Research Article

Salmonella Status of Eggs on the Market in Southwestern Nigeria

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ABSTRACT

The increasing prevalence of *Salmonella enteritidis* and the corresponding rise in human health risks necessitate the need to know the status of Salmonella in eggs sold in a major market: Bodija in Southwestern Nigeria.

A total of 360 eggs were randomly bought from Bodija market. Six egg shells were pooled together to form 60 egg composites and six egg content were pooled together to form another 60 egg composites making a total of 120 egg composites. Biochemical screening, gram staining, serological identification, antimicrobial sensitivity test, coagulase, oxidase test were done in bacterial egg culture to identify *Salmonella enteritidis*.

In all composites, 4 (3.33%) were suspected to be positive to Salmonella. Only 2 of the 4 isolates were subjected to serology and proved positive after testing with Poly "O" antiserum and Salmonella antisera "D", so that the final proportion of egg composite sample isolated to be Salmonella was (1.66%). The strain 5v8 was very sensitive to Nitrofurantion with mean inhibition zone of (9.5mm) and totally resistant to Nalidixic acid while 3s2 was very sensitive to Nalidixic acid with mean inhibition of (12.5mm). 3s2 was resistant to Cotrimoxazole and Nitrofurantion.

The two isolated strains and their replicates showed antibiotic sensitivity with a marked variation suggesting different strains of *Salmonella enteritidis* or genetic drift.

Resistance of the two isolated strains of *Salmonella enteritidis* to antibiotics is of great concern to public health in treatment of infections.

Keywords: commercial eggs, health risks, salmonella, strains, antimicrobial agent.

INTRODUCTION

Salmonellosis includes a large group of bacterial diseases caused by different species of the micro- organisms belonging to the genus Salmonella and the family Enterbacteriacea (Javeed and Hameed, 1989). Salmonellosis is one of the most frequently reported foodborne diseases worldwide (FAO/WHO, 2002). A wide range of foods has been implicated in such disease. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis (FAO/WHO 2002).

Although primarily an intestinal bacteria, salmonellae are widespread in the environment and commonly found in farm effluents, human sewage and in any material subject to faecal contamination (Todar, 2005). Salmonella organisms are etiological agents of diarrhoea and systemic infections in humans, most commonly as secondary contaminants of food originating from the environment, or as a consequence of septicaemia in food animals. Human salmonellosis is the most common and important zoonotic disease caused by Salmonella organisms. These organisms are also found in feedstuffs, causing infectious disease in animals, particularly poultry and pig (OIE, 2004).

A team of scientists developed a quantitative model to characterize the risk associated with the consumption of eggs contaminated internally with *S.enteritidis*. However undertaking a Microbiological Risk Assessment particularly a quantitative Microbiological Risk Assessment is a resource intensive task requiring a multidisciplinary

approach and it is not currently within the capacity of many countries (Cahill, 2002).

Egg is an important source of animal protein to many people globally (FAO, 2001). It was reported that egg production and consumption is fast gaining ground in Nigeria (Adesola, 2005). However, eggs have been consistently implicated in human salmonellosis (FAO/WHO, 2002). In addition, the increasing prevalence of *Salmonella enteritidis* and the corresponding rise in human health risks necessitate the need to know the status of Salmonella in eggs. Salmonella often shows multiple drug resistance, but the predominant patterns vary among isolates from different countries, serotypes, antibiotics and from different animal species. This multiple drug resistance has been attributed to the uncontrolled use of anti microbial agents as growth promoters or in the treatment of bacterial infection with the farmers having unlimited access to these agents (Adesiyun 2005).

Information on research findings to determine the extent of the role of poultry shell eggs as reservoir for salmonella spp in Nigeria is scarce. This study therefore assessed the prevalence Salmonella enteritidis in composites egg samples to determine the level of public risk.

METHOD

A total of 360 eggs were randomly bought from major wholesale and retail egg sellers at Bodija market, Ibadan. Thirty six (36) eggs were bought from each of the 10 randomly selected wholesalers and retailers and were taken to the laboratory immediately for analysis.

EGG SAMPLE PREPARATION

The exact point of the egg shell surface to be cracked was aseptically cleaned using methanol and tissue paper and then aseptically cracked open and poured into sterile jam jars. The egg shells were collected separately in polythene bags. In order to reduce laboratory expenses and have a sensitive detection, eggs were pooled because egg contamination occurs very infrequently (Ebel and Scholosser, 2000). Six egg shells were pooled together to form a composite, there were sixty composites for egg shell. Six egg content (yolk and albumin) were pooled together to form a composite sample; there were sixty composites for egg content. In all, there were total of 120 egg sample composites from both the egg content and shell. The pools were incubated for one day to allow bacterial multiplication to proceed sufficiently (Gast, 1993a). Each pooled sample was mixed and between each pool the glass rod used for mixing the sample was sterilized.

PREPARATION OF EGG CULTURES AND MEDIA

The isolation and identification of Salmonella from eggs was performed following a similar principle as described in the (Proposed Official Method MFO-21, 2002)

Egg pools were incubated for 48hrs at 37°C. One ml of each pool was diluted in 10mls of sterile broth also 20mls of this broth was dispended into each of the polythene bags containing 6 egg shells and incubated at 37°C for 24hrs.

The following media were made according to revered standard, selenite broth, desoxycholate citrate agar, macconkey agar (without salt), triple sugar iron slants (tsi), urea agar base slants, plate count agar.

BIOCHEMICAL SCREENING

Biochemical screening involved testing isolates using determinant biochemical reactions. The pure isolates from MacConkey agar which were incubated in triple sugar iron slants and urea slants for 24hr and 48hrs respectively at 37˚C. **S**amples that showed no growth on DCA were not subjected to any further microbiological test. Samples that showed colourless to grayish growth were further culture on MacConkey. Some of the samples that showed growth on DCA when cultured on MacConkey agar appear as yeast therefore, only those samples that showed off pink, colourless and grayish colonies on MacConkey agar were further subjected to oxidase, catalase and coagulase test.

GRAM STAINING

Gram stain was also carried out so as to confirm salmonella organism. Smears containing thin and thick areas from the suspects plates were prepared on clean glass slides, air dried and fixed by quickly passing the slide three times through flame. The smear was stained with crystal violet for 1 minute and then washed with tap water for 30seconds. It was then flooded with iodine solution for 1min and washed in tap water again then carefully blot dried to remove excess water 0.95% ethyl alcohol was used to decolourize for 30 seconds then the smear was counter stained with safranin solution for 10 seconds. The smear was finally rinsed in tap water and carefully blot dried. Oil immersion objective was used to view the slides for red short rods.

SEROLOGICAL IDENTIFICATION

Salmonella poly "O" antiserum and Salmonella antiserum group "D" (Difco) were used to support the identification of isolates as members of Salmonella spp. A drop of each of the antisera was mixed with the isolated colonies from freshly made DCA plates on two separate spots on a clean slide. Isolated colonies taken were also mixed with the distilled water this serves as control. The mixtures were rocked gently and strong agglutination within 1 or 2 minutes is a positive reaction while non agglutination is taken as negative.

ANTIMICROBIAL SENSITIVITY TEST

The culture of selenite broth was poured into Petri dishes containing plate count agar in such a way that the broth covered the agar plate the excess broth culture was decanted. The surface of the agar was allowed to dry, and then standard antibiotic sensitivity disc impregnated with known agents and strength were firmly placed on the surface of each of the agar plates and incubated at 37˚C for 24hrs**.** Antibiotic sensitivity test was carried out on the two isolated strains (5v8 and 3s2).

Characterization of stains as sensitive or resistant was based on the size of the zone of inhibition around the disc. Antimicrobial susceptibility tests were performed using the Kirby Bauer Disc Diffusion method (Bauer et al. (1966) consisting of multidisc (Abtek) of Ampicillin (25µg), Cotrimazole (25µg), Gentamycin (10µg), Nalidixic acid (30µg) Nitrofurantion (200 µg), Colistin (25µg), Streptomycin (25µg), Tetracycline (25µs). Results of the zones of inhibition were interpreted in mm by measuring inhibition zones using a millimeternscale rule to measure the diameter of zones of inhibition.

RESULTS

In all 120 egg composite sampled, 4 (3.33%) were suspected to be positive to Salmonella. The final proportion of egg composite sample isolated to be Salmonella was (1.66%).

Table 1 showed the summary of the egg composite samples that are suspected to be positive to salmonella after undergoing the microbiological test as stated in the above tables. In Plate 2 the blackening of the agar indicates the presence of gas (H_2S), while table 9 showed the proportion(1.66%) of egg samples from both the egg shell and egg content(yolk and albumen) were positive to Salmonella from a total (120) egg composite sample.

Table 2: Out of the 120 egg composite samples tested only 1.66% were positive to salmonella with equal proportion from the egg content and shell, these means no difference whatsoever in the route of infection.

Microbiological test summary for recovered positive strains

Table 3: The recovered strain (5v8 and 3s2) were further subjected to serology, gram staining and oxidase test. Table 3 shows the summary of all the microbiological tests that were carried out on the two recovered strains.

ANTIBIOTIC SENSITIVITY TEST

The strain 5v8 was observed to be most sensitive to Nitrofurantion with a mean inhibition zone of (9.5mm) and least sensitive to Tetracycline with mean inhibition zone of (0.5mm) and totally resistant to Nalidixic acid while 3s2 was most sensitive to Nalidixic acid with mean inhibition of (12.5mm) and least sensitive to Ampicillin with mean inhibition zone of (1mm). 3s2 was resistant to Cotrimoxazole and Nitrofurantion. When the two strains were replicated and tested with the sensitivity disc, there was different resistance and sensitivity rate to the drugs (Table 3, 4).

DISCUSSION

The isolation of *Salmonella enteritidis* in egg samples in this study is an egg borne pathogen causing human salmonellosis, a zoonosis.

The two isolated strains showed similar antibiotic sensitivity profile with those of their replicates, however between them there was a marked variation in the profile and this might indicate variation within the same strains of *Salmonella enteritidis* or might be due to genetic drift. The strains were however fully sensitive to most of the drugs. One of the two strain (5v8) was most sensitive (in descending order) to Nitrofurantoin, Cotrimoxazole, Streptomycin, Ampicillin, Gentamicin Colistin and Tetracycline while it was resistant to Nalidixic acid. The other strain (3s2) showed sensitivity to Nalidixic acid, Tetracycline Streptomycin, Colistine, Gentamicin and Ampicilline while it was resistant to Cotrimoxazole.and Nitrofurantoin.

In conclusion, two strains of Salmonella enteritidis were isolated from the commercial eggs and they possessed different pattern of antibiotic sensitivity and resistance. The first was very sensitive to nitrofurantoin and had resistance to nalidixic acid while the second strain was very sensitive to nalidixic acid and had resistance to both cotrimoxazole and nitrofurantoin. The prevalence of Salmonella in eggs sold in the market is significant and the

resistance of harvested strains to the antibiotic sensitivity test is of great significance.
Public awareness through special program focus on hygienically display of commercial food products like egg in market places is highly recommended to Ministry of Health and related agencies in the fight against drug resistance.

Table 1: Total number of suspected egg sample composites from Batches 1 and 2

Total number of egg sample composite suspected to be positive	Desoxycholate citrate agar (DCA)	MacConkey agar	Triple sugar agar	Urea agar
4v2	Colourless and pinkish colonies	Colourless to grayish colonies	Red/Yellow+blackening of agar	Orange!
3s2	Colourless colonies	Colourless to grayish colonies	Red + darkening of agar	Pinkish!
5v8	Pinkish colonies+ creamish colony on one side	Creamish /light grayish colonies	Red	Orange!
6s9	Creamish colonies	Creamish to grayish colonies	Yellow	Orange/yellow!

Table 2: Proportion of egg samples positive to Salmonella

Total number positive to Salmonella		% positive to Salmonella	Total number negative to Salmonella	% negative to Salmonella	Total number of egg sample composite
Pooled egg(content)	1	0.83	59	49.17	60
Pooled egg(shell)	1	0.83	59	49.17	60
Total	2	1.66	118	98.34	120

Table 3: Summary of all microbiological tests carried out on recovered strains

Media or Test		Description		Strains	
		Standard colour	Standard interpretation	5v8	3s2
Media	Desoxycholate citrate agar	Clear colonies to grayish	+ve	+ve	+ve
	MacConkey agar	Colourless to grayish colonies	+ve	+ve	+ve
Screening media Serological identification	TSI	Red slant/yellow butt (+-) H2S	+ve	Red	Yellow+ darkening of agar
	Urea	Orange-red	-ve	-ve	-ve
	Poly "O"	Agglutination	+ve	+ve	+ve
	Antiserum "D"	Agglutination	+ve	+ve	+ve
Test	Gram stain	Red rods	-ve	-ve	-ve
	Oxidase test	No colour change	-ve	-ve	-ve
	Catalase test	No effervescence	-ve	-ve	-ve
	Coagulase test	No agglutination	-ve	-ve	-ve

Table 4: 11 Antibiotic sensitivity test after 24hrs showing zones of inhibition (in mm)

Positive egg composite samples with replicates	TET	AMP	COT	GEN	NAL	NIT	COL	STR
5V8	-	1	8	3	-	10	1	5
5V8(replicate)	1	7	8	3	-	9	4	5
3s2	4	2	-	4	13	13	5	9
3s2(replicate)	12	-	-	5	12	-	6	7

(-) means zero reading AMP-Ampicillin (25µg), COT-Cotrimazole (25µg), GEN-Gentamycin (10µg), NAL-Nalidixic acid (30µg) NIT-Nitrofurantion (200µg), COL-Colistin (25µg), STR-Streptomycin (25µg), TET- Tetracycline (25µs)

Table 5: 12 Mean values in (mm) of zones of inhibition to the antibiotic sensitivity disc

Positive egg composite samples with replicates	TET	AMP	COT	GEN	NAL	NIT	COL	STR
5v8	0.5	4	8	3	0	9.5	2.5	5
3s2	8	1	0	4.5	12.5	0	5.5	7

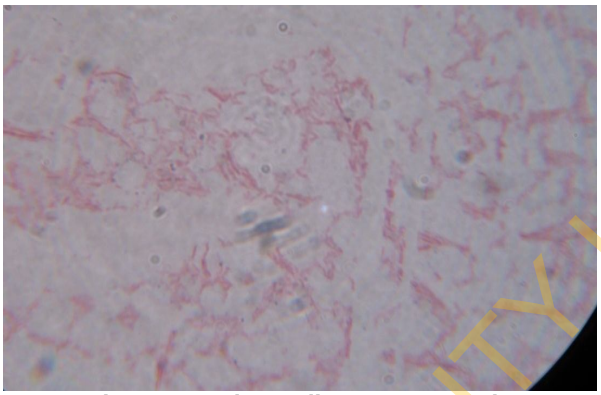

Plate 1: 5 Salmonella on Gram Stain

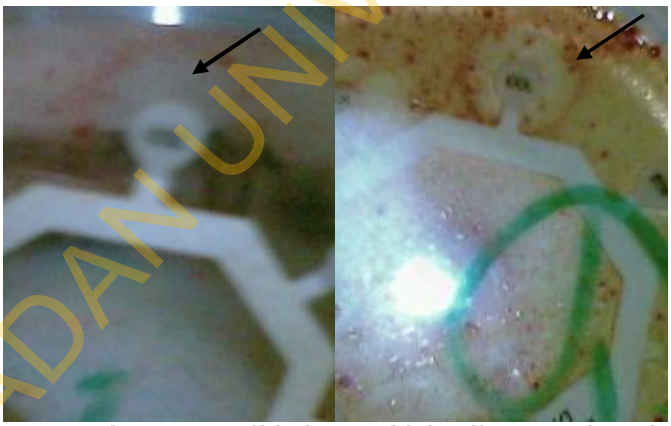

Plate 2: 6 Antibiotic sensitivity disc on cultured salmonella on plate count agar plate

Arrow ( ) indicates zone of inhibition to antibiotic sensitivity disc

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