BENHA VETERINARY MEDICAL JOURNAL, VOL. 33, NO. 2:160-173, DECEMBER,2017

A real-time PCR, an accurate method for detection of salmonella in chickens

Ashraf A. Abd El-Tawab[1],Wafaa M. Hassan[2], Fawzi R. El-Saidi[3], DaliaM. Ayoup[4]

1 Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University. 2Animal Health Research Institute, Dokki Lab., Giza. 3Faculty of Veterinary Medicine, BaniSweif University. 4 Directorate of Veterinary Medicine in Cairo.

ABSTRACT

A total of 100 sample were obtained from different slaughter houses located in cairo, Living chickens(20 cloacal swabs), Viscera (20 cecum and 20 internal organs), 20 swabs from Chilling water and 20 swabs from final product, The samples were examined bacteriologically and serologically for Salmonella. where was 4% from total samples . The serotyping was two S.kentuky , one S.Tamilandu and one S.Anatum. The four isolated salmonella serovars were tested for antibiotic sensitivity and was found one isolate of Salmonella Kentuky was sensitive to all antibiotics while the other one was resistant to all antibiotics except (neomycin) was intermediate , S.Tamilandu was sensitive to all antibiotics except (neomycin and colistin sulphate) was resistant , S. Anatum was sensitive to neomycin, norfloxacin and ciprofloxacin , while resistant to nalidixic acid , cefatoxine ,ceftriaxon , gentamycin , amoxicillin and colistin sulphate and intermediate to ampicillin.Real time PCR was used for detection of salmonella, the positive Salmonella reference strain was ATCC 14028 and negative control was E.coli ATCC 25922. The number of positive Salmonella samples which was detected by RT-PCR was six samples while was four samples was No. of positive samples detected by stander method.

Keywords: Salmonella, Real-time PCR, Chickens, slaughter houses

(http://www.bvmj.bu.edu.eg)**(BVMJ-33(2): 160-173, 2017)**

1.INTRODUCTION

Salmonella is a Gram-negative, non-spore forming rod and facultative anaerobe which can ferment glucose belonging to the family Enterobacteriaceae .Most strains are motile with peritrichous flagella and can reduce nitrate to nitrite (Grimont *et al*., 2000).Salmonella is the etiologic agent of Salmonellosis in humans causing severe illness in infants, the elderly, and immune compromised patients and cause typhoid fever or enteric fever (Baumler *et al*., 2000). The largest number of foodborne diseased cases attributed to poultry and poultry products are caused by paratyphoid serotypes of Salmonella. Salmonella infection occurs by direct contact with clinically diseased or symptomless birds by the consumption of contaminated water or feed and through the environment. Salmonella infected birds shed the microorganism, causing contamination of the environment and of other birds. Subsequent contamination of the transport vehicles at the time of harvest participates to the contamination of the carcass or meat product during slaughter and processing. Poultry arrive at the slaughter processing plant with various amounts of fecal contamination on the feathers and skin. Evisceration contributes to the contamination of carcasses, although the viscera are removed in such a way that contact of intestinal contents with the carcass is minimized. Plant workers or equipment can cross-contaminated carcasses (Sutmoller, 1997).So rapid detection methods are required for the diagnosis as well as for the prevention of food contamination and food borne outbreaks (NG *etal*. 1996). The development of novel chemistries and instrumentation platforms enabling detection of PCR products on a real-time basis has led to widespread adoption of real-time PCR as the method of choice for detection of Salmonella (Espy *et al.* 2006). So, this study aimed to compare between Real time PCR as an accurate method and standard method for detection of salmonella in chickens.

2.MATERIALS AND METHODS

2.1. Sample collection

A total of 100 sample were obtained from different slaughter houses located in Cairo Living birds (20 cloacal swabs), Viscera (20 cecum and 20 internal organs), 20 swabs from Chilling water and 20 swabs from Final product were aseptically collected to prevent cross contamination and transferred immediately in ice box to Reference Laboratory for Veterinary Quality Control on Poultry Production.

2.2. Isolation of Salmonella

It was done according to ISO 6579 (2002).

2.3. Biochemical identification of isolated Salmonellae

Oxidase reaction, hydrolysis of urea, H2S production, Lysine decarboxylation, Indole test, MR test, VP test and Citrate utilization test were done according to (Cruickshank et al, 1975).

2.4. Serotyping of Salmonella organism

The isolates that were identified biochemically as Salmonella was subjected to serological identification according to (Modified Kauffman - White scheme as described by WHO(2007) for determination of somatic (O) and flagellar (H) antigens (Cruickshank *et al*, 1975).

2.5.Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for salmonella isolates was applied by the agar disc diffusion method as described by Fine gold and Martin (1982). The following antimicrobial discs were tested (Oxoid, UK): ampicillin (10 µg), amoxycillin (25µg), ceftriaxone (30 µg), gentamicin (10 µg), neomycin (30 µg), ciprofloxacin (5 µg), Nalidixic acid (30 µg), Cefatoxine (µg), colistin sulphate (25µg) and Norfloxacin (10µg). The inhibition zone diameter around each disc was measured and the isolates were categorized as susceptible or resistant based upon the interpretative criteria developed by Clinical and Laboratory Standards Institute (CLSI, 2007).

2.6. Application of Real-Time PCR

Application of RT- PCR for isolation of Salmonella, the positive Salmonella reference strain was ATCC 14028 and negative control was E.coli ATCC 25922,Extraction of DNA according to QIAamp DNA mini kit instructions, Preparation of PCR Master Mix, Cycling conditions for taqman real time PCR of invA gene (Daum et al, 2002).

3. RESULTS

3.1.Cultural and staining characters of the isolated Salmonellae

The cultural characters of the isolated Salmonellae appeared on XLD agar as smooth pink colonies with or without black center (H_2S production), while it appeared on Brilliant green agar as reddish color and translucent colony and slightly convex, However on Hektonenteric agar colonies have green color with black center.

The staining characters of the isolated Salmonella revealed a Gram negative, non spore forming short bacilli (2-3x0.5µ).

3.2.Biochemical characters of the isolated Salmonellae

The application of different biochemical tests revealed a negative result (colorless) inoxidase reaction, a negative result on urea agar (yellowish coloration), also a negative Indole reaction (Yellow ring) and negative VP test (no bright red color), while a positive reaction on TSI agar (alkaline red slant, acid yellow butt with H2S and gas), a positive reaction on LI agar (alkaline deep purple slant and alkaline butt with no gas or H2S),a positive reaction on MR test (red color at surface) and a positive blue color on Simmon's Citrate agar.

3.3.Incidence of Salmonella isolation from different poultry slaughter houses

The incidence rate was 4% (4 out of 100).the incidence was the highest in Chilling water where 2 isolates out of 20 (10%),also 2 isolates out of 20 with percentage of (10%) isolated from final product. While the incidence of salmonella in Living chickens (cloacal swabs) and viscera (cecum and internal organs) was (0%).

3.4. Serotyping of isolated salmonella

The serotyping of the isolated salmonella was two S.kentuky (O8,20., I., Z6)(50%), one S.Tamilandu (O6,7 ., Z41 ., Z35) (25%) and one S. Anatum (O3,10 ., e ,h ., 1 , 6) (25%).

3.5. Sensitivity of salmonella isolates to different antibiotics

The four isolated salmonella serovars were tested for antibiotic sensitivity and were found to be (75%) resistant to colistin sulphate, (50%) resistant to amoxicillin, gentamycin, nalidixic acid , cefatoxine , ceftriaxon also (25%) resistant to ampicillin, neomycin , norfloxacin , ciprofloxacin while (75%) of isolates serovars were found to be sensitive to norfloxacin and ciprofloxacin. One isolate of Salmonella Kentuky was sensitive to all antibiotics while the other one was resistant to all antibiotics except (neomycin) was intermediate, S.Tamilandu was sensitive to all antibiotics except (neomycin and colistin sulphate) was resistant , S. Anatum was sensitive to neomycin, norfloxacin and iprofloxacin , while resistant to nalidixic acid , cefatoxine ,ceftriaxon , gentamycin , amoxicillin and colistin sulphate and intermediate to ampicillin.

3.6.Detection of salmonella by real time -PCR

The number of positive Salmonella samples which was detected by real time PCR was six samples while the positive Salmonella samples which were detected by traditional method were four samples.

Table (1) Results of biochemical identification of isolated salmonella by standard laboratory tests.

Medium	Result
Triple sugar iron agar	Positive: alkaline slant (red), acid butt (yellow) with H2S and gas production
Urea agar	Negative , no change of yellow colour
Simmon’s citrate	Positive ,blue colour
Lysine iron broth	Positive ,deep purple
Vogasproskauer	Negative , no bright red colour
Methyl red test	Positive, red colour at the surface
Indole reaction	Negative, yellow ring

Table (2) The incidence of salmonella infection in different point in chicken slaughter house

Type of samples	No of examined samples	No of positive samples	Percent of salmonella
1-Living chickens (cloacal swabs)	20	0	0%
2-viscera		0	0%
*cecum	20		
*Spleen	10		
*liver	10		
3- chilling water	20	2	10%
4- final product	20	2	10%
Total	100	4	4%

Table (3) Serotyping of isolated salmonella strain in slaughtered chicken.

Salmonella strain	No. of isolates	%	Serotyping
S.kentuky	2	50%	(O8,20., I., Z6)
S.Tamilandu	1	25%	(O6,7 ., Z41 ., Z35)
S.Anatum	1	25%	(O3,10 ., e ,h ., 1 , 6)

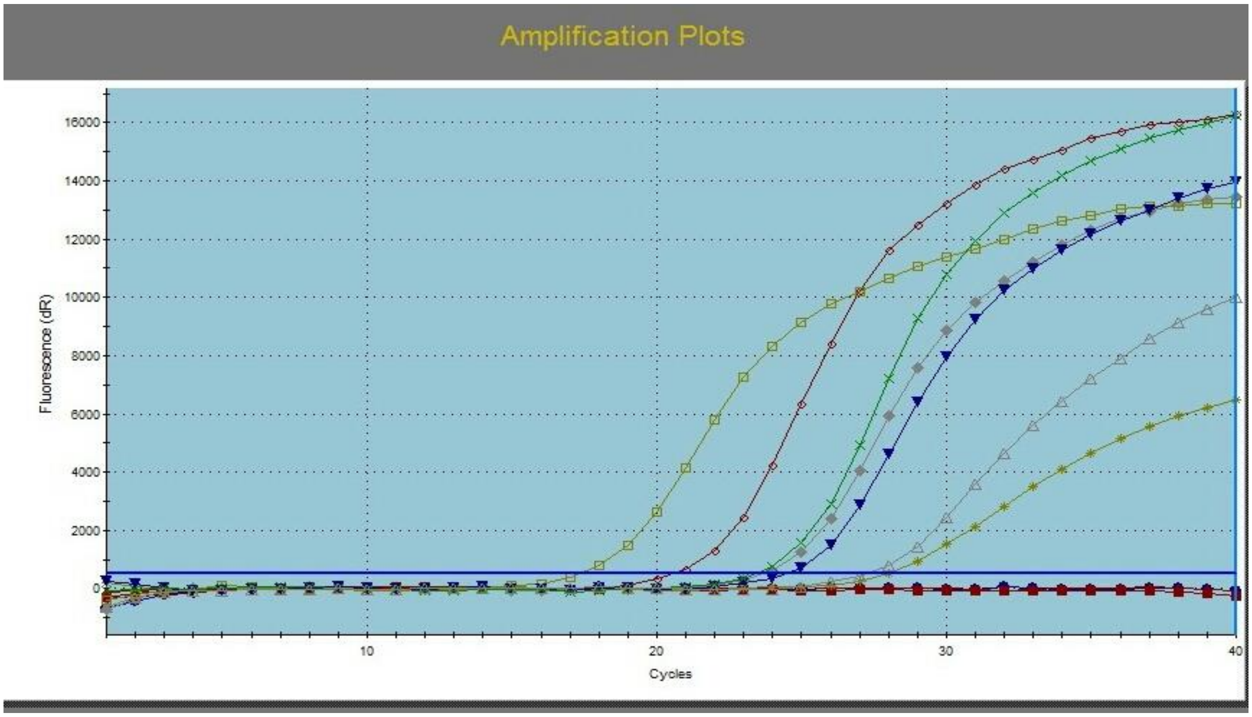
Table (4) Show results of antibiotic sensitivity test of Salmonella isolates.

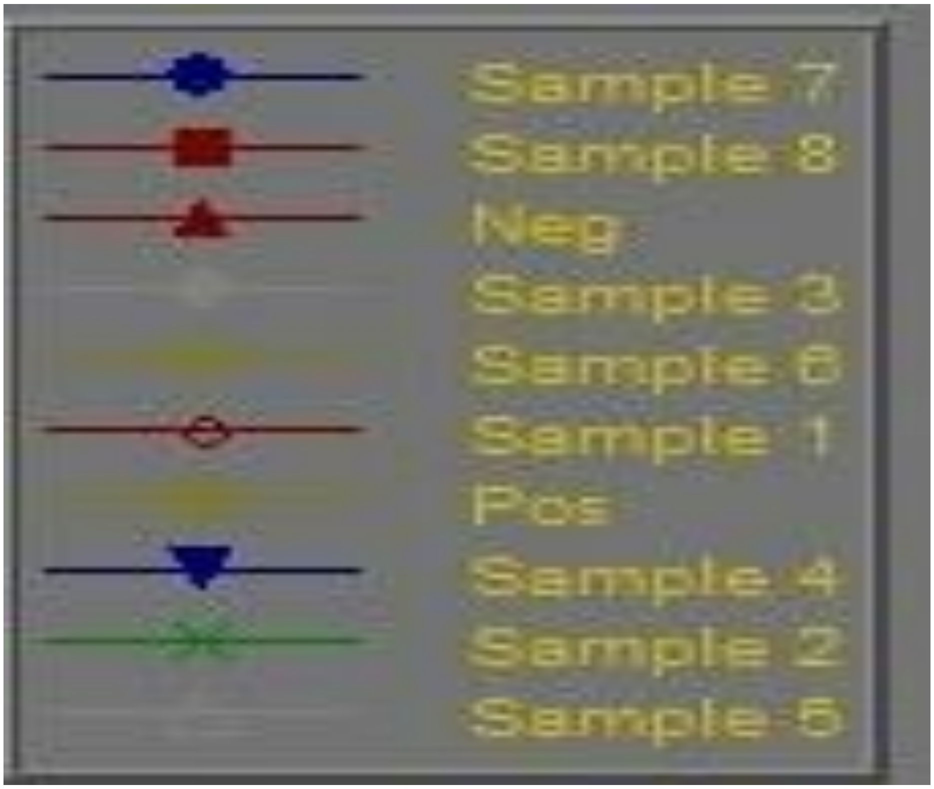
Antibiotic	S.Kentuky(1)	S.Tamilandu	S.Anatum	S.Kentuky(2)
Ampicillin	32 (S)	37 (S)	15 (I)	6 (R)
Nalidixic acid	30 (S)	30 (S)	6 (R)	6 (R)
Cefatoxine	30 (S)	26 (S)	18 (R)	6 (R)
Ceftriaxon	28 (S)	36 (S)	12 (R)	6 (R)
Gentamycin	24 (S)	23 (S)	6 (R)	6 (R)
Neomycin	22 (S)	14 (R)	18 (S)	15 (I)
Norfloxacin	26 (S)	26 (S)	20 (S)	6 (R)
Ciprofloxacin	27 (S)	27 (S)	25 (S)	14 (R)
Amoxicillin	23 (S)	27 (S)	6 (R)	14 (R)
Colistin sulphate	16 (S)	6 (R)	6 (R)	6 (R)
(S)=Sensitive & (R)=Resistant & (I) = Intermediate				

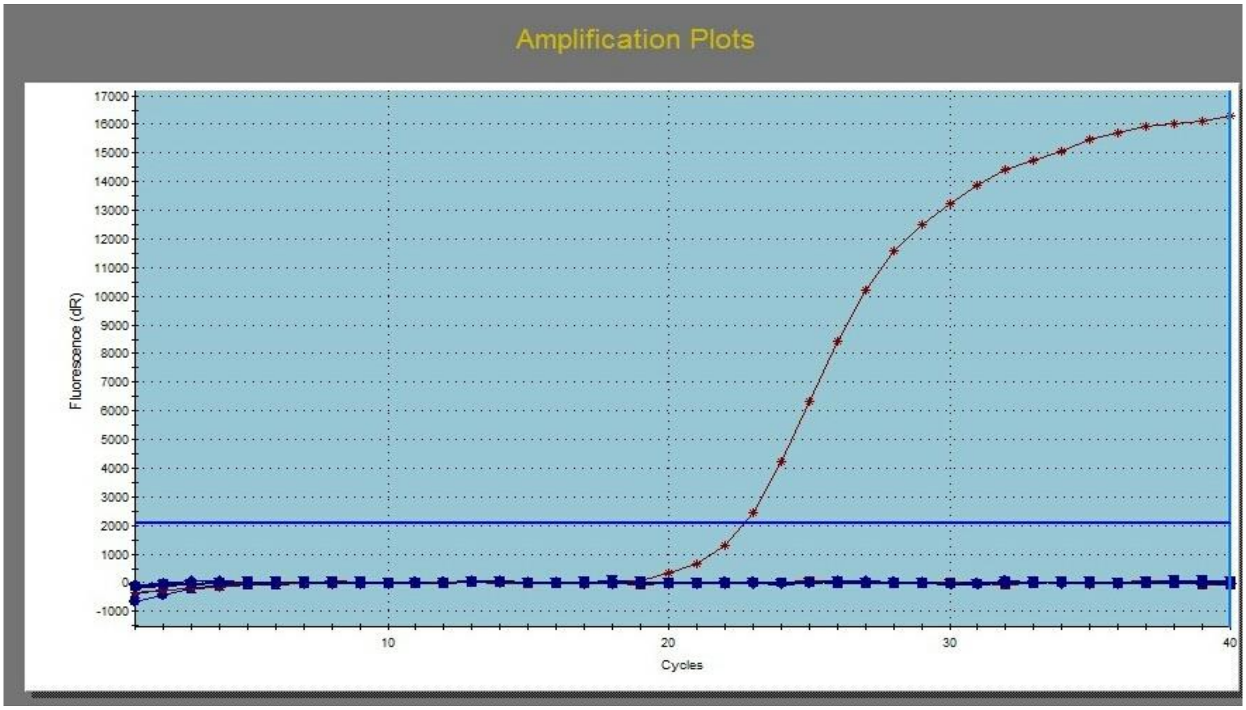
Table (5): Result of RT-PCR

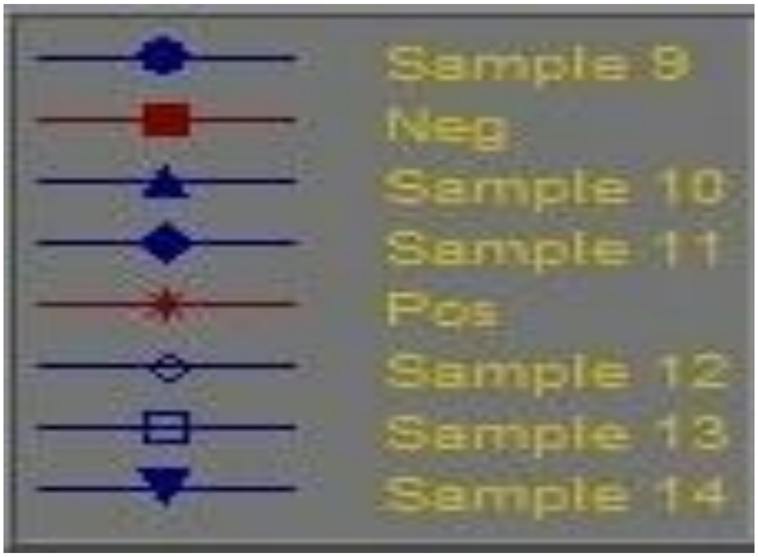
Type of sample	No. of Sample	No. of positive samples	No. of negative samples
Living chickens (cloacal swabs)	5	0	5
Viscera (cecum, liver and spleen)	5	0	5
Chilling water	5	3	2
Final product	5	3	2
Total	20	6	14

Figure (1) Amplification curves of Salmonella invArt-PCR assay




Sample 7
Sample 8
Neg
Sample 3
Sample 6
Sample 1
Pos
Sample 4
Sample 2
Sample 5


Amplification Plots
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
Fluorescence (dR)
10
20
30
40
Cycles



Amplification curves show positive results for some Salmonella strains and negative results for other strains. Amplification plot generated by Strata gene MX3005 software. The fluorescence emission intensity is plotted on the Y axis versus the cycle number on the axis.

4. DISCUSSION

In this study a total of 100 samples from chickens and chilling water were tested and the incidence of salmonella was (4%) , this results agreed with Balala et al . (2006) who found the incidence of salmonella in the 325 samples was (4.9%) . and agreed with Ziada (2007) who isolated the salmonella from (2.94%) of samples , Also Abd El-Gany et al. (2012) who examined four broiler chicken flocks in kalubia governorate , Egypt, found the incidence of salmonella ranged in between (3.84% to 5.18 %). On the other hand, the results in this study lower than the rate of isolation of Harison et al. (2001) who collected 300 raw chickens samples from three supermarket chains and three local butchers' shops and found the incidence of salmonella was (29%), and Cardinal et al. (2003) who examined 300 chicken carcasses from retail shops in Dakar and found the prevalence of Salmonella was (32%), also Dahal (2007) examined 400 samples for detection the prevalence of Salmonella and it was (13%), Kim et al. (2012) who collected 210 samples from retail supermarkets in Seoul, South Korea, and analyzed for the presence of Salmonella and found the Salmonella incidence was (22.4%), Phagoo and Neetoo (2015) who analyzed Thirty poultry samples for Salmonella and found the prevalence of Salmonella was (16.67%), Hassan et al. (2016) who tested 75chicken samples and found the salmonella isolates were 55 isolates and the prevalence was (73.3 %), Yadav, et al. (2017) who collected twenty three rectal swabs from 23 Captive Ostrich reared in a park situated in Banskhali, Chittagong and found the prevalence of Salmonella was (78.3%).

Serotyping of isolated salmonella from chickens and chilling water were 2 S. kentuky, 1 S. Tamilandu and 1 S.Anatum with a percentage of (50%) , (25%) and (25%) respectively. And that was agreed with Bada-Alambedji *et al.* (2006) who reported that S.

Kentuchy was the most frequently isolated (30%) , also he reported other serotypes as S. Muenster (13.3%), S. Brancaster (8.8%), S. Enteritidis and S. Hadar (6.6%).

Moreover Korashy and Mohammed (2012) revealed that 5 different serotypes were identified as S. typhimurium (42.9 %) , followed by S. enteritidis (21.4%) , S. virchow (21.4%) , S.anatum (7.1%) and Salmonella type II (7.1%), also Abd El- Tawab et al. (2015) found that Salmonella isolates were serotyped as S. Typhimurium, S. Apeyeme, S. Kentucky, S.Daula, S. Newport, S. Tamale, S. Molade, S.Colindale, S. Lexington, S. Bargny, S. Enteritidis,S. Papuana, S. Labadi, S. Santiago, S. Magherafelt, S. Rechovot, S. Takoradi, S. Angers and S.Shubra were isolated from chickens. While S. Inganda, S. Infantis and S. Larochelle were isolated from ducks but S. Virchow and S. Vejle were isolated from turkeys. S. Shangani and S.Jedburgh were isolated from quails while S. Alfort and S. Wingrove were isolated from pigeons, Ammar et al. (2016) conducted that the most frequently encountered serotype was Salmonella Enteritidis (56.25%), other serotypes as Salmonella Typhimurium (18.75%), Salmonella Labadi and Salmonella Kentucky (12.5%) for each, On the other hand Cardinal et al. (2003) who recorded the most predominant Salmonella serovars were Salmonella Hadar (41.6%) and Salmonella Brancaster (20.8%), while Balala et al . (2006) found The most predominant Salmonella serovars was S. weltevreden followed by S. derby, S. enteritidis PT1, S. enteritidis phage type untypable, S. new port , S. albany and S. Lexington, Saeed (2010) isolated 3 isolates belong to S. enteritidis and isolate belongs to S. arizonae, Fallah et al.(2013) found that 34 of 44 isolates of Salmonella were Salmonella infantis (79.5 %), one strain (2.3%) of group C and 8 strains (18.2%) of group D, also Nidaullah et al.(2017) found the predominant serovars were S. Albany (57/161), S. Corvallis (42/161), and S. Brancaster (37/161).

In this study, the antibiogram was carried out against different Salmonella serotypes using 10 different antibiotic discs. The results revealed that (75%) of isolates were resistant to colistin sulphate, (50%) resistant to amoxicillin, gentamycin, nalidixic acid , cefatoxine , ceftriaxon also (25%) resistant to ampicillin, neomycin , norfloxacin , ciprofloxacin while (75%) of isolates serovars were found to be sensitive to norfloxacin and ciprofloxacin and this results nearly were agreed with El-jakee et al . (2010) who found that (80 %) of the isolates were sensitive to ciprofloxacin and (70%) for enrofloxacin and norfloxacin, also agreed with Selvaraj et al . (2010) who detected that the sensitivity of ceftriaxon was (62.50%) for 5 isolates (37.50%) for 3 isolates and sensitivity of cafataxime was (50%) for 4 isolates,(37.5%), also similar to Khallaf et al.(2014) who reported that the resistance to tetracycline and nalidixic acid was the most common (50%), followed by resistance to ampicillin (39.47%), streptomycin (34.21%) and ciprofloxacin (26.31%),and Yadav et al . (2017) who tested Salmonella positive samples for 12 different antimicrobials and resistance was found to colistin sulfate (83.33%) ,

The results of this study were differ from Ruzauskas et al. (2005) who reported that 8.1% were resistant to aminoglycosides (neomycin and gentamicin), also SOOMRO et al . (2010) observed that all the Salmonella isolates showed resistance to ampicillin, and sensitivity to streptomycin, cefotaxime, gentamicin, tobramycin and ciprofloxacin, the results were different from Fallah et al . (2013) who observed that all isolates strains were sensitive to cefotaxime and 100% were resistant to nalidixic acid, tetracycline and streptomycin, also differ from Tessema et al . (2017) who found the resistance of ampicillin (72.7%).

In this study one isolate of Salmonella kentuky was sensitive to all antibiotics while the other one was resistant to all antibiotics except (neomycin) was intermediate and this agreed with Ammar et al .(2016) who observed that all Salmonella kentucky sensitive to ciprofloxacin and gentamicin and Hassan et al . (2016) who found Salmonella Kentucky isolates exhibited high rates of resistance against the majority of the used antimicrobials, where 100% (14 of 14) were resistant to ciprofloxacin, ampicillin, nalidixic acid and tetracycline, moreover 85.7% (12 of 14) showed resistance against both of cefotaxime and ceftazidime.

In this study the number of positive Salmonella samples which was detected by Real time PCR was 6 samples while the positive Salmonella samples which were detected by Traditional method were 4 samples and that was agreed with Kimura et al .(1999) who analyzed 100 samples by TaqMan and found (10) were positive for Salmonella with both the kit and conventional culture methods and (89) were negative with both. One sample was negative by the culture method but positive by the kit assay, and Somyanontanagul (2009) who tested 906 samples were taken from 48 finishing pig farms by bacteriological methods and real-time PCR and found (20.64%) of samples were positive for Salmonella by bacteriological methods and (39.51%) and (37.19%) of samples were found Salmonella positive by real-time PCR (default setting) and real-time PCR (modified setting), Moreover Temelli et al . (2010) who collected a total of 259 samples from 50 layer flocks and tested by real -PCR and ISO culture methods and found the incidence of Salmonella in layer flocks by rPCR and culture was (61.0 and 55.6%) respectively, and Yan et al . (2014) who collected 16 samples of retail whole poultry from markets and tested by real time -PCR , PCR and traditional method and found 7 of 16 samples were positive by real time -PCR which were also tested positive by PCR ,while only 5 samples were positive by traditional method.

On the other hand the results in this study were different from Nam et al . (2005) who collected 93 environmental samples including fecal slurry, feed /silage , drinking water , lagoon water , bulk milk tank , bedding soil and farm soil and analyzed for presence of salmonella by conventional culture methods and real time PCR and found all samples analyzed were negative for salmonella by both real time PCR and standard culture method , no false negative or false positive results were detected.

5. REFERENCES

Abd El-Ghany , W.A. ; El-Shafii , S.S.A. and Hatem M.E. (2012): A survey on Salmonella species Isolated from Chicken flocks in Egypt. Asian Journal of Animal and Veterinary Advances .7(6):489-501.

Abd El- Tawab , A.A.; Ammar , A.M.; NasefS.A.; El- Hofya , F.I. and Nabil , N.M. (2015): Studies on different salmonella serotypes isolated from poultry in different governorates in Egypt . Benha veterinary medical journal, 28(2):169-175 .

Ammar,A. M.; Abd El-Aziz , N.K.; Hanafy , M.S. and Ibrahim , O.A.(2016) : Serotypes Profile of Avian Salmonellae and Estimation of Antibiotic Residues in Chicken Muscles using High-Performance Liquid Chromatography. Advances in Environmental Biology, 10(7) Pages: 173-179. AENSI Journals

Bada-Alambedji, R. ;Fofana, A.; Seydi, M. andAkakpo , A.J.(2006): Antimicrobial resistance of Salmonella isolated from poultry carcasses in Dakar (senegal). Brazilian Journal of Microbiology 37:510-515.

Balala ,L.M ; Rovira ,H.G ; Vizmanos ,M.F.C. ; Bernardo ,F.A. E.M. and Divina ,B.P. (2006): Isolation, Serologic Identification and Antibiotic Sensitivity Testing of Salmonella spp. in Chickens .Philipp. J. Vet. Med., 43 (2): 63-70

Baumler, A.J.; Tsolis , R.M. and Heffron F. (2000): Virulence mechanisms of Salmonella and their genetic basis. In: C Wray and A Wray (eds), Salmonella in Domestic Animals,(CAB International, Wallingford, UK), pp.52-57.

Cardinale, E.; Gros-Claude, J.D.P.;Tall,F.; Cissé,M.; Guèye ,E.F. and Salvat,G. (2003): Prevalence of Salmonella and Campylobacter in Retail Chicken Carcasses in Senegal .Revue Elev. Méd. vét. Pays trop 56 (1-2) : 13-16.

Clinical and Laboratory Standards Institute (CLSI) 2007 Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100-S17 [ISBN 1-56238-625-5]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Cruickshank, R.; Deguid, J.P.; Marimon, B.P. and Swain, H.A.(1975): Medical Microbiology . The practice of medical microbiology.12th , Vol. II. Churchill, Edinbourgh

Dahal, N.(2007): prevalence and antimicrobial resistance of Salmonella in imported chicken carcasses in Bhutan. Master degree

Daum, L.T.; Barnes, W.J.; McAvin, J.C.; Neidert, M.S.; Cooper, L.A.; Huff, W.B.; Gaul, L.; Riggins, W.S.; Morris, S.; Salmen, A. and Lohman, K.L. (2002): Real-Time PCR Detection of Salmonella in Suspect Foods from a Gastroenteritis Outbreak in Kerr County, Texas. J ClinMicrobiol. 2002 August; 40(8): 3050–3052.

El-Jakee , J.; Abd El-Moez , S.I.; Mohamed , Kh .F.; Effat , M.M.; Samy , A.A. and Gad El-said , W.A. (2010): Restriction Enzyme, Plasmid profile analysis and Antibiotic Resistance of Salmonella Typhimurium of poultry origin Isolated from Egyptian farms . International Journal of Microbiological Research , 1(3): 137-146 .

Espy, M.J.; Uhl, J.R.; Sloan, L.M.; Buckwalter, S.P.; Jones, M.F.; Vetter,E.A.; Yao, J.D.; Wengenack, N.L.; Rosenblatt, J.E.; Cockerill, F.R.,3rd& Smith, T.F. (2006): "Real-time PCR in clinical microbiology: applications for routine laboratory testing", Clinical Microbiology Reviews, 19 (1): 165-256 .

Fallah , S.H.; Asgharpour , F.; Naderian , Z. and Moulana , Z. (2013):Isolation and Determination of Antibiotic Resistance Patterns in Nontyphoid Salmonella spp isolated from chicken . Int J EntricPathog.1(1).

Finegold, S.M. and Martin, W.J.(1982): Diagnostic microbiology 6th C.V. Mosby Co. St. Louis, Toronto, London.

Grimont, P.A.D.; Grimont ,F. and Bouvet P.(2000): Taxonomy of the genus Salmonella. In Hagens S., and Loessner M. Application of bacteriophages for detection and control of foodborne pathogens. ApplMicrobiolBiotechnol (2007) 76:513–519.

Harrison,W.A.;Griffith,C.J.;Tennant, D. and Peters, A.C.(2001): Incidence of Campylobacter and Salmonella isolated from retail chicken and associated packaging in South Wales. Letters in Applied Microbiology. 33, 450-454.

Hassan , A.H.A.; Salam , H.S.H. and Abdel-Latef , G.K. (2016): Serological identification and antimicrobial resistance of Salmonella isolates from broiler carcasses and human stools in Beni-Suef, Egypt . Beni – suef university journal of basic and applied sciences 5: 202–207.

ISO 6579(2002): Microbiology of food and animal feeding stuffs- horizontal method for the detection of Salmonella species International standard . (4th edition) .

Kim , M.S. ; Lim ,T.H. ; Jang ,J.H. ; Lee ,D.H.; Kim ,B.Y.; Kwon ,J.H. ; Choi ,S.W. ; Noh ,J.Y.; Hong , Y.H.; Lee , S.B. ; Yang , S.Y.; Lee ,H.J.; Lee ,J.-B. ; Park , S.Y.; Choi ,I.S. and Song , C.S. (2012): Prevalence and antimicrobial resistance of Salmonella species isolated from chicken meats produced by different integrated broiler operations in Korea. Poultry Science 91 : 2370–2375 .

Kimura, B. ; Kawasaki, S. ; Fujii, T. ; Kusunoki, J. ; Itoh, T. and Flood,S.J.A.(1999): Evaluation of TaqMan PCR Assay for Detecting Salmonella in Raw Meat and Shrimp.Journal of Food Protection. 62(4): 329–335.

Korashy, N.T. and Mohammed ,G.M.O. (2012): incidence of salmonellae in chilled chicken carcasses in retails Port-Said city .AssiutVet.Med. J. 58 (134).

Nam ,H.M.; Srinivasan, V. ; Gillespie, B.E.; Murinda, S.E. and Olver, S.P.(2005):Application of SYBR green real-time PCR assay for specific detection of salmonella spp. In dairy farm environmental samples.int. J. food Microbial .102 (2):161-71

NG, S.P.; Tsui, C.O.; Roberts, D.; Chau, P.Y. and NG, M.H. (1996): Detection and serogroup differentiation of Salmonella spp. in food within 30 hours by enrichment-immunoassay with a T6 monoclonal antibody capture enzyme-linked immune sorbent assay. Appl. Environ. Microbiol, 62: 2294-2302.

Nidaullah,H.; Abirami,N.; Shamila-Syuhada, A.K.; Chuah, L.; Nurul, H.; Tan,T.P.; Abidin, F.W.Z and Rusul, G.(2017): Prevalence of Salmonella in poultry processing environments in wet markets in Penang and Perlis, Malaysia. Veterinary World, 10(3): 286-292.

Phagoo , L. and Neetoo , H. (2015) : Antibiotic Resistance of Salmonella in Poultry Farms of Mauritius . J. World's Poult. Res. 5(3): 42-47.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.C. and leonard, F.C. (2002): Veterinary Microbiology and Microbial diseases .salmonella serotypes. Great Britain by HPG, Books Ltd; Bodmin, Cornwall, UK.P.114-118.

Ruzauskas, M.; Virgailis , M. and Špakauskas , V. (2005): Serological diversity and antimicrobial resistance of Salmonella isolated from different sources in Lithuania . Vet. arhiv 75, 211-221.

Saeed , H.I.M.A.(2010): Isolation and identification of Salmonella from the environment of traditional poultry farms in Khartoum North. Master thesis. Khartoum university

Somyanontanagul , N. (2008) : Comparison between detecting Salmonella spp. by bacteriological method and Real-Time PCR assay in samples from pig herds. In: Oral Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa, 2008, 176

Soomro, A.H.; Khaskheli,M.; Bhutto,M.B.; Shah,G.; Memon,A. and Dewani,P. (2010): Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry meat in Hyderabad, Pakistan . J. Vet. Anim. Sci. 34(5): 455-460.

Sutmoller, P.(1997): Contamination of Animal Products: Prevention and Risks for Public Health.Volume 16(2).

Temelli, S.; Kahya, S.; Eyigor, A.andCarli,K.T.(2010):Incidence of SalmonellaEnteritidis in chicken layer flocks in Turkey: Results by real-time polymerase chain reaction and International Organization for Standardization culture methods. Poultry Science .89:1406–1410.

Tessema , K.; Bedu , H.; Ejo , M. and Hiko , A. (2017) : Prevalence and Antibiotic Resistance of Salmonella Species Isolated from Chicken Eggs by Standard Bacteriological Method . J .Vet SciTechnol 8:1, DOI: 10.4172/2157-7579.1000421.

Waltman, W.D.; Gast, R.K. and Mallinson, E.T.(1998): Salmonellosis in: A

Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th Edition, American Association of Avian Pathologists, 2-13.

World Health organization (WHO) (2007):"Salmonella epidemiology. http://www.safepoultry.com/Salmonellaepidemiology.asp

Yadav, S.K.; Ahaduzzaman, Md.; Shafiqul ,I.Md.; Siddiki and Zonaed , A. (2017): Isolation and identification of Salmonella spp. from captive ostriches in Bangladesh by InvA gene and SefA gene - specific PCR and detection of their antimicrobial resistance patterns . Research article ,4(1) .

Yan, L.; Wang, X.; Guo, Y.; Pei, X.; Yu, D. and Yang, D.(2014): Study on detection of Salmonella in poultry samples by real-time PCR with Taqman probes. Zhonghua Yu Fang Yi XueZaZhi. 48(8):731-5.

Ziada, H.H.M.A. (2007): Isolation and Identification of Salmonella species from Chickens in Khartoum State. Master thesis. Khartoum University