Asian Journal of Biological Sciences 6 (3): 168-174, 2013
ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.168.174

Correlation of Traditional Knowledge with Laboratory Based *Salmonella* Detection in Egg Shell and Contents of Market Sold Egg

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ABSTRACT

Salmonelosis is a leading food borne disease worldwide and Africa at large. Eggs were one of the sources *Salmonella* for the food. So, this study was aimed to evaluate the traditional knowledge with scientific methods for detection of *Salmonella* in egg shell and content. Randomly collected egg samples were categorized based upon traditional knowledge and S*almonella* metabolic recovery and proliferation of cells were done with primary and secondary enrichment media. From a total of 60 samples analyzed, 31 isolates of *Salmonella* were detected. Moreover, Conventional biochemical and serological test methods were used to identify the suspected *Salmonella*. The isolates were checked for their susceptibility to different antibiotics on Muller-Hinton agar in standardized inoculum and disc diffusion technique with 11 Oxoid drug discs. The result of study shows that 71% and 29% of *Salmonella* isolates were detected from egg shell and contents, respectively. All the isolates were resistant to ampicillin; Amoxicillin, Tetracycline and also 45% of the isolates were susceptible to Gentamicin and 16% for ciprofloxacin. Most *Salmonella* isolates show multi-drug resistance for at least five antibiotics. The laboratory results were supported the traditional egg spoilage identification knowledge, hence most of *Salmonella* isolates were detected from movable, floating and opaque eggs. On the other hand, the detection of high number of *Salmonella* isolate from the egg shell could be due to poor sanitation of the laid egg environment. Hence, the appropriate care and awareness should be needed before and/or after the egg laid to minimize contamination of egg by *Salmonella*.

Key words: Egg, MDR, penetration, prevalence, *Salmonella*

INTRODUCTION

Food-borne bacterial pathogen, with poultry and poultry products in connection to *Salmonella* sp. were primary source of infection to humans (Baggesen *et al*., 2000). The entry of *Salmonella* into the egg was occurring through vertical and horizontal method (Cox *et al*., 2000), that *Salmonella* come from an infected Hen and invade the egg through the shell after the egg is laid, respectively. Disease causing bacteria like, *Salmonella* and other can enter the egg shell at different stage (Aragaw *et al*., 2007). This is done directly when the egg is in the ovary and also indirectly after the egg laid. A disease due to *Salmonella* has most often been associated with consumption of contaminated foods of animal origin, such as poultry, swine, dairy products and eggs (Baggesen *et al*., 2000). In Ethiopia, traditionally people consume uncooked egg as food and medicine to remedy from respiratory disease. On the contrary, Consumption of raw egg is one of

the routes of transmission for *Salmonella* disease. So, Egg is considered an important source of foodborne disease and the illnesses were associated with the consumption of raw contaminated eggs (Aragaw *et al*., 2007).

A better understanding of the relations and appliances between *Salmonella* and poultry eggs is necessary to reduce disease caused by *Salmonella*. Bacterial contamination of the egg shell and contents were reported by different researchers (Zhang *et al*., 2011). This study was hypothesized to bridge the attitude of traditional knowledge of testing the egg safety with scientifically detection of *Salmonella* from the egg.

MATERIALS AND METHODS

Study area and period: Study duration was from August 2012 to March 2013. The total of 60 egg samples were collected from the market found in Kersa woreda (Fig. 1) Jimma town and around Kochi road. The experimental works were conducted in Biology department, Jimma University College of Natural Science in microbiology and biomedical science laboratory in main campus. Jimma town is located at 335 km from Addis Ababa at 1720 m above sea level, 1000 mm average rainfall. May to September is the main rainy season in Jimma zone. The temperature of Jimma ranges 28 to 8°C from maximum to minimum in a year (Alemu *et al*., 2011).

Bacteriological analysis: On market and street sold chicken eggs were collected from different site in Jimma town around Kochi. The Unwashed eggs were collected aseptically in sterile

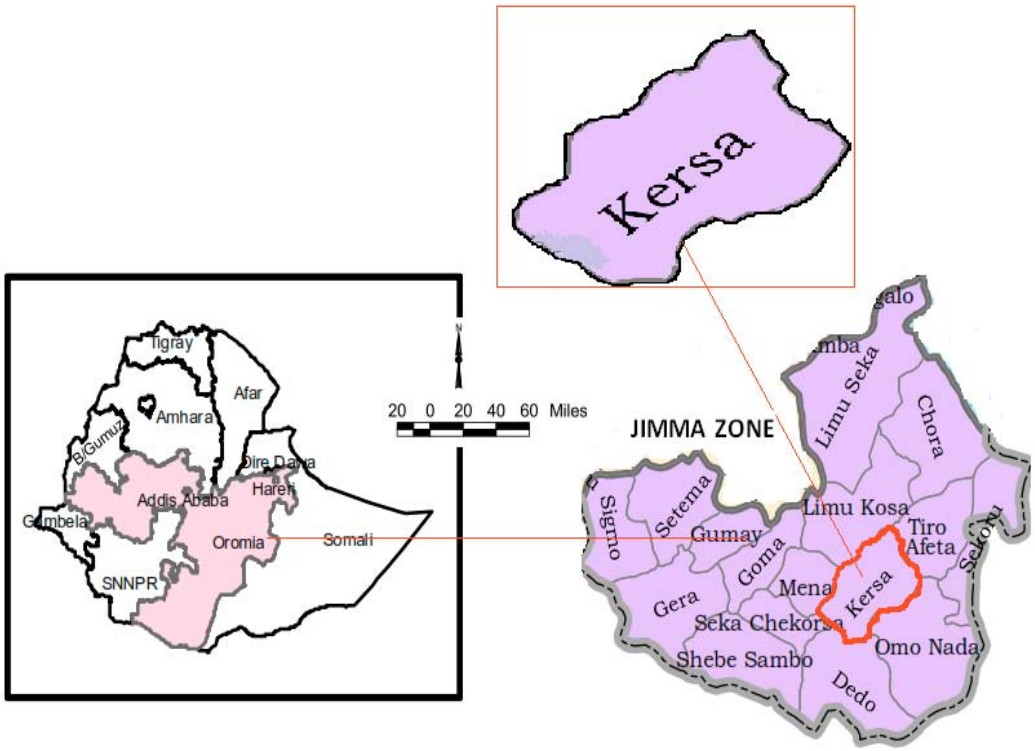

Fig. 1: Map the study area, thee figure shows that Ethiopia, Oromia and study area, Jimma town where the egg contamination survey took place for this study

polyethylene bags and transported to the microbiology laboratory, Jimma University, Department of biology. The entire surface of the eggshell were swabbed with sterile cotton swabs dipped in sterile peptone broth and then added to the primary enrichment broth (buffered peptone water) and subsequently incubated for 24 h at 37°C for bacterial proliferation. For the egg content collection, the eggs were surface sterilized with 75% alcohol for two minute, air dried in laminar flow hood for 10 min and cracked with sterile blade. Each egg's content was mixed thoroughly and 10 mL of the mixed egg content was aseptically inoculated into 90 mL of the buffered peptone water and incubated at 37°C for 20-24 h.

Bacteriological identification: From each pre-enriched egg surface and content sample, 1 mL was inoculated to 10 mL of the RappaportVassiliadis medium (RV) Bio-Rad (Marne-la-Coquette-France) incubated at 37°C for 24 h. Form the young culture a loopful of bacterial isolation were streaked on XLD media and incubated at 37°C for 24 h. The Lactose-negative colonies were kept for further studies and were examined for colonies typical of *Salmonella*. Suspect colonies were streaked on nutrient agar plates to obtain pure cultures which were subjected to oxidase testing, gram-staining and motility testing. Gram-negative short-motile rods and non-motile rods with characteristic red slope/yellow butt reaction on TSI either with the production of H_2S or not were taken presumptively as *Salmonella* (Akoachere *et al*., 2009). They were further confirmed with using serological agglutination test according to Gruenewald *et al*. (1990).

Antimicrobial susceptibility test: Antibiotic sensitivity test was conducted using antibiotic disc (Oxoid, UK) according to Kirby-Bauer antibiotic disc diffusion techniques. Briefly described, Mueller-Hinton agar was prepared in petri-dishes (Bibby Sterilin, UK). Pure colonies of the isolated organisms were emulsified in normal saline and the turbidity matched against McFarland No. 0.5 turbidity standard. The bacteria were plated on the Mueller-Hinton agar and antibiotic disc was placed centrally using the antibiotic disc dispenser (Oxoid, UK). The Petri-dish and its content were incubated for 24 h in a humidified incubator at 37°C. The organisms were observed for antibiotic sensitivity by measuring the zone of inhibition on the plate.

Standardization of inocula: The 0.5 McFarland turbidity standards was prepared by adding 0.5 mL of a 1.175% (wt/vol) barium chloride dehydrate ($BaCl_2 \bullet 2H_20$) solution to 99.5 mL of 1% (vol/vol) sulfuric acid (H_2SO_4). This mix was considered to be equivalent to cell density of about 3×10^8 cfu mL^{-1} (Andrews, 2001). The turbidity standard is then aliquoted into test tubes identical to those used to prepare the inoculum suspension. McFarland turbidity standard tubes were Seal with Parafilm, to prevent evaporation.

RESULTS AND DISCUSSION

The egg samples collected for analysis were categorized based upon the egg traditionally selected for consumption as, movable content when shacked around the ear and transparency with sunlight in-bounded with finger and floating or sinking in water (Table 1) when immersed.

From the isolated *Salmonella* 22 (71%) were from egg surface, from which 7 (31.8%) were movable content surface, 3 (13.6%) immovable content surface, 5 (22.7%) opaque surface, 2 (9.1%) transparent surface, 2 (9.1%) floating and 3 (13.6%) from sinking egg surface samples. The content has a total of 9 (29%) bacterial infection from which 3 (33.3%) from movable contents, 1 from

Table 1: Frequency of *Salmonella* detection from various grades of chicken eggs

Egg type	No. of sample examined	% of content infection	% of frequency *Salmonella* egg shell	*Salmonella* in both cont and on shell
Content movable	10	33.3	31.8	Present
Content immovable	10	11.1	13.7	Present
Opaque content	10	33.3	22.7	Present
Transparent content	10	0.0	9.1	Absent/present
Floating egg	10	11.1	9.1	Present
Sink egg	10	11.1	13.6	Present

Table 2: Biochemical analysis results for *Salmonella* isolated from the egg surface and contents, in Jimma town South western Ethiopia

Test	Positive or negative reaction	Reactions/enzymes	Results
TSI glucose (acid formation)	Positive	Acid production	Butt yellow
TSI glucose (gas formation)	Positive	Acid production	Surface yellow
TSI lactose	Negative	Gas production	No air bubbles in butt
TSI hydrogen sulfide	Positive	H_2O production	Black color
Urea splitting	Negative	Urease	Yellow
Lysine decarboxylation	Positive	Lysine decarboxylase	A purple colour
B-Galactosidase reaction-β	Negative	Galactosidase	Yellow
Voges-Proskauer reaction	Negative	Acetoin production	A pink
Indole reaction	Negative	Indole production	Pink ring

immovable content, 3 (33.3%) from opaque, 1 (11.1%) from floating and 1 (11.1%) from sinking egg contents. From *Salmonella* infected egg 70.9% were from the egg surface and 29.1% were from the egg contents.

Egg contamination with *Salmonella* or other bacteria could make the egg to decay and have different in appearance and important to identify the infected from the normal egg traditionally. *Salmonella* penetrations to the egg shell were done during the egg hatch or cross-contamination (Berrang *et al*., 1998; Cox *et al*., 2000). Movable content, opaque appearance and floating indicate the egg to be contaminated with bacteria. Report of Berrang *et al*. (1998) supported these results, in that percentage of weight loss through incubation shows the egg contamination.

Subsequently *Salmonella* species were confirmed with different biochemical tests method for the colonies resembling *Salmonella* on XLD are *Salmonella*. According to the ISO 6579, 2002 standard (ref. 1) and (ref. 2) recommends using the Urea agar, L-lysine decarboxylase, ß-galactosidase (ONPG) and Voges Proskauer and indole tests in this order and Triple Sugar Iron (TSI) agar, mannitol, urea, ornithine decarboxylase and lysine decarboxylase were the suitable methods used to confirm *Salmonella* suspect colonies with the following results (Table 2).

The presumptive *Salmonella* were positive for TSI glucose (acid formation), TSI glucose (gas formation), TSI hydrogen sulfide and Lysine decarboxylation (Table 2) and negative for, TSI lactose, Urea splitting, B-Galactosidase reaction, Voges-Proskauer reaction and Indole reaction with negative results. Procop *et al*. (2008) results supports the idea that *Salmonella* were positive for TSI for gas and acid production and negative for Urea and lactose. In addition, the *Salmonella* serological tests were used for further confirmation.

Akoachere *et al*. (2009) supplemented that, gram-negative short-motile rods and non-motile rods with characteristic red slope/yellow butt reaction on TSI either with the production of H_2S or not were taken presumptively as *Salmonella*. Subsequently, with their biochemical test the antimicrobial resistance and susceptibility of *Salmonella* were evaluated, as it was the biggest public health concern (Table 3) worldwide.

Table 3: Antimicrobial susceptibility test for salmonella isolated from retail egg shall and contents in Jimma town South western Ethiopia

	Sensitivity		Resistance	
Antibiotics	No.	%	No.	%
Amikacin	12	38.7	19	61.3
Amoxicillin	0	0.0	31	100.0
Ampicillin	0	0.0	31	100.0
Ceftriaxone	11	35.5	20	65.5
Chloramphenicol	2	6.5	29	93.5
Ciprofloxacin	5	16.1	26	83.9
Gentamicin	14	45.2	15	54.8
Kanamycin	4	2.9	27	87.1
Nalidixic acid	10	32.3	21	67.7
Sulfisoxazole	3	9.7	28	91.3
Tetracycline	0	0.0	31	100.0

Table 4: Pattern of multiple drug resistance observed in *Salmonella* isolates from the egg shell and contents in Jimma town south western Ethiopia, in 2013

No. of drug	Multidrug resistance pattern	Percentage
	Amik/Amo/Amp/Salf/Tet	45.00
5	Amo/Amp/Ceft/Chlo/Tet	38.00
	Amik/Amo/Amp/Ceft/Tet	38.00
	Amik/Amo/Amp/Chlo/Salf/Tet	35.00
6	Amo/Amp/Chlo/Kan/Nal/Tet	29.00
	Amik/Amo/Amp/Chlo/Salf/Tet	19.00
	Amo/Amp/Cepr/Nal/Salf/Tet	25.80
	Amik/Amo/Amp/Chlo/Cepr/Nal/Salf/Tet	29.00
8	Amik/Amo/Amp/Chlo/Cepr/Kan/Salf/Tet	32.30
	Amo/Amp/Chlo/Cepr/Kan/Nal/Salf/Tet	29.00
10	Amik/Amo/Amp/Ceft/Chlo/Cepr/Kan/Nal/Salf/Tet	9.70
	Amik/Amo/Amp/Ceft/Chlo/Gent/Kan/Nal/Salf/Tet	3.20

The results of the study showed that most *Salmonella* isolates were resistant to different antibiotics. The frequencies of susceptible isolate were low in number. *Salmonella* isolates were resistant to all amoxicillin, ampicillin and tetracycline (100%), Chloramphenicol and Sulfisoxazole, respectively. The *Salmonella* isolate were susceptible to gentamicin and Amikacin. In this study, sensitivity to the entire antibiotic was less than 50%. On the contrary, Prajapati *et al.* (2008) shows that the *Salmonella* isolates were sensitive to Amoxycillin, Ciprofloxacin, Chloramphenicol, Ceftriaxone, Cotrimoxazole and Cefotaxime. Asghar *et al.* (2002) results also that *Salmonella* isolates were found to be susceptible to gentamicin, cefotaxime and amikacin, while resistant to ampicillin, cefamendole, chloramphenicol, gentamicin and cefuroxime.

Almost all, *Salmonella* spp. isolated from the egg were resistance to more than five antibiotics. This indicates that *Salmonella* infection needs special care in the developing world, because of the poor hygienic conditions and their modes of consumption of uncooked egg for different reason that favour its spread. The reports from Swanenburg *et al.* (2001) strengthen the idea that the spread of drug resistance *Salmonella* in the developing world were very common. This also confirmed that *Salmonella* isolates are present in our most egg samples the study area and are seriously becoming

a public health concern due to their highest prevalence of multidrug resistance pattern observed (Table 4). Nath *et al.* (2000) also supports the idea that dissemination of multidrug resistance *Salmonella* is because of indiscriminate use of the antibiotics.

The high percentage of multi-drug resistance could be indicating for unwise usage and abuse of antibiotics for human and animal at the study area. Reports from Onyango *et al.* (2008), Nath *et al.* (2000) and Chandra *et al.* (2006) also supports that indiscriminate use of antibiotics has led to the emergence of multidrug-resistant strains. These results sound for intensive observation/examination of microbial spp. to control the high prevalence of resistant *Salmonella* strain.

CONCLUSION

Contamination of an egg can be either though vertical transmission or horizontal transmission. Traditional ways of egg spoilage determination is importantly indicator for bacterial infection of the egg. In addition, egg with movable contents, opaque egg when seen in sunlight by rounding with your finger and the floating on water surface indicate egg contamination/spoilage. Hence, the infected hens (flocks) were the major contamination source for eggs. Thus, the egg producers should have to kept the hen in good environmental hygiene, feed and water safety should be ensured and implement effective management strategies to guard hens against *Salmonella* infection. Furthermore, consumption of uncooked egg critically aggravates *Salmonella* dissemination in the community. So, good manufacturing and handling practices were needed to diminish the potential risk of salmonellosis that results from consumption of egg and egg products. Egg handlers also have to make a proper care in light of their practical values coupled with human health concern. After the eggs are laid, it could also be stored at low-temperature storage to reduce the microbial multiplication. Most importantly, eggs have to be well cooked for enough time in case *Salmonella* recover from washing and physical damage.

ACKNOWLEDGMENT

Authors thank to Department of Biology, Jimma university, Natural Science College for his support in facility and material to accomplish this work successfully.

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