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ORIGINAL PAPER

Biofilm Production Potential of *Salmonella* Serovars Isolated from Chickens in North West Province, South Africa

STEPHEN ABIOLA AKINOLA[1, 2], MPINDA EDOAURD TSHIMPAMBA[3], MULUNDA MWANZA[3] and COLLINS NJIE ATEBA[1]

[1] Department of Microbiology, Bacteriophage Therapy and Phage Bio-Control Laboratory, Faculty of Natural and Agricultural Sciences, North West University, Mmabatho, South Africa
[2] Phytochemical Food Network Research Group, Department of Crop Sciences, Tshwane University of Technology, Pretoria, South Africa
[3] Center for Animal Health Studies, Food Security and Safety Niche, Faculty of Natural and Agricultural Sciences, North West University, Mmabatho, South Africa

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Abstract

Bacterial biofilms have recently gained considerable interest in the food production and medical industries due to their ability to resist destruction by disinfectants and other antimicrobials. Biofilms are extracellular polymer matrices that may enhance the survival of pathogens even when exposed to environmental stress. The effect of incubation temperatures (25°C, 37°C, and 40°C) and *Salmonella* serotype on biofilm-forming potentials was evaluated. Previously typed *Salmonella* serotypes (55) isolated from the gut of chickens were accessed for biofilms formation using a standard assay. *Salmonella* Typhimurium ATCC 14028™ and *Salmonella* Enteritidis ATCC 13076™ (positive controls), *Escherichia coli* (internal control) and un-inoculated Luria Bertani (LB) broth (negative control) were used. The isolates formed no biofilm (11.86–13.56%), weak (11.86–45.76%), moderate (18.64–20.34%), strong biofilms (23.73–54.24%) across the various temperatures investigated. Serotypes, *Salmonella* Heidelberg and *Salmonella* Weltevreden were the strongest biofilm formers at temperatures (25°C, 37°C, and 40°C, respectively). The potential of a large proportion (80%) of *Salmonella* serotypes to form biofilms increased with increasing incubation temperatures but decreased at 40°C. Findings indicate that average temperature favours biofilm formation by *Salmonella* serotypes. However, the influence of incubation temperature on biofilm formation was greater when compared to serotype. A positive correlation exists between *Salmonella* biofilm formed at 25°C, 37°C and 40°C ($p \geq 0.01$). The ability of *Salmonella* species to form biofilms at 25°C and 37°C suggests that these serotypes may present severe challenges to food-processing and hospital facilities.

Key words: *Salmonella*, biofilm, biofilm production potential, crystal violet microtitre

Introduction

Biofilms exist as summative clusters of microorganisms that could be from a single or multiple species. Biofilms are densely populated microbial communities comprising microorganisms of the same or different species that live close to each other and therefore facilitate social interaction (Davey and O'Toole 2000; Li and Tian 2012). The multicellular properties of biofilms assist in the survival of microorganisms when exposed to undesirable environmental and stressful conditions. The attachment of planktonic microorganisms to surfaces is critical for biofilm formation (Arunasri and Mohan 2019). Biofilms can be formed on food contact surfaces, contaminated food materials, natural environments such as water bodies, and on human tissues (Hall-Stoodley et al. 2004). The formation of biofilms is an important virulence factor that enhances the pathogenicity of most microbes that cause infections in humans and animals and therefore alleviate their public health significance (Costerton et al. 1999). The formation of biofilms by bacteria has resulted in increasing rates of antimicrobial resistance emerging from the potential to prevent the penetration of antibacterial agents into cells during treatment (Patel 2005) thus making biofilm control medically important. However, very few data has been reported on a substantial correlation that could exists between *Salmonella* serotypes isolated from chickens, the multiple antibiotic resistance behavior, incubation/storage temperature, and

* Corresponding author: S.A. Akinola, Department of Microbiology, North West University, Mmabatho, South Africa; **e-mail:akinolastephen3@gmail.com**

their ability to form biofilms (Díez-García et al. 2012; Wang et al. 2013; Borges et al. 2018).

Similarly, the strive to achieve food safety through the inactivation of pathogenic microorganisms from food and food products is important and often faced with challenges such as biofilm formation (Sadekuzzaman et al. 2015). Microbial biofilms on food and food processing plants constitute a threat to food safety and health of consumers due to the huge tolerance to exogenous stress that results in ineffective disinfection process during plant sanitation and reduced options of antibiotics treatment, which could lead to food poisoning (Hall-Stoodley and Stoodley 2009; Sofos and Geornaras 2010). The abilities of bacteria to form biofilms have been investigated using the qualitative or the quantitative assays. In recent times, the qualitative biofilm assays have given way to the quantitative assays, which give more precise results than just findings based on observation. The quantitative biofilm assays allow for a numerical evaluation of the ability of bacteria to form biofilms. In this study, the quantitative assays were adopted based on its accuracy, reliability, and potential to enable precise quantification instruments.

Biofilm forming pathogens (*Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) have been isolated in food and food processing plants in developed and developing countries (Dourou et al. 2011; Cook et al. 2012; Wang et al. 2013; Li et al. 2017; Papa et al. 2018). Some pathogenic bacteria are capable of growing at low temperatures on food and contact surfaces. Recently, according to Webber et al. (2019) *Salmonella* Enteritidis have been reported to form biofilms on industrial food surfaces at relatively low temperatures (3°C). This provokes concerns for safety in cold store food preservation. Therefore it is important to research into the biofilm formation potentials of *Salmonella* serotypes colonizing chickens reared for food in the North West province, South Africa, which is an agricultural hub of the nation to ensure safety of foods and encourage regional trade.

Food poisoning may ensue from consuming contaminated raw, fresh, and minimally processed food commodities. *Salmonella* borne infection outbreaks have been associated with the ingestion of *Salmonella* infected livestock products such as eggs, poultry meat and pork (Hur et al. 2012; EFSA-ECDC 2018). In the European Union and the United State of America, *Salmonella* spp. has been implicated as the causative agent for food poisoning, which results in ill-health with many cases of the outbreak in recent years. Based on previous epidemiological studies, salmonellosis outbreaks have been traced to the food of animal origin, and research interest has been geared at investigating the occurrence of pathogenic strains of *Salmonella* in animal food products (Dallal et al. 2010). The rate of deaths among humans resulting from non-typhoidal salmonellosis has been increasing, especially in developing countries, and the mortality rate among children and adults in Africa ranges from 22–47% (Gordon et al. 2008). *Salmonella* Typhimurium is known as the main cause of foodborne salmonellosis globally, including South Africa; however, in recent years *Salmonella* Enteritidis have soon become the dominant cause of Salmonellosis in South Africa (Muvhali et al. 2017). From 2003 to 2007, 2013 to 2015, and October 2019 an outbreak of foodborne salmonellosis emanating from national food programme was reported in the rural areas of the Kwazulu Natal province and North West province, South Africa causing severe conditions in humans (Niehaus et al. 2011; Motladiile et al. 2019). Malangu and Ogunbanjo (2009) reported an acute *Salmonella* poisoning in 2005 emanating from South African Hospitals. Biofilm production was reported in drinking water (Mulamattathil et al. 2014), while Isoken (2015) reported the isolation of biofilm-forming *Salmonella* species in cabbage and spinach sold in South Africa. The presence of *Salmonella* species in food and water provides opportunities for cross-contamination along the food chain and accounts for diseases in susceptible individuals (Karkey et al. 2016; Byrd-Bredbenner, 2017). Unfortunately, investigation along the critical control points on the food value chain has not been comprehensive. Most research has focused on the retail stores, processing utensils, and processing environment (Cook et al. 2012) as a source of *Salmonella* contamination while few focus on the livestock rearing environment, which is critical to an effective epidemiological survey. Therefore, this research hypothesized that the incubation temperature and type of *Salmonella* serotypes would affect the biofilm-forming potentials of *Salmonella* pathogens. This will help identify the biofilm formation status of microbial communities colonizing the food environment and possibly give an explanation to the observed cases of antibiotic resistance of *Salmonella* serotypes so as to develop informed strategies to counteract the menace of food poisoning that could emanate from such microbial communities. The study investigated the effect of incubation temperature on biofilm-forming potentials of selected *Salmonella* serotypes isolated from Chickens in North-West Province, South Africa.

Experimental

Materials and Methods

Materials. The following reagents and materials were used in the study; analytical grade absolute ethanol (95%), Luria Bertani broth medium (Merck, South

Africa), phosphate buffer saline tablets (Merck, South Africa), Crystal violet (Merck, South Africa) and sterile 96 well Eppendorf polystyrene flat-bottom microtitre plate (Greiner bio-one, Hamburg, Germany). All the reagents used were of analytical grades. Typed *Salmonella* cultures used were isolated from live Chickens in Mafikeng, North West Province, South Africa, and previously identified (Akinola et al. 2019). *Salmonella* Typhimurium ATCC 14028™ and *Salmonella* Enteritidis ATCC 13076™ were used as positive controls, un-inoculated media broth (negative control), and an environmental strain of *E. coli* was used as an internal control in the experiment.

Methods. Culturing of *Salmonella* isolates. Luria Bertani (LB) broth was prepared following the manufacturer's instruction and was sterilized in an autoclave at 121°C for 15 minutes. Presumptive *Salmonella* strains were isolated using the International Organization for Standardization (2002) ISO 6579:2002 protocols, characterized and serotyped as previously reported by Akinola et al. (2019). Individual *Salmonella* serotypes (55) were inoculated into sterile LB broth and were incubated aerobically at 37°C overnight. Re-activated cultures were then used to investigate the biofilm-forming potentials of the isolates.

Determination of biofilm formation by *Salmonella* isolates. The biofilm production abilities of *Salmonella* isolates was determined using the crystal violet based microtitre plate assay method as described by Silagyi et al. (2009) and Stepanović et al. (2000). A loop full of *Salmonella* cultures were inoculated and grown overnight in LB (Balbontin et al. 2014) broth at 25°C, 37°C, and 40°C. The turbidimetry method was used to determine the concentration of *Salmonella* serotypes in a UV-spectrophotometer through the instrument of absorbance at 600 nm (Moosdeen et al. 1988). Dilution was made till an average of 5×10^6 CFU/ml concentration was reached and confirmed using the pour plating techniques on prepared *Salmonella Shigella* agar plates. One hundred microliters of grown culture was diluted in 10 ml sterile LB broth (1:100). Then, 200 µl of diluted culture was dispensed in 96 wells microtitre plate and was incubated at 25°C, 37°C, and 40°C for 24 hours. *Salmonella* Typhimurium ATCC 14028™, and *Salmonella* Enteritidis ATCC 13076™ (positive control) and the environmental strain of *E. coli* was used as an internal positive control in the experiment. Un-inoculated sterile LB broth was used as a negative control in the experiment. The experiment was done in three replicated wells. After 24 hours of incubation, LB broth was discarded by turning upside down and shaking off the liquid broth prior to washing of the plate in a tub of phosphate buffer saline solution. The washing process was repeated twice to enable the removal of unattached cells. A 200 µl of crystal violet dye (1% w/v) was added to each well and plates were incubated at room temperature for 1 h. After incubation, the dye was discarded, and wells were washed five times in phosphate buffer saline solution. The microtitre plate was blot dry with laboratory paper towels and was allowed to dry at room temperature. After, 200 µl of 95% ethanol was added to each well and was incubated at room temperature for 5 min. The resulting solution was thereafter transferred into a new 96 well microtitre plate. The optical density (OD) of the resulting solution was quantified in terms of absorbance at a wavelength of 630 nm in an automatic Enzyme-Linked Immunosorbent Assay (ELISA) microtitre plate reader (MB-580, Zhengzhou, China). Sterile LB broth was used as blank in the determination, while the optical densities was used to investigate the biofilm formation potential of *Salmonella* isolates using the following conditions as stated by Papa et al. (2018); $OD_S < OD_C$ = No biofilm formation, $OD_C < OD_S < 2OD_C$ = Weak biofilm formation, $2OD_C < OD_S < 4OD_C$ = Moderate biofilm formation, $4OD_C < OD_S$ = Strong biofilm formation; Where: OD_C = OD of negative control, OD_S = OD of sample. Optical densities were obtained in triplicates, and the mean obtained was regarded as optical densities for each *Salmonella* serotype.

Statistical analysis. The statistical analysis was done using percentages and central tendency measures such as mean and frequencies using Statistical Package for Social Sciences. The significance of the effect of incubation temperatures on biofilm formation was evaluated using the one-way analysis of variance (ANOVA). The relationship between incubation temperature and biofilm-forming potentials of *Salmonella* isolates was evaluated using Pearson correlation analysis. The significance of variables was evaluated at a 90% confidence interval using the Statistical Package for Social Sciences (SPSS version 17, Illinois USA).

Results and Discussion

In Table I, the identity of *Salmonella* serotypes used in this study is presented. The isolates were from chickens reared in North West Province, South Africa, as earlier reported by Akinola et al. (2017). The optical densities and degree of biofilm formation by *Salmonella* serotypes isolated from chickens as influenced by incubation temperature is as presented in Table II. The values obtained represent the optical densities obtained from the crystal violet biofilm microtitre plate assay using various *Salmonella* serotypes as inoculum. At incubation temperature of 25°C, the optical density of *Salmonella* serotypes ranged from 0.008 to 1.048 while at 37°C (0.04–1.02) and 40°C (0.023–1.509). At 37°C the OD of CHG16 (*Salmonella enterica* subsp.

Table I
Identities of *Salmonella* isolates used for biofilm assay.

Isolate number	Sources	Accession number	Organism
CHG1	Broiler	MG663456	*Salmonella enterica* subsp. *enterica*
CHG2	Broiler	MG663457	*Salmonella enterica* subsp. *enterica*
CHG3	Broiler	MG663458	*Salmonella enterica* subsp. *enterica*
CHG4	Broiler	MG663459	*Salmonella enterica ser.* Weltevreden
CHG5	Broiler	MG663460	*Salmonella enterica ser.* Chingola
CHG6	Broiler	MG663461	*Salmonella enterica ser.* Arizonae
CHG7	Broiler	MG663462	*Salmonella enterica ser.* Bovismorbificans
CHG8	Layer	MG663463	*Salmonella enterica* subsp. *enterica*
CHG9	Layer	MG663464	*Salmonella enterica* subsp. *enterica*
CHG10	Layer	MG663465	*Salmonella enterica ser.* Typhimurium
CHG11	Layer	MG663466	*Salmonella enterica ser.* Salamae
CHG12	Layer	MG663467	*Salmonella enterica ser.* Houten
CHG13	Layer	MG663468	*Salmonella enterica* subsp. *enterica*
CHG14	Indigenous Venda	MG663469	*Salmonella enterica ser.* Bareilly
CHG15	Indigenous Venda	MG663470	*Salmonella enterica* subsp. *enterica*
CHG16	Indigenous Venda	MG663471	*Salmonella enterica* subsp. *enterica*
CHG17	Indigenous Venda	MG663472	*Salmonella enterica* subsp. *enterica*
CHG18	Indigenous Venda	MG663473	*Salmonella enterica ser.* Heidelberg
CHG19	Indigenous Venda	MG663474	*Salmonella enterica ser.* Arizonae
CHG20	Indigenous Venda	MG663475	*Salmonella enterica* subsp. *enterica*
CHG21	Indigenous Venda	MG663476	*Salmonella enterica ser.* India
CHG22	Indigenous Venda	MG663477	*Salmonella enterica ser.* Crossness
CHG23	Indigenous Venda	MG663478	*Salmonella enterica ser.* Albany
CHG24	Indigenous Venda	MG663479	*Salmonella enterica ser.* Yovokome
CHG25	Indigenous Venda	MG663480	*Salmonella enterica ser.* Pullorum
CHG26	Indigenous Venda	MG663481	*Salmonella enterica ser.* Infantis
CHG27	Broiler	MG663482	*Salmonella enterica ser.* Arizonae
CHG28	Broiler	MG663483	*Salmonella enterica ser.* Heidelberg
CHG29	Broiler	MG663484	*Salmonella enterica* subsp. *enterica*
CHG30	Broiler	MG663485	*Salmonella enterica* subsp. *enterica*
CHG31	Broiler	MG663486	*Salmonella bongori*
CHG32	Broiler	MG663487	*Salmonella bongori*
CHG33	Broiler	MG663488	*Salmonella enterica ser.* Arizonae
CHG34	Layer	MG663489	*Salmonella enterica* subsp. *enterica*
CHG35	Layer	MG663490	*Salmonella enterica ser.* Wandsworth
CHG36	Layer	MG663491	*Salmonella enterica* subsp. *enterica*
CHG37	Layer	MG663492	*Salmonella bongori*
CHG38	Layer	MG663493	*Salmonella enterica ser.* Kentucky
CHG39	Layer	MG663494	*Salmonella bongori*
CHG40	Layer	MG663495	*Salmonella enterica ser.* Blockley
CHG41	Layer	MG663496	*Salmonella enterica ser.* Newport
CHG42	Layer	MG663497	*Salmonella enterica ser.* Typhimurium
CHG43	Indigenous koekoek	MG663498	*Salmonella bongori*
CHG44	Indigenous koekoek	MG663499	*Salmonella enterica ser.* Manchester
CHG45	Indigenous koekoek	MG663500	*Salmonella enterica* subsp. *enterica*
CHG46	Indigenous koekoek	MG663501	*Salmonella enterica* subsp. *enterica*
CHG47	Indigenous koekoek	MG663502	*Salmonella enterica ser.* Typhimurium

Table I. Continued

Isolate number	Sources	Accession number	Organism
CHG48	Indigenous koekoek	MG663503	*Salmonella enterica* subsp. *enterica*
CHG49	Indigenous koekoek	MG663504	*Salmonella enterica ser.* Typhimurium
CHG50	Indigenous koekoek	MG663505	*Salmonella enterica ser.* Typhimurium
CHG51	Indigenous koekoek	MG663506	*Salmonella enterica ser.* Typhimurium
CHG52	Indigenous koekoek	MG663507	*Salmonella enterica ser.* Koessen
CHG53	Indigenous koekoek	MG663508	*Salmonella bongori*
CHG54	Indigenous koekoek	MG663509	*Salmonella enterica ser.* Blegdam
CHG55	Indigenous koekoek	MG663456	*Salmonella enterica* subsp. *enterica*

Akinola et al. (2019)

Table II
Optical densities and degree of biofilms formed by *Salmonella* serotypes as influenced by incubation temperatures.

ID	*Salmonella* isolates	Incubation temperature			Degree of biofilms formed		
		25°C	37°C	40°C	25°C	37°C	40°C
CHG1	*Salmonella enterica* subsp. *enterica*	0.107 ± 0.003	0.312 ± 0.089	0.132 ± 0.020	Moderate	Weak	Moderate
CHG2	*Salmonella enterica* subsp. *enterica*	0.075 ± 0.009	0.969 ± 0.065	0.342 ± 0.106	Moderate	Strong	Strong
CHG3	*Salmonella enterica* subsp. *enterica*	0.023 ± 0.018	0.946 ± 0.123	0.063 ± 0.032	No biofilm	Strong	Weak
CHG8	*Salmonella enterica* subsp. *enterica*	0.247 ± 0.099	0.271 ± 0.030	0.300 ± 0.071	Strong	Weak	Strong
CHG9	*Salmonella enterica* subsp. *enterica*	0.120 ± 0.052	0.291 ± 0.015	0.082 ± 0.041	Moderate	Weak	Weak
CHG13	*Salmonella enterica* subsp. *enterica*	0.095 ± 0.017	0.319 ± 0.058	0.261 ± 0.081	Moderate	Weak	Strong
CHG15	*Salmonella enterica* subsp. *enterica*	0.037 ± 0.013	1.006 ± 0.031	0.167 ± 0.136	Weak	Strong	Moderate
CHG16	*Salmonella enterica* subsp. *enterica*	0.067 ± 0.009	1.022 ± 0.108	0.085 ± 0.033	Moderate	Strong	Weak
CHG17	*Salmonella enterica* subsp. *enterica*	0.405 ± 0.222	1.010 ± 0.045	0.082 ± 0.060	Strong	Strong	Weak
CHG20	*Salmonella enterica* subsp. *enterica*	0.278 ± 0.071	0.885 ± 0.120	0.083 ± 0.027	Strong	Strong	Weak
CHG29	*Salmonella enterica* subsp. *enterica*	0.149 ± 0.061	0.961 ± 0.180	0.077 ± 0.024	Strong	Moderate	Weak
CHG30	*Salmonella enterica* subsp. *enterica*	0.303 ± 0.085	0.591 ± 0.174	0.112 ± 0.006	Strong	Strong	Moderate
CHG34	*Salmonella enterica* subsp. *enterica*	0.039 ± 0.032	1.227 ± 0.273	0.010 ± 0.005	Weak	Weak	No biofilm
CHG36	*Salmonella enterica* subsp. *enterica*	0.026 ± 0.024	0.704 ± 0.220	0.065 ± 0.046	No biofilm	Weak	Weak
CHG45	*Salmonella enterica* subsp. *enterica*	0.800 ± 0.572	0.983 ± 0.177	1.098 ± 0.736	Strong	Weak	Strong
CHG46	*Salmonella enterica* subsp. *enterica*	0.937 ± 0.668	1.017 ± 0.244	1.089 ± 0.803	Strong	Weak	Strong
CHG48	*Salmonella enterica* subsp. *enterica*	0.259 ± 0.308	1.248 ± 0.080	0.407 ± 0.447	Strong	Moderate	Strong
CHG55	*Salmonella enterica* subsp. *enterica*	0.341 ± 0.115	1.605 ± 0.066	0.395 ± 0.098	Strong	Weak	Strong
CHG31	*Salmonella bongori*	0.276 ± 0.037	0.561 ± 0.150	0.034 ± 0.012	Strong	Moderate	No biofilm
CHG32	*Salmonella bongori*	0.012 ± 0.007	0.422 ± 0.191	0.034 ± 0.017	No biofilm	No biofilm	No biofilm
CHG37	*Salmonella bongori*	0.030 ± 0.001	0.700 ± 0.204	0.066 ± 0.013	No biofilm	Weak	Weak
CHG39	*Salmonella bongori*	0.277 ± 0.094	1.075 ± 0.340	0.489 ± 0.192	Strong	Weak	Strong
CHG43	*Salmonella bongori*	0.077 ± 0.003	0.812 ± 0.288	0.129 ± 0.012	Moderate	Weak	Moderate
CHG53	*Salmonella bongori*	0.769 ± 0.205	1.244 ± 0.104	1.020 ± 0.207	Strong	Weak	Strong
Serovars							
CHG4	*Salmonella enterica ser.* Weltevreden	0.138 ± 0.042	0.817 ± 0.273	1.509 ± 0.453	Strong	Strong	Strong
CHG5	*Salmonella enterica ser.* Chingola	0.181 ± 0.107	0.308 ± 0.055	0.446 ± 0.011	Strong	Weak	Strong
CHG6	*Salmonella enterica ser.* Arizonae	0.108 ± 0.090	0.287 ± 0.035	0.198 ± 0.044	Moderate	Weak	Strong
CHG19	*Salmonella enterica ser.* Arizonae	0.151 ± 0.045	0.574 ± 0.145	0.152 ± 0.036	Strong	Moderate	Moderate
CHG27	*Salmonella enterica ser.* Arizonae	0.026 ± 0.011	0.901 ± 0.040	0.472 ± 0.040	No biofilm	Strong	Strong
CHG33	*Salmonella enterica ser.* Arizonae	0.038 ± 0.017	0.848 ± 0.453	0.064 ± 0.031	Weak	Weak	Weak
CHG7	*Salmonella enterica ser.* Bovismorbificans	0.586 ± 0.116	0.220 ± 0.032	0.163 ± 0.147	Strong	No biofilm	Moderate

Table II. Continued

ID	*Salmonella* isolates	Incubation temperature			Degree of biofilms formed		
		25°C	37°C	40°C	25°C	37°C	40°C
CHG10	*Salmonella enterica ser.* Typhimurium	1.028 ± 0.507	0.230 ± 0.059	0.167 ± 0.166	Strong	No biofilm	Moderate
CHG42	*Salmonella enterica ser.* Typhimurium	0.069 ± 0.064	0.089 ± 0.038	0.039 ± 0.018	Moderate	Weak	No biofilm
CHG47	*Salmonella enterica ser.* Typhimurium	0.024 ± 0.011	0.920 ± 0.315	0.053 ± 0.026	No biofilm	Weak	Weak
CHG49	*Salmonella enterica ser.* Typhimurium	0.167 ± 0.107	0.468 ± 0.142	0.163 ± 0.071	Strong	No biofilm	Moderate
CHG50	*Salmonella enterica ser.* Typhimurium	0.116 ± 0.084	0.310 ± 0.098	0.099 ± 0.007	Moderate	No biofilm	Weak
CHG51	*Salmonella enterica ser.* Typhimurium	0.098 ± 0.041	1.132 ± 0.333	0.185 ± 0.051	Moderate	Weak	Strong
CHG11	*Salmonella enterica ser.* Salamae	0.008 ± 0.004	0.284 ± 0.024	0.173 ± 0.019	No biofilm	Weak	Moderate
CHG12	*Salmonella enterica ser.* Houten	0.327 ± 0.059	0.360 ± 0.053	0.248 ± 0.118	Strong	Weak	Strong
CHG14	*Salmonella enterica ser.* Bareilly	0.182 ± 0.061	0.906 ± 0.163	1.009 ± 0.642	Strong	Strong	Strong
CHG18	*Salmonella enterica ser.* Heidelberg	1.048 ± 0.915	0.976 ± 0.104	0.064 ± 0.022	Strong	Strong	Weak
CHG28	*Salmonella enterica ser.* Heidelberg	0.098 ± 0.012	0.695 ± 0.167	0.038 ± 0.019	Weak	Strong	No biofilm
CHG21	*Salmonella enterica ser.* India	0.390 ± 0.091	1.024 ± 0.077	0.238 ± 0.094	Strong	Strong	Strong
CHG22	*Salmonella enterica ser.* Crossness	0.097 ± 0.008	0.640 ± 0.154	0.402 ± 0.366	Moderate	Moderate	Strong
CHG23	*Salmonella enterica ser.* Albany	0.212 ± 0.088	0.700 ± 0.108	0.303 ± 0.108	Strong	Moderate	Strong
CHG24	*Salmonella enterica ser.* Yovokome	0.107 ± 0.011	0.906 ± 0.277	0.041 ± 0.014	Weak	Strong	No biofilm
CHG25	*Salmonella enterica ser.* Pullorum	0.183 ± 0.082	0.733 ± 0.035	0.729 ± 0.082	Strong	Moderate	Strong
CHG26	*Salmonella enterica ser.* Infantis	0.320 ± 0.115	0.754 ± 0.124	0.743 ± 0.137	Strong	Moderate	Strong
CHG35	*Salmonella enterica ser.* Wandsworth	0.056 ± 0.018	0.723 ± 0.240	0.101 ± 0.031	Weak	Weak	Moderate
CHG38	*Salmonella enterica ser.* Kentucky	0.214 ± 0.088	1.012 ± 0.224	0.304 ± 0.255	Strong	Weak	Strong
CHG40	*Salmonella enterica ser.* Blockley	0.057 ± 0.030	0.387 ± 0.077	0.077 ± 0.037	Weak	No biofilm	Weak
CHG41	*Salmonella enterica ser.* Newport	0.245 ± 0.376	0.604 ± 0.310	0.388 ± 0.554	Strong	Weak	Strong
CHG44	*Salmonella enterica ser.* Manchester	0.078 ± 0.012	1.107 ± 0.172	0.128 ± 0.020	Moderate	Weak	Moderate
CHG52	*Salmonella enterica ser.* Koessen	0.206 ± 0.038	1.021 ± 0.169	0.290 ± 0.034	Strong	Weak	Strong
CHG54	*Salmonella enterica ser.* Blegdam	0.155 ± 0.078	0.584 ± 0.194	0.135 ± 0.027	Strong	Weak	Moderate
BLNK	Blank (LB broth)	0.089 ± 0.009	0.278 ± 0.017	0.0385 ± 0.036	–	–	–
CNTRL1	Negative control (un-inoculated broth)	0.025 ± 0.038	0.267 ± 0.002	0.023 ± 0.017	No biofilm	No biofilm	No biofilm
CNTRL2	Positive control *(Salmonella enterica ser.* Typhimurium ATCC 14028™)	0.352 ± 0.106	1.397 ± 0.107	0.493 ± 0.167	Strong	Moderate	Strong
CNTRL3	Positive control (*Salmonella enterica ser.* Enteritidis ATCC 13076™)	0.410 ± 0.017	1.725 ± 0.009	0.602 ± 0.059	Strong	Moderate	Strong
CNTRL4	Internal Control (*E. coli* 0157)	1.031 ± 0.072	1.236 ± 0.030	1.309 ± 0.076	Strong	Moderate	Strong

Values represents means of triplicate determinations.
No biofilm formation (if $OD_S < OD_C$), weak biofilm formation (if $OD_C < OD_S < 2OD_C$), moderate biofilm formation ($2OD_C < OD_S < 4OD_C$) and strong biofilm formation ($4OD_C < OD_S$). Optical density (OD) ± standard deviation at 630 nm.
CNTRL1 – Negative control (un-inoculated nutrient broth), CNTRL2 – Positive control (*Salmonella enterica ser.* Typhimurium), CNTRL3 – Positive control 2 (*Salmonella enterica ser.* Enteritidis), CNTRL4 – Positive Internal Control (*Escherichia coli*), BLNK – Luria Bertani broth.

enterica) was highest while CHG18 (*Salmonella* Heidelberg) at 25°C and CHG4 (*Salmonella* Weltevreden) at 40°C. As expected, the negative control (un-inoculated broth) had low OD (0.267 ± 0.002) hence did not form biofilm, while the positive controls *Salmonella* Typhimurium (1.397 ± 0.107) and *Salmonella* Enteritidis (1.725 ± 0.009), and the internal control *E. coli* (1.236 ± 0.030) were positive to biofilm production at 24 hours of incubation. As obtained in this study, biofilm formation was greatly influenced by the *Salmonella* serotype colonizing the substrates than the temperature of incubation at 24 hours of incubation. The optical density of eighty percent *Salmonella* serotypes increased at increasing incubation temperatures of 25°C to 37°C but decreased at a higher incubation temperature of 40°C. However, the optical densities of samples CHG4, CHG5, CHG14, CHG25, CHG26, CHG45, and CHG46 increased with increasing incubation temperature. The optical density of the *Salmonella* serotype was optimum at incubation temperatures of 37°C except in isolates CHG7, CHG10 and CHG18 that were optimum at 25°C. Similarly, the incubation temperatures had a significant effect on the optical density obtained in the positive and internal controls, while

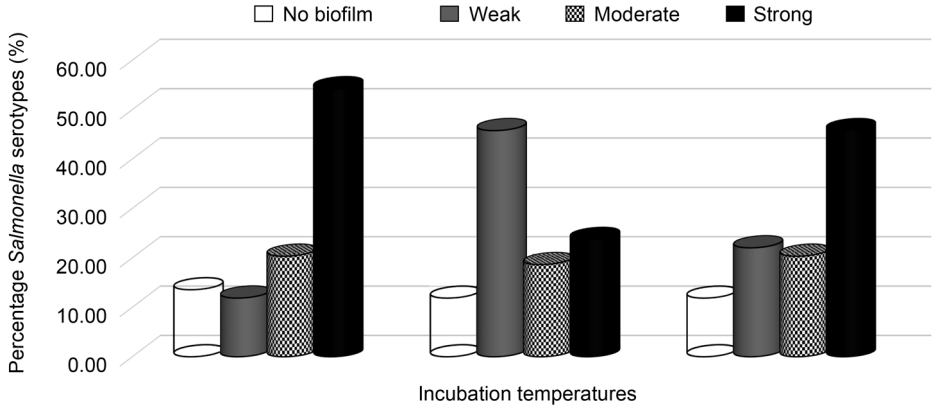

Fig. 1. Effect of incubation temperatures on biofilm-forming potentials of *Salmonella* serotypes.

there was no effect on the negative control. Hence, incubation temperature and type of *Salmonella* serotype influences the biofilm-forming abilities of *Salmonella*. Biofilm formation by *Salmonella* serotypes are well favored at an incubation temperature of 37°C.

The degree of biofilm formed by test *Salmonella* serotypes is as presented in Table II. The degree of biofilms formed by the *Salmonella* serotypes ranged from no biofilm, weak, moderate to strong biofilm. Fig. 1 presents the percent distribution of the degree of biofilm formed by selected pathogens. *Salmonella* serotypes that produced no biofilms ranged from 11.86% to 13.56%. The percent *Salmonella* serotypes that produced weak biofilms at varying temperatures ranged from 11.86% to 45.76%, and this observation was optimum at an incubation temperature of 37°C (45.76%). The percent distribution of moderate *Salmonella* biofilm producers at varied incubation temperatures ranged from 18.64 to 20.34% and was highest at both 25°C and 40°C (20.34%). The percent *Salmonella* serotype that produced strong biofilms ranged from 23.73 to 54.24% and was highest at 25°C incubation temperatures.

This study observed that biofilm production by selected *Salmonella* serotypes was influenced by the incubation temperature and type of *Salmonella* serotypes. A strong *Salmonella* biofilm can be produced at 25°C (room temperature) within 24 hours of incubation. An incubation temperature of 25°C favors *Salmonella* biofilm formation than at much higher temperatures. The ability of *Salmonella* serotypes to form strong biofilms at room temperatures could pose a threat to food safety and hygiene practices especially in food processing facilities. Public health pathogens, including *Salmonella*, has been identified to have the ability to form biofilms on food contact surfaces (Bridier et al. 2014), which supports the findings in this study. The occurrence of this *Salmonella* serotype in food or food contact surfaces could incur extra cost in plant sanitation, thereby increasing the overhead cost of food production, which in turn results in high food prices. Biofilm formation has been identified as one of the mechanisms of bacterial pathogens to evade antimicrobial treatment (Floyd et al. 2017). Bacteria biofilms are able to tolerate harsh conditions and resist antibiotics treatments due to a unique biofilm matrix (Sharma et al. 2019). Microbial cells can sense the extracellular environment and cause the cellular response's triggering in favor of biofilm formation (Koo and Yamada 2016). Biofilm matrices act as both physical and chemical barriers (Khan et al. 2017) that could prevent antimicrobials from reaching their targets in microbes, thus preventing the control of pathogens and increasing resistance among microorganisms implicated in biofilm formation or infections. Besides the barrier to penetration, the depletion of nutrient sources and triggering of stress response and development of biofilm resistant phenotypes in microorganism have been proved as mechanisms that aid antibiotic resistance of pathogens (Mah and O'Toole 2001). Similarly, *Salmonella* pathogens have been reported to contain the alternative sigma factor (RpoS) and flagella architectures that could enable its biofilm formation (Lee et al. 1995; Kroupitski et al. 2009) which supports the biofilm formation in this study. Hence, *Salmonella* biofilms could pose a serious threat to the effective treatment of salmonellosis through antimicrobial.

Fig. 2, 3 and 4 presents the behavioral patterns of *Salmonella* serotypes to biofilm production at 25°C, 37°C, and 40°C, respectively. At 25°C, 50% of the total *Salmonella enterica* subsp. *enterica* produced strong biofilms while at 37°C and 40°C only 38.7% had strong biofilm formation. *Salmonella bongori* (50%) produced strong biofilm at 25°C and 40°C (33.3%) whereas could not produce strong biofilms at 37°C. Only 33.3% of *Salmonella* Typhimurium produced strong biofilms at 25°C, while 16.7% at 40°C. However, none of the isolate produced strong biofilms at 40°C. Furthermore, 27.8% of *Salmonella enterica* subsp. *enterica, Salmonella* Typhimurium (50%) and *Salmonella bongori* (16.7%) produced moderate biofilms. Also, at 37°C, *Salmonella* Arizonae (25%) and *Salmonella bongori* (16.7%) produced

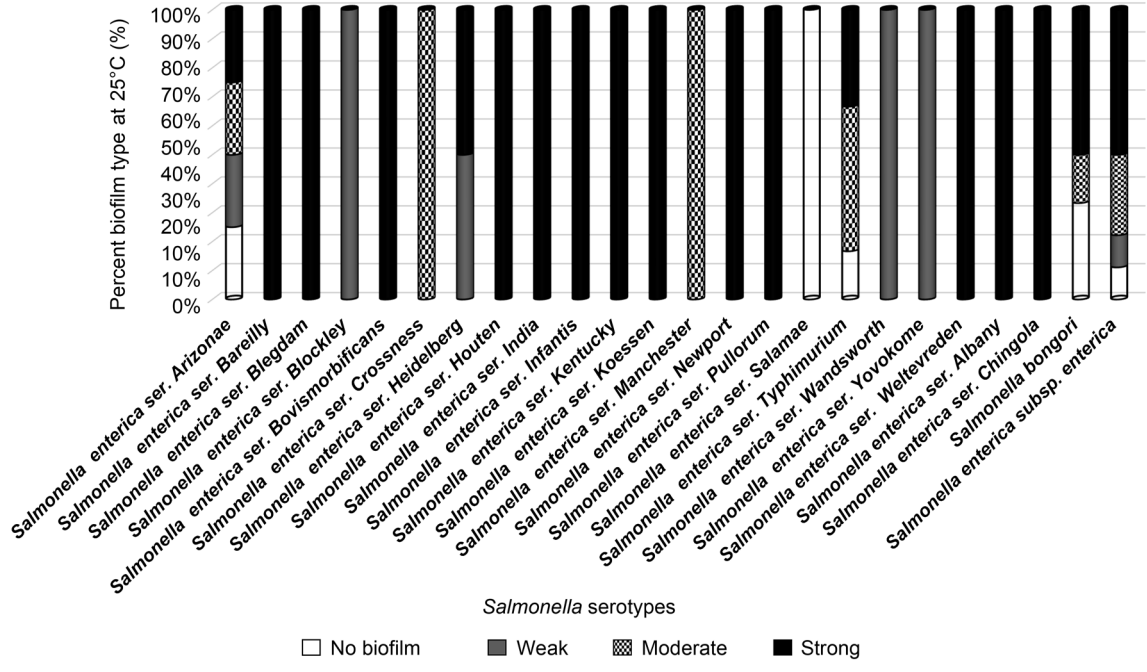

Fig. 2. Behavioral pattern of *Salmonella* serotypes to biofilm production at 25°C incubation temperature.

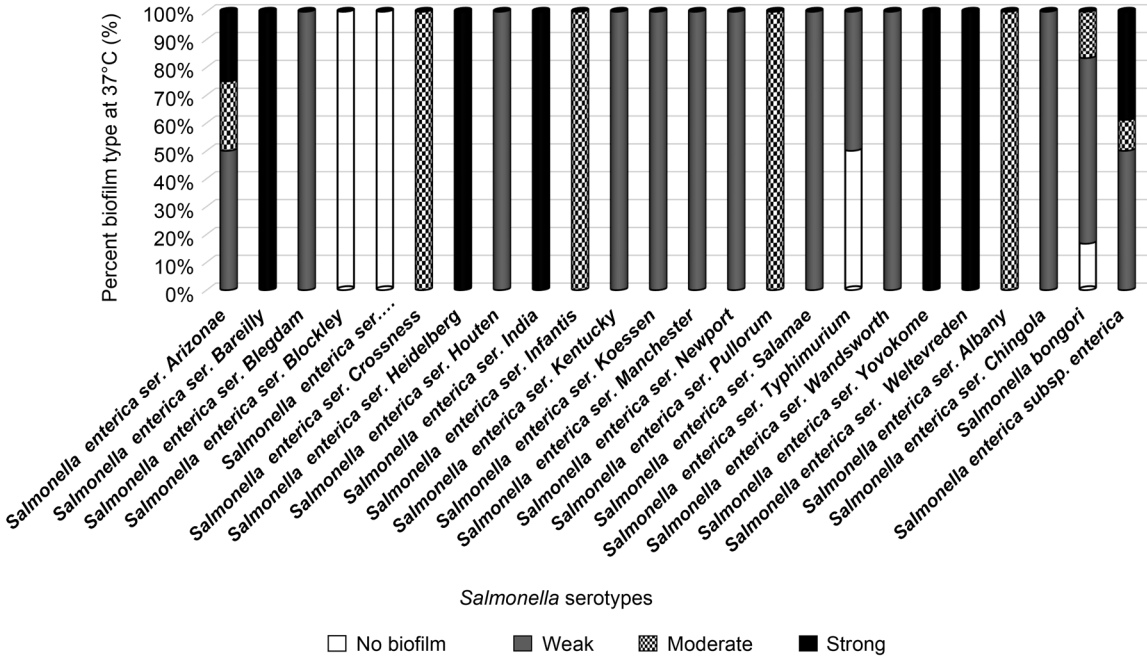

Fig. 3. Behavioral pattern of *Salmonella* serotypes to biofilm production at 37°C incubation temperature.

moderate biofilms. However, *Salmonella* serotypes Crossness and Manchester could only produce moderate biofilms at 25°C and 37°C. *Salmonella* Pullorum, *Salmonella* Albany, and *Salmonella* Infantis could only produce moderate biofilms at 37°C, while *Salmonella* Bovismobifacens, *Salmonella* Kentucky, and *Salmonella Salamae* produced moderate biofilms at 40°C.

Furthermore, fifty percent of total *Salmonella* Heidelberg, *Salmonella* Arizonae, *Salmonella* Typhimurium, and *Salmonella* Arizonae (25%) were weak biofilms producers at 25°C, 37°C, and 40°C, while *Salmonella* Yovokome, *Salmonella* Wandsworth, and *Salmonella* Blockley were all weak biofilm producers at 25°C. Weak biofilm formation by *Salmonella* serotypes is indicative of decreased potentials of adherence to surfaces, auto-aggregation among cells, and increased sensitivity to biocides treatments (Rendueles et al. 2013). Eleven percent of *Salmonella enterica* subsp. *enterica*, *Salmonella*

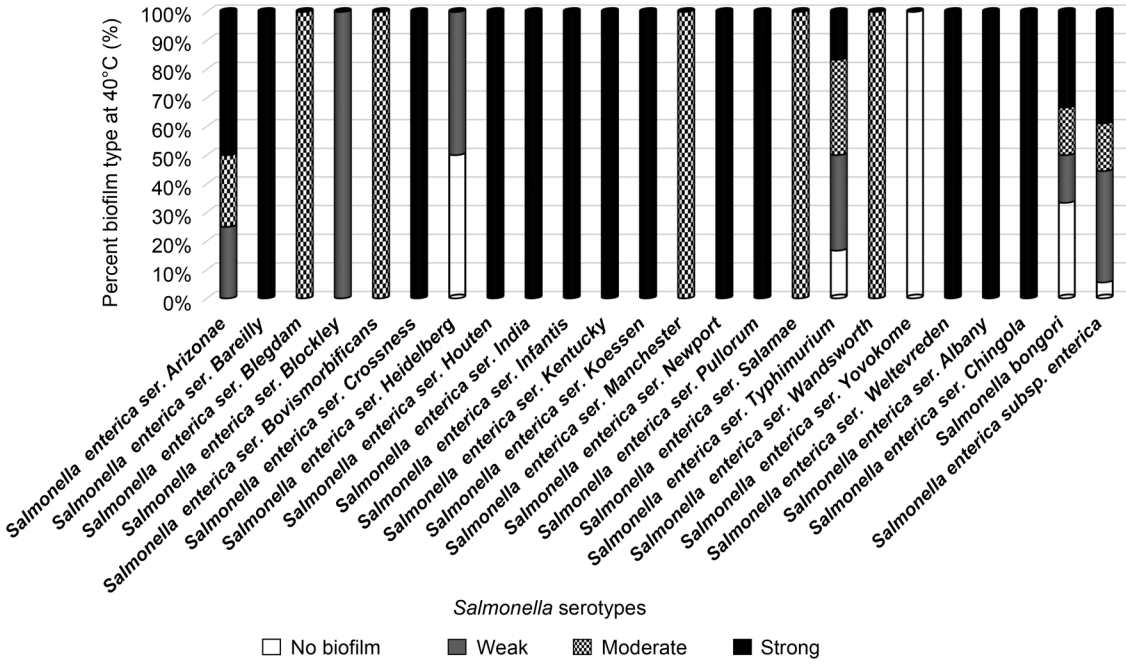

Fig. 4. Behavioral pattern of *Salmonella* serotypes to biofilm production at 40°C incubation temperature.

Typhimurium (16.7%) and *Salmonella bongori* (33.3%) isolates were non-biofilm producers at 25°C and 40°C while at 37°C *Salmonella* Typhimurium (50%) lost their biofilm producing abilities. The potentials of bacteria to form biofilms on food contact surfaces have been related to the type of media or substrate, incubation time, and type of microorganisms (Díez-García et al. 2012). The detection of biofilm-producing *Salmonella* serotypes isolated from chicken in this study corroborates the previous reports of Wang et al. (2013) on the occurrence and isolation of biofilm-forming *Salmonella* isolated from chicken processing surfaces in China. Similarly, biofilm-forming *Salmonella* has been isolated from tomatoes (Iturriaga et al. 2007), cereals (Cui et al. 2015), and almond (Suehr et al. 2015). The dependence of temperature and *Salmonella* type on the quality of biofilm formation agrees with the report of Shi and Zhu (2009) on the dependence of *Salmonella* type and environmental factors on the quality, quantity, and ability of *Salmonella* to form biofilms.

Similar to the observation made in this study, Almaguer-Flores (2013) has reported the influence of nutrient medium and bacterial cell characteristics on biofilm formation. In this study, the quality of biofilm formed by *Salmonella* serotypes was a function of the *Salmonella* serotype involved in biofilm formation. The process of biofilm formation is such a vibrant process whereby bacterium attaches itself to another cell of similar or different strains or onto surfaces, thereby producing an exopolysaccharides matrix through which they achieve survival against antibiotics or detergents (Tanaka et al. 2017). This process is affected by factors such as availability of nutrient/growth medium, pH, temperature, hydrodynamics of cells, and the hydrophobicity of contact surfaces (Irie and Parsek 2008; Dourou et al. 2011). Biofilms are extracellular polymeric substances that facilitate the interaction between bacterial cells and surfaces, which are important for the stability and survival of bacteria colonies (Olaya et al. 2013). Several authors have reported the production of biofilms in bacteria such as *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *E. coli* O157:H7, *Campylobacter* spp. and *Salmonella* Typhimurium, *Salmonella* Enteritidis to mention but a few (Zogaj et al. 2001; Solano et al. 2002; Olaya et al. 2013; Chen et al. 2015; Yang et al. 2016; Li et al. 2017). Some strains of *Salmonella* Typhimurium isolated in this study do not produce biofilms, contrary to the previous report of Solano et al. (2002) on biofilm production in *Salmonella* Typhimurium. This observation may be due to genetic variation within the genetic make-up of *Salmonella* serotypes used in the investigation.

Table III presents the Pearson correlation between biofilm-forming potentials of *Salmonella* serotypes as influenced by incubation temperatures. The Pearson correlation coefficients ranged from 0.17 to 0.50. The correlation coefficient ($r = 0.50$) was highest between the biofilm-forming potentials obtained at 25°C and 40°C, indicating a significant temperature-dependent association. A positive correlation existed between the biofilm-forming potentials of *Salmonella* serotypes incubated at 25°C, 37°C, and 40°C. A significant positive correlation exists between *Salmonella* biofilm production at 25°C and 37°C ($p \leq 0.05$), while a positive and

Table III
Pearson correlations between biofilm production potential of *Salmonella* serotypes incubated at varied temperatures.

Incubation temperatures		25°C	37°C	40°C
25°C	Pearson correlation	1	0.170*	0.501**
	p-value		0.021	0.000
37°C	Pearson correlation	0.170*	1	0.263**
	p-value	0.021		0.000
40°C	Pearson correlation	0.501**	0.263**	1
	p-value	0.000	0.000	

* – correlation is significant at the 0.05 level (2-tailed)
** – correlation is significant at the 0.01 level (2-tailed)

moderate correlation exists between biofilms formed at 25°C and 40°C ($p \leq 0.01$). Similarly, a positive correlation exists between biofilm formed at 37°C and 40°C at $p \leq 0.01$ with a Pearson correlation coefficient of 0.263. The closer the correlation coefficient to unity the higher the relationship that exists between variables (Benesty et al. 2009; Mukaka 2012). However, a positive correlation, as observed in this study between *Salmonella* biofilm formed at different incubation temperatures, is implicative of a temperature-dependent association; hence, biofilm formation in *Salmonella* serotypes are temperature dependent.

Microbial biofilms are composed of exopolysaccharide matrices that aid the survival and breeding of new bacteria when exposed to harsh environments (Ikuma et al. 2013). Biofilm formation is an adaptation strategy to evade antibiotics or disinfectant treatment in biofilm, producing virulent strains (Patel 2005). Biofilm formation by microorganisms could enhance pathogenicity and provoke food safety issues. Bacterial biofilms make stronger the defense systems of bacterial pathogens to antibiotic treatments (Stewart and Costerton 2001; Patel 2005). Antibiotic resistance could threaten good health, increase economic burden and poverty on both processors and consumers of food products, especially in the developing countries. The presence of selected *Salmonella* serotypes in foods could cause the development of biofilms, which could resist antimicrobial treatment and, thereby, cause ill-health. The control of biofilm through the use of processing plant cleaning and sanitation operations in the poultry industries has become a difficult task due to the associative resistance of *Salmonella* to disinfectants and antimicrobials (Merino et al. 2019). Also, the inaccessibility of antimicrobials to equipment crevices and parts has limited plant sanitation; hence, the use of well-designed and cleaning efficient equipment is important to effectively control biofilm formation (Chmielewski and Frank 2004; Merino et al. 2019). The prevention of biofilm formation still remains the best strategy to control *Salmonella* biofilms (Merino et al. 2019). The combined use of antimicrobials and disinfectant having a broad spectrum has been recommended for *Salmonella* biofilm control in the poultry plants, which resulted in the use of triclosan, nanomaterials, halogenated furanones, antibiotics, disinfectants, and quaternary ammonium salts (Bridier et al. 2011; Steenackers et al. 2012). However, *Salmonella* biofilms formation on food contact surfaces and food processing equipment could increase the cost of cleaning operations in plants. The increased cost of production could lead to an increased cost of food products, which affects consumers' purchasing power, thereby casting a burden on the low- and middle-class income earners. Thus the inactivation of biofilm producers is important to ensure food safety and public health.

Conclusions

Salmonella serotypes isolated from chickens do have the potential to produce biofilms ranging from strong to no biofilm. *Salmonella* Heidelberg, *Salmonella enterica* subsp. *enterica* and *Salmonella* Weltevreden were the highest producers of strong biofilms at 25°C, 37°C and 45°C. A significant positive correlation exists between *Salmonella* biofilm production at 25°C, 37°C, and 40°C. The biofilm production potentials of *Salmonella* are both serotypes and temperature dependent. Ambient temperature (25°C) favors *Salmonella* biofilm formation than at a much higher temperature. This poses a concern to food quality and safety in homes, small and medium scale food enterprises where there is a limit to the power supply, especially in developing countries. The findings from this study are quite important for global tracking on the state of *Salmonella* serotypes biofilms formation and develop effective control strategies as some similar serotypes isolated from this study have been reported in other countries. The detection of strong *Salmonella* biofilm formers in chickens found within the North West province, South Africa, also calls for concern as biofilms forming pathogens are capable of evading antimicrobial treatment. However, a broader screening will be important to further provide information on this subject in other provinces within South Africa. Similarly, the investigation on the relationship between pathogenicity, multiple antibiotic resistance behaviors of *Salmonella* serotypes, and biofilm formation might be necessary to further knowledge in this field.

ORCID
Stephen Abiola Akinola https://orcid.org/0000-0001-9361-7680
Mpinda Edoaurd Tshimpamba
https://orcid.org/0000-0003-4469-3200
Mulunda Mwanza https://orcid.org/0000-0002-9311-6517
Collins Njie Ateba https://orcid.org/0000-0003-1230-5138

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Authors' contributions

Conceptualization: Stephen Abiola Akinola; Methodology: Stephen Abiola Akinola, Collins Njie Ateba, Mulunda Mwanza; Formal analysis and investigation: Stephen Abiola Akinola; Writing – original draft preparation: Stephen Abiola Akinola, Mpinda Edoaurd Tsimpamba, Collins Njie Ateba, Mulunda Mwanza under Original draft preparation.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Akinola SA, Mwanza M, Ateba CN. Occurrence, genetic diversities and antibiotic resistance profiles of *Salmonella* serovars isolated from chickens. Infect Drug Resist. 2019;12:3327–3342. https://doi.org/10.2147/idr.s217421

Almaguer-Flores A. Biofilms in the oral environment. In: Yan Y, editor. Bio-tribocorrosion in biomaterials and medical implants. Duxford (UK): Woodhead Publishing. 2013 Jan 1; p. 169–186. https://doi.org/10.1533/9780857098603.2.169

Anderson TC, Nguyen TA, Adams JK, Garrett NM, Bopp CA, Baker JB, McNeil C, Torres P, Ettestad PJ, Erdman MM, Brinson DL. Multistate outbreak of human *Salmonella* Typhimurium infections linked to live poultry from agricultural feed stores and mail-order hatcheries, United States 2013. One Health. 2016 Dec 1;2: 144–149. https://doi.org/10.1016/j.onehlt.2016.08.002

Arunasri K, Mohan SV. Biofilms: microbial life on the electrode surface. In: Mohan SV, Pandey A, Varjani S, editors. Microbial electrochemical technology sustainable platform for fuels, chemicals and remediation, biomass, biofuels and biochemicals. Amsterdam (Netherlands): Elsevier. 2019 Jan 1; p. 295–313. https://doi.org/10.1016/b978-0-444-64052-9.00011-x

Balbontín R, Vlamakis H, Kolter R. Mutualistic interaction between *Salmonella enterica* and *Aspergillus niger* and its effects on *Zea mays* colonization. Microb Biotechnol. 2014 Nov;7(6):589–600. https://doi.org/10.1111/1751-7915.12182

Benesty J, Chen J, Huang Y, Cohen I. Pearson correlation coefficient. In: Noise reduction in speech processing. Springer Topics in Signal Processing, vol 2. Berlin, Heidelberg (Germany): Springer. 2009; p. 1–4. https://doi.org/10.1007/978-3-642-00296-0_5

Borges KA, Furian TQ, Souza SN, Menezes R, Tondo EC, Salle CT, Moraes HL, Nascimento VP. Biofilm formation capacity of *Salmonella* serotypes at different temperature conditions. Pesqui Vet Brasil. 2018 Jan;38(1):71–76. https://doi.org/10.1590/1678-5150-pvb-4928

Bridier A, Briandet R, Bouchez T, Jabot F. A model-based approach to detect interspecific interactions during biofilm development. Biofouling. 2014 Aug 9;30(7):761–771. https://doi.org/10.1080/08927014.2014.923409

Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Resistance of bacterial biofilms to disinfectants: a review. Biofouling. 2011 Oct 15;27(9):1017–1032. https://doi.org/10.1080/08927014.2011.626899

Byrd-Bredbenner C. Food Safety. In: Rippe J, editor. Nutrition in Lifestyle Medicine. Nutrition and Health. Humana Press, Cham. 2017; p. 413–422. https://doi.org/10.1007/978-3-319-43027-0_23

Chen D, Zhao T, Doyle MP. Single-and mixed-species biofilm formation by *Escherichia coli* O157:H7 and *Salmonella*, and their sensitivity to levulinic acid plus sodium dodecyl sulfate. Food Control. 2015 Nov 1;57:48–53. https://doi.org/10.1016/j.foodcont.2015.04.006

Chmielewski RA, Frank JF. A predictive model for heat inactivation of *Listeria monocytogenes* biofilm on buna-N rubber. LWT-Food Sci Technol. 2006 Jan 1;39(1):11–19. https://doi.org/10.4315/0362-028x-67.12.2712

Cook A, Odumeru J, Lee S, Pollari F. *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, verotoxigenic *Escherichia coli*, and *Escherichia coli* prevalence, enumeration, and subtypes on retail chicken breasts with and without skin. J Food Protect. 2012 Jan;75(1):34–40. https://doi.org/10.4315/0362-028x.jfp-11-206

Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999 May 21;284(5418): 1318–1322. https://doi.org/10.1126/science.284.5418.1318

Cui Y, Walcott R, Chen J. Attachment of various serovars of *Salmonella enterica* to vegetable seeds with different surface characteristics. Presented at: International Association for Food Protection (IAFP) Annual Meeting. IAFP 2015; Portland, OR.

Dallal MM, Doyle MP, Rezadehbashi M, Dabiri H, Sanaei M, Modarresi S, Bakhtiari R, Sharifiy K, Taremi M, Zali MR, Sharifi-Yazdi MK. Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolated from retail chicken and beef, Tehran, Iran. Food Control. 2010 Apr 1;21(4):388–392. https://doi.org/10.1016/j.foodcont.2009.06.001

Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol R. 2000 Dec 1;64(4):847–867. https://doi.org/10.1128/mmbr.64.4.847-867.2000

Díez-García M, Capita R, Alonso-Calleja C. Influence of serotype on the growth kinetics and the ability to form biofilms of *Salmonella* isolates from poultry. Food Microbiol. 2012 Sep 1;31(2):173–180. https://doi.org/10.1016/j.fm.2012.03.012

Dourou D, Beauchamp CS, Yoon Y, Geornaras I, Belk KE, Smith GC, Nychas GJ, Sofos JN. Attachment and biofilm formation by *Escherichia coli* O157:H7 at different temperatures, on various food-contact surfaces encountered in beef processing. Int J Food Microbiol. 2011 Oct 3;149(3):262–268. https://doi.org/10.1016/j.ijfoodmicro.2011.07.004

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 2018 Dec;16(12):e05500. https://doi.org/10.2903/j.efsa.2011.2090

Floyd KA, Eberly AR, Hadjifrangiskou M. Adhesion of bacteria to surfaces and biofilm formation on medical devices. In: Deng Y, Lv W, editors. Biofilms and implantable medical devices. Duxford (UK): Woodhead Publishing. 2017; p. 47–95. https://doi.org/10.1016/b978-0-08-100382-4.00003-4

Giaouris E, Chorianopoulos N, Skandamis P, Nychas GJ. Attachment and biofilm formation by *Salmonella* in food processing environments. In: Mahmoud BSM, editor. *Salmonella*: A dangerous foodborne pathogen. InTech. 2012; p. 157–180. https://doi.org/10.5772/28107

Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS, Hart CA, Molyneux ME. Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* Serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. Clin Infect Dis. 2008 Apr 1;46(7):963–969. https://doi.org/10.1086/529146

Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004 Feb;2(2):95–108. https://doi.org/10.1038/nrmicro821

Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cell Microbiol. 2009 Jul;11(7):1034–1043. https://doi.org/10.1111/j.1462-5822.2009.01323.x

Hur J, Jawale C, Lee JH. Antimicrobial resistance of *Salmonella* isolated from food animals: A review. Food Res Int. 2012 Mar 1;45(2): 819–830. https://doi.org/10.1016/j.foodres.2011.05.014

Ikuma K, Decho AW, Lau BL. The extracellular bastions of bacteria – a biofilm way of life. Nature Education Knowledge. 2013;4(2):2–19.

International Organization for Standardization. Microbiology of food and animal feeding stuffs: horizontal method for the detection of *Salmonella* spp. Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. Amendment 1, Annex D. Geneva (Switzerland): ISO. 2007.

Irie Y, Parsek MR. Quorum sensing and microbial biofilms. In: Romeo T, editor. Bacterial Biofilms. Current Topics in Microbiology and Immunology, vol 322. Berlin, Heidelberg (Germany): Springer. 2008; p. 67–84. https://doi.org/10.1007/978-3-540-75418-3_4

Isoken HI. Biofilm formation of *Salmonella* species isolated from fresh cabbage and spinach. JAppl Sci Environ Manage. 2015;19(1): 45–50. https://doi.org/10.4314/jasem.v19i1.6

Iturriaga MH, Tamplin ML, Escartin EF. Colonization of tomatoes by Salmonella Montevideo is affected by relative humidity and storage temperature. J Food Protect. 2007 Jan;70(1):30–34. https://doi.org/10.4315/0362-028x-70.1.30

Karkey A, Jombart T, Walker AW, Thompson CN, Torres A, Dongol S, Tran Vu Thieu N, Pham Thanh D, Tran Thi Ngoc D, Voong Vinh P, Singer AC. The ecological dynamics of fecal contamination and *Salmonella* Typhi and *Salmonella* Paratyphi A in municipal Kathmandu drinking water. PLoS Neglect Trop D. 2016 Jan 6;10(1):e0004346. https://doi.org/10.1371/journal.pntd.0004346

Khan MS, Altaf MM, Ahmad I. Chemical nature of biofilm matrix and its significance. In: Ahmad I, Husain FM, editors. Biofilms in Plant and Soil Health. Hoboken (USA): John Wiley & Sons Ltd. 2017. https://doi.org/10.1002/9781119246329.ch9

Koo H, Yamada KM. Dynamic cell-matrix interactions modulate microbial biofilm and tissue 3D microenvironments. Curr Opin Cell Biol. 2016 Oct 1;42:102–112. https://doi.org/10.1016/j.ceb.2016.05.005

Kroupitski Y, Golberg D, Belausov E, Pinto R, Swartzberg D, Granot D, Sela S. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. Appl Environ Microb. 2009 Oct 1;75(19):6076–6086. https://doi.org/10.1128/aem.01084-09

Li J, Feng J, Ma L, de la Fuente Núñez C, Gölz G, Lu X. Effects of meat juice on biofilm formation of *Campylobacter* and *Salmonella*. Int J Food Microbiol. 2017 Jul 17;253:20–28. https://doi.org/10.1016/j.ijfoodmicro.2017.04.013

Li YH, Tian X. Quorum sensing and bacterial social interactions in biofilms. Sensors. 2012 Mar;12(3):2519–2538. https://doi.org/10.3390/s120302519

Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001 Jan 1;9 (1):34–39. https://doi.org/10.1016/s0966-842x(00)01913-2

Malangu N, Ogunbanjo GA. A profile of acute poisoning at selected hospitals in South Africa. South Afr J Epidemiol Infect. 2009 Jan 1;24(2):14–16.

Merino L, Procura F, Trejo FM, Bueno DJ, Golowczyc MA. Biofilm formation by *Salmonella* sp. in the poultry industry: Detection, control and eradication strategies. Food Res Int. 2019 May 1;119:530–540. https://doi.org/10.1016/j.foodres.2017.11.024

Moosdeen F, Williams JD, Secker A. Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. J Antimicrob Chemoth. 1988 Apr 1;21(4): 439–443. https://doi.org/10.1093/jac/21.4.439

Motladiile TW. *Salmonella* food-poisoning outbreak linked to the National School Nutrition Programme, North West province, South Africa. South Afr J Infect Dis. 2019;34(1):1–6. https://doi.org/10.4102/sajid.v34i1.124

Mukaka MM. A guide to appropriate use of correlation coefficient in medical research. Malawi Med J. 2012;24(3):69–71.

Mulamattathil SG, Bezuidenhout C, Mbewe M. Biofilm formation in surface and drinking water distribution systems in Mafikeng, South Africa. S Afr J Sci. 2014 Dec;110(11–12):01–09. https://doi.org/10.1590/sajs.2014/20130306

Muvhali M, Smith AM, Rakgantso AM, Keddy KH. Investigation of *Salmonella* Enteritidis outbreaks in South Africa using multilocus variable-number tandem-repeats analysis, 2013–2015. BMC Infect Dis. 2017 Dec 1;17(1):661. https://doi.org/10.1186/s12879-017-2751-8

Niehaus AJ, Apalata T, Coovadia YM, Smith AM, Moodley P. An outbreak of foodborne salmonellosis in rural KwaZulu-Natal, South Africa. Foodborne Pathog Dis. 2011 Jun 1;8(6):693–697. https://doi.org/10.1089/fpd.2010.0749

Papa R, Bado I, Iribarnegaray V, Gonzalez MJ, Zunino P, Scavone P, Vignoli R. Biofilm formation in carbapenemase-producing *Pseudomonas* spp. and *Acinetobacter baumannii* clinical isolates. Int J Infect Dis. 2018 Aug 1;73:119–120. https://doi.org/10.1016/j.ijid.2018.04.3688

Patel R. Biofilms and antimicrobial resistance. Clin Orthop Relat R. 2005 Aug 1;437:41–47. https://doi.org/10.1097/01.blo.0000175714.68624.74

Rendueles O, Kaplan JB, Ghigo JM. Antibiofilm polysaccharides. Environ Microbiol. 2013 Feb;15(2):334–346. https://doi.org/10.1111/j.1462-2920.2012.02810.x

Römling U, Sierralta WD, Eriksson K, Normark S. Multicellular and aggregative behaviour of *Salmonella typhimurium* strains is controlled by mutations in the agfD promoter. Mol Microbiol. 1998 Apr;28(2):249–264. https://doi.org/10.1046/j.1365-2958.1998.00791.x

Sadekuzzaman M, Yang S, Mizan MF, Ha SD. Current and recent advanced strategies for combating biofilms. Compr Rev Food Sci F. 2015 Jul;14(4):491–509. https://doi.org/10.1111/1541-4337.12144

Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist In. 2019 Dec;8(76):1–10. https://doi.org/10.1186/s13756-019-0533-3

Shi X, Zhu X. Biofilm formation and food safety in food industries. Trends Food Sci Tech. 2009 Sep 1;20(9):407–413. https://doi.org/10.1016/j.tifs.2009.01.054

Silagyi K, Kim SH, Lo YM, Wei CI. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. Food Microbiol. 2009 Aug 1;26(5):514–519. https://doi.org/10.1016/j.fm.2009.03.004

Sofos JN, Geornaras I. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. Meat Sci. 2010 Sep 1;86(1):2–14. https://doi.org/10.1016/j.meatsci.2010.04.015

Solano C, García B, Valle J, Berasain C, Ghigo JM, Gamazo C, Lasa I. Genetic analysis of *Salmonella enteritidis* biofilm formation: critical role of cellulose. Mol Microbiol. 2002 Feb;43(3):793–808. https://doi.org/10.1046/j.1365-2958.2002.02802.x

Soo Lee I, Lin J, Hall HK, Bearson B, Foster JW. The stationary-phase sigma factor σS (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella* Typhimurium. Mol Microbiol. 1995 Jul;17(1):155–167. https://doi.org/10.1111/j.1365-2958.1995.mmi_17010155.x

Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SC. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. Food Res Int. 2012 Mar 1;45(2):502–531. https://doi.org/10.1016/j.foodres.2011.01.038

Stepanović S, Ćirković I, Ranin L, Svabić-Vlahović M. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol. 2004 May;38(5):428–432. https://doi.org/10.1111/j.1472-765x.2004.01513.x

Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Meth. 2000 Apr 1;40(2):175–179. https://doi.org/10.1016/s0167-7012(00)00122-6

Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001 Jul 14;358(9276):135–138. https://doi.org/10.1016/s0140-6736(01)05321-1

Suehr Q, Jeong S, Marks BP. Modeling of cross-contamination of *Salmonella* during almond processing. Presented at: International Association for Food Protection (IAFP) Annual Meeting; IAFP 2015; Portland, OR.

Tanaka MH, Lima GM, Ito CK. Biology of the oral environment and its impact on the stability of dental and craniofacial reconstructions. In: Spencer P, Misra A, editors. Material-tissue interfacial phenomena. Duxford (UK): Woodhead Publishing. 2017; p. 181–202. https://doi.org/10.1016/b978-0-08-100330-5.00007-8

Wang H, Ye K, Wei X, Cao J, Xu X, Zhou G. Occurrence, antimicrobial resistance and biofilm formation of *Salmonella* isolates from a chicken slaughter plant in China. Food Control. 2013 Oct 1;33(2):378–384. https://doi.org/10.1016/j.foodcont.2013.03.030

Webber B, Oliveira AP, Pottker ES, Daroit L, Levandowski R, Santos LR, Nascimento VP, Rodrigues LB. *Salmonella* Enteritidis forms biofilm under low temperatures on different food industry surfaces. Ciênc Rural. 2019;49(7):e20181022. https://doi.org/10.1590/0103-8478cr20181022

Yang Y, Mikš-Krajnik M, Zheng Q, Lee SB, Lee SC, Yuk HG. Biofilm formation of *Salmonella* Enteritidis under food-related environmental stress conditions and its subsequent resistance to chlorine treatment. Food Microbiol. 2016 Apr 1;54:98–105. https://doi.org/10.1016/j.fm.2015.10.010

Zogaj X, Nimtz M, Rohde M, Bokranz W, Römling U. The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. Mol Microbiol. 2001 Mar;39(6):1452–1463. https://doi.org/10.1046/j.1365-2958.2001.02337.x