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Evaluation of Oxidative Stress Adaptability of Typhoidal Salmonella from Patients Suspected of Enteric Fever in Bauchi, Nigeria

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Abstract—***Introduction.*** *Salmonella typhi has the ability to effectively resist stress damage due to exposure to stressors, it may also be observed in may bacteria in their response to some cytotoxic agents. The aim of the study was to evaluate the oxidative stress adaptability of typhiodal salmonella and molecular detection of the regulon gene involved in adaption of the oxidative stress in clinical specimen in Tertiary Hospital of Bauchi State, Nigeria.* ***Methodology****. The blood specimens were cultured in thioglycollate broth and sub-cultured onto deoxycholate citrate agar (DCA), Salmonella-Shigella agar (SSA) followed by confirmation of presumptive colonies using different biochemical tests. Serologic identification of Salmonella was performed by slide agglutination test using polyvalent O and H Salmonella antisera. Stress effect on growth was done with the supplementation of 2.5ml hydrogen peroxide (H_2O_2) at the late log phase and the cell viability was simultaneously retraced through culture in agar plates for up to 72hr Subsequent changes in cell morphology and arrangements were monitored. HotStart Polymerase chain reaction (PCR) was also done to detect the gene involve in the stress adaptability.* ***Result.*** *Seven isolates of salmonella typhi employed and impact of H_2O_2 were insignificant on their growth measuring the turbidity. Growth and colony morphology was also observed by culture after treatment of H_2O_2 and was found to be unaffected by the H_2O_2 treatment. Molecularly, the gene (RpoE) was detected in the seven isolates suggestive of a stringent defense mechanism against oxidative stress.* ***Conclusion****. Data obtained clearly showed new information on the defense strategies of Salmonella typhi against oxidative stress. Salmonella formed CFUs with the absence of aggregates in culture media, the cell morphology of the salmonella found to be completely unaffected by the oxidant which supported the maintenance of cell culturability of Salmonella. While the bacteria resist the oxidative stress, molecular detection on rpoE in all the seven salmonella typi isolates shows the presence of the regulatory gene in them.*

Keywords— *Salmonella, Gene, Typhoidal, Superoxide, Primer, Polymerase.*

I. INTRODUCTION

Oxidative stress adaptability of *salmonella typhi* (*S. typhi*) is referred to its ability to effectively resist stress damage by exposure to a few amount of stress; it may be observed in many bacteria in response to a numbers of cytotoxic agents. Demonstration of a various critical genes for enzymatic defense and DNA repair in especially in *Escherishia colli (E. colli)* has proven the involvement of certain gene in resistance and adaptation to phagocytes-derived oxidant stress (PreetiMalik-Kale *et al.*, 2011). Some *Salmonella* strains have developed an array of sophisticated tools to manipulate the host cell and establish an intracellular niche, for successful propagation as a facultative intracellular pathogen. Salmonella affects its host cell in a variety of ways, but only the cell biology of the traditional "trigger" -mediated invasion process and the subsequent growth of the *Salmonella*-containing vacuole have been well studied (PreetiMalik-Kale *et al.,* 2011). These processes are dependent on cohorts of effector proteins translocated into host cells by two type III secretion systems (T3SS), although T3SS-independent mechanisms of entry may be important for invasion of certain host cell types. Recent studies into the intracellular lifestyle of Salmonella have provided new insights into the mechanisms used by this pathogen to modulate its intracellular environment (PreetiMalik-Kale *et al.*, 2011).

The extended intracellular survival in macrophages is the main distinction between Typhi and the other serotypes. This is due to the organism's ability to inhibit the oxidative metabolic burst and continue to multiply. As the bacterial population grows over time, it finally starts to overflow into the bloodstream. The onset of the fever is caused by the introduction of Gram-negative bacteria and their lipopolysaccharide (LPS) endotoxin into the blood; the fever then gradually rises and persists as long as *S. typhi* is still being sown this sometimes results in metastatic infection of other organs including the urinary tract and the biliary tree. The latter causes reinfection of the bowel. This cycle beginning and ending in the small intestine takes approximately 2 weeks to complete (Kenneth *et al*., 2014). In the cause of phagocytosis, bacterial are faced with different toxic such as superoxide and other reactive oxygen species generated from immune cells. Extensive studies has been done on temperature stress, bile-salt and nutrient starvation but Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are the forms of reactive oxygen species (ROS)

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that were least studied, they are constantly produced endogenously through the autoxidation of O_2 on a range of both aerobic and non-aerobic respiratory flavoproteins (Imlay, 1995; Messner and Imlay, 1999; Kussmaul and Hirst, 2006; Korshunov and Imlay, 2010), as well as on non-respiratory flavoproteins (Massey *et al.*, 1969; Geary and Meister, 1977; Grinblat *et al.,* 1991). To protect the cells against these harmful compounds, bacteria evolved enzymes termed superoxide dismutases (SODs) to convert O_2^- into O_2 and H_2O_2, as well as catalyses and peroxides to remove H_2O_2 to continuously neutralize the endogenously produced ROS (Imlay, 2008). In most bacteria sigma factor -32 encoded by the RpoH gene regulates the responses to temperature stress, although RpoE, which encodes sigma factor σ^E, was reported to be a very important regulator in the maximal survival of enteric pathogens Most work on the RpoE regulon has been in the closely related enterobacterial species *Escherichia coli*. It remain unclear whether RpoE is present in *S. typhi* and involve in the oxidative stress resistance. Several studies involving *E. coli* RpoE gene has been done on stress including cold shock but there are few data to show for typhoidal *Salmonella* and oxidative stress involving RpoE (David *et al.,* 2019).

We hypothesized that there is no significant effect between the *Salmonella typhi* isolated and hydrogen peroxide on the survival of the bacterial isolated from patients suspected of enteric fever in the study area as well the resistance gene, based on the above hypothesis. Therefore we examined the oxidative stress adaptability of typhoidal salmonella in-vitro and the presence of its regulatory gene (RpoE) in patients who presented symptomes of enteric fever using molecular method.

II. Materials and Methods

Specimen collection and processing. A total of 150 samples were collected from patients that presented the symptomes of enteric fever in Abubakar Tafawa Balewa University Teaching Hospital Bauchi, Nigeria (ATBUTH). Blood were drawn aseptically using 5 ml syringe and needle (Walia *et al.,* 2006). After disinfecting the site with methylated spirit. The antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was applied a few centimeters above the antecubital fossa to distend the vein; about 4ml of blood was collected from patients. The blood was aseptically transferred into 8ml tetrathionate broth tube and incubated (Walia *et al.,* 2006). For stool specimen, 2g of the stool were transferred unto tubes containing 8ml of Selenite F Broth and then incubated at 37 ºC for 24 hours (Walia *et al.,* 2006).

Processing of blood and stool specimen. The blood collected in tetrathionate broth were incubated overnight at 37 ºC for 18 to 24 hours. Tubes that showed turbidity were sub-cultured each from each of the containers unto freshly prepared and dried Salmonella-Shigella agar (SSA) and MacConkey agar, Brilliant Green agar and incubated at 37 °C for 18-24 hours. For stool samples, they were first inoculated into the enrichment medium (selenite–F broth) and after incubation for 24 hours at 37 ºC, each were sub-cultured in SSA, Brilliant Green agar (BGA), and MacConkey agar (MCA). The SSA, BGA, and MCA plates were incubated overnight at 37 ºC (Sur *et al.,* 2007).

Biochemical characterization of isolates; sugar fermentation tests, Nutrient broth cultures were prepared. Bijou bottles containing the basal medium and appropriate prepared carbohydrate (mannitol, maltose, dulcitol, sucrose and glucose) were inoculated with the drop of nutrient broth suspension of the test isolate and then loosely capped and incubated at 35 ºC overnight. Each of the bottles was observed for change in color from amber to red and for gas production (in the medium filled inverted Durham tube) (Hansen, 2017).

Urease test. The test bacteria were inoculated heavily on the entire slope surface of the urea agar slants prepared in caped tubes. The tubes were placed in racks and incubated at 37 ºC up to 48 hours. Tubes were thereafter examined for change of color from plain to pink (Sur *et al.,* 2007).

Indole test. Tube containing 5 ml of the tryptone/tryptophan medium was inoculated with the suspected colony and Incubated at 37 °C for 24 h. After incubation, One (1) ml of the Kovacs reagent was added. Formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction (Sur *et al.,* 2007).

Hydrogen sulphide production. The Test bacterial were inoculated into the triple sugar iron agar slants contained in the test tubes. These were incubated at 35- 37 °C for up to 48 hours. After incubation, the triple sugar ion agar (TSI) agar media was checked for blackening and change in colour from amber to red at the bottom of the tube (Sur *et al.,* 2007).

*Serological identification (serotyping).*Suspected colonies were picked and sub-cultured unto moist nutrient agar slopes in MacCartney bottles. These were then incubated for minimum of 4 hours. One to two loop-full of the agar cultures was mixed with normal saline on clean microscope slides to form a paste. A drop each of the O and H polyvalent sera was added and further mixed with the organisms on the slide. Positive results are indicated by visible agglutination within 30 seconds. Slide tests were repeated for the positive cultures using single factor sera (Walia *et al.,* 2006).

Oxidative stress response invitro method for salmonella typhi. Confirmation of oxidative stress adaptability phenotype was done by phenotypic confirmatory H_2O_2 invitro method, 2.5ml H_2O_2 was aseptically added into one set of NB and the other set without H_2O_2 were considered as control (Kabir*et al.,* 2004; Munna *at al*., 213). At every 12 hours of interval, cell growth was monitored by measuring optical density (OD) and the formation of colony forming unit (CFU) was estimated counting the colony up to 72 hours at every 24 hours intervals (Munna *at al.,* 2013; Noor *et al*., 2013).

Genomic DNA extraction and purification. Glycerol stock of *Salmonella typhi* was streaked on Salmonella-shigella agar plate and incubated at 37º C for 24 hours. A single colony was inoculated in 10mL nutrient broth and incubated at 37º C with shaking at 210 rpm for 24 hours. Genomic DNA was extracted using the Accu prep Genomic DNA extraction kit from Bioneer according to the manufacturer's instruction as follows: 1ml of an overnight cultured was added to a 1.5ml micro centrifuged tube.600µl of Nuclei Lysis Solution was added and mixed immediately by vortex mixer then incubated at 80°C for 5 minutes to lyse the cells, and cooled to room temperature. 3µl of RNase solution was added to the cell lysate. The tubes were

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inverted 2–5 times to mixed and incubated at 37°C for 15–60 minutes and cooled at room temperature. 200µl of Protein precipitation solution was added to the RNase-treated cell lysate and vortex vigorously at high speed for 20 seconds to mix the Protein precipitation solution with the cell lysate, then incubated on ice for 5 minutes and centrifuged at 13,000–16,000× g for 3 minutes. The supernatant containing the DNA was transferred to a clean 1.5ml micro centrifuged tube containing 600µl of room temperature isopropanol. The later was gently mixed by inversion until the thread-like strands of DNA form a visible mass then centrifuged at 13,000–16,000 × g for 2 minutes**.** The supernatant was carefully pure off and drain the tube on clean absorbent paper, 600µl of room temperature70% ethanol was added and the tubes were gently inverted several times to wash the DNA pellet an centrifuged at 13,000–16,000 × g for 2 minutes and the ethanol was carefully aspirated.The tubes were drained on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.100µl of DNA Rehydration Solution was added to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. The solution was periodically mixed by gently tapping the tube and eluted genomic DNA was stored at 4℃.

Genomic DNA Purification. Microbial DNA was purified using the Mo Bio Power Clean Pro DNA Clean-Up Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions as follows:150 µl of DNA sample was added to 2 ml Collection Tube. 70 µl of PowerClean®DNA Solution1 was added to DNA and gently inverted 3-5 times to mix, 20 µl of PowerClean®DNA Solution 2 was added and inverted 3-5 times to mix.85 µl of PowerClean®DNA Solution 3 was added and inverted 3-5 times to mix and incubated at 4°C for 5 minutes. The **t**ubes were centrifuged at 10,000 x g for 1 minute at room temperature and the entire volume of supernatant was transfered to a clean 2 ml Collection Tube (provided)**.** 70 µl of PowerClean®DNA Solution 4 was added and inverted 3-5 times to mix and then Incubated at 4°C for 5 minutes and the tubes were centrifuged at 10,000 x g for 1 minute at room temperature. The entire volume of supernatant was transfered to a clean 2 ml Collection Tube (provided)800µl of PowerClean®DNA Solution 5 was added to the supernatant and vortex for 5 seconds. Approximately 600 µl was loaded onto Spun Filter and centrifuged at 10,000 xgfor 1 minute at room temperature, flow through was discarded and the remaining 600µl supernatant was added to Spun Filter and centrifuged at 10,000 x g for 1 minute at room temperature**.** 500 µl of PowerClean®DNA Solution 6 was added to Spun Filter and centrifuged at 10,000 x g for 30 seconds at room temperature. Spun filter was centrifuged at 13,000 x g for 1 minute at room temperature. The spun Filter was carefully placed in new 2 ml Collection Tube (provided).50 µl of PowerClean®DNA Solution 7 was added to center of white filter membrane and centrifuged at 10,000 x g for 30 seconds at room temperature. The spun Filter was discarded. The DNA in 2 ml Collection Tube application ready and frozen -20ºC.

Primer design.

The sequence of the genes was taken from NCBI. Specific primers targeting the full length of resistant gene to oxidative stress 181(5′-GTCTACAACATGACAAACAAAAACAAATGC) and 182(5′-TTTTCCAGTATCCCGCTATCGTCAACGC) with 599 base pair and 56ºC melting temperature was adopted from a research journal (Güssow, D., and T. Clackson. 2012) and validated using Fast PCR software (Kalendar *et al*., 2017). 3.12 Genes Amplification. The resistant gene to oxidative stress from the genomic DNA of *Salmonella typhi* was amplified using polymerase chain reaction (PCR). A total reaction volume of 50 µL was prepared, which composed of 2 µL of genomic DNA, 0.4 µM each of forward and reverse specific primers 25 µL of 2 X PCR Bestaq Master Mixes (Applied Biological Materials, Canada), and 19 µL of sterile de-ionised water. PCR amplification was carried out in a S1000TM thermal cycler (BIORad, USA) using an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of amplification with denaturation at 94 °C for 10 sec, annealing at 61 °C for 30 sec, and extension at 72 °C for 1 min, ending with a final extension at 72 °C for 5 min. (Kalendar*et al*., 2010).

*Sequencing and Analysis of 16srRNA.*Sequencing of 16S rRNA gene was conducted using genetic analyzer (ABI Prism 3130 and 3130 xl Genetic Analyzer) The sequencing was used universal primers. The sequences were edited to exclude the PCR primer binding sites and was manually corrected using MEGA 5.2 version software. The full gene sequences of the strains was compared automatically using BLAST against the sequences of bacteria available in databanks (http://www.ncbi.nlm.nih.gov/). The phylogenetic analysis was constructed using neighbor-joining algorithm (Kalendar *et al*., 2010).

Resistance Genes to Oxidative Stress from the Isolates. The presence of genes encoding rpoE type was detected by multiplex PCR using universal primers and conditions previously reported (Fang *et al.,* 2008). The PCR was conducted in a Thermal Cycler PXE-0.5 (THERMO; Electron Corporation) and the resulting PCR products were subjected to electrophoretic separation in 1.5% agarose gel. Visualization of amplicons was completed by staining with ethidium bromide (Sigma-Aldrich) (1 µg/ml) under UV transilluminator and photographed. DNA bands of each amplicon was compared with 100-bp DNA mass marker. (Fang *et al.,* 2008). Statistical analysis regarding bacterial growth was performed by determining the P-value through a t-test. Standard deviations were also measured.

III. Results

*S. Salmonella typhi in the presence of H_2O_2.*The growth of the seven (7) confirmed isolated bacteria upon treatment with 2.5ml of H_2O_2 is shown in table 1-7. Oxidative stress adaptability was done by phenotypic confirmatory H_2O_2 in-vitro method, 2.5ml H_2O_2 was aseptically added into one set of NB and the other set without H_2O_2 were considered as control (Kabir *et al*., 2004; Munna *at al*., 213). At every 12 hours of interval, cell growth was monitored by measuring optical density (OD) The test media showed steady growth up to 72 hour and confirmative demonstration of culturability and survival was done on nutrient media and *Salmonella typhi* continued to form colonies, the nonreactivity of these cells against H_2O_2 was

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confirmed by their growth sustainability (plate 1). All the data gotten from this study estimated as significant at P<0.1.

Table 1, shows that a total of 150 patients attending ATBUTH participated in the study. 95(63.3%) were males and 55(36.7%) were females. The mean age was 21.6 years and standard deviation (SD), 6.0. Nearly, one-third of the study patients 45(30%) were in the age category of 21-30 years. The majority of the study participants were urban 102(68.0%) residents. Concerning the marital status 84(56%) were married and 85(56.7%) had completed secondary school and above. The highest prevalence of *S. typhi* infection was observed among patients in the age group <10 years old was 5(20.8%). Based on marital status, 4(8.9%) single patients were positive for *S. typhi* infection. With regard to place of residence, 5% of patients who came from rural area were positive for *S. typhi* infection. Those patients with no formal education was 1(8.3%) for *S. typhi.* While Table 2, shows the morphological, cultural and biochemical characteristics of *Salmonella typhi.* For the 7 positive isolates, on MCA it shows pale, colorless, transparent, smooth and round colonies while on SSA its shows a characteristic opaque, translucent, smooth and raised (black colonies). The positive isolates shows no reaction with indole, urease but utilze citrate where color changed from green blue. For carbohydrate, only dextrose, maltose and mannose where fermented leaving lactose and sucrose unfermented.

Table 3, is antigen of *S. typhi* which the titer 1:160 is significant, the titer of 160 in each of the antigen of the bacteria in table is an indicative of presence of *S. typhi* However, the agglutination titer of 1:160 appears more at H antigen which is more reliable and is an indicative of the presence of the *S. typhi.* This is also in agreement with the findings of Adeleke *et al.* (2006) in which, Salmonella Typhi 'H' titers were higher than those of 'O'.

*S. Salmonella typhi in the presence of H_2O_2.*The growth of the seven (7) confirmed isolated bacteria upon treatment with 2.5ml of H_2O_2 is shown in table 1-4. Oxidative stress adaptability was done by phenotypic confirmatory H_2O_2 in-vitro method, 2.5ml H_2O_2 was aseptically added into one set of NB and the other set without H_2O_2 were considered as control (Kabir *et al*., 2004; Munna *at al*., 213). At every 12 hours of interval, cell growth was monitored by measuring optical density (OD) The test media showed steady growth up to 72 hour and confirmative demonstration of culturability and survival was done on nutrient media and *Salmonella typhi* continued to form colonies, the nonreactivity of these cells against H_2O_2 was confirmed by their growth sustainability (plate 1). All the data gotten from this study estimated as significant at P<0.1.

TABLE 1: Socio-demographic characteristics and its distribution of *S. typhi* of suspected enteric fever patients visiting ATBUTH Bauchi

Variables	No. of patients (%)	Number of positive for S. typhi (%)
Sex		
Male	95(63.3)	4(4.2)
Female	55(36.7)	3(5.5)
Age group		
Less than 10	24(16)	5(20.8)
11-20		0(0.0)
21-3031		2(4.4)
31-40	(20.7)	0(0.0)
More than 40	23(15.3)	0(0.0)
Residence		
Urban	102(68.0)	2(2.0)
Rural	48(32.0)	5(10.4)
Marital status		
Married	84(56)	2(2.4)
Single	27(18)	4(8.9)
Widowed and divorced	45(30)	1(4.8)
Educational level		
No formal education	12(12.7)	1(8.3)
Primary	53(35.3)	4(7.5)
Secondary and above	85(56.7)	2(2.4)

TABLE 2: Morphological, cultural and biochemical characteristics of Salmonella typhi isoted from blood and stool samples

Colonial characteristics									
MAC					SSA				
Pale, colorless, transparent, Smooth and round colony					Opaque, translucent, smooth and raised, colorless colonies with black center				
Biochemical Test									
Dex	Mal	Lac	Suc	Mann	indole	citrate	urease	TSI	
+	+	-	-	+	-	+	-	R/Y	

Key: MaC=MacConkey, SSA=Salmonella-Shigella agar, Dex= Dextrose, Mal= Maltose, Lac= Lactose, Suc= Sucrose, Mann= mannose, += Positive, - =Negative TSI= Tripple Sugar Iron Agar.

TABLE 3: Distribution of Antibody Titers for *Salmonella* Serotypes among Patients who presented symptoms of enteric fever in ATBUTH

Antigen Dilution	Dilution		
	1:40 (%)	1:80 (%)	≥ 1:160 (%)
***S. typhi* O**	NA	4(57.1)	3(42.9)
***S. typhi* H**	NA	1(14.3)	6(85.7)
***S. paratyphi* AH**	2(28.6)	3(42.9)	2(28.6)
***S. paratyphi* BH**	1(14.3)	3(42.9)	3(42.9)
***S. paratyphi* AO**	NA	5(71.4)	2(28.6)
***S. paratyphi* BO**	1(14.3)	3(42.9)	3(42.9)
***S. paratyphi* CH**	1(14.3)	NA	6(85.7)
***S. paratyphi* CO**	NA	4(57.1)	3(42.9)

Key: O = *S. typhi* – O Ag, H= *S. typhi* – H A, AH= *S. paratyphi* A- H Ag, BH= *S. paratyphi* B- H A, CH= *S. paratyphi* C- H Ag NA=No Agglutinati

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TABLE 4: Assessment of cell culturability through the examination of growth of S*almonella typhi* 1-7. upon treatment with 2.5ml H_2O_2

Isolates		Time (hours) /Optical density (OD) 12hr	24hr	36hr	48 hr	60hr	72hr	SD
	Control	0.88	1.16	1.16	1.17	1.17	1,17	0.127554
1	**Test**	0.83	1.14	1.16	1.17	1.17	1.17	0.136039
	Control	0.49	1.46	1.46	1.48	1.48	1.48	0.40102
2	**Test**	0.42	1.45	1.47	1.47	1.49	1.49	0.430554
	Control	0.83	1.15	1.17	1.18	1.18	1.18	0.140107
3	**Test**	0.83	1.12	1.15	1.17	1.17	1.17	0.134524
	Control	0.67	1.12	1.12	1.13	1.14	1.14	0.188007
4	**Test**	0.66	1.11	1.12	1.13	1.13	1.13	0.189596
	Control	0.91	1.51	4.71	4.81	4.81	4.81	0.233723
5	**Test**	0.91	1.43	1.46	1.47	1.47	1.48	0.226009
	Control	0.79	1.16	1.18	1.19	1.19	1.19	0.160458
6	**Test**	0.75	0.13	0.13	1.15	1.15	1.16	0.501348
	Control	0.86	1.46	1.46	1.48	1.48	1.48	0.25004
7	**Test**	0.86	1.44	1.46	1.47	1.48	1.48	0.189596

Key: SD= Standard Deviation

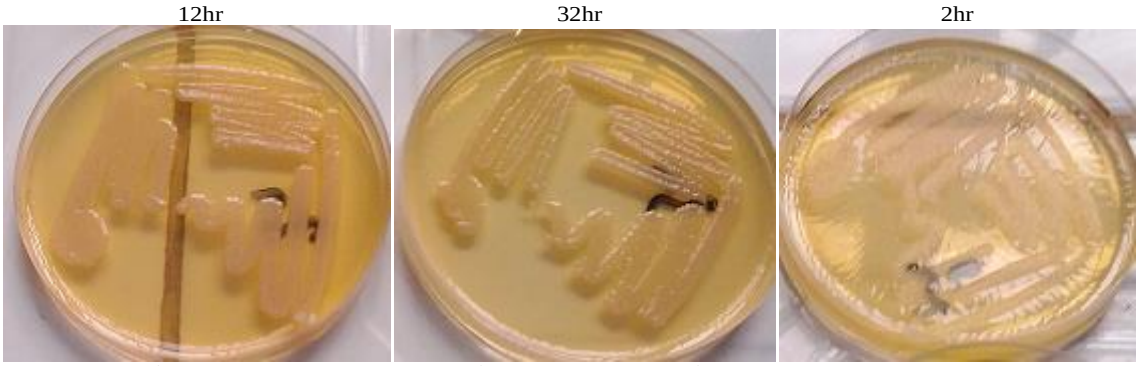

Plate 1. Shows demonstration of culturability and survival potential of Salmonella typhi cells after 2.5ml H_2O_2 treatments. Bacterial cells were grown in nutrient broth and 2.5ml H_2O_2 was applied after 12 h of bacterial growth. In agreement with previous observations *Salmonella typh* remained unaffected.

Table 4, shows data distribution from seven confirmed *S. salmonella* isolates which were used for the assay of growth. After 24 h of incubation on NA plates at 37 °C, at every 12-hr interval, cell growth was monitored by measuring OD_{600} up to 72h.The growth of *S.typhi* was found to be unaffected by the H_2O_2 treatment.

Sequencing and analysis of 16srRNA. We conducted Sequencing of 16S rRNA gene using genetic analyzer (ABI Prism 3130 and 3130 xl Genetic Analyzer) The sequencing used universal primers. The sequences were edited to exclude the PCR primer binding sites and was manually corrected using MEGA 5.2 version software. The full gene sequences of the strains were compared automatically using BLAST against the sequences of bacteria available in databanks (http://www.ncbi.nlm.nih.gov/).

Detection of resistance genes to oxidative stress from the isolates. The presence of genes encoding rpoE type was detected by multiplex PCR using universal primers and conditions previously reported (Fang *et al.,* 2008). The PCR was conducted in a Thermal Cycler PXE-0.5 (THERMO; Electron Corporation) and the resulting PCR products were subjected to electrophoretic separation in 1.5% agarose gel (plate 2). DNA bands of each amplicon was compared with 100-bp DNA mass marker. (Sabry and Mohammed., 2015).

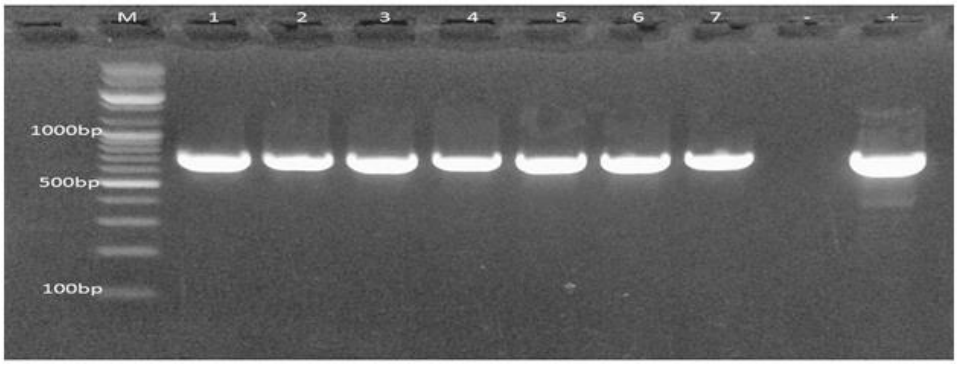

Plate 2. DNA polymerase chain reaction for the seven *S. typhi* showing different band in detection of the gene (RpoE) involved in oxidative stress adaptability in the bacteria.

Key: bp = base pair, + = positive control, - = negative control, M = Ladder, RpoE = RNA polymerase extra cytoplasmic E

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Discussion-Substantial studies has been done on temperature stress, nutrient starvation, and even hypertonicity though, oxidative stress is of prime interest for molecular microbiologist. In former experiments by some researchers, *E. coli* strain also showed the typical stress response towards heat shock (Noor *et al.,* 2013). The results of this current study indeed portrayed a phenotypic characteristic of *Salmonella typhi* specie in response to external oxidative stress imposed by 2.5ml H_2O_2. However, the gene involved in this phenotype was also confirmed in the bacterial.

In the growth observation of *S. typhi* upon H_2O_2 treatment up to 72 hours, steady figures obtained in alliance with the control figures in turbidity measurement through optical density indicate growth of the bacteria not affected and the culture shows no alteration in the colony phenotypes (i.e. flatness), this revealed the nonresponsive trait of this bacterium against external oxidative stress. Shiny convex colonies with entire margins were observed at up to 72 hours of incubation, which remained uninterrupted, a steady growth was monitored all along the incubation period (plate 1) this is in line with previous study on assessment of cell culturability through the examination of growth of *Salmonella* spp. upon treatment with 6 mM H_2O_2. Bacterial growth retardation was observed for *Salmonella* spp. and was found to be unaffected by the H_2O_2 treatment. (Rungrassamee, 2018; Rungrassamee *et al.,* 2019). It's an evidence of adaptive ability of *S. typhi* that after 2.5ml H_2O_2 treatments and grown in nutrient broth and 2.5ml H_2O_2 was applied, there were growth on the culture plate. This is in agreement with previous work done by Rungrassamee (2018) who observed in his study that *S. typhi* remained unaffected after all the treatment with H_2O_2. *S. typhi* continued to form colonies; the non reactivity of these cells against H_2O_2 was confirmed by their growth sustainability. (Rungrassamee, 2018; Rungrassamee *et al.,* 2019).

The role of the σE regulon has been examined in stationary-phase cells where it has been proposed that σE controls the lysis of dead cells (Nitta *et al.,* 2000). However, these studies were performed on cultures at much higher cell densities and cells being subjected to multiple stress conditions simultaneously, e.g. pH stress, oxygen stress or excreted toxic metabolites, depending on the medium used. Though, it was predicted that limited understanding of the roles of these proteins in *Salmonella* exists, because when single deletions are made no phenotypes are observed. To counter this, in previous study, generation of mutants incorporating deletions in up to four of the genes of interest to enable a better understanding of their roles during *Salmonella* stress survival and infection and confirm its role.

In line with the above study we decided to further check the presence of the regulatory gene in the 7 isolated *salmonella* and the positive band of DNA polymerase chain reaction (plate 2) for seven *S. typhi* confirmed the presence of the gene (rpoE) involved in oxidative stress adaptability in the bacterial. This approach is indeed young to this field. Although, the current study focused only on the adaptive ability of the bacteria upon oxidant sensitization, an extension of the study examined the presence of genetic regulation particularly gene encode for sigma factor E.

Nevertheless, our data clearly showed no physiological influence of external oxidative stress in the bacteria which is in agreement with studies by Kabir *et al.* (2013) and provided new information on the defense mechanism of *Salmonella typhi* against oxidative stress.

In conclusion, the current study detailed the adaptive strength in the isolated bacteria in one of the reactive oxygen stress (H_2O_2) which is in agreement with the existing knowledge and the gene for oxidative stress regulation. The current study would be of significance in terms of cellular survival and adaptability mechanism. The presented results may provide further information on activating phase of oxidative stress commencement, rate of damage at cellular level, and, finally, the consequences of bacteria that have lost the ability to be grown on routine media (VBNC) in a family of other bacterial populations.

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