International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 30-42**
http://www.ijcmas.com

Original Research Article

Assessment of the Prevalence of Salmonellae in Food

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K e y w o r d s

Salmonella serovar; food poisoning; animal origin; Heat shock proteins by SDS; antibiotic sensitivity.

A B S T R A C T

In the present study standard ISO 6579 method was used to investigate the presence of salmonellae in milk and meat products. Salmonellae were detected in 5% of minced meat samples, 10% of the 20 burger samples, 35% of sausage samples and 25% of poultry products. *Salmonella* isolates were identified as *S. infantis, S. lagos, S. bolombo, S. cerro, S. enteritidis, S. kentucky, S. newlands, S. newport, S. saintpaul, S. sandiego, S. senftenberg* and *S. typhimurium.* Most isolates were sensitive to colistine sulphate and ciprofloxacin (94.1% each), amoxicillin (82.4%), sulfa-trimethoprim (70.6%) and 64.7% of the isolates were sensitive to ampicillin, danofloxacin and gentamicin (each). On the other hand, all isolates were resistant to lincomycin and 64.7% were resistant to penicillin. Most *Salmonella* isolates were multiple drug resistance. The role of heat stresses among the isolates was carried out using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of heat treated salmonellae. The phage shock protein A gene, (pspA) encodes a 25.4 kDa protein was detected in 8 heat treated isolates. The four major classes of heat shock proteins (Hsp90, Hsp70, GroE, and small heat shock proteins) were detected among the heat treated salmonellae. The present work indicated poor sanitation of the environment under which animals are slaughtered, transported, processed and sold. Thus, it is recommended to manage meat safety risks based on an integrated effort and approach that applies to all stages; from the producer to the consumer. Also, there is an emergency need to control the use of growth promoters and antimicrobial agents in animals to minimize the emergence of resistant strains.

Introduction

Salmonella remains an important food-borne pathogen throughout the world and food products of animal origin are considered to be the major source of infection. Moreover; human-to-human and animal-to-human transmission can occur

(Torlak *et al*., 2012). Apart from the food-borne infections, the other major epidemiological development in salmonellosis is the emergence of multiple-antibiotic resistant salmonellae. The antibiotic resistance of *Salmonella* strains of avian origin is attributed to chromosomal mutation, gene transfer mechanisms like conjugation, transduction and transformation. Avian *Salmonella* shows resistance against many antimicrobials; tetracycline, oxytetracycline, penicillin, aminoglycosides, sulpha drugs and fluoroquinolones (Rajagopal and Mini, 2013). Exposure to heat has been resulted in the rapid production of a number of proteins termed heat shock proteins (HSPs). HSPs are ubiquitous chaperones that bind and help fold nascent or denatured polypeptides and are also recognized as major immunogens in the immune response against pathogens (Colaco *et al*., 2013). HSPs were inducible upon exposure to a range of environmental stresses including oxygen deprivation, pH extremes, and nutrient deprivation demonstrating a more general function in providing protection against cellular stress, by limiting protein aggregation and denaturation, and they are thus now more commonly referred to as stress proteins (Colaco *et al*., 2013). In view of the above considerations, the present study was carried out to determine the prevalence of *Salmonella* in meat and dairy products and study the antibacterial sensitivity test of the isolates. Also, the present work analyzed *Salmonella* heat shock proteins by SDS PAGE and studies the effect of heat stress on different *Salmonella* serovars.

Materials and Methods

Samples collection

A total number of 200 samples of meat products, poultry products and milk products were collected randomly from different markets, retail shops and butcher shops of Cairo and Giza governorates as shown in Table (1) and examined to detect *Salmonella* species. Meat and meat products were cooled freshly or frozen and packed. Milk product samples were refrigerated. Samples were collected under possible aseptic conditions individually and transported in sealed cold icebox from the retail to the destination of analysis in the lab.

Identification of Salmonellae

Detection of *Salmonella* was carried out according to ISO 6579: 2002. The suspected colonies on Xylose Lysine Deoxycholate and Brilliant Green agar plates were picked up and examined microscopically by Gram's stain before being transferred into semisolid and slope agar for preservation and further identification. *Salmonella* isolates were identified biochemically (Quinn *et al*., 2002) and serologically (Popoff *et al*., 2000).

Antimicrobial susceptibility test

The antimicrobial susceptibility testing for 16 antimicrobial discs (Oxoid) was carried out using agar disc diffusion method as described by **NCCLS (2002)**.

Analysis of Salmonellae Heat Shock Proteins

Preparation of *Salmonella* Cultures (Hassani *et al*., 2009)

Salmonella isolates were routinely cultured on Trypticase soy agar and incubated at 37°C. Using Trypticase soy broth (Difco) aliquot tubes (10 ml) of

working culture (10^8 CFU/ml) were heat stressed by immersion (3cm above medium level in bottle) into 55 °C water bath. After 10 min, the tubes were removed from water bath and heat stressed cells were prepared after incubation of the cultures at 40 °C for 18h. Control not heat treated salmonellae grown in TSB at 37 °C for 18h were also investigated.

Sample preparation

The bacterial cultures were centrifuged at 5000xg for 5 minutes. Then the pellets were resuspended in buffer one and centrifuged at 10000xg for 10 minutes. The supernatant was discarded and the pellets were vortexed in 100µl of buffer two then the phosphate buffer was added for resuspension. The samples were incubated with 33mg/ lysozyme for 30 minutes. Samples were sonicated for 20 second for each one. Then samples were centrifuged at 15000 xg for 15 minutes. The total proteins concentration for each sample was determined using the method described by Bradford (1976).

PAGE procedure (Laemmli, 1970)

The gel constituents were prepared from stock solution of 9ml of 30% polyacrylamide and 4.5 ml of Resolving gel buffer and 4.5 ml dist, 50 µl APS and 20 µl TEMED. The prepared solution was pipetted for 2/3 of the gel surface and kept undisturbed over night for good polymerization. The stacking gel was prepared from the stock solution of the following ingredients and then was pipetted and the comp was inserted. The protein samples were added to the sample buffer in concentration 100µg per well. The gel was run at 100 voltage until the dye reached to the anode end of the gel, then the power was turned off and the gel were extracted from the equipment. The gel was removed from the electrophoresis unit, and then gel was soaked in commassie blue staining solution, sealed in a plastic box and left for two hours at 60ºC. The gel was washed several times with distaining solution till the back ground become clear.

Results and Discussion

Prevalence of *Salmonella* in retail food products

Of the 100 meat product samples tested, 15(15%) samples were positive for *Salmonella*. The isolates were detected from minced meat (5%), burger (10%), sausage (35%) and poultry products (25%). On the other hand, no *Salmonella* isolates could be isolated from luncheon samples or milk products. The recovered serovars were, Logos from minced meat; Newlands and Cerro from burger; Kentucky, Saintpaul, Sandiego, Lagos, Infantis and Bolombo, from sausage; Typhimurium, Newport, Senftenberg, Enteritidis and Infantis from poultry products as shown in Table 2.

The presence of salmonellae in food products presents a potential health hazard that has been studied for nearly 90 years (CDC, 1983). Food products of animal origin are considered to be the major source of human salmonellosis (Torlak *et al*., 2012). In the present investigation, ISO 6579 method was used to detect the presence of salmonellae in milk and meat products. Salmonellae could not be detected from the collected milk products. While twenty nine *Salmonella* outbreaks were identified by Buyser *et al.* (2001), 14 were associated with milk, of which 6 with raw milk, 1 with cream from pasteurized milk, and 14 with cheese of which 8 were

made from raw milk and 3 from unpasteurized milk.

The presence of salmonellae in meat is of serious public health concern. Consumption of contaminated meat and meat products is the main cause for salmonellosis outbreaks (Torlak *et al.,* 2012). Of the tested samples, *Salmonella* was isolated from the examined minced meat, burger, sausage and poultry products. The main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs (Tafida *et al*., 2013). In Egypt, Abd El-Atty and Meshref (2007) detected *Salmonella* with a prevalence of 4% in sausages and 2% in spiced minced meat. The overall prevalence of *Salmonella* in meat and meat products samples found in this study was consisted with the previous studies conducted in different countries (Pui *et al.,* 2011; Cetinkaya *et al*., 2012).

In the present study, the incidence of salmonellae in poultry product was 25%. Poultry products are frequent vehicles in the transmission of *Salmonella*, dominating other foods of animal origin as potential source of infection (Antunes *et al.,* 2003).

Contaminated poultry products are widely accepted as a major source of *Salmonella* infections (Cogan and Humphrey, 2003). The results of Antunes *et al*. (2003) can be compared to the findings reported in 1990 by Machado and Bernardo, where 57% of chicken carcasses were contaminated with *Salmonella*, suggesting that poultry products are one of the potential vehicles of transmission of *Salmonella* in Portugal. However, in surveys performed by other authors, the prevalence of *Salmonella* in poultry products were 8% in Albania (Beli *et al.,* 2001), 23–34% in Belgium (Uyttendaele *et al*., 1998), and 43% in USA (Bokanyi *et al*., 1990). The difference in the prevalence rates may be due to socio-economic factors. High ambient temperature and humidity coupled with poor handling practices predispose the meat products to massive microbial contamination.

In the present work, the most commonly recovered serovars from different retail meats were: Lagos from minced meat; Newlands and Cerro from burger; Kentucky, Saintpaul, Sandiego, Lagos, Infantis and Bolombo, from sausage; Typhimurium, Newport, Senftenberg, Enteritidis and Infantis from poultry products. The serovars involved in salmonellosis vary geographically, but frequently include *S. typhimurium, S. enteritidis, S. heidelberg, S. agona, S. newport, S. infantis, S. panama, S. saintpaul* and *S. weltevreden* (Uyttendaele *et al*., 1998). In the early 1900s, *Salmonella enterica* serovars pullorum and gallinarum caused widespread diseases in poultry, but vaccination helped in eradication of Pullorum disease and fowl typhoid from commercial flocks. However, the niche created by the eradication of these serovars was likely filled by *S. enteritidis* and *S. typhimurium*, which proliferated in the bird populations. This pathogen remains a significant problem in commercial egg and poultry production. Coinciding with the decrease of *S. enteritidis* and *S. typhimurium*, *S. heidelberg* and *S. kentucky* have emerged as the predominant serovars in commercial broilers (Foley *et al.,* 2011).

S. enteritidis and *S. typhimurium* were identified from the examined poultry products (5% each). Antunes*et al.* (2003) reported that the most prevalent serotype

identified from poultry product was *S.* enteritidis (44%). Also, this serotype predominates in *Salmonella* from poultry products in other surveys in Spain (Carramin˜ana *et al*., 1997), Belgium (Uyttendaele *et al*., 1998) and United Kingdom (Plummer *et al.*, 1995). this serotype was the most frequently implicated in outbreaks of food borne diseases, as revealed in the seventh report of the WHO Surveillance Programme for Control of Food borne Infections and Intoxications in Europe (WHO, 2000). *Salmonella* contamination of meat and meat products in this study verified that meat and meat products should be monitored regularly for the *Salmonella* contamination.

Antimicrobial susceptibility testing of *Salmonella* serovars isolated from retail products

A total of 15 isolates and the two reference strains were tested against sixteen commonly used antimicrobial agents as shown in Table 3. It is clear that most of isolates were sensitive to colistine sulphate and ciprofloxacin (94.1% each) followed by amoxicillin (82.4%), sulfa-trimethoprim (70.6%), ampicillin, danofloxacin and gentamicin (64.7% each). On the other hand, 100% of isolates were resistant to lincomycin followed by penicillin (64.7%). Also the isolates were resistant and intermediate resistant to tetracycline and streptomycin (76.5% each) oxytetracycline (70.6%) and nalidixic acid (53%).). Multidrug resistance was observed among the isolated *Salmonella* serovars: Lagos (12.5 & 68.8 %), Newlands (37.5%), Cerro (18.8%), Infantis (12.5, 37.5 & 50%), Bolombo (18.8%), Typhimurium (50%), Senftenberg (56.3%), Kentucky (87.5%), Saintpaul (81.3%), Newport (50%), enteritidis (87.5%) and Sandiego (50%) as shown in Table 4.

In veterinary medicine, antimicrobial agents are used in therapy, prophylaxis, and as growth promoters. This kind of use may be responsible for generate resistant bacteria. It is clear that most of isolates were sensitive to colistinesulphate and ciprofloxacin (94.1% each) followed by amoxicillin (82.4%), sulfa-trimethoprim (70.6%), ampicillin, danofloxacin and gentamicin (64.7% each). All *Salmonella* strains investigated by Fallah *et al*. (2013) were sensitive to cefotaxime and ciprofloxacin, and 100% were resistant to nalidixic acid, tetracyclin and sterptomycin. *Salmonella* isolates showed sensitivity to ceftazidime, gentamicin, tobramycin, ciprofloxacin, ofloxacin and chloramphenicol (Shah and Korejo, 2012). On the other hand, 100% of isolates were resistant to lincomycin followed by penicillin (64.7%).

Also, the isolates were resistant and intermediate resistant to tetracycline (76.5%), streptomycin (76.4%) oxytetracycline (70.6%) and nalidixic acid (53%). Multiple drug resistance was observed in all the isolates of *Salmonella* tested in this study. Significant increases were seen for *Salmonella* resistance to ampicillin: chicken isolates, 16.7% to 40.5%; ground turkey isolates, 16.2% to 58.4% (Roos, 2013). Reports from different parts of Nigeria have observed temporal trends in the prevalence of resistance among enteric organisms (Tafida *et al*., 2013); resistance to commonly used antimicrobials, including trimethoprimesulphamethoxa zole, ampicillin, tetracycline and chloramphenicol has shown increasing prevalence in the last 25 years (Iwalokun *et al.*, 2001).

The present work illustrated that76.5% of the isolates were resistant and intermediate resistant to streptomycin. Streptomycin resistance was observed by Duffy *et al*. (1999) among *Salmonella* isolated from poultry products. During the two past decades, the emergence of antibiotic-resistant *Salmonella* has become a serious problem worldwide and wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings. The problem of resistant strains to multiple drugs (MDR) is increasing and most studies in different countries have shown high resistance of *Salmonella* strains to several antibiotics (Fallah *et al*., 2013). Bacterial gain of antimicrobial resistance is an alarming situation where treatment is getting limited to currently sensitive antibiotics. The prophylactic use of many antimicrobials in poultry feed can also lead to acquired antibiotic resistance (Rajagopal and Mini, 2013). Resistance to *Salmonella* transmitted by contaminated foods of animal origin is undesirable, but it can be prevented with the rational use of antimicrobials in animal production (Tessari *et al*., 2013).

Most pathogens change their environmental challenges in order to establish it in the host. In addition to adaptation mechanisms specific to their host, elaboration of the heat shock response appears to be a common survival strategy among all pathogens that play an important role in helping bacteria cope with stressful host environment, thereby aiding bacterial pathogenesis (Colaco *et al.,* 2013). Heat shock proteins (HSPs) comprise a subgroup of molecular chaperones that are induced in response to adverse environmental conditions (Neckers and Tatu, 2008). Given that the high cellular protein concentration (200–400 mg/ml) naturally favors inappropriate protein-protein interactions, leading to insolubility and protein denaturation, molecular chaperones, by transiently associating with hydrophobic surfaces of "client" proteins, are key to maintaining cellular homeostasis and a functional intracellular milieu (Ellis, 2006). Expression of HSPs in all cells strongly suggests that these proteins play a more fundamental role in protein housekeeping within the cell, chaperoning the folding of nascent polypeptides and prevention of protein aggregation (Colaco *et al.*, 2013).

Results of Heat shock proteins by SDS

In the present study, the role of heat stresses was investigated among the isolates using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It is clear that 8 heat treated isolates had a 25.4 kDa protein which is peripherally associated with the cytosolic membrane. Hassani*et al.* (2009) recorded that the phage shock protein A gene (*pspA*) encodes a 25.4 kDa protein. *S*. typhimurium after heat stresses at 40–70^{o}C revealed different patterns, after stress induction at 40^{o}C, the pspA was not observed in protein samples but at 45^{o}C the narrow protein band was seen (Hassani *et al.*, 2009). At 50, 55, and at 60^{o}C, the quantity of pspA protein was increased, respectively, while at 65^{o}C, the bacteria had maximum expression of pspA, but this band was disappeared in recovered bacteria at 70^{o}C (Hassani *et al.*, 2009). Three and ten heat treated isolates had

Table.1 Number and types of the collected samples

Type of samples	No. of examined samples
Meat and Meat products	
Burger	20
Sausage	20
Luncheon	20
Minced meat	20
Poultry products	20
Total meat products	100
Milk products	
White cheese	30
Hard cheese	20
Processed cheese	30
yoghurt	20
Total milk products	100
Total	200

Table.2 *Salmonella* prevalence and serovars isolated from retail meat.

Meat products		No. %*	Serovars	No. of Isolates	%*
Burger	20	2 10	*S. newlands*	1	5
			S. cerro	1	5
Sausage	20	7 35	*S. kentucky*	1	5
			S. saintpaul	1	5
			S. sandiego	1	5
			S. lagos	1	5
			S. infantis	2	10
			S. bolombo	1	5
Luncheon	20	0 0	-	0	0
Minced meat	20	1 5	*S. lagos*	1	5
Poultry meat	20	5 25	*S. typhimurium*	1	5
			S. newport	1	5
			S. senftenberg	1	5
			S. enteritidis	1	5
			S. infantis	1	5
Total meat products	100	15 15		15	15
Milk products	100	0 0		-	0

*% calculated according to the total number of examined samples.

Table.3 Antimicrobial resistance among the examined strains (n=17).

Antimicrobial	Resistant		Sensitive		Intermediate	
	No	%	No	%	No	%
AMP10	4	23.5	11	64.76	2	11.8
AML25	3	17.6	14	82.4	0	0
CT10	0	0	16	94.1	1	5.9
CIP5	0	0	16	94.1	1	5.9
DFX5	6	35.3	11	64.7	0	0
DO30	6	35.3	9	53	2	11.8
GM10	4	23.5	11	64.7	2	11.8
L15	17	100	0	0	0	0
NA30	8	47.1	8	47.1	1	5.9
N30	7	41.2	9	52.9	1	5.9
NOR10	4	23.5	11	64.7	2	11.8
OT30	8	47.1	5	29.4	4	23.5
P10	11	64.7	1	5.9	5	29.4
S10	9	53	4	23.5	4	23.5
SXT5	3	17.6	14	70.6	0	0
T30	8	47.1	4	23.5	5	29.4

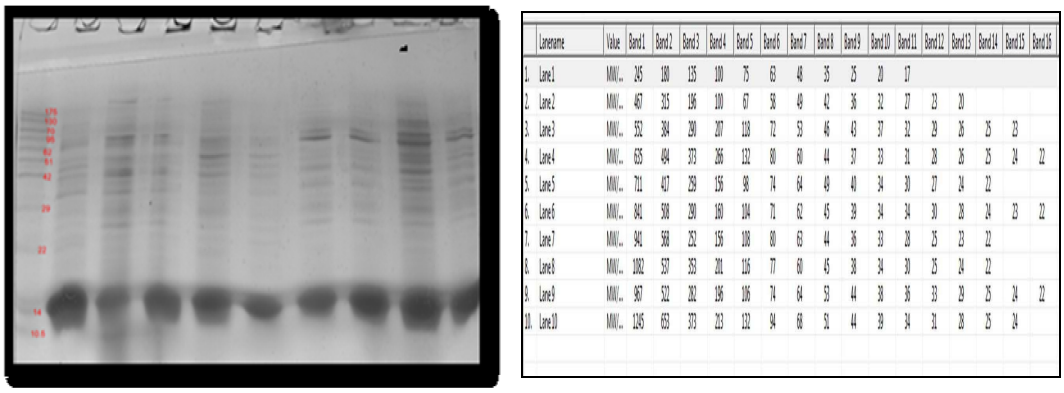
Ampicillin=AMP10, Amoxicillin=AML25, Ciprofloxacin=CT10, Colistinsulphate=CIP5, Danofloxacin=DFX5, Doxycycline=DO30, Gentamicin=GM10, Lincomycin=L15, Nalidixic Acid=NA30, Neomycin=N30, Norfloxacin=NOR10, Oxytetracycline=OT30, Penicillin=P10, Streptomycin=S10, Sulfa-trimethoprim=SXT5, Tetracycline=T30.

[

Table.4 Percentage of multidrug resistance (MDR) *Salmonella* strains among the 16 tested antimicrobial agents

Salmonellae	MDR	MDR%
S. newlands	6/16	37.5
S.cerro	3/16	18.8
S. lagos	2/16	12.5
S. infantis	2/16	12.5
S.bolombo	3/16	18.8
S. lagos	11/16	68.8
S. infantis	6/16	37.5
S. kentucky	14/16	87.5
S. saintpaul	13/16	81.3
S. sandiego	8/16	50
S. typhimurium	8/16	50
S. newport	8/16	50
S. senftenberg	9/16	56.3
S. enteritidis	14/16	87.5
S. infantis	8/16	50
S. paratyphi B (Local isolated)	8/16	50
S. typhimurium (ATCC 14028)	7/16	43.8

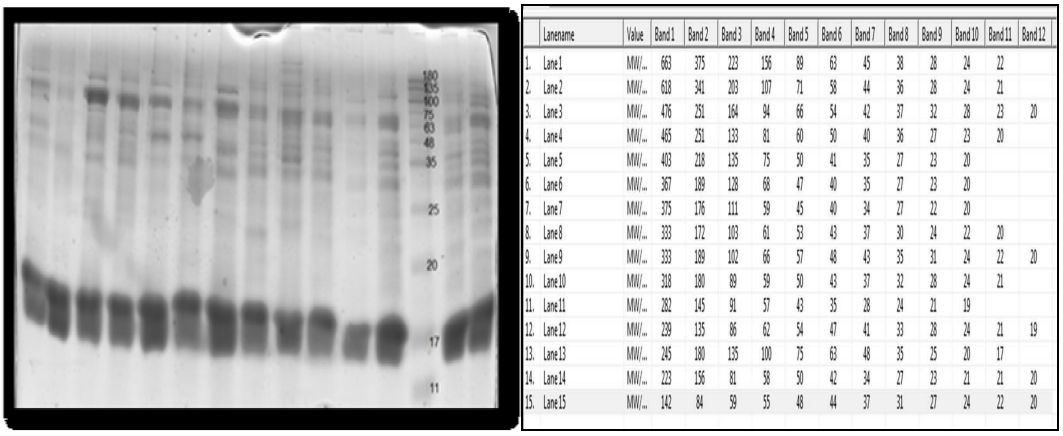
Figure.1 SDS profile analysis of heat shock protein among salmonellae isolates



	Lanename	Value	Band1	Band2	Band3	Band4	Band5	Band6	Band7	Band8	Band9	Band10	Band11	Band12	Band13	Band14	Band15	Band16
1.	Lane1	MW/..	245	188	135	100	75	63	48	35	25	20	17					
2.	Lane2	MW/..	457	315	196	100	67	58	49	42	36	32	27	23	20			
3.	Lane3	MW/..	552	384	290	207	110	72	53	46	42	37	32	29	26	25	23	
4.	Lane4	MW/..	655	494	373	286	122	80	60	44	37	33	31	28	26	25	24	22
5.	Lane5	MW/..	721	417	259	156	98	74	64	49	40	34	30	27	24	22		
6.	Lane6	MW/..	841	508	290	160	104	71	62	45	39	34	34	30	28	24	23	22
7.	Lane7	MW/..	941	568	252	156	108	80	63	44	36	33	28	25	23	22		
8.	Lane8	MW/..	1082	577	353	201	116	77	60	45	38	34	30	25	24	22		
9.	Lane9	MW/..	907	521	202	196	106	74	64	52	44	38	36	33	29	25	24	22
10.	Lane10	MW/..	1245	655	373	213	122	94	68	51	44	39	34	31	28	25	24	

Lane 1: marker (Vivantis. Chromatein pre stained protein ladder), Lane 2: heat treated *S.* bolombo, Lane 3: non heated *S. infantis*, Lane 4: heat treated *S. infantis*, Lane 5: non heated *S. lagos*, Lane 6: heat treated *S. lagos*, Lane 7: non heated *S. cerro*, Lane 8: heat treated *S. cerro* ,Lane 9: heat treated *S. newlands* and Lane 10: non heated *S. newlands.*

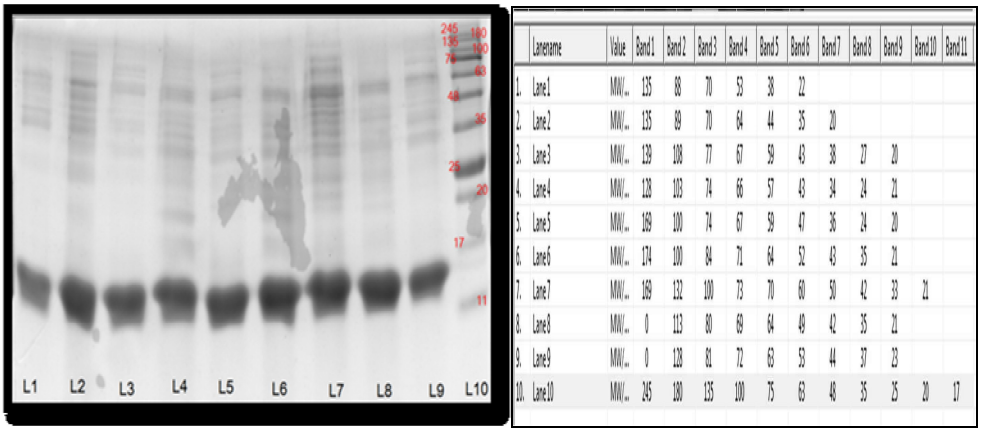
Figure.2 SDS profile analysis of heat shock protein among salmonellae isolates Gel_2



	Lanename	Value	Band1	Band2	Band3	Band4	Band5	Band6	Band7	Band8	Band9	Band10	Band11	Band12
1.	Lane1	MW/..	663	375	223	156	89	63	45	38	28	24	22	
2.	Lane2	MW/..	618	341	203	107	71	58	44	36	28	24	21	
3.	Lane3	MW/..	476	251	164	94	66	54	42	37	32	28	23	20
4.	Lane4	MW/..	465	251	133	81	60	50	40	36	27	23	20	
5.	Lane5	MW/..	403	218	135	75	50	41	35	27	23	20		
6.	Lane6	MW/..	367	189	128	68	47	40	35	27	23	20		
7.	Lane7	MW/..	375	176	111	59	45	40	34	27	22	20		
8.	Lane8	MW/..	333	172	103	61	53	43	37	30	24	22	20	
9.	Lane9	MW/..	333	189	102	66	57	48	43	35	31	24	22	20
10.	Lane10	MW/..	318	180	89	59	50	43	37	32	28	24	21	
11.	Lane11	MW/..	282	145	91	57	43	35	28	24	21	19		
12.	Lane12	MW/..	239	135	86	62	54	47	41	33	28	24	21	19
13.	Lane13	MW/..	245	180	135	100	75	63	48	35	25	20	17	
14.	Lane14	MW/..	223	156	81	58	50	42	34	27	23	21	21	20
15.	Lane15	MW/..	142	84	59	55	48	44	37	31	27	24	22	20

Gel_2. Lane 1: non heated *S.* Lagos, Lane 2: heat treated *S. typhimurium*, Lane 3: heat treated *S. sandiago*, Lane 4: non heated *S. sandiago*, Lane 5: heat treated *S. paratyphi* B, Lane 6: non heated *S. paratyphi* B, Lane 7: non heated *S. saintpaul*, Lane 8: heat treated *S.* saintpaul, Lane 9: heat treated *S. kentucky*, Lane 10: non heated , *S.* Kentucky, Lane 11: non heated , *S. infantis*, Lane 12: heat treated *S. infantis*, Lane 13: Marker (Jena Bioscience. Blue Elf pre stained protein marker), Lane 14: non heated *S.* Logos and Lane 15: non heated *S.* Bolombo.

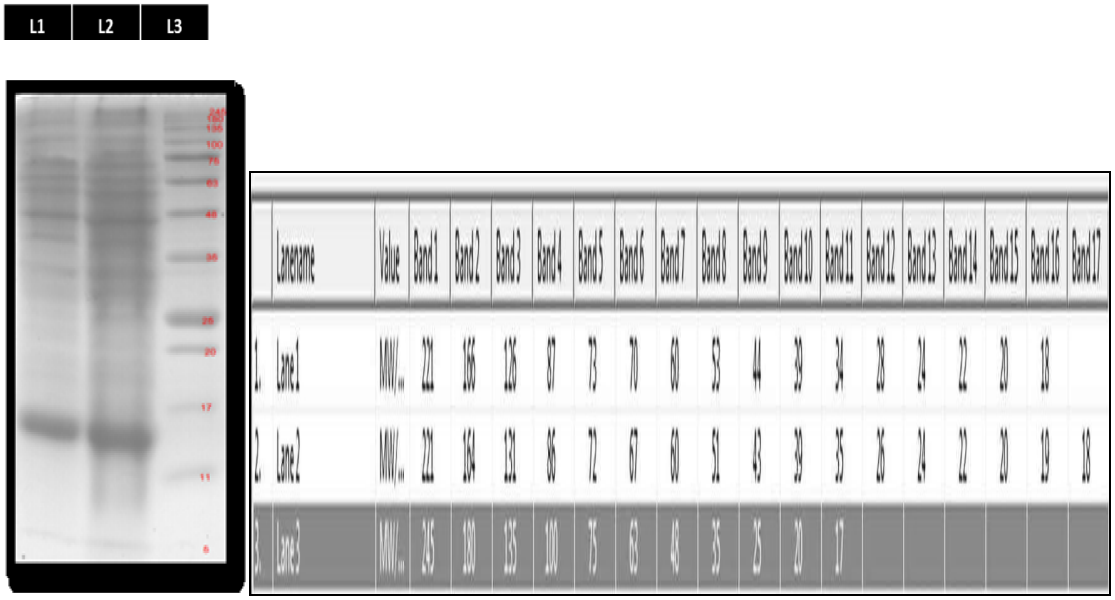
Figure.3 SDS profile analysis of heat shock protein among salmonellae isolates



	Lanename	Value	Band1	Band2	Band3	Band4	Band5	Band6	Band7	Band8	Band9	Band10	Band11
1.	Lane1	MW(...	135	88	70	53	38	22					
2.	Lane2	MW(...	135	89	70	64	44	35	20				
3.	Lane3	MW(...	128	108	77	67	59	45	38	27	20		
4.	Lane4	MW(...	128	103	74	66	57	43	34	24	21		
5.	Lane5	MW(...	126	100	74	67	59	47	36	24	20		
6.	Lane6	MW(...	174	100	84	71	64	52	43	35	21		
7.	Lane7	MW(...	169	132	100	73	70	60	50	42	33	21	
8.	Lane8	MW(...	0	113	80	69	64	49	42	35	21		
9.	Lane9	MW(...	0	128	81	72	65	53	44	37	23		
10.	Lane10	MW(...	245	180	135	100	75	63	48	35	25	20	17

Gel$_3$. Lane 1: non heated *S.* Newport, Lane 2: heat treated *S.* Newport, Lane 3: non heated *S. infantis*, Lane 4: heat treated *S. infantis*, Lane 5: non heated *S. enteritidis*, Lane 6: heat treated *S. enteritidis*, Lane 7: heat treated *S. seftenberg*, Lane 8: non heated *S. seftenberg*, Lane 9: non heated *S. typhimurium* and Lane 10: Marker (Jena Bioscience. Blue Elf pre stained protein marker).

Figure.4 SDS profile analysis of heat shock protein among salmonellae isolates



	Lanename	Value	Band1	Band2	Band3	Band4	Band5	Band6	Band7	Band8	Band9	Band10	Band11	Band12	Band13	Band14	Band15	Band16	Band17
1.	Lane1	MW(...	228	165	128	97	73	70	60	53	44	39	34	28	24	22	20	18	
2.	Lane2	MW(...	228	164	128	86	72	67	60	51	43	39	35	28	24	22	20	19	18
3.	Lane3	MW(...	245	180	135	100	75	63	48	35	25	20	17						

Lane 1: non heated *S. typhimurium*, Lane 2:heat treated *S. typhimurium* and Lane 3: Marker (Jena Bioscience. Blue Elf pre stained protein marker).

a protein band at 90-98 and 70-77 kDa respectively. Four major classes of heat shock proteins (Hsp90, Hsp70, GroE, and small heat shock proteins) are known to function as molecular chaperones, proteins that help other proteins adopt a biologically active conformation, without themselves becoming part of the final structure (Korber *et al*., 2010).

HtpG is an Hsp90 homolog usually present as a dimer involved in protein folding and interacts with substrates in their near native conformations, regulating their biological activities. Inhibiting Hsp90 function provides a possible new approach to overcome drug resistance. The HSP90 families are found predominantly in the cytoplasm and are thought to mediate the folding of specialized proteins such as steroid receptors and protein kinases (Colaco *et al.*, 2013). Hsp70 is mainly involved in supporting the early phase of protein folding (Neckers and Tatu, 2008). Ten heat heat treated isolates had a protein band at 100-130 kDa. The Hsp100 family is able to disassemble protein aggregates (Neckers and Tatu, 2008). Thermal tolerance, disaggregation, and unfolding of aggregated proteins for enzymatic digestion are handled by the larger HSP100 chaperones (Kress*et al*., 2009).

Eleven heat heat treated isolates had a band at 59-64 kDa. GroEL (HSP60) exists in a double heptameric ring structure, presenting a protected cavity within which newly synthesized proteins can fold. There are several reports that suggest a role for bacterial chaperones at the cell surface, as adhesins for invading the host cell or in signaling the immune system (Colaco *et al.*, 2013).

Fourteen and 15 heat heat treated isolates had a band at 40-44 kDa and 19-22 kDa respectively. The Hsp40 family presents substrates to the Hsp70 group of chaperones. The Hsp20 family is involved in stabilization of cytoskeletal proteins. Heat shock proteins of the classes Hsp20, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100 have been well characterized from a large number of organisms (Neckers and Tatu, 2008). The Hsp60, Hsp70, and Hsp90 families are involved in protein folding (Neckers and Tatu, 2008). The DnaK (Hsp70) and DNA J (Hsp40) pair is involved in protein folding and is also associated with survival under stress conditions (Genevaux *et al.*, 2007). DnaK results in compromised growth in macrophages or inability to colonize mice (Takaya *et al.*, 2004). These data support the notion that heat shock proteins are critical for survival of these bacteria in host environments. All the above heat shock proteins are inducible in nature and have been implicated in helping bacteria override stressful environmental conditions (Arnold *et al.*, 2007). HSPs are found throughout the cell, but different HSP families can be localized to specific cellular locations and can be divided into broad families based on size. The HSPs involved in protein folding can be separated into differing functional systems, with some overlap (Colaco *et al*., 2013).

Recommendation

The present work indicated poor sanitation of the environment under which animals are slaughtered, transported, processed and sold. Thus, Authors recommended managing meat safety programs based on an integrated effort that applies to all stages; from the producer to the consumer. Also, there is an emergency need to control the use of growth promoters and antimicrobial agents in animals to

minimize the emergence of resistant strains.

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