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Genetic virulence of biofilm-forming *Salmonella* recovered from chicken sausages and nuggets

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ABSTRACT

Aims: *Salmonella* is one of the most common foodborne illnesses worldwide. Poultry meat and products are the main sources of human infection. Therefore, the main objective of the current study was to assess the genetic virulence of biofilm-forming *Salmonella* isolated from chicken sausage and nuggets.
Methodology and results: Isolation of *Salmonella* was carried out using XLD agar; suspected colonies were identified biochemically and then serotyped using the Kauffman-White scheme for detection of somatic (O) and flagellar (H) antigens. Congo red (CR) medium was used for the assessment of biofilm formation of the isolated strains. The invasion gene (*inv*A), the heat-labile *Salmonella* enterotoxin gene (*stn*), plasmid-encoded fimbriae (*pef*A) genes, the protein effectors *sop*B, *sop*D and biofilm genes in six *Salmonella* isolates were investigated using mPCR, following QIAamp® DNA Mini Kit instructions and 1.5% agarose gel electrophoreses. *Salmonella* was detected in 12%, 8% and 4% of the examined frozen packaged raw chicken sausage, frozen packaged raw chicken nuggets and ready-to-eat sausage. The isolated strains were *S*. Typhimurium, *S*. Enteritidis, *S*. Essen and *S.* Montevideo. Moreover, mPCR indicated the presence of biofilm gene *(csg*D gene), *stn*, *sop*B and *sop*D virulence genes in all isolated strains (100%); however, *pef*A gene failed to be detected.
Conclusion, significance and impact of study: The current findings showed that every *Salmonella* isolate examined was capable of creating biofilm at room temperature. As a result, these isolates are more likely to persist on abiotic surfaces, which raises the danger of cross-contamination and foodborne outbreaks.

Keywords: Salmonella, chicken sausage, nuggets, virulence genes

INTRODUCTION

Sausage and nuggets are among the most popular food products worldwide. Being quick and easily prepared meals at a relatively low price, sausage and nuggets are popular chicken meat products solving the problem of the shortage of fresh meat. Despite their high popularity, they are of serious concern to public health authorities, owing to the associated risks of bacterial food poisoning, especially *Salmonella* and *Escherichia coli* (Beli *et al.*, 2001).

Salmonella ranks number one worldwide among the potentially foodborne bacteria in terms of importance as a foodborne pathogen. Furthermore, it is considered number one in the most reported deaths and hospitalizations yearly in the United States due to bacterial foodborne illness (CDC, 2015). They could be found in many types of contaminated foods, including meat products and ready-to-eat foods. Contamination of these products generally occurs during their processing from fecal contaminants, internal contaminants such as contaminated tissues and environmental contamination of raw foods (Cox *et al.*, 2013).

There are more than 2600 serotypes of *S. enterica* based on the Kauffmann-White scheme for subtyping and strain differentiation. The most prevalent serotypes that cause foodborne illnesses are *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis (Lee *et al.*, 2015).

Virulence genes and the secreted virulence effectors are known to play a significant role in the pathogenesis of *Salmonella* and its interaction with the host cells. They produce toxins that are important to human health because it is a significant cause of food poisoning (Haraga *et al.*, 2008). *S. enterica* produces more than 40 types of virulence agents. The *inv*A gene enables

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Salmonella to invade epithelial cells. The *pef*A gene, *Salmonella* encoded fimbriae aids in adhesion to the host cell (Murugkar *et al*., 2003; Nayak *et al*., 2004). Moreover, *stn* gene is responsible for enterotoxin production, which is the main cause of diarrhea, while *sop* genes are mainly related to virulence (Huehn *et al.*, 2010).

Salmonella spp. has great adapting ability to adverse conditions than other pathogenic bacteria due to developing biofilms. Biofilm formation is controlled by both genetic factors and environmental signals (Davey *et al.*, 2003; Sereno *et al.*, 2017).

In *Salmonella enterica* serovars, the co-expression of thin aggregative fimbriae and cellulose leads to an aggregative colony phenotype (red, dry and rough [rdar]) when grown on a medium containing the dye Congo red (Zogaj *et al*., 2001; Solano *et al*., 2002).

Salmonella contaminating chicken and chicken products is widely investigated in many countries of the world, but its prevalence varies (Tibaijuka *et al*., 2003; Soomro *et al.*, 2010). Egypt is a developing country that lacks routine *Salmonella* surveillance (Barbour *et al*., 2015). So, this study was designed to determine the *Salmonella* contamination rate in some marketed sausage and nuggets in Egypt using bacteriological and molecular techniques and also to detect the types, virulence and biofilm-forming ability of the isolates.

MATERIALS AND METHODS

Collection of samples

A total of 100 randomly frozen and ready-to-eat chicken meat products (sausage and nuggets) were aseptically collected from 10 supermarkets for frozen samples and 10 food restaurants for ready-to-eat samples in Assuit city in the period from October 2019 to June 2020. Samples were classified into 50 frozen and 50 ready-to-eat sausages and nuggets sandwiches (25 each). Collection was done every 15 days, 10 samples were collected in every single visit (5 frozen and 5 ready-to-eat sandwiches). Each sample was wrapped in a sterile polyethylene bag and transported in its package in an insulated icebox at 4 ± 1 °C. All samples were examined promptly upon their arrival at the laboratory for detection and identification of *Salmonella*.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was made according to the technique recommended by ISO (2002). Briefly, 25 g of each sample were aseptically homogenized using lab blender (400; Seward Medical Ltd., London, UK) with 225 mL of buffered peptone water (BPW, HiMedia, M14941) and incubated at 37 °C for 18 ± 2 h. One-hundred µL of the pre-inoculated BPW was transferred to 10 mL of Rappaport Vassiliadis soyabean meal broth (RVSM) (Himedia, India) and incubated at 42 °C for 24 h. A loopful from the incubated broth was streaked onto the surface of XLD (HiMedia, M031) agar and incubated at 37 °C for 24 ± 3 h, characteristic colonies (Black center - Transparent zone of radish color colonies) from XLD agar were transferred into nutrient agar slant and incubated at 37 °C for 24 h for further identification.

Suspected colonies of *Salmonella* were morphologically and biochemically identified by Gram staining and biochemical characteristics. Triple sugar iron agar (BD Difco-226540), lysine iron agar (BD Difco-284920), urea agar (BD BBL-221096) and Simmons citrate agar (Neogen-7156) were inoculated and incubated at 37 °C for 24 h.

Serotyping of *Salmonella*

Serological identification of Salmonellae was carried out according to Kauffman-White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Identification of somatic (O) antigen "Slide agglutination test"

In 0.5 mL of saline solution, a dense suspension of the organism was prepared. Two circles (1 cm each) were marked on a microscopic slide; in one of the marked circles, one drop of *Salmonella* Polyvalent "O" antiserum (positive control) was added. Then one drop of bacterial suspension (0.05 mL) was transferred into each of the circles and mixed thoroughly for 1-2 min. The positive reaction was adopted by rapid and complete agglutination.

Identification of flagellar (H) antigen "Tube agglutination test"

A loopful of Polyvalent H antiserum was added to one drop of the bacterial suspension in a small agglutinating tube and mixed gently by a sterile loop. The agglutination tube was gently agitated for one minute and observed for agglutination under normal lighting conditions**.**

Detection of biofilm formation

The biofilm-forming ability of S*almonella* was determined by Congo red agar (CRA) method (Nachammai *et al*., 2016). CRA plates were inoculated with test organisms and incubated aerobically at 37 °C for 24 h. Black colonies with a dry crystalline consistency indicated biofilm production.

Detection of some virulence factors of *Salmonella* using polymerase chain reaction (PCR)

The isolated *Salmonella* strains were sent to the Reference Laboratory for veterinary quality control of poultry production in Animal Health Research Institute, Dokki, Giza, Egypt, for detection of the invasion gene (*inv*A); the heat-labile *Salmonella* enterotoxin gene (*stn*); plasmid-encoded fimbriae (*pef*A) genes, the protein

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Table 1: Primer set sequences.

Gene			Primer sequence (5'-3') [Metabion, Germany]	Amplicon size (bp)	Reference
Salmonella spp.	*Id.*	*Inv*A	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	Oliveira *et al.* (2003)
	Virulence genes	*sop*D	ACCATGCGCTGGAAGTGTTA GCGGGACGCATCATCTCATA	430	Designed
		stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	Murugkar *et al.* (2003)
		*pef*A	TGT TTC CGG GCT TGT GCT CAG GGC ATT TGC TGA TTC TTC C	700	Murugkar *et al.* (2003)
		*sop*B	TCA GAA GRC GTC TAA CCA CTC TAC CGT CCT CAT GCA CAC TC	517	Huehn *et al.* (2010)
	BF	*csg*D	ACCATGCGCTGGAAGTGTTA GCGGGACGCATCATCTCATA	651	Bhowmick *et al.* (2011)

Id.: Identification gene; *BF*: Biofilm gene.

effectors *sop*B, *sop*D and biofilm gene in six *Salmonella* isolates using mPCR.

DNA extraction

DNAs were extracted according to the QIAamp DNA Mini Isolation Kit. Two hundred µL of the sample suspension and 200 µL of lysis buffer were incubated with 20 µL QIAGEN protease at 56 °C for 10 min. After incubation, 200 µL of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µL of elution buffer provided in the kit.

Oligonucleotide primer

Primers used were supplied from Metabion (Germany); target genes and oligonucleotide primers sequences used for PCRs are listed in Table 1.

PCR amplification

Primers were utilized in a 25 µL reaction containing 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µL of each primer of 20 pmol concentration, 4.5 µL of water and 6 µL of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Positive and negative controls were included in every PCR reaction.

Analysis of the PCR products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1× TBE buffer at room temperature using gradients of 5 V/cm. Twenty µL of each PCR product sample, negative control and positive control were loaded into the gel. A DNA ladder of 100-1,500 bp size (RTU, Cat. No. DM001-R500, 11 bands) was used as DNA molecular weight marker. The ladder was mixed gently by up and down pipetting, then 5 µL were directly loaded to the gel. Then, the gel was electrophoresed at 50 V. The run was stopped after about 30 min and the gel was transferred to a UV cabinet. The data was analyzed through computer software**.**

RESULTS AND DISCUSSION

Prevalence of *Salmonella* in sausage and nuggets

Despite the exerted food hygiene efforts to eliminate agents responsible for foodborne illness, *Salmonella* remains a great worldwide public health danger, particularly in developing countries. This might be attributed to relevant implications (enteric diseases, mortality and morbidity) and meat and meat products play an important role, as a reservoir, in *Salmonella* dissemination (Normanno *et al.*, 2007; Gunasegaran *et al.*, 2011).

The prevalence of *Salmonella* in sausage and nuggets samples (frozen packaged raw chicken sausage, frozen nuggets, ready-to-eat sausage sandwiches and ready-to-eat nuggets sandwiches) collected and examined from October 2019 to June 2020 was illustrated in Table 2. The overall prevalence of *Salmonella* contamination rate was 6% (6/100), in which each product was implicated with at least one type of *Salmonella* serovars. Twelve percent (12%) of the frozen packaged raw chicken sausage samples (3/25) and 8% (2/25) of the frozen packaged raw chicken nuggets were contaminated with *Salmonella* spp., while 4% (1/25) of the ready-to-eat sausage samples were contaminated. No *Salmonella* (0/25) was detected in the ready-to-eat nuggets. The results obtained in the frozen packaged raw chicken nuggets correspond with the results of Eglezos *et al*. (2008) in Queensland, Australia and Samaha *et al*. (2012) in Alexandria, Egypt, who recorded similar results of 8% and 8.7%, respectively. Likewise, the results recorded in the ready-to-eat nuggets samples agree with Amin and Abd El-Rahman (2015). However, lower rates were obtained by Abd El-Tawab *et al*. (2015) who recorded *Salmonella* in 2.5% of sausage samples in Gharbeia governorate, Egypt. On the other hand, higher results were recorded in Rio De Janeiro, Brazil by Costa Lima *et al*. (2011) who

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Table 2: Incidence of *Salmonella* in the examined frozen and ready-to-eat- sausage and nuggets.

	No. of examined samples	Positive samples	
		No.	%
Frozen packaged Sausage	25	3	12
Frozen packaged Nuggets	25	2	8
Ready-to-eat Sausage	25	1	4
Ready-to-eat Nuggets	25	0	0
Total	100	6	6

Table 3: Serotypes of *Salmonella* isolated from frozen and ready-to-eat sausage and nuggets.

Serotype	Sausage				Nuggets				Total	
	Frozen packaged		Ready-to- eat		Frozen packaged		Ready-to-eat			
	No	%	No	%	No	%	No	%	No	%
S. Typhimurium	1	4	1	4	-	-	-	-	2	8
S. Enteritidis	1	4	-	-	1	4	-	-	2	8
S. Montevideo	-	-	-	-	1	4	-	-	1	4
S. Essen	1	4	-	-	-	-	-	-	1	4
Total	3	12	1	4	2	8	0	0	6	6

found that 53% of sausage samples were contaminated with *Salmonella* spp. Differences in the prevalence of *Salmonella* spp. could be attributed to the number of samples, method of sampling, experimental methodology, geographical area and climate differences in the areas where the samples were collected.

The Salmonellae serovars isolated from frozen packaged raw chicken sausage were *S.* Typhimurium (4%), *S.* Enteritidis (4%) and *S.* Essen (4%), while the serovars detected in the frozen packaged raw chicken nuggets were *S.* Enteritidis (4%) and *S.* Montevideo (4%). *Salmonella* Typhimurium was the only recorded serovar (4%) in the ready-to-eat sausage (Table 3). These results may be attributed to cross-contamination from multiple sources and poor hygienic measures during meat cutting and handling.

Different levels of *Salmonella* in sausage and nuggets were obtained by many investigators in different localities in Egypt. Ammar *et al*. (2009) isolated *S.* Enteritidis for the first time in chickens and other sources in Dakhlia governorate, Egypt. Rabie *et al.* (2012) obtained a *Salmonella* contamination rate of 4% in raw frozen chicken meat in Toukh, Egypt. The most encountered serovars were *S*. Enteritidis and *S.* Typhimurium. Furthermore, Amin and Abd El-Rahman (2015) found that the incidence of *S*. Typhimurium, *S*. Kentucky and *S.* Enteritidis in raw retail chicken meat samples were 2.5%, 0.5% and 0.5%, respectively. The authors failed to isolate *Salmonella* from ready-to-eat chicken meat products.

The encountered *S.* Enteritidis and *S.* Typhimurium in the examined chicken products are alarming to public health. In this respect, Crump *et al.* (2011) pointed out that the most common *Salmonella* spp. causing human disease in the United States are *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, *S.* Javiana and *S.* Heidelberg. The relatively higher prevalence of *Salmonella* in frozen packaged products in the current study may be attributed to the imperfect hygienic measures and bad quality of the meat used in sausage and nuggets processing. Moreover, *Salmonella* can grow over a wide temperature range (7-48 °C), but growth is slow below 10 °C; nevertheless, it is able to survive for extended periods in chilled and frozen foods representing a hazard in low-temperature preserved meat (Lawely, 2013). On the other hand, the low *Salmonella* prevalence among ready-to-eat products might be ascribed mainly to the exposure to a cooking temperature sufficient to kill the bacteria. Most *Salmonella* strains are usually killed by temperatures >50 °C, with the death rate increasing as the temperature increases (Doyle and Mazzotta, 2000; Mattick *et al*., 2002). Moreover, adding acidic sauces (ketchup and mayonnaise) and spices to the ready-to-eat sausage and nuggets sandwiches provides unfavorable conditions for *Salmonella.* Furthermore, commonly used spices, herbs and smoke have antimicrobial effects. These factors can prevent the growth of common foodborne pathogens such as *Salmonella* (Työppönen *et al.*, 2003).

Based on the prevalence of the bacteria in retail-level poultry, *Salmonella* prevalence in different areas could be separated into two subjective groups (Bohaychuk *et al*., 2006). One of them was the ‘‘high’’ group, isolating *Salmonella* at the levels of 30 to 60% (Canada, Spain, Belgium, Maryland as a state of U.S.A. and Portugal) and the second ’low’ group, isolating *Salmonella* at the levels of 4.2 to 11% (Washington, D.C.; Wales; Italy; and Northern Ireland). In this survey, the prevalence of *Salmonella* in raw chicken sausage and nuggets fall within the low grouping.

Virulence of the obtained *Salmonella* isolates

Analysis of virulence genes showed that the prevalence of *inv*A, *stn*, *sop*B and *sop*D was 100% (n=6), while the *pef*A gene was not detected.

Salmonella virulence varies not only among different species but also among strains of the same species; therefore, the present study was directed mainly to the genotypic detection of *Salmonella* and its virulence genes

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Table 4: The identified virulence genes in *Salmonella* serotypes in the examined frozen and ready-to-eat sausage and nuggets.

Salmonella	*inv*A	*csg*D (Biofilm gene)	*sop*D	*stn*	*Pef*A	*sop*B
S. Essen	+	+	+	+	-	+
S. Enteritidis	+	+	+	+	-	+
S. Typhimurium	+	+	+	+	-	+
S. Montevideo	+	+	+	+	-	+
S. Enteritidis	+	+	+	+	-	+
S. Typhimurium	+	+	+	+	-	+
Total %	100%	100%	100%	100%	0%	100%

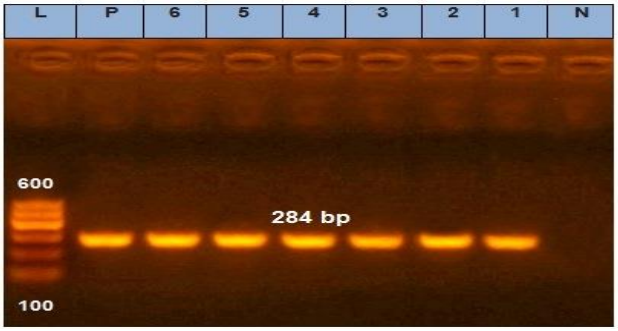

Figure 1: Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of identification gene *inv*A gene (284 bp) in isolated *Salmonella.* Lane L: 100-600 bp ladder as molecular size DNA marker; Lane P: Positive control *Salmonella* for *inv*A gene; Lane N: Negative control *Salmonella* for *inv*A gene; Lanes 1 to 6: Positive *Salmonella* for *inv*A gene.

that constitute the main factors in Salmonella virulence. The *Salmonella* invasion gene (*inv*A) is a unique gene for *Salmonella* species amplified at 284 bp (Jamshidi *et al*., 2009), which encodes a protein in the inner membrane of bacteria, it is necessary for invasion of the intestinal mucosa of the host and a common unique marker gene in all strains of *Salmonella* spp. (Liu *et al.*, 2012; Singh *et al*., 2013).

In this study, the *Salmonella inv*A gene was detected in all *Salmonella* isolates (100%) (Table 4, Figure 1). These results agreed with Malorny *et al*. (2003), Diarra *et al.* (2014), who reported this gene in 97.9% of their isolated *Salmonella enterica* serovars, Amin and Abd El-Rahman (2015) and Abd El-Tawab *et al*. (2017).

Focusing on *Salmonella* virulence genes, *stn* gene encodes effector proteins, which are involved in the pathogenesis of salmonellosis and diarrhea (Murugkar *et al*., 2003; Singh *et al*., 2013). The *Salmonella* outer protein B (*sop*B) gene and *sop*D promote systemic disease and act in concert to induce fluid secretion and inflammation (neutrophil influx) during gastroenteritis by directly promoting *Salmonella* invasion (Jones *et al.*, 1998). The *pef*A gene, is encoded by the plasmid-associated *pef* operon and produces fimbriae that enhance bacterial attachment to intestinal epithelial cells potentiating pathogenicity (Castilla *et al*., 2006). The *stn*, *sop*B and *sop*D virulence genes were detected in all the studied strains (100%), but *pef*A gene could not be detected (Table 4, Figure 1-3).

Salmonella virulence factors were assessed by many authors. Amin and Abd El-Rahman (2015) found that 617 bp *stn* specific gene fragment in all tested strains of *Salmonella* isolated from poultry meat in Alexandia, Egypt. However, Abd El-Tawab *et al.* (2015) did not find *sop*B in their isolates of *S*. Enteritidis, while Li *et al*. (2017) detected *pef*A, a specific gene band, in 58.7% of the recovered *Salmonella* isolates from retail raw chickens. This variation among different studies is a warning sign of probable acceptance of *Salmonella* to more genetic traits.

Biofilm forming ability of the isolated *Salmonella*

Biofilm formation is a natural lifestyle in most microorganisms for long-term persistence in various environments and resistance to antibiotics. It is vital for some pathogenic bacteria for host infection (Anderson *et al.*, 2003; Cvitkovitch *et al.*, 2003; Garcia-Medina *et al.*, 2005; Tajbakhsh *et al*., 2016). Biofilm forming abilities of bacteria depend on multiple factors, including bacterial cell surface, culture conditions (Dewanti and Wong, 1995) and nutrient availability (O' Toole *et al.*, 2000; Jackson *et al.*, 2002) and environmental stress (Scher *et al.*, 2005).

Three different colony morphotypes, saw (smooth and white), bdar (brown, dry and rough) and rdar (red, dry and rough) of *Salmonella* isolates, were determined on CR agar, which is caused by the co-expression of curli fimbriae and cellulose. This is a typical colony morphology that was observed between biofilm producer isolates. The bdar and saw morphotypes were determined with a deletion mutation in *csg* genes required for curli fimbriae synthesis or in both *csg* and *bcs* genes coding for cellulose synthesis, respectively. The production of cellulose and curli fimbriae is vital in biofilm formation and its persistence on various surfaces (Römling *et al*., 2000; Cookson *et al.*, 2002, Solano *et al.*, 2002).

The current results indicated that all the isolated *Salmonellae* (100%) are able to form biofilm with a rdar phenotype within 24-48 h (Table 4; Figure 4). Sereno *et al.* (2017) assessed 72.7% capability of Salmonellae to produce biofilms on polystyrene microplates. Ziech *et al*. (2016) reported that 100% of *Salmonella* sp. strains

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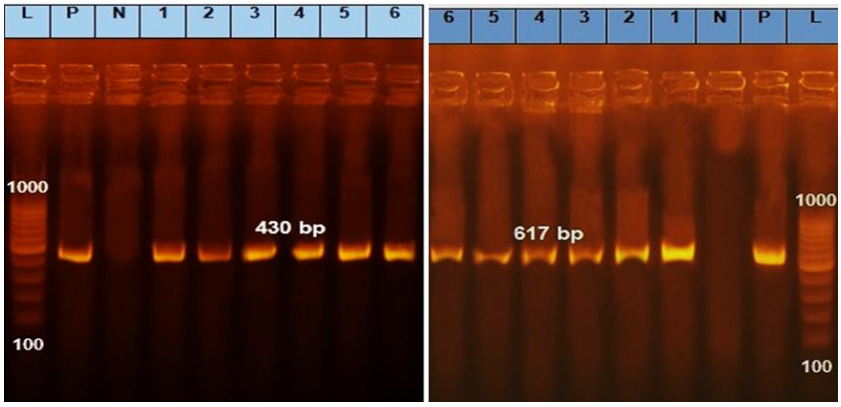

Figure 2: Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of *Salmonella sop*D and *stn* virulence genes illuminate (430 bp and 617 bp, respectively) detected in molecularly positive isolates. Lane L: 100-600 bp ladder as molecular size DNA marker; Lane P: Positive control; Lane N: Negative control; Lanes 1 to 6: Positive isolates.

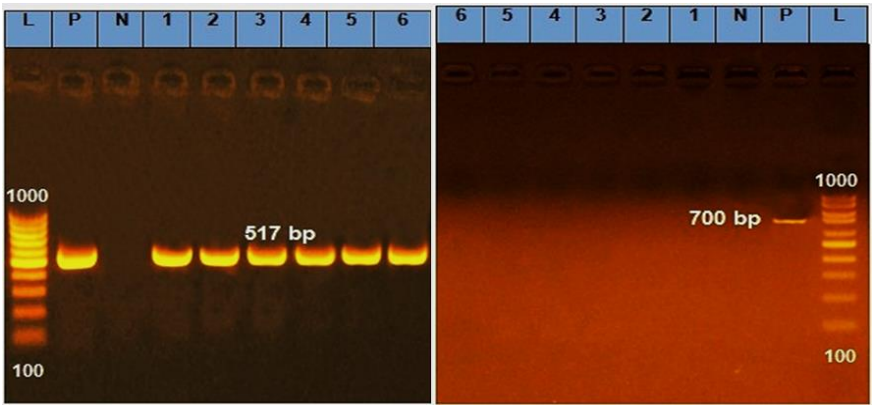

Figure 3: Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of *Salmonella sop*B and *pef*A virulence genes illuminate (517 bp and 700 bp, respectively) detected in molecularly positive isolates; Lane L: 100-1000 bp ladder as molecular size DNA marker; Lane P: Positive control; Lane N: Negative control; Left Lanes: 1 to 6: Positive isolates for *sop*B gene; Right lanes: Negative isolates for *pef*A gene.

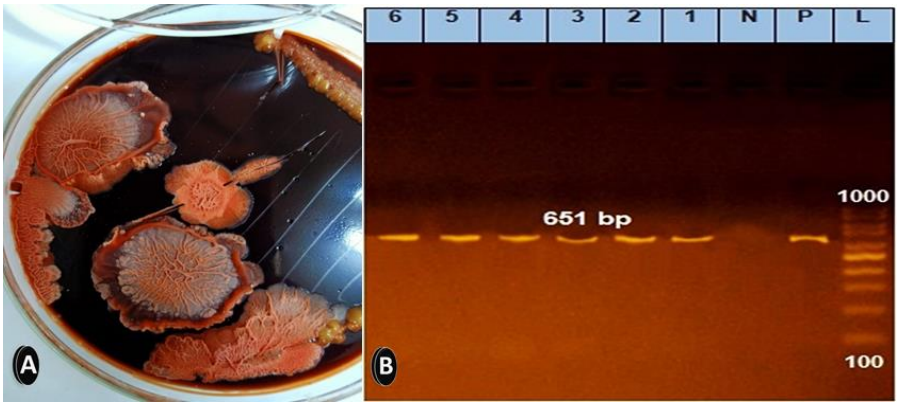

Figure 4: (A) Biofilm formed by *Salmonella* on CR agar. (B) Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of *Salmonella* biofilm gene illuminate (651 bp) detected in molecularly positive isolates. Lane L: 100-1000 bp ladder as molecular size DNA marker; Lane P: Positive control; Lane N: Negative control; Lanes 1-6: Positive isolates.

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isolated in poultry slaughterhouses and processing plants in Brazil were able to produce biofilm. Moreover, De Oliveira *et al.* (2014) in Brazil and Solano *et al.* (2002) in Spain found rates of 98.3% and 97%, respectively.

In this study, the *csg*D was identified in all obtained *Salmonella* isolates (100%). Transcription of the *csg*D gene potentiates curli and cellulose production that increases nutrient-limiting conditions in *Salmonella* (Gerstel and Römling, 2003). The observations in our study are consistent with such a notion, strongly suggesting that cellulose overproduction in a *csg*D in all strains of *Salmonella* positively affects biofilm formation.

Biofilms increase the tolerance of microorganisms to stress, reducing their sensitivity to disinfectants and antimicrobials, favor equipment corrosion and aid the adhesion of bacteria that are less able to form biofilms. Consequently, biofilms contribute to the persistence of resistant microorganisms both in the food industry environment and in processed foods, mainly due to cross-contamination (Sereno *et al.*, 2017). The present results indicated that all *Salmonella* isolates produced biofilm at room temperature. As a result, the persistency of these isolates on abiotic surfaces increases, which in turn increases the cross-contamination risk of the isolates. This can also be an explanation for the wide distribution of *Salmonella* isolates in food. It is also an important public health concern because poor sanitation of surfaces that come in contact with food causes foodborne outbreaks. Moreover, there is growing evidence suggesting that some genes involved in biofilm formation are also involved in adherence and colonization of host tissues (Latasa *et al.*, 2005; Manetti *et al.*, 2007; Muñoz-Elías *et al.*, 2008). Therefore, a link between biofilm formation and adherence to host tissues is being studied.

CONCLUSION

Salmonella was recovered from frozen packaged raw chicken sausage, frozen packaged raw chicken nuggets, and ready-to-eat sausage by 12%, 8% and 4%, respectively. However, it was not detected in ready-to-eat nuggets. Therefore, cooking poultry meat and products at a core temperature above 70 °C and preventing cross-contamination between raw and cooked items is very important in reducing *Salmonella*. The isolated strains were *S*. Typhimurium, *S*. Enteritidis, *S*. Essen and *S.* Montevideo. Additionally, mPCR indicated the presence of biofilm gene *(csg*D gene), *stn*, *sop*B and *sop*D virulence genes in all the isolated strains (100%); however, the *pef*A gene was not detected. The variation in genetic virulence of *Salmonella* among different studies is a warning sign of probable acceptance of the *Salmonella* to more genetic traits, which need to be ascertained in large-scale studies.

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