, endotoxin can produce fever. Intravenously administered *S. enteritidis* endotoxin caused liver and spleen lesions in 2-week-old chickens (Turnbull and Snoeyenbos, 1974; Rezania *et al*., 2011).

Lipopolysaccharide also contributes to the resistance of the bacterial cell wall to attack and digestion by host phagocytes. Loss of the ability to synthesize complete LPS has been associated with an impaired ability of *S. typhimurium* to colonize the ceca and to invade the spleen in broiler chicks (Craven, 1994; Rezania *et al*., 2011).

Two proteinaceous toxins have also been identified in Salmonella. Enterotoxin activity by salmonellae induces a secretory response by epithelial cells that results in fluid accumulation in the intestinal lumen (Koupal and Deibel, 1975). A heat-labile enterotoxin was detected in 44% of 123 *S. typhimurium* strains from animal sources (McDonough *et al*., 1998).

Enterotoxin-deficient mutants caused less mucosal damage in cell culture and less mortality in mice (Chen *et al*., 1998). The heat-stable cytotoxin of salmonellae causes structural damage to intestinal epithelial cells, perhaps by inhibiting protein synthesis (Koo *et al*., 1984; Lucas and Lee, 2000).

The adherence of PT salmonellae to intestinal epithelial cells is the pivotal first step in the sequence of events that produces disease. Strains of Salmonella with reduced ability to colonize the intestinal tract of chicks also had severely attenuated virulence (Tuner *et al*., 1990; Lucas and Lee, 2000). Both flagella and fimbria of salmonellae have been investigated extensively as potential mediators of attachment. Mutants of *S. enteritidis* lacking flagella were reported by Allen-Vercoe and Woodward to exhibit reduced adherence to cultured avian intestinal cells (Allen-vercoe and Woodward, 1999) and did not compete effectively with wild-type strains to colonize the ceca of chicks (Allen-vercoe and Woodward, 1999). Similarly, Thiagarajan *et al*., (1996) found that *S. enteritidis* strains lacking fimbria were less often isolated from the ceca of inoculated chicks than were fimbriated strains. However, other investigators concluded that neither flagella nor fimbria were essential for *S. enteritidis* to colonize the avian intestinal tract (Dibb-fuller and Woodward, 2000).

The overall virulence of salmonellae also depends heavily on the initial degree of mucosal invasiveness (Amin *et al*., 1994). Adherence and invasion

appear to be separately regulated activities. Mutations that affected the intestinal colonization of chicks after oral infection with *S. enteritidis* and *S. typhimurium* did not affect virulence after intraperitoneal administration (Porter and Curtiss, 1997). Although adherence may not involve ongoing bacterial metabolic activity, the subsequent invasion of host cells requires protein synthesis by live salmonellae (Kusters *et al*., 1993). The expression of some invasion-related bacterial proteins evidently is induced by contact with epithelial cell surfaces (Zierler and Galan, 1995). Dibb-Fuller and Woodward determined (Dibb-fuller and Woodward, 2000) that flagella and some types of fimbria played a role in invasion and dissemination to internal organs of chicks by *S. enteritidis*. Allen-Vercoe *et al*. (1999) found that flagella-deficient (but not fimbria-deficient) mutants of *S. enteritidis* were less able to invade to the livers and spleens of chicks. However, other researchers were unable to identify any significant effect on the invasion of enterocytes, ingestion by macrophages, or virulence for chickens when fimbrial genes were inactive (Rajashekara *et al*., 2000).

Adherence and invasiveness of salmonellae can be influenced by culture growth conditions. Logarithmically growing Salmonella cells are more invasive in tissue culture than are cells in the stationary phase of growth, and salmonellae grown anaerobically have been shown to be both more adherent and more invasive than salmonellae grown aerobically (Ernst *et al*.,

1990). The infectivity of Salmonella cultures for chicks was lost fairly quickly during combined starvation and desiccation (Lesne *et al*., 2000).

The changing environmental conditions to which an enteric pathogen is exposed during the course of infection in an avian host may induce corresponding changes in the expression of virulence-related genes (Durant *et al*., 2000). For example, the high oxygen level and nutrient availability experienced in the gut might promote an invasive bacterial phenotype, but lower oxygen levels and nutrient availability after invasion might induce a different set of virulence proteins (Guniey *et al*., 1995). Several characterized virulence genes are indeed apparently induced following invasion into cells (Pfeifer *et al*., 1999). Different patterns of protein synthesis by *S. typhimurium* have been observed within intestinal ephithelial cells, macrophages, and liver cells (Burns-keliher *et al*., 1998).

The replication of salmonellae within host cells has also been found to be necessary for the full expression of pathogenicity (Leung and Finlay, 1991). Mutants of *S. typhimurium* that were unable to survive within host macrophages (Fields *et al*., 1986; Hathaway and Kraehenbuhl, 2000) or to resist the antimicrobial effects of host peptides (Groisman *et al*., 1992) were reported to exhibit reduced virulence in mice. Both growth and killing of Salmonella seemingly occur simultaneously within macrophages (Buchmeier and Libby, 1997). Salmonellae that survive after phagosome/ lysosome fusion

in the macrophage (Oh *et al*., 1996) eventually may destroy the macrophage itself (Lindgren *et al*., 1996). The production of iron-chelating siderophores may also contribute to the in vivo survival of salmonellae (Yancey *et al*., 1979). A cluster (or "pathogenicity island") of genes, which affect Salmonella survival inside macrophages, has been identified (Ochmam *et al*., 1996).

Plasmids are extrachromosomal DNA elements that have often been associated with bacterial pathogenicity. Serotype-specific plasmids of characteristic molecular weights have been directly linked with virulence for a number of salmonellae. Considerable homology has been demonstrated between virulence-associated plasmids of different serotypes (Chu *et al*., 1999). Strains of *S. typhimurium* and *S. enteritidis* cured of their virulence-associated plasmids have been found to be significantly less lethal for mice (Chart *et al*., 1991). Plasmid-mediated virulence among *S. typhimurium* and *S. enteritidis* isolates has been associated variously with invasion of mesenteric lymph nodes, the liver, and the spleen (Gulig and Curtiss, 1987), in vivo growth within cells of infected mice, survival and multiplication in serum (Chart *et al*., 1996), lysis of macrophages (Guilloteau *et al*., 1996), and immunosuppression (Hoertt *et al*., 1989).

The pathogenicity of salmonellae, however, does not always require the presence of the serotype-specific plasmids. Some strains of *S. typhimurium*, for example, have been shown to retain their invasiveness in cell culture

assays (Horiuchi *et al*., 1991) and their lethality for infected mice (Ou and Baron, 1991) in the absence of virulence-associated plasmids. Moreover, although a serotype-specific plasmid was found to be essential for the full expression of virulence by *S. enteritidis* in mice, curing this plasmid did not affect *S. enteritidis* colonization and invasion of the tissues of orally inoculated chickens (Halavatkar and Barrow, 1993).

2.8. Sources, vectors, and transmission

PT salmonellae can be introduced into poultry flocks from many different sources. Contaminated feeds, particularly those containing animal proteins, have often been identified as likely sources of Salmonella (Davies *et al*., 1997; Meerburg *et al*., 2006) . Contamination by salmonellae has been reported in up to 42% of feed mill samples in the United Kingdom (Davies and Wray, 1997) and in 58% of finished feed (mash) and 92% of meat and bone meal samples in the United States (Cox *et al*., 1983). Meal or mash feeds are more often implicated as sources of salmonella than are pelleted feeds (Rose *et al*., 1999). The serotypes of salmonellae isolated from live poultry and carcasses have sometimes (but not always) been correlated with the serotypes found in feedstuffs (Mackenzie and Bains, 1976). Experimental inoculation studies have demonstrated that chicks can be infected readily by very low levels of PT salmonellae in their feed (Hinton, 1988). Salmonellae

have survived for two years in artificially inoculated feeds (Davies and Wary, 1996).

The extremely wide host range of PT salmonellae creates an equally large number of reservoirs of infectious organisms. Biologic vectors can both disseminate and amplify salmonellae in poultry flocks. Insects, including cockroaches (Kopanic *et al*., 1994), lesser mealworms (McAllister *et al*., 1994), flies (Olsen and Hammack 2000), and darkling beetles (Goodwin and Waltman, 1996) can carry salmonella organisms internally and externally. Mice have been identified as particularly important vectors for *S. enteritidis* in laying flocks (Schlosser *et al*., 1999). Henzler and Opitz (1992) detected *S. enteritidis* in 24% of mice from environmentally contaminated laying farms, but in none of the mice from farms with environments free of *S. enteritidis*. They noted that a single mouse fecal pellet could contain 105 *S. enteritidis* cells. Wild birds can carry salmonella infections (Daoost *et al*., 2000), and contact with wild birds or their droppings has sometimes been identified as a risk factor for commercial poultry (Craven *et al*., 2000). Humans can also be a source of salmonellae transmissible to poultry, as shown by a California sewage treatment plant that apparently spread infection to both wild animals and a commercial laying flock (Kind *et al*., 1997).

Vertical transmission of PT salmonellae to the progeny of infected breeder flocks can result from internal or external contamination of eggs. Egg

shells are often contaminated with PT salmonellae by fecal contamination during oviposition (Gast and bread, 1990). The penetration of salmonellae into or through the shell and shell membranes can result in direct transmission of infection to the developing embryo or can lead to exposure of the chick to infectious Salmonella organisms when the shell structure is disrupted during hatching. Some PT serotypes, particularly *S. enteritidis,* can be deposited in the contents of eggs before oviposition. The resulting transovarian transmission of infection to progeny is an important aspect of the epidemiology of *S. enteritidis* in chickens. Egg-borne transmission has long been known to play a major role in spreading *S. arizonae* infections in turkeys (Hinshaw and McNeil, 1946; Gast and bread, 1990) . The same salmonella serotypes responsible for mortality in naturally infected chicks and poults have often also been isolated from their parent flocks. In a survey of 10 farms in France, Lahellec *et al*., (1986) concluded that the greatest contribution to the eventual distribution of Salmonella serotypes in broiler houses came from the chicks themselves and not from their environment.

Any PT salmonellae carried in or on eggs can be spread extensively in the hatchery. As chicks or poults pip through egg shells, salmonellae are released into the air and circulated around hatching cabinets on contaminated fluff and other hatching debris. Bailey *et al*. (1994) found Salmonella on 17% of egg shell samples and 21% of chick rinse samples obtained from

commercial broiler hatcheries in the United States (Cox *et al*., 1990). Likewise isolated salmonellae (of 12 different serotypes) from more than 75% of samples of egg fragments, belting material, and paper pads from broiler hatcheries. Newly hatched birds, lacking protective intestinal microflora, are highly susceptible to intestinal colonization by salmonellae. (Cason *et al*., 1994) observed that nearly 44% of chicks from uncontaminated eggs became infected with *S. typhimurium* when hatched along with surface-contaminated eggs. Bhatia and McNabb (1980) found the same Salmonella serotypes in hatchery fluff and meconium as were later detected in broiler house litter and finished broiler carcasses.

The crop has been implicated as an important source of carcass contamination within the processing plant (Ramirez *et al*., 1997). Higher incidence of salmonella in crops than in ceca and a higher incidence of salmonella in crops than ruptured ceca during commercial evisceration have been reported by Hargis *et al*. (1995). Feed withdrawal increases the incidence of salmonella in broiler crops prior to slaughter and provide further evidence that the crop may be an important critical control point for reducing salmonella contamination of broiler carcasses (Ramirez *et al*, 1997). Ramya *et al*. (2012) studied the incidence of *S. enteritidis* in poultry and meat samples by culture and PCR method. Out of 130 samples, 30 samples (10 from spleen) collected from different sources were subjected to cultural and PCR methods

for the presence of *S. enteritidis*. 4 (40%) and 3 (30%) collected from spleen were positive for *S. enteritidis* by culture and PCR, respectively (Ramya *et al*., 2012).

Salmonella can also spread horizontally within and between flocks. Snoeyenbos *et al*. (1969) noted that 10 salmonella serotypes spread rapidly from infected day-old chicks to penmates reared on litter. Gast and Beard (1990) reported that *S. enteritidis* was found in the feces and internal organs of uninoculated laying hens housed in cages adjacent to those of orally inoculated birds. Contaminated poultry house environments are often implicated as leading sources of PT salmonellae (Lahellec and Colin, 1985) concluded that Salmonella serotypes present in broiler houses or introduced into houses by vectors during the rearing period were more likely to appear on processed carcasses than were serotypes originating in the hatchery. Studies in Dutch and Japanese laying flocks have likewise suggested that infection was more likely acquired from farm environments than from breeding stocks (Vande Gessen *et al*., 1997).

Horizontal transmission can be mediated by mechanisms including direct bird-to-bird contact, ingestion of contaminated feces or litter, contaminated water, or personnel and equipment. Hoover *et al*. (1997) reported that salmonella isolation from the environment of turkey poults reached peak levels by 2 weeks after the placement of infected birds in the

house. Davies and Wray (1996) reported that *S. enteritidis* persisted for at least 1 year in dust in an empty poultry house (even after cleaning and disinfection). In a French study, 70% of flocks had Salmonella- positive dust or litter samples. Perhaps mediated by contaminated dust, airborne transmission of experimental *S. enteritidis* infection has been observed on several occasions. Negative air ionization has been proposed as a mechanism for reducing salmonella transmission in poultry flocks by limiting the circulation of contaminated dust particles. In experimental settings, ionizers have reduced airborne levels of *S. enteritidis* and airborne transmission of *S. enteritidis* infection in chicks (Gast *et al*., 1999).

2.9. Clinical Signs

PT infection of poultry is usually associated with disease only in very young birds. The contamination of eggs with salmonellae may lead to a high level of embryo mortality and the rapid death of newly hatched birds before clinical signs are observed. Signs of disease are rarely observed after the first 2 weeks of life, although morbidity and mortality can be high during that period, and significant growth retardation can occur. The course of illness is normally relatively brief in individual birds. Signs of severe PT infection in young poultry are generally similar to those observed in connection with other avian Salmonella infections (pullorum disease and fowl typhoid) and with other bacteria that can cause acute septicemia. Although clinical disease is not

normally associated with PT infections in mature poultry, some *S. enteritidis* strains have been found to cause anorexia, diarrhea, and reduced egg production in experimentally infected laying hens (Gast and Beard, 1990).

Typical signs of PT infection in chicks and poults include progressive somnolence with closed eyes, drooping wings, and ruffled feathers. Anorexia and emaciation are common. Affected birds are often seen to shiver and huddle near heat sources. Profuse watery diarrhea is frequently observed, often resulting in dehydration and pasting of the vent area. Blindness and lameness occasionally have been reported.

2.10. Disease in humans

Signs of salmonellosis in human beings include diarrhoea, nausea, abdominal pain, mild fever, chills, vomiting, prostration, headache, malaise. The diarrhoea varies from thin vegetable soup like stools to a massive evacuation with accompanying dehydration (Forshell *et al*., 2006).

A wide array of faster alternative strategies for detecting and identifying salmonellae have also been proposed in recent years. Serologic detection of specific antibodies often is used effectively as a rapid preliminary screening device to identify flocks that have been exposed to salmonellae.

2.11. Isolation and Identification of Causative Agent

2.11.1. Sample Selection

To identify PT infection in poultry flocks, samples are obtained and cultured from a variety of sources, principally tissues, eggs, and the poultry house environment. The number of samples that must be processed to achieve a predetermined level of confidence of detection of PT infection in a flock is directly related to the size of the flock and inversely related to the actual prevalence of infection (Aho, 1992). In very large flocks estimated to have very low prevalence of salmonella infection, samples from more than one bird are often pooled together before culturing to allow an adequate sample size to be attained within the limitations of existing laboratory resources.

As many PT salmonella serotypes are highly invasive and can be disseminated systemically to numerous internal tissues, a diversity of different sites (including the liver, spleen, ovary, oviduct, testes, yolk sac, heart, heart blood, kidney, gall bladder, pancreas, synovia, and eye) can provide samples for diagnostic culturing. As lesions cannot be relied upon to indicate infected tissues, several different organs should be cultured from each bird (separately or together). Some highly invasive PT serotypes, particularly *S. enteritidis*, can be deposited in the contents of eggs before oviposition (Gast and Beard, 1990). Culturing eggs for *S. enteritidis*, therefore, has been applied as a test for assessing the potential threat to public health posed by infected laying flocks. (Gast, 1993) reported that culturing pools of egg contents for *S. enteritidis* detected experimentally infected hens at a frequency similar to

culturing fecal samples or testing for specific serum antibodies during the first 2 weeks after inoculation.

Because infections of poultry with PT salmonellae almost invariably involve colonization of the intestinal tract, samples of intestinal tissues and contents are frequently the focus of Salmonella-culturing efforts. In a survey of birds submitted to a diagnostic laboratory (Faddol and Fellows, 1966), salmonellae were found exclusively in intestinal samples in 78% of the chickens and 70% of the turkeys. In experimentally inoculated laying hens, *S. enteritidis* was recovered more often from the intestinal tract than from any other tissue sampled (Gast and Beard, 1990). The caudal ileum, ceca, cecal tonsils, and cecal contents are the intestinal sites most often recommended for recovering salmonellae. Cloacal swabs or samples of voided feces have been used to provide evidence of persistent intestinal colonization by salmonellae in individual birds. The often intermittent pattern of shedding of salmonellae in the feces of infected birds tends to diminish the overall reliability of cloacal swabs for diagnosing infection (Williams and Whittemore, 1976; Lucas and Lee, 2000).

Fecal shedding of salmonellae into the poultry house environment by infected birds makes culturing environmental samples a useful diagnostic tool. Moreover, environmental samples also provide an opportunity to monitor the introduction of salmonellae into poultry houses by vectors, personnel,

equipment, and other sources. Although sampling fresh feces themselves likely provides the most sensitive test for the shedding of salmonellae (Higgins *et al*., 1996), sampling litter can sometimes provide a comparable level of detection (Sato, *et al*., 1971). Olesiuk *et al*. (1969) reported that experimental *S. typhimurium* infection in laying flocks was detected more consistently over a period of 1 year by culturing floor litter than by any other testing approach. Nest litter samples have been identified as particularly productive samples for recovering salmonellae (Davies and Wray, 1996). Drag-swab samples, obtained by dragging moistened gauze pads across the floor of poultry houses, have been reported to detect salmonellae with greater sensitivity than litter sampling (Kingston, 1981). The use of multiple-swab assemblies can further improve the sensitivity of this method (Carr *et al*., 1995). Swabs dragged through wet areas of manure appear to be more productive than swabs from dry areas. Foot covers worn in poultry houses can also provide an effective sample for detecting environmental salmonellae (Cadwell *et al*., 1998).

Numerous other environmental sampling approaches, including the culturing of cage surfaces, water sources, egg belts, trapped rodents, and dust have also been suggested. Dust can remain contaminated with salmonellae even after cleaning and disinfection of poultry houses. Air sampling has detected Salmonella in both hatching cabinets and rooms containing infected

chickens. Hatchery fluff is frequently contaminated with salmonellae, offering an opportunity for early detection of infection in flocks (Mine, 1997). Culturing poultry feed for salmonellae is often important in establishing the source of infection of a flock with a particular serotype (Snoeyenbos *et al*., 1967).

2.11.2. Culture Media

A diverse array of media has been developed and recommended for isolating and identifying salmonellae. Although some evidence has suggested that proper selection of culture media is somewhat contingent upon the type of sample being tested, several commercially available formulations have been consistently effective in a variety of applications.

Suggested broth media for the pre-enrichment of samples for salmonellae include buffered peptone water and trypticase soy broth. (Stephenson *et al*., 1991) reported that, of five pre-enrichment media tested, trypticase soy broth provided the greatest sensitivity of detection of *S. enteritidis* in artificially contaminated egg yolks. The selective broth media most often used for isolating PT salmonellae in recent years are tetrathionate (TT) broth and Rappaport-Vassiliadis (RV) broth. Tetrathionate broth preparations have been found to yield a higher frequency of salmonella detection than RV broth or selenite cystinebroth from a variety of types of samples, including cloacal swabs, intestinal tissues, pooled egg contents, and

poultry feeds (Cox *et al*., 1982). Rappaport-Vassiliadis broth has been effectively used to isolate salmonellae from raw chicken and egg contents pools (Humphrey and Whitehead, 1992). Concern about selenium toxicity for human laboratory workers has led to the diminished use of selenite-cystine broth.

Numerous agar media are available for the isolation of PT salmonellae. Among the most commonly used plating media are brilliant green (BG) agar, XLD agar, XLT4 agar, bismuth sulfite agar, and Hektoen enteric agar. Brilliant green agar is perhaps the most widely used medium for salmonella isolation from poultry sources and has been shown to be effective in application to diverse tissue, environmental, egg, and feed samples. XLT4 agar has been applied successfully to detect salmonellae efficiently from poultry house environmental drag swabs (Miller *et al*., 1991). The addition of novobiocin to agar plating media has been demonstrated to improve salmonella recovery by suppressing the growth of some competing organisms (notably Proteus) that might otherwise overgrow the salmonellae (Tate *et al*., 1992). Samples should always be streaked onto two different media, preferably with dissimilar indicator systems for differentiating salmonellae from other organisms.

2.11.3. Serologic Diagnosis of Infection

Specific antibodies to PT salmonellae have been found in the sera of infected poultry with a high degree of sensitivity using diverse agglutination and enzyme immunoassay methods. Detectable serum antibody titers are often still present long after the clearance of all salmonellae from tissues and the cessation of fecal shedding (Hassan *et al*., 1990). Antibodies to Salmonella have been detected in both naturally and experimentally (Barrow, 1992) infected poultry. Because antibody tests document only prior exposure to salmonellae and do not provide unequivocal evidence of a currently ongoing infection in a flock, positive serologic results generally must be followed by bacteriologic culturing for confirmation. Other problems with serologic testing include the possibility that subclinical infections will lead to fecal shedding without sufficient invasion and dissemination to elicit a detectable antibody response (Olesiuk *et al.*, 1969), the general immunologic unresponsiveness of very young birds to Salmonella infection, and cross-reactions between antibodies to similar PT serotypes (Nicholas and Cullen, 1991).

Agglutination tests have detected both natural and experimental infections of chickens with PT salmonellae (Gast and Beard, 1990). The principal agglutination assay formats include rapid whole-blood plate, serum plate, tube, and microwell plate tests. All of these tests rely on the ability of specific antibodies to cause visible agglutination when mixed with antigen

preparations of killed whole Salmonella cells. Except for the tube test, all agglutination assays use stained antigens to improve the ease of visualization of the agglutination reaction. An additional incubation period with a secondary antibody (antiglobulin) directed against chicken immunoglobulins by increasing the overall agglutination of the target antigen (William and Whittemre, 1972) has been reported to provide greater sensitivity for detecting PT infections than other agglutination test methods.

PT salmonella infections in poultry have also been detected using numerous ELISA approaches. For example, ELISA tests with antigens including LPS, flagella, or outer membrane proteins have identified chickens infected naturally or experimentally with *S. typhimurium* or *S. enteritidis* (Barrow, 1992). An international collaborative effort reported a generally high degree of correspondence in the performance of a wide assortment of ELISA formats and antigens for detecting *S. enteritidis* infections (Barrow *et al*., 1996). By using very precisely defined antigens, ELISA tests often achieve a high degree of specificity and are frequently associated with fewer false-positive results due to cross-reactions between serotypes than are agglutination reactions. Assays using fimbrial antigens have shown a particularly high degree of specificity for identifying *S. enteritidis* infections in chickens. The discriminatory potential of ELISA tests often depends on judicious selection of positive/negative cut-off values. Screening for serum

antibodies using a flagella-based ELISA test has been applied successfully for controlling *S. enteritidis* in Dutch breeder flocks. Antibodies deposited in egg yolks can also be used to detect poultry infected with PT salmonellae. Both microantiglobulin and ELISA tests have been applied to find antibodies to *S. enteritidis* and *S. typhimurium* in eggs from naturally and experimentally infected chickens. Egg yolk antibodies have been consistently detected by flagella-based ELISA in egg yolks from hens inoculated orally with as few as 10^3 cfu of *S. enteritidis*. Gast and Beard (1991) reported that the presence of specific antibodies in eggs from commercial laying flocks in the United States was directly correlated with the presence of *S. enteritidis* in tissue samples from those flocks (Van de Giessen *et al*., 1992) found a direct relationship between specific egg-yolk antibody titers and the incidence of shedding of *S. enteritidis* in the feces of laying flocks in the Netherlands. Egg yolk antibody detection was found to be slightly more effective than bacteriological culturing of voided feces for predicting *S. enteritidis* contamination of eggs laid by experimentally infected hens (Gast *et al*., 1997) .

2.12. Intervention Strategies

2.12.1. Management Procedures

The diversity of sources from which salmonellae can be introduced into flocks or houses complicates efforts to establish specific critical control points for preventing PT infections in poultry (Fris and Van den Bos, 1995). The

infection or contamination status of the parent flock, the hatchery, and the poultry house before placement of chicks or poults have been identified as principal risk factors for salmonellosis in broiler flocks (Chril *et al*., 1999). Effective prevention and control programs must involve coordinated and simultaneous attacks on the problem from several directions. Eggs and chicks or poults should be secured only from demonstrably Salmonella-free breeding flocks. Hatching eggs should be disinfected properly and hatched according to stringent sanitation standards. Poultry houses should be thoroughly cleaned and disinfected by recommended procedures between flocks. Rodent and insect control measures should be incorporated into house design and management and verified by periodic testing.

Rigidly enforced biosecurity practices should be implemented to restrict the movement of personnel and equipment onto poultry housing premises and between houses. Only pelleted feed or feed containing no animal protein should be used to minimize the likelihood of using contaminated rations. Water provided to poultry should come only from sources treated to ensure purity. Treatments such as medication, competitive exclusion cultures, or vaccination can be applied to reduce the susceptibility of birds to salmonella infection. Finally, the Salmonella status of poultry and their environment should be tested frequently. Such multifaceted prevention and

control programs have reportedly been successful in addressing Salmonella problems in both chickens and turkeys (Pomroy *et al*., 1989) .

Increased international interest in controlling *S. enteritidis* in poultry has led to the development and implementation of numerous testing and monitoring programs in recent years (Engvall and Anderson, 1999) . In the United States, the National Poultry Improvement Plan (NPIP) defines stringent sanitation and testing standards for breeder flocks to prevent the transmission of *S. enteritidis* infection to egg laying stock (Rhorer *et al*., 1999). Participation in this plan requires compliance with standards for feed selection and handling, disinfection of hatching eggs, and hatchery sanitation. NPIP testing for *S. enteritidis* involves bacteriologic monitoring of the environment and serologic monitoring of birds, with culturing of tissues from selected birds used for confirmation. A proposed national *S. enteritidis* testing protocol for U.S. laying flocks, similar to a successful risk reduction program in Pennsylvania (White *et al*., 1997), would screen for infection with drag-swab environmental samples and then confirm the threat posed to public health by culturing eggs (President's council on food safety, 1999).

3. MATERIALS AND METHODS

3.1. Sampling

The study included three regions of Tripoli namely South, East and West regions. Five chicken slaughterhouses were selected from each region.

Every chicken slaughterhouse was visited 3 times for sample collection with 2 weeks interval. Samples collected from each slaughterhouse included swabs from neck skin, crop and spleen from 5 chickens. Total of the samples from all regions were 675. Every 5 samples were pooled prior to isolation and identification. Therefore, a total of 135 samples were processed for isolation and identification and antibiotic sensitivity test (Table 1).

Table 1. Number and type of samples collected from different regions of Tripoli.

Regions*	Number of samples									Total	
	Crop			Neck			Spleen				
	Collection			Collection			Collection				
	1	2	3	1	2	3	1	2	3	Before pooling	After pooling
West	25	25	25	25	25	25	25	25	25	225	45
South	25	25	25	25	25	25	25	25	25	225	45
East	25	25	25	25	25	25	25	25	25	225	45
Total	75	75	75	75	75	75	75	75	75	675	135

*Samples were collected from 5 slaughterhouses in each region.

3.2. Isolation and identification

FDA/AOAC BAM Salmonella Isolation Procedure was used (Waltman *et al*., 1993). Following collection, the swabs were inoculated in pre-enrichment media (peptone water) at 35-37°C for 24 hours. A loopful of the pre-enrichment medium was then inoculated in the selective-enrichment broth (tetrathionate broth, selenite cysteine and Rappaport vassilidis broth) at 35-37°C for 24 hours. A loopful of the selective-enrichment broth was then

streaked on the selective media Xylose lysine desoxycholate (XLD) agar (Figure 1) at 35-37°C for 24 hours. The morphology of the bacteria was tested by Gram stain (Figures 2 & 3). The isolates were then identified biochemically and serologically.

3.2.1. Biochemical identification

A pure colony was selected and inoculated in triple sugar iron (TSI) agar (Figure 4), Citrate, Lysine, Indol, Urea, and Oxidase.

3.2.2. Serotyping

Slide agglutinations – O1, O9 and H antigens were used for serotyping of Salmonella isolates (Figure 5 & 6).

3.3. Antimicrobial Sensitivity Test (The Kirby-Bauer Disc Method)

Antibiotic susceptibility of the isolates were tested by using disc diffusion method described by Bauer *et al*. (1966) with minor modifications. Fresh 3-5 colonies of the isolate were collected and suspended in sterile saline. The suspension was then standardized to match that of a 0.5 McFarland standard (corresponds to approximately 1.5×10^8 CFU/ml). The adjusted suspensions were used as inocula within 15 minutes. The suspension was swabbed with sterile non-toxic cotton swab and streaked on Mueller-Hinton agar plates (Merk, Darmstadt, Germany) and left to dry for 2 to 4 min. The antimicrobial sensitivity discs (Oxoid, Hamphire, United Kingdom) were then

placed on the culture by using a Disk Diffusion Dispenser (Oxoid). Antibiotic discs tested were Ciprofloxacin, Trimethoprim, Chloramphenicol, Amoxicillin/Clavulanic acid, Sulphamethazone-Trimethoprim, Ampicillin, Gentamycin, Doxycyclin, Colistin, Neomycin, Tetracycline, Nitrofurantouin, Lincomycin, Erithromycin and Cefuroxime. After incubation at 37°C for 24 hours, the size of the inhibition zone was measured and the level of susceptibility (sensitive, intermediate, or resistant) was determined. The multiple antibiotic resistance (MAR) index was calculated by using the formula: a/b where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested (Krumperman, 1983).

3.4. Statistical analysis

Salmonella prevalence data were subjected to Pearson's chi-square test using SPSS software (SPSS Inc. Chicago, Illions, USA) to determine the significant variation, if any, among different regions (west, south and east) and organs (crop, neck and spleen). The value of ($P<0.01$) was taken as the cut-off value to consider differences statistically significant.

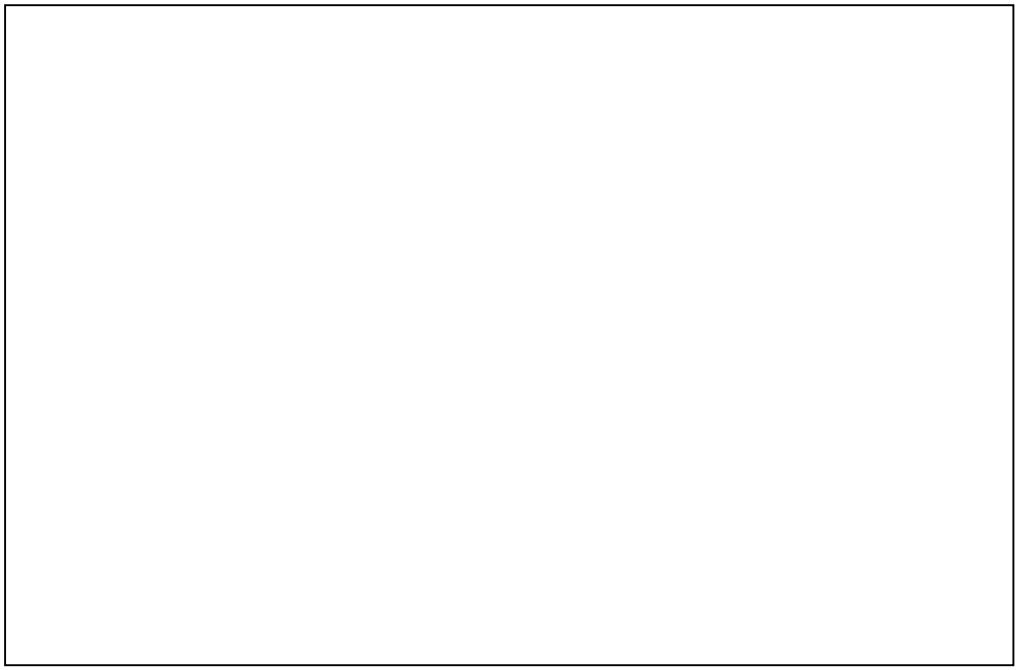

Figure 1. Growth of Salmonella on the selective media Xylose lysine desoxycholate (XLD) agar.

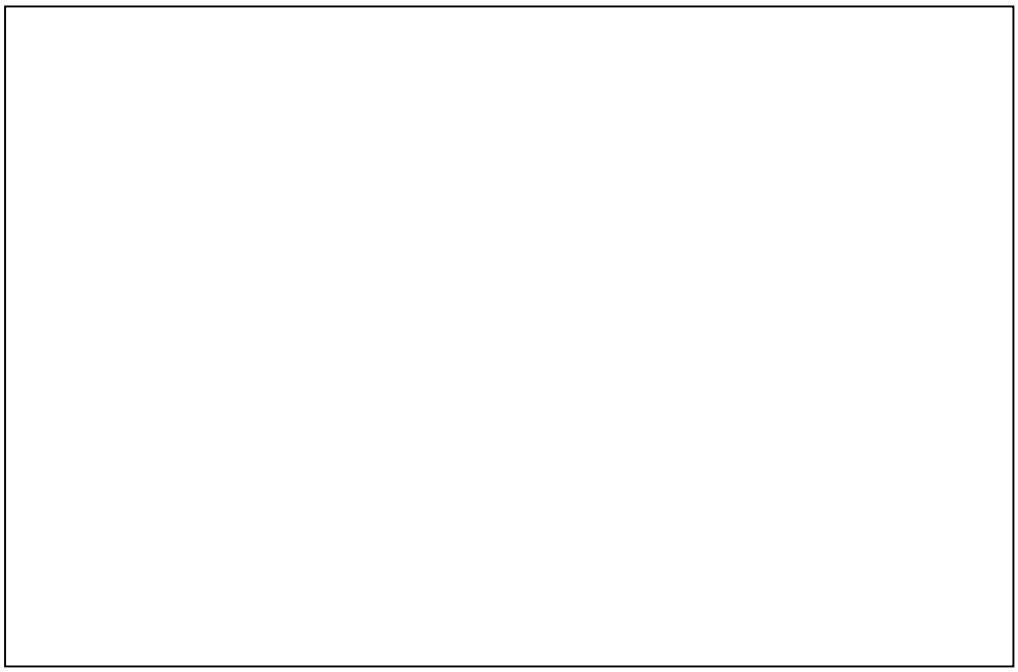

Figure 2. The use of Gram stain for bacterial classification.

Figure 3. Salmonella bacteria appear as short red bacilli (gram negative).

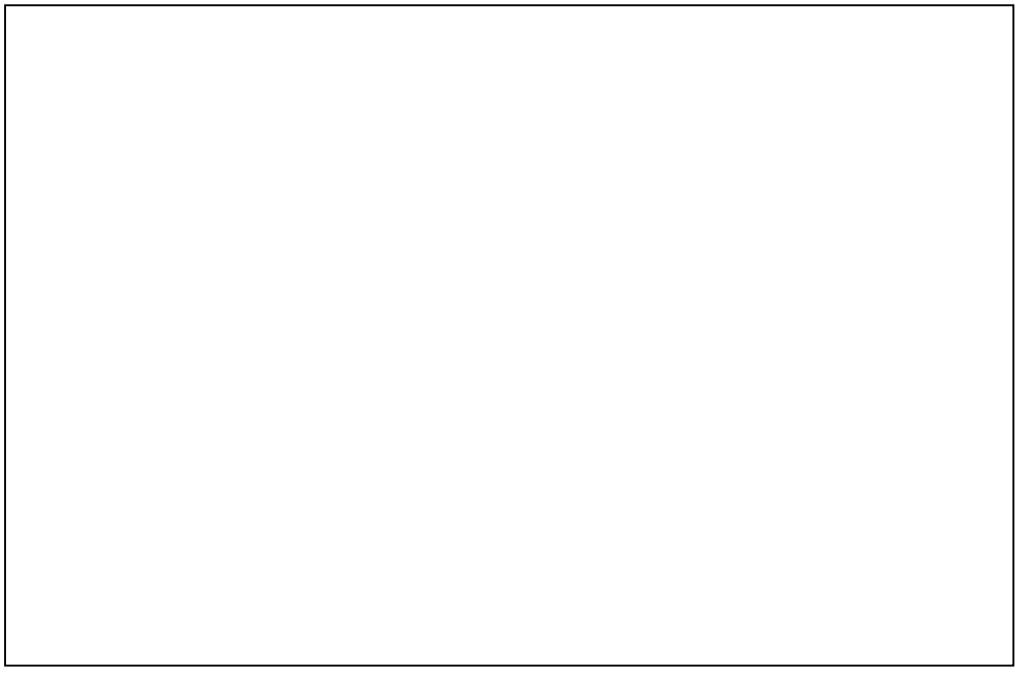

Figure 4. Growth of Salmonella on triple sugar iron (TSI) agar.

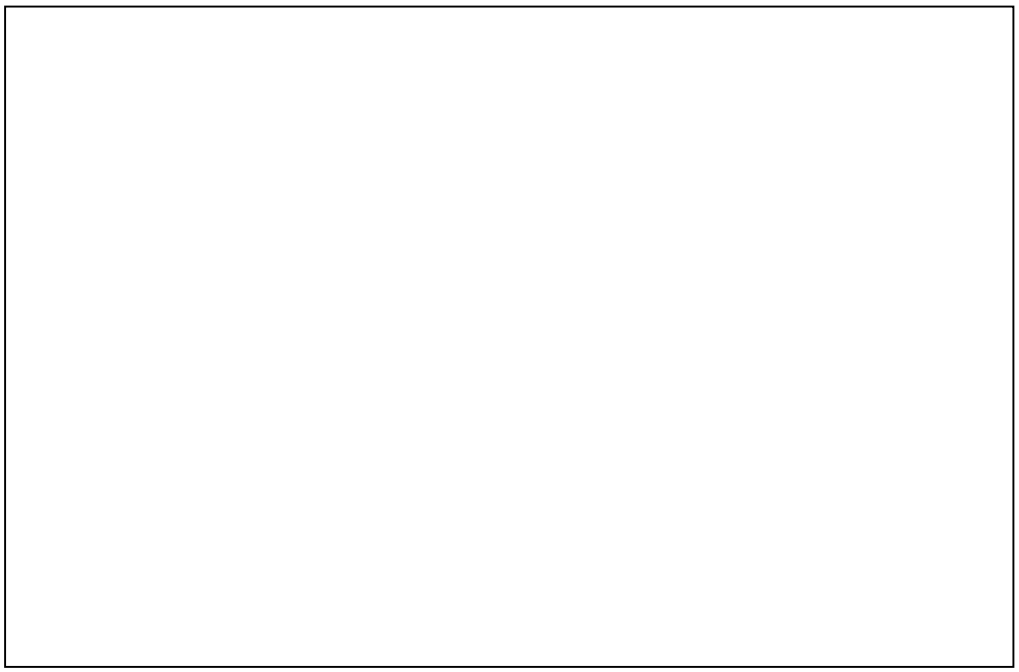

Figure 5. Serotyping of Salmonella isolates (Slide agglutination – O1, O9 and H antigens).

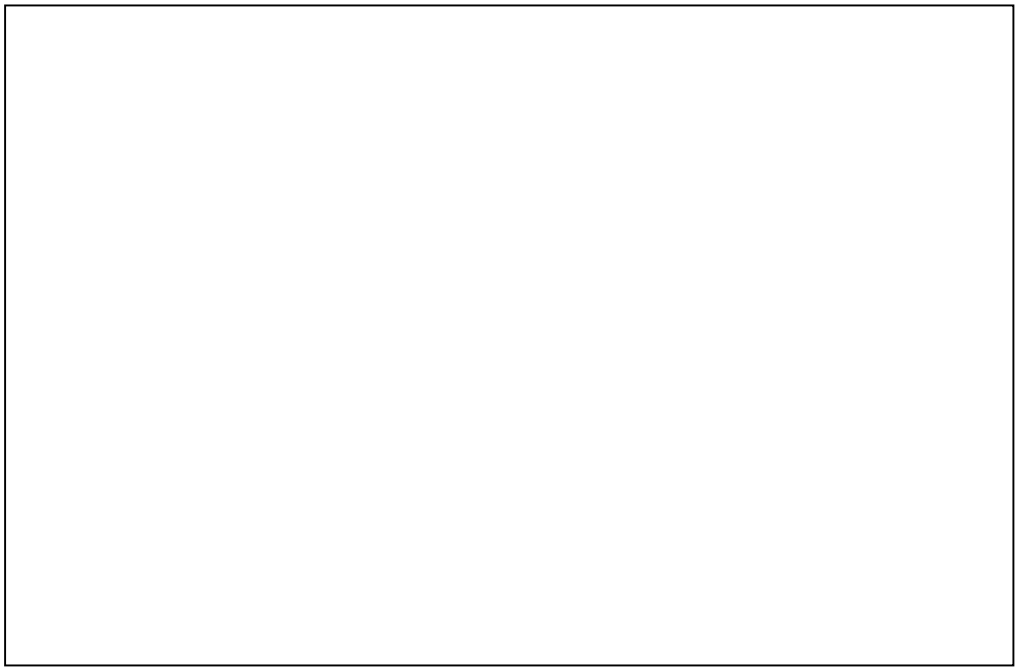

Figure 6. Positive slide agglutination of Salmonella.

4. RESULTS

4.1. Isolation and identification of Salmonella

In the current study, *Salmonella spp* and *S. enteritidis* was isolated from 29 out of 135 samples collected from three regions of Tripoli (Table 2 and Figure 7). The overall prevalence was 21%. The prevalence of *Salmonella spp* was 15% whereas the prevalence of *S. enteritidis* was 7%. The highest prevalence (9%) of *S. enteritidis* was recorded in the South region of Tripoli. However, the highest prevalence (22%) of *Salmonella spp* was found in the West region (Figure 7). Statistically, there were no significant differences ($P>0.05$) in prevalence of total salmonella between the regions (Table 2). In general, the prevalence of salmonella was significantly ($P<0.01$) higher in spleen (13%) as compared with crop and neck where the prevalence of salmonella in these organs were 4% and 5%, respectively. In spleen, 12 (9%) of isolated salmonella were *Salmonella spp* and only 5 (4%) were *S. enteritidis* (Table 6).

In the West region, a total of 13 (29%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for salmonella (Table 2 and 5). Among those, 3 (7%) were positive for *S. enteritidis* and 10 (22%) were positive for *Salmonella spp* (Table 5). High prevalence of salmonella was found in slaughterhouse 1 then slaughterhouse 2 followed by 4 and 5. The highest prevalence (40%) of salmonella was found in spleen followed by neck

(27%) then crop (20%). Two isolates of *S. enteritidis* was found in the neck and one isolate was found in spleen whereas the crop was negative. For *Salmonella spp*, the number of isolates from neck, spleen and crop was 2, 5 and 3, respectively (Table 6).

In the south region, a total of 11 (24%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for salmonella (Table 2 and 3). Among those, 4 (9%) were positive for *S. enteritidis* and 7 (16%) were positive for *Salmonella spp* (Table 3). High prevalence of salmonella was found in slaughterhouse 1 and 2 then slaughterhouses 4 and 5 followed by slaughterhouse 3. The highest prevalence (47%) of salmonella was found in spleen followed by neck (13%) and crop (13%). One isolates of *S. enteritidis* was found in the crop and 3 isolates were found in spleen whereas the neck was negative. For *Salmonella spp*, the number of isolates from neck, spleen and crop was 2, 4 and 1, respectively (Table 6).

In the East region, a total of 5 (29%) out of 45 chicken organs collected from 5 slaughterhouses, were positive for salmonella (Table 2 and 4). Among those, 2 (4%) were positive for *S. enteritidis* and 3 (7%) were positive for *Salmonella spp* (Table 5). High prevalence of salmonella was found in slaughterhouse 3 then slaughterhouses 1, 2 and 5. The highest prevalence (27%) of salmonella was found in spleen followed by neck (7%) and crop (0%). One isolate of *S. enteritidis* was found in the neck and one isolate was

found in spleen whereas the crop was negative. For *Salmonella spp*, the number of isolates from neck, spleen and crop was 0, 3 and 0, respectively (Table 6).

Table 2. Prevalence of *Salmonella spp*. and *S. enteritidis* isolated form different regions of Tripoli.

Type of Salmonella	Number of salmonella isolates			Total
	West	South	East	
Salmonella spp.	10/45 (22%)	7/45 (16%)	3/45 (7%)	20/135 (15%)
Salmonella enteritidis	3/45 (7%)	4/45 (9%)	2/45 (4%)	9/135 (7%)
Total	13/45 (29%)[a]	11/45 (24%)[a]	5/45 (11%)[a]	**29/135 (21%)**

[a] Within a row, data labeled with letters indicate no significant differences (P> 0.05).

Table 3. Number and percentage of *Salmonella spp*. and *S. enteritidis* isolated form different slaughterhouses of South region of Tripoli.

Slaughterhouse	Number of positive samples for Salmonella									Total
	Crop Collection			Neck Collection			Spleen Collection			
	1	2	3	1	2	3	1	2	3	
1	0/1	0/1	**1/1***	0/1	0/1	0/1	1/1	0/1	1/1	3/9
2	0/1	0/1	0/1	1/1	0/1	0/1	**1/1***	1/1	0/1	3/9
3	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	1/9
4	0/1	0/1	0/1	0/1	0/1	0/1	**1/1***	0/1	**1/1***	2/9
5	1/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	2/9
Total	1/5	0/5	1/5	1/5	0/5	1/5	3/5	2/5	2/5	**11/45 (24%)**
	2/15 (13%)			2/15 (13%)			7/15 (47%)			

**Salmonella enteritidis*

Table 4. Number and percentage of *Salmonella spp*. and *S. enteritidis* isolated form different slaughterhouses of East region of Tripoli.

Slaughterhouse	Number of positive samples for Salmonella									Total
	Crop			Neck			Spleen			
	Collection			Collection			Collection			
	1	2	3	1	2	3	1	2	3	
1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	1/9
2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	1/9
3	0/1	0/1	0/1	**1/1***	0/1	0/1	**1/1***	0/1	0/1	2/9
4	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/9
5	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	1/9
Total	0/5	0/5	0/5	1/5	0/5	0/5	2/5	2/5	0/5	**5/45 (11%)**
	0/15 (0%)			1/15 (7%)			4/15 (27%)			

**Salmonella enteritidis*

Table 5. Number and percentage of *Salmonella spp*. and *S. enteritidis* isolated form different slaughterhouses of West region of Tripoli.

Slaughterhouse	Number of positive samples for Salmonella									Total
	Crop			Neck			Spleen			
	Collection			Collection			Collection			
	1	2	3	1	2	3	1	2	3	
1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	1/1	**1/1***	4/9
2	0/1	0/1	0/1	0/1	**1/1***	0/1	1/1	0/1	1/1	3/9
3	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	2/9
4	0/1	0/1	0/1	0/1	0/1	**1/1***	0/1	1/1	0/1	2/9
5	0/1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	2/9
Total	1/5	1/5	1/5	0/5	2/5	2/5	2/5	2/5	2/5	**13/45 (29%)**
	3/15 (20%)			4/15 (27%)			6/15 (40%)			

**Salmonella enteritidis*

Table 6. Number and percentage of *Salmonella spp*. and *S. enteritidis* isolated form different organs.

Organs	Salmonella isolates						Total /135	
	West		South		East			
	SS	SE	SS	SE	SS	SE	SS	SE
Crop	3/15	0/15	1/15	1/15	0/15	0/15	4(3%)	1(1%)
							5(4%)[a]	
Neck	2/15	2/15	2/15	0/15	0/15	1/15	4(3%)	3(2%)
							7(5%)[a]	
Spleen	5/15	1/15	4/15	3/15	3/15	1/15	12(9%)	5(4%)
							17(13%)[b]	
Total	10/45 (22%)	3/45 (7%)	7/45 (16%)	4/45 (9%)	3/45 (7%)	2/45 (4%)	**29/135 (21%)**	

SS= *Salmonella spp*., SE= *Salmonella enteritidis*

Data within a column lacking a common superscript differ at (P<0.01)

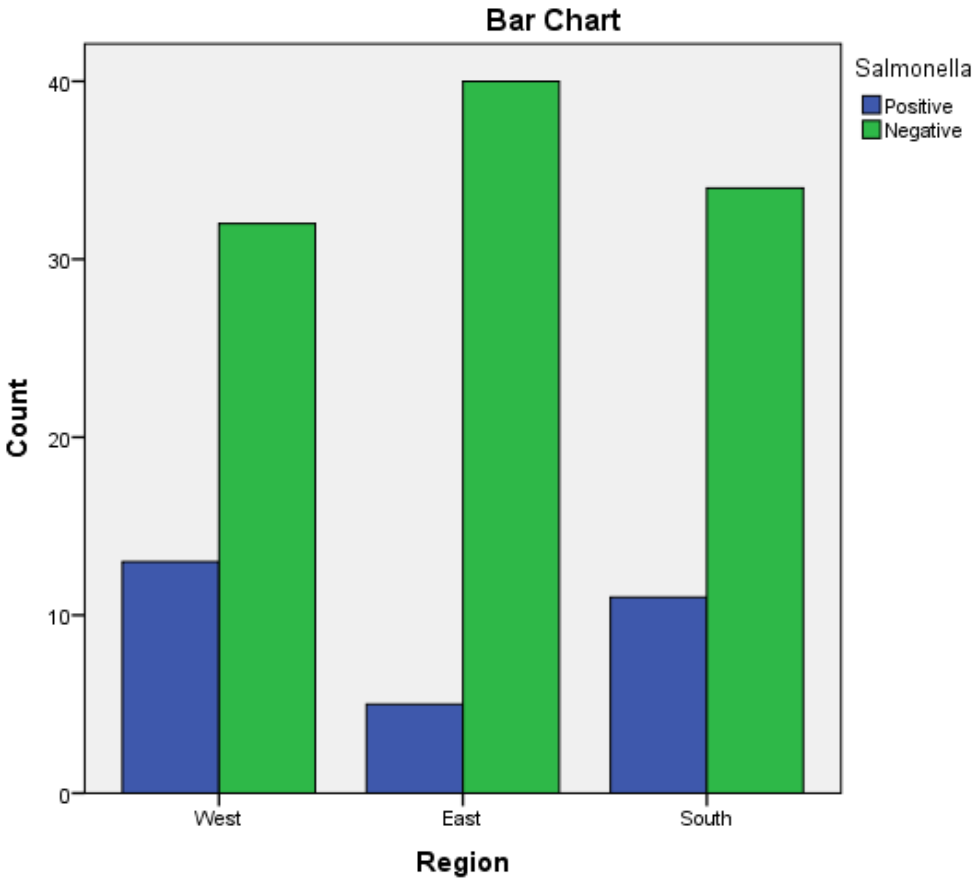

Figure 7. Number of *Salmonella spp*. and *Salmonella enteritidis* isolated form different regions of Tripoli.

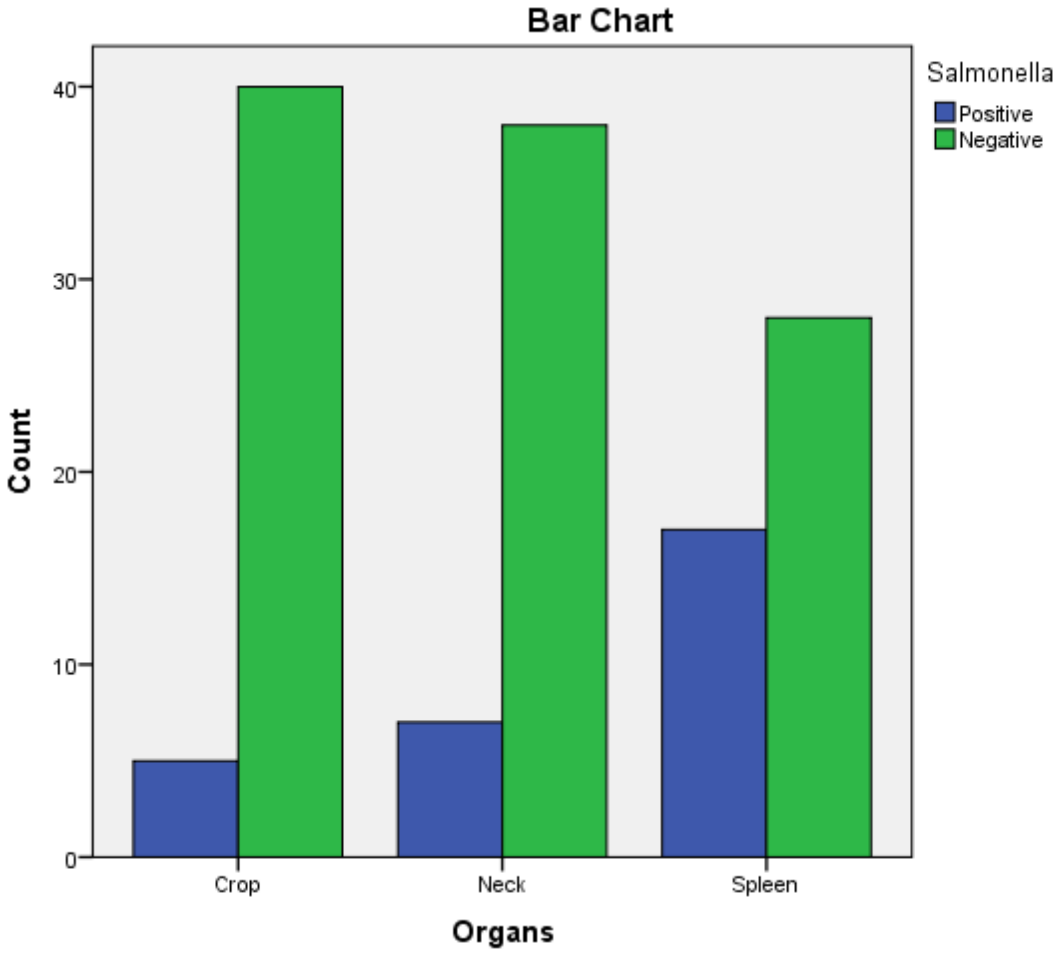

Figure 8. Number of *Salmonella spp*. and *Salmonella enteritidis* isolated form different organs.

4.2. Result of antibiotic sensitivity test

All the isolates were resistance to ampicillin, lincomycin, erythromycin, and cefuroxime. However, low level of resistance was observed to colistin 8 (28%), and sulphamethazon-trimethoprim 12 (41%). High level of sensitivity was showed by colistin 21 (72%) followed by neomycin 18 (62%), whereas low level of sensitivity was showed by nitrofurantion 4 (14%) and doxytetracyclin 4 (14%).

Based on the resistance pattern, *Salmonella spp* isolated from spleen had highest MAR index value of 0.86 in South region (Table 8) followed by MAR index value of 0.8 in the West region (Table 7) and MAR index value of 0.46 in the East region (Table 9). The highest MAR index of *S. enteritidis* isolated from spleen and crop was 0.53 in South region (Table 8).

Table 7. Antimicrobial susceptibility pattern of *S. enteritidis* and *Salmonella spp* isolated from chickens in West region.

Isolate no.	Salmonella serovar	Organ	Antibiotic resistance profiles	(MAR) index
1	*Salmonella spp*	Crop	AM CN MY E CXM	0.33
2	*Salmonella spp*	Neck	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
3	*Salmonella spp*	Spleen	CIP TMP AMC AM CN DO TE F MY E CXM	0.73
4	*Salmonella enteritidis*	Spleen	AMC AM MY E CXM	0.33
5	*Salmonella enteritidis*	Neck	AMC AM MY E CXM	0.33
6	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
7	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO TE F MY E CXM	0.8
8	*Salmonella spp*	Crop	AM CN F MY E CXM	0.4
9	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8
10	*Salmonella enteritidis*	Neck	AMC AM MY E CXM	0.33
11	*Salmonella spp*	Spleen	CIP TMP AMC AM CN DO TE F MY E CXM	0.73
12	*Salmonella spp*	Crop	AM CN F MY E CXM	0.4
13	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8

MAR index = number of resistance antibiotic /total number of antibiotic tested.

CIP - Ciprofloxacin; TMP - Trimethoprim; AMC - Amoxycillin/clavulinic acid; SXT - Sulphamethazone trimethoprim; AM - Ampicillin; CN - Gentamycin; CT – Colistin; DO - Doxytetracyclin; TE - Tetracycline; MY - Lincomycin; E - Erythromycin; CXM - Cefuroxime; N - neomycine; F – Nitrofurantoin

Table 8. Antimicrobial susceptibility pattern of *S. enteritidis* and *Salmonella spp* isolated from chickens in South region.

Isolate no.	Salmonella serovar	Organ	Antibiotic resistance profiles	(MAR) index
14	*Salmonella enteritidis*	Crop	AMC AM DO TE F MY E CXM	0.53
15	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
16	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
17	*Salmonella spp*	Neck	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86
18	*Salmonella enteritidis*	Spleen	AMC AM DO TE F MY E CXM	0.53
19	*Salmonella spp*	Spleen	TMP SXT AM CN DO CT F MY E CXM	0.66
20	*Salmonella spp*	Neck	CIP TMP AMC CXT AM CN DO CT F MY E CXM	0.8
21	*Salmonella enteritidis*	Spleen	AMC AM DO TE F MY E CXM	0.53
22	*Salmonella enteritidis*	Spleen	AMC AM DO TE F MY E CXM	0.53
23	*Salmonella spp*	Crop	CIP TMP AMC SXT AM CN DO CT F MY E CXM	0.8
24	*Salmonella spp*	Spleen	CIP TMP AMC SXT AM CN DO CT TE F MY E CXM	0.86

MAR index = number of resistance antibiotic /total number of antibiotic tested.

CIP - Ciprofloxacin; TMP - Trimethoprim; AMC - Amoxycillin/clavanic acid; SXT - Sulphamethazon trimethoprim; AM - Ampecillin; CN - Gentamycin; CT – Colistin; DO - Doxytetracyclin; TE - Tetracycline; MY - Lincomycin; E - Erythromycin; CXM - Cefuroxime; N - neomycine; F – Nitrofurantoin

Table 9. Antimicrobial susceptibility pattern of *S. enteritidis* and *Salmonella spp* isolated from chickens in East region.

Isolate no.	Salmonella serovar	Organ	Antibiotic resistance profiles	(MAR) index
25	*Salmonella spp*	Spleen	AM CN DO TE MY E CXM	0.46
26	*Salmonella spp*	Spleen	AM CN DO TE MY E CXM	0.46
27	*Salmonella enteritidis*	Neck	AMC AM MY E CXM	0.33
28	*Salmonella enteritidis*	Neck	AMC AM MY E CXM	0.33
29	*Salmonella spp*	Spleen	AM CN MY E CXM	0.33

MAR index = number of resistance antibiotic /total number of antibiotic tested.

CIP - Ciprofloxacin; TMP - Trimethoprim; AMC - Amoxycillin/clavanic acid; SXT - Sulphamethazon trimethoprim; AM - Ampecillin; CN - Gentamycin; DO - Doxytetracyclin; TE - Tetracycline; MY - Lincomycin; E - Erythromycin; CXM - Cefuroxime; N - neomycine; F – Nitrofurantion

Table 10. Antimicrobial susceptibility pattern of *S. enteritidis*, and *Salmonella spp* isolated from chicken organs samples tested by disc diffusion method

no.	Antimicrobial agent	No. of isolates tested	Antibiogram pattern of Salmonella enteritidisand s.spp		
			Resistant(%)	Intermediat(%)	Sensitive(%)
1	Ciprofloxacin	29	13(45)	-	16(55)
2	Trimethoprim	29	14(48)	-	15(52)
3	Chlorophenicol	29	-	15(52)	14(48)
4	Amoxycillin/clavanic acid	29	22(76)	7(4)	-
5	Sulphamethazon trimethoprim	29	12(41)	-	17(59)
6	Ampecillin	29	29(100)	-	-
7	Gentamycin	29	20(69)	-	9(31)
8	Doxytetracyclin	29	20(69)	5(17)	4(14)
9	Colistin	29	8(28)	-	21(72)
10	Neomycine	29	-	11(38)	18(62)
11	Tetracycline	29	15(52)	6(20)	8(28)
12	Nitrofurantion	29	20(69)	5(17)	4(14)
13	Lincomycin	29	29(100)	-	-
14	Erythromycin	29	29(100)	-	-
15	Cefuroxime	29	29(100)	-	-

5. DISCUSSION

In the current study, *Salmonella spp* and *S. enteritidis* was isolated from 29 out of 135 samples collected from three regions of Tripoli. The overall prevalence was 21%. The prevalence of *Salmonella spp* was 15% whereas the prevalence of *S. enteritidis* was 7%. This prevalence is considered high and might reflect a poor hygienic and biosecurity measures in poultry houses, slaughter houses, and live birds markets. Similar results were reported by Paiao *et al*. (2013) in Brazil and by Karim *et al*. (2017) in Bangladesh. Lower prevalence of *Salmonella spp* (0.39%) and *S. enteritidis* (1.18%) was reported in Poland (Witkowska et al., 2018). In Turkey, Goncag *et al*. (2005) reported prevalence of 8.57% for *S. enteritidis* in chicken carcass skins of the wing parts. In Algeria, Djeffal *et al*. (2018) also reported prevalence of 8% for *Salmonella spp* isolated from the skin of the nick. However, Ramya *et al*. (2012) reported higher incidence of *Salmonella spp* and *S. enteritidis* in chickens in India. They reported prevalence of 64% (16 out of 25) and 56% (14 out of 25) for *Salmonella spp* by PCR and culture, respectively and prevalence of 48% (12 out of 25) for *S. enteritidis* by PCR. Salmonellosis is very important zoonotic disease in human beings causing diarrhoea, nausea, abdominal pain, mild fever, chills, vomiting, prostration, headache, and malaise. The diarrhoea varies from thin vegetable soup like stools to a massive evacuation with accompanying dehydration (Forshell *et al*., 2006).

Among the regions included in this study, the highest prevalence (9%) of *S. enteritidis* was recorded in the South region of Tripoli and the highest prevalence (22%) of *Salmonella spp* was found in the West region. These two regions are known for intensive poultry production. Low biosecurity measures of poultry farms could be one of the reasons for high prevalence of Salmonella although there is a lack of studies on the level of biosecurity measures in poultry farms of Tripoli. A study conducted by Kammon *et al*. (2017) showed low level of biosecurity in poultry farms located in Aljabal Al-Gharbi especially the ground of the houses, distance between farms, the presence of disinfectants at the farm entry, the use of coverall cloths, disposal of dead birds and control of wild birds and rodents . 63% of poultry houses has a ground of soil and 44% of them has uncoated walls which may influence the proper cleaning and disinfection. In the current study while visiting the slaughterhouses for sampling, low level of biosecurity measures were observed including the absence regular use of disinfectants, absence of coverall cloths, present of multiple clots of blood on walls and ground, and dirty chicken feather removing machine and cutting knives. Moreover, some of slaughterhouse are located nearly to the accumulation of municipal sewage just in front of the main door. Mostly there was no program to control the flies, wild birds and rodents. Making sure that proper biosecurity and sanitation procedures are followed will reduce the possibility of Salmonella contamination. Sanitizing water lines, keeping wild birds and other animals

out of the houses, reducing farm visitors to necessary personnel, the routine use and maintenance of foot baths, either the use of shoe covers or dedicated shoes, rodent and insect control programs are all common biosecurity practices performed on poultry farms (Tablante, 2002; Dorea, 2010). Several studies have shown that having inadequate biosecurity practices impacts the incidence of disease and flock performance (Tablante, 2002; Dorea, 2010; Van Steenwinkel, 2011). Salmonella can also be introduced into poultry flocks from contaminated feeds, particularly those containing animal proteins, have often been identified as likely sources of Salmonella (Davies *et al*., 1997). Contamination by salmonella has been reported in up to 42% of feed mill samples in the United Kingdom (Davies and Wray, 1997) and in 58% of finished feed (mash) and 92% of meat and bone meal samples in the United States (Cox *et al*., 1983). Meal or mash feeds are more often implicated as sources of salmonella than are pelleted feeds (Rose et al., 1999). The serotypes of salmonellae isolated from live poultry and carcasses have sometimes (but not always) been correlated with the serotypes found in feedstuffs (Mackenzie and Bains, 1996). Biologic vectors can both disseminate and amplify salmonellae in poultry flocks. Insects, including cockroaches (Kopanic *et al*., 1994), lesser mealworms (McAllister *et al*., 1994), flies (Olsen and Hammack, 2000), and darkling beetles (Goodwin and Waltman, 1996) can carry salmonella organisms internally and externally. Mice have been identified as particularly important vectors for *S. enteritidis* in laying flocks (Schlosser et

al., 1999). Henzler and Opitz (1992) detected *S. enteritidis* in 24% of mice from environmentally contaminated laying farms, but in none of the mice from farms with environments free of *S. enteritidis*. They noted that a single mouse fecal pellet could contain 105 *S. enteritidis* cells. Wild birds can carry salmonella infections (Daoost *et al*., 2000), and contact with wild birds or their droppings has sometimes been identified as a risk factor for commercial poultry (Craven *et al*., 2000). Humans can also be a source of salmonellae transmissible to poultry, as shown by a California sewage treatment plant that apparently spread infection to both wild animals and a commercial laying flock (Kind *et al*., 1997).

In general, the prevalence of Salmonella was significantly ($P<0.01$) higher in spleen (13%) as compared with crop and neck where the prevalence of Salmonella in these organs were 4% and 5%, respectively. In spleen, 12 (9%) of isolated *Salmonella spp* and only 5 (4%) were *S. enteritidis*. Prevalence of salmonella in spleen was 47%, 40% and 27% in South, West and East regions, respectively. This result may indicate systemic infection of chickens with Salmonella. After ingestion, salmonella adhere to intestinal epithelial cells which is the pivotal first step in the sequence of events that produces disease. Strains of salmonella have ability to colonize the intestinal tract of chicks (Tumer *et al*., 1990) and ability to survive after phagosome/lysosome fusion in the macrophage (Oh *et al*., 1996). *S. enteritidis*

isolates have been associated variously with invasion of liver, and the spleen (Gulig and Curtiss, 1987). In contrast to other studies when the crop has been implicated as an important source of carcass contamination within the processing plant (Ramirez *et al*, 1997). Higher incidence of salmonella in crops than in ceca have been reported during commercial evisceration by Hargis *et al*. (1995). However, the presence of Salmonella in the intestinal tract, skin and among the feathers of chickens may cause carcasses contamination during slaughtering and processing and possibly it is responsible for the introduction of this microorganism in the slaughterhouses (Paiao *et al*., 2013).

High resistance of *S. enteritidis* and *Salmonella spp* to ampicillin, lincomycin, erythromycin, cefuroxime was reported in the current study with high multible antibiotic resistance (MAR) index of 0.86 and 0.53 for *Salmonella spp* and *S. enteritidis* isolated from spleen in South region, respectively. Resistance to erythromycin has been reported as most common resistance profile in retail meat production (Sallam *et al*., 2014). Thung *et al*. (2016) have found 100% resistance of salmonella to erythromycin, 69% to gentamycin, 100% to ampicillin, 45% to ciprofloxacin, and 52 % to tetracycline. In another study, Bhuvaneswari *et al*. (2015) reported 60.7%, 92.1%, 100%, 23.5% and 92.1% resistance of salmonella for erythromycin, gentamycin, ampicillin, ciprofloxacin and tetracycline in India, respectively. Antimicrobial resistance in *S. enteritidis* and other *Salmonella spp* is an

increasing problem leading to serious health hazards in the world (Singh *et al*., 2013). The reason of this problem could be due to overuse and misuse of antibiotics in developing countries (Ikwap *et al*., 2014). In contrast, our study showed that isolated *S. entritidis* was susceptible to ciprofloxacin, trimethoprim, chlorophenicol, sulphamethazon trimethoprim, gentamycin, colistin and neomycin. In a study of Thung *et al*. (2016), *S. enteritidis* was susceptible to trimethoprim and gentamycin.

6. CONCLUSION

Twenty-nine Salmonella (20 *Salmonella spp* and 9 *Salmonella enteritidis*) were isolated from broiler chickens during processing in slaughter

houses located in Southern, Eastern and Western regions of Tripoli, Libya with prevalence of 21%. The highest prevalence was recorded in the South region which might be due to high intensive of poultry production in this region and lack of application of biosecurity measures.

The prevalence of Salmonella was significantly higher in spleen as compared with crop and neck which may indicate systemic infection of chickens with Salmonella in the poultry farm.

High resistance of isolated *Salmonella enteritidis* and *Salmonella spp* to some antibiotics was reported in the current study with high multible antibiotic resistance (MAR) index. The reason of this problem could be the overuse and misuse of antibiotics as treatment and/or growth promotion.

7. RECOMMENDATIONS

It is highly recommended to implement hygiene applications from farm to fork such as hazard analysis and critical control point (HACCP) in slaughter

houses of Libya to guarantee food safety and to ensure the protection of products and to prevent disease transmission to man and to provide a safe wholesome meat for his consumption especially when the meat is considered as an essential food and a kind of high quality animal protein. Implementation of biosecurity measures in poultry farms is must to reduce the risk of introduction and later spread of disease agents to humans. Hygiene must be improved, first by educating workers to adhere to personal hygiene and slaughter facilities, equipment and personnel garments should be cleaned and disinfected.

The use of antibiotics should be considered if necessary after isolation and identification of pathogenic bacteria and conducting sensitivity test. Research projects to find alternatives to antibiotics are recommended such as using probiotics, prebiotics and other alternatives to combat multiple resistance of bacteria.

The Veterinary authority should adapt and implement a National Plan to control salmonella in poultry farms and slaughter houses. Network between competent authorities is also important for rapid response to foodborne outbreaks.

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