Transcriptomic Responses of *Salmonella enterica* Serovars Enteritidis in Sodium Hypochlorite

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**Specialty section:**
This article was submitted to Molecular Bacterial Pathogenesis, a section of the journal Frontiers in Cellular and Infection Microbiology

**Received:** 12 January 2022  
**Accepted:** 24 March 2022  
**Published:** 20 April 2022

**Citation:**
Wang S, Xiao X, Qiu M, Wang W, Xiao Y, Yang H, Dang Y and Wang W (2022) Transcriptomic Responses of *Salmonella enterica* Serovars Enteritidis in Sodium Hypochlorite. Front. Cell. Infect. Microbiol. 12:853064. doi: 10.3389/fcimb.2022.853064

*Salmonella enterica* serovars Enteritidis (S. Enteritidis) can survive extreme food processing environments including bactericidal sodium hypochlorite (NaClO) treatments generally recognized as safe. In order to reveal the molecular regulatory mechanisms underlying the phenotypes, the overall regulation of genes at the transcription level in S. Enteritidis after NaClO stimulation were investigated by RNA-sequencing. We identified 1399 differentially expressed genes (DEG) of *S. Enteritidis* strain CVCC 1806 following treatment in liquid culture with 100 mg/L NaClO for 20 min (915 upregulated and 484 downregulated). NaClO stress affects the transcription of genes related to a range of important biomolecular processes such as membrane damage, membrane transport function, energy metabolism, oxidative stress, DNA repair, and other important processes in *Salmonella enterica*. First, NaClO affects the structural stability of cell membranes, which induces the expression of a range of outer and inner membrane proteins. This may lead to changes in cell membrane permeability, accelerating the frequency of DNA conversion and contributing to the production of drug-resistant bacteria. In addition, the expression of exocytosis pump genes (*emrB, yceE, ydhE*, and *ydhC*) was able to expel NaClO from the cell, thereby increasing bacterial tolerance to NaClO. Secondly, downregulation of genes related to the Kdp-ATPase transporter system (*kdpABC*) and the amino acid transporter system (*aroP, brnQ* and *livF*) may to some extent reduce active transport by bacterial cells, thereby reducing their own metabolism and the entry of disinfectants. Downregulation of genes related to the tricarboxylic acid (TCA) cycle may drive bacterial cells into a viable but non-culturable (VBNC) state, resisting NaClO attack by reducing energy metabolism. In addition, significant upregulation of genes related to oxidative stress could mitigate damage caused by disinfectants by eliminating alkyl hydroperoxides, while upregulation...
of genes related to DNA repair could repair damage to bacterial cells caused by oxidative stress. Therefore, this study indicated that *S. Enteritidis* has genomic mechanisms to adapt to NaClO stress.

**Keywords:** cell membrane damage, membrane transport function, energy metabolism, oxidative stress response, DNA repair

## INTRODUCTION

*Salmonella enterica* is one of the most common causes of human salmonellosis in China among the > 2600 known *S. enterica* serovars (Zhang et al., 2020). *S. Enteritidis* primarily causes foodborne gastroenteritis that is characterized by diarrhea, fever, headache, abdominal pain, nausea and vomiting (Daniel et al., 2015). A previous study utilizing samples from 5 pig slaughterhouses in Sardinia (Italy) had identified *Salmonella* isolates from 26/85 (30.5%) mesenteric lymph nodes, 14/85 (16.4%) colonic contents, 12/85 (14.1%) carcasses and livers from 462 samples (Piras et al., 2011). A recent study in China found that 37.5% of poultry samples were contaminated with *Salmonella* (Yang et al., 2020).

*Salmonella* can survive in a variety of adverse environments in which they have adapted to different environmental stressors ranging from nutrient starvation, acidity, osmosis and temperature fluctuations to the presence of antimicrobials (Spector et al., 2012; Bai et al., 2021). Chlorine is the antimicrobial chemical most widely used by the poultry industry due to its antimicrobial efficacy, convenience and low cost. Chilling water treatment facilities typically add between 50 and 100 mg/L of sodium hypochlorite (NaClO) to disinfect and combat microbial contamination in industrial production facilities.

**Materials and Methods**

### Bacteria Preparation

*S. Enteritidis* CVCC 1806 was obtained from the China Veterinary Culture Collection Center and was stored in brain heart infusion broth (BHI, Becton Dickinson, Franklin Lakes, NJ, USA) containing 20% glycerol at -80°C until use. For use in experimental procedures, strain were incubated in 5 ml BHI at 37°C with 150 rpm for 24 h to approximately 9 log CFU/mL. Bacterial cells were collected by centrifugation (5424R high-speed frozen centrifuge, Eppendorf, Germany) at 8000 rpm for 5 min and washed three times with normal sterile saline.

### NaClO Treatment

NaClO stock solutions contained 56.8 mg/mL chlorine (Sangon Biotech, Shanghai, China) that was diluted with sterile Milli-Q water (Pall, Buckinghamshire, UK). Chlorine concentrations were 100
mg/L (pH = 11) as determined using a ChlorSense meter (Palintest, Gateshead, Tyne & Wear, UK). In bacterial suspensions, add 0.5 ml of NaClO to 0.5 ml of cell suspension for 20 min as the treatment group and treat with 0.5 ml of 0.9% NaCl as the control group, each in triplicate, and the suspension was then added to a test tube containing 100 ul of 0.1 mol/L Na2S2O3 to instantly burst the residual disinfectant. 0.9% NaCl can maintain the stability of intracellular osmotic pressure, while Na2S2O3 neutralizes the residual chlorine after the reaction to achieve the purpose of stopping the reaction (Weerasooriya et al., 2021; Xiao et al., 2022). In China, 50 to 100 mg/L NaClO without pH adjustment is commonly used in poultry chilling process and previous study has been reported that 100 mg/L NaClO treatment could promote bacteria disinfectant resistance. Therefore, 100 mg/L NaClO without pH adjustment was chosen to further investigate the transcriptomic changes to uncover the molecular regulatory mechanisms that underlie the phenotypes.

**cDNA Library Construction and Sequencing**

The cDNA library construction and sequencing were performed by LC-Biotech. Hangzhou, China. RNA-Seq strand-specific libraries were prepared using the TruSeq RNA sample preparation kit from Illumina (San Diego, CA, USA) using 5 μg of total RNA. In brief, rRNA removal by Ribozero rRNA removal kit, fragmented using fragmentation buffer. cDNA synthesis, end repair, A-base addition and ligation of the Illumina-indexed adapters were performed according to Illumina’s protocol, all of which are performed according to the manufacturer’s specifications. Screening of 200-300 bp cDNA target fragments by 2% Low Range Ultra agarose electrophoresis (BioRad, Hercules, CA, USA), followed by 15 PCR cycles of PCR amplification using Phusion DNA polymerase (New England Biolabs, Beverly, MA, USA). The amplicons were quantified using a Turner Tbs-380 fluorometer (Promega, Madison, WI, USA) and paired-end libraries were sequenced using the Illumina NovaSeq 6000 sequencing platform.

**Bioinformatic Data Analysis**

Transcripts that were altered with a [fold change (FC) ≥ 2] were identified using Bioconductor edgeR (http://www.r-project.org/) and a threshold false discovery rate (FDR) of < 0.05 were considered as significant DEGs. In brief, a negative binomial distribution statistical model is built to test the original hypothesis on the data and obtain the pvalue information for gene comparisons. DEGs were subjected to enrichment analysis using Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways Goatools (https://github.com/tanghaibao/Goatools) and Kobas (http://kobas.cbi.pku.edu.cn/home.do), respectively. DEGs were significantly enriched in GO terms and metabolic pathways when their Bonferroni-corrected P-value was < 0.05. Pathway-based database like KEGG helps to further analyze the biological functions of genes. Pathway enrichment analysis identified significantly enriched metabolic pathways or signal transduction pathways in DEGs comparing with the whole genome background.

**Quantitative Real-Time Reverse Transcription PCR Validation (qRT-PCR)**

Representative DEGs were additionally verified using qRT-PCR using a 7300 Plus Real-Time PCR System (Thermo Fisher, Pittsburg, PA, USA). Total RNA was reverse-transcribed into cDNA using an All-in-One RT Master Mix and qPCR reactions utilized Eva Green qPCR Master Mix (Applied Biological Materials, Vancouver, Canada) with the following cycles: 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 63°C for 30 s. The relative expression level of target genes was measured with the 2^ΔΔCt method (Kenneth et al., 2001) and 16S rRNA was used as the reference gene. All tests were performed in triplicate using primers listed in Table 1.

**Sequence Accession Numbers**

The raw data reported in this article are available in the NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA758059 accessed on 22 September 2021 under BioProject accession number PRJNA758059.

**RESULTS**

**DEG Distributions**

The whole genome sequencing of our samples cultured for 20 min in the presence and absence of 100 mg/L NaClO generated 31710116 and 32912302 clean raw reads with an average length of 100 nt, respectively. A total of 4650 genes were identified using RNA-Seq and 1399 were significant DEGs. These included 915 upregulated and 484 downregulated genes and the relative levels of expression varied. As shown in Figure 1 volcano plot diagrams revealed upregulated and downregulated DEGs of the NaClO treatment group in contrast. Among them, yhdG, ileT and ileU are most significantly revised upwards by 3.7 log2(FC), 3.5 log2(FC) and 3.5 log2(FC), fixA, puta and ndy are most significantly revised downwards by 5.4 log2(FC), 4.2 log2(FC) and 4.0 log2(FC), respectively.

**GO Analyses**

To obtain an overview of the changes of gene functions after NaClO treatment, the DEGs were annotated by BLAST analysis of the GO database. Within these samples we identified 363 DEGs that were annotated in biological process, 296 in cellular components and 228 in molecular function. A ranking of these 3 GO lists according to their enrichment scores indicated that the biological process DEGs were gathered at the terms ‘cellular process’, ‘metabolic process’, ‘response stimulus’, ‘localization’ and ‘biological regulation’. The cellular component terms were primarily ‘cellular anatomical entity’, ‘intracellular’, ‘protein-containing complex’ while the molecular function terms gathered at ‘catalytic activity’, ‘binding’ and ‘transporter activity’ (Figure 2). In the enrichment map of differentially expressed gene pathways, we found that “inorganic molecular entity transmembrane transporter activity”, “metal cluster binding” and “iron-sulfur cluster binding” were the most significantly expressed pathways (Figure 3).
KEGG Analyses

KEGG analysis was performed to explore gene biological functions and identify pathways for the DEGs. This analysis indicated that most DEGs were involved in metabolism, genetic information processing, cellular processes and environmental information processing. The metabolism category revealed groupings distributed in carbohydrate, amino acid and energy metabolism. Two enriched terms ‘membrane transport’ and ‘signal transduction’ were the primary elements for environmental information processing and the most enriched term in the cellular process category was ‘cellular community’ (Figure 4).

qRT-PCR Validation

We validated the RNA-Seq results using qRT-PCR on five randomly selected DEGs. Five genes including one upregulated genes and four downregulated genes were selected for qRT-PCR analysis and used to validate the RNA-seq data from preliminary experiments. In general, the qRT-PCR results were consistent with the RNA-Seq results ($R^2 = 0.93$) indicating the transcriptome data were reliable (Table 1).

DISCUSSION

Our experiments revealed that gene regulation at the transcriptional level in S. Enteritidis CVCC 1806 was dramatically altered following a brief exposure to NaClO. The pathways most enriched were related to crucial cellular processes and we further analyzed these for each category (Figure 5).

Cell Membrane Damage

In Table 2, DEGs encoding outer (sthB, invG, yohG, apeE, yaiU) and inner membranes (ydgK, yddG, yeiH, yhjD) were upregulated. This may be due to the cellular response induced

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### TABLE 1 | The qPCR verification of differentially expressed genes of S. Enteritidis CVCC 1806.

| Genes | Primer sequence | Description | qPCR | RNA-seq |
|-------|-----------------|-------------|------|---------|
| 16S RNA | 5′-AGAGTTTGATCCTGGCTCAG-3′ | -- | -- | -- |
| argG | 5′-GATGGCAGCTCCTGCAATTG-3′ | argininosuccinate synthetase | -1.4 ↓ | -1.5 ↓ |
| sucB | 5′-GAGCGCTCTAGGAAGGAAA-3′ | 2-oxoglutarate dehydrogenase | -1.7 ↓ | -1.5 ↓ |
| kluB | 5′-CAACTGCATCAGCTGCTTATC-3′ | 3-isopropylmalate dehydrogenase | -2.5 ↓ | -1.8 ↓ |
| dmsB | 5′-GTGCGAACCAAGGACCTGTCT-3′ | dimethylsulfoxide reductase subunit B | -1.7 ↓ | -1.8 ↓ |
| cydB | 5′-TCCTGAGTGGCGGGCCTT-3′ | cytochrome d ubiquinol oxidase subunit I | 2.3 ↑ | 1.2 ↑ |

| Genes | Primer sequence | Description | qPCR | RNA-seq |
|-------|-----------------|-------------|------|---------|
| 16S RNA | 5′-ACGGCGGTGCTT-3′ | -- | -- | -- |
| argG | 5′-CTGATATCACTTCCTGCTT-3′ | argininosuccinate synthetase | -1.4 ↓ | -1.5 ↓ |
| sucB | 5′-CGTGATATCACTTCCTGCTT-3′ | 2-oxoglutarate dehydrogenase | -1.7 ↓ | -1.5 ↓ |
| kluB | 5′-GCTCTATCCTTGGCGCTGAA-3′ | 3-isopropylmalate dehydrogenase | -2.5 ↓ | -1.8 ↓ |
| dmsB | 5′-GATGGCAGCTCCTGCAATTG-3′ | 2-oxoglutarate dehydrogenase | -1.7 ↓ | -1.8 ↓ |
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### FIGURE 1 | Volcano plot of S. Enteritidis CVCC 1806 treated with 100 mg/L NaClO. Red spots in the right part represent upregulated genes; blue spots in the left part represent downregulated genes; black spots in the middle part represent genes with insignificant changes between the stressed and unstressed.
by NaClO stress on *Salmonella* leading to cell membrane disruption and thus stimulating the expression of genes associated with cell membrane proteins (Zhang et al., 2021). Xiao et al. (2022) found that 100 mg/L NaClO caused damage to *Salmonella* cell membranes by flow cytometry combined with fluorescent dye technique. Rajkowski et al. (2012) also found that treatment of *Salmonella* with chlorine at a concentration of 300 ppm for 3 minutes resulted in the destruction of the outer cell membrane.

**FIGURE 2** | GO functional classification of differentially expressed genes between control and NaClO treated group. The x-axis denotes the subcategories and the left y-axis denotes the number of DEGs.

**FIGURE 3** | Enrichment maps of differentially expressed gene pathways between the control and NaClO treatment groups.
membrane of Salmonella by transmission electron microscopy. Membrane permeability is a primary barrier to the uptake of foreign or extracellular DNA. In particular, a previous study reported that 100 mg/L NaClO exposure altered the transcription of cell membrane-related genes and especially those regulating cell membrane permeability in the bacterium Pseudomonas sp. (Tong et al., 2021). Additionally, following chlorine disinfection, increased membrane permeability can increase the frequency of DNA conversion (Jin et al., 2020). This releases antibiotic resistance genes into the water as free...
### TABLE 2 | Major metabolic pathways involved in differentially expressed genes.

| Gene ID   | Gene Name     | Log2 (FC) | NR top hit description                                      |
|-----------|---------------|-----------|------------------------------------------------------------|
| STM4593   | sthB          | 1.1↑      | outer membrane usher protein                               |
| STM2698   | invG          | 1.6↑      | type III secretion system outer membrane ring protein InvG |
| STM2172   | yorG          | 1.6↑      | multidrug resistance outer membrane protein MdtQ           |
| STM0570   | aoeE          | 1.2↑      | outer membrane esterase                                     |
| STM0329   | yaeU          | 1.1↑      | autotransporter outer membrane beta-barrel domain-containing protein |
| STM0196   | stfC          | 2.0↑      | fimbral biogenesis outer membrane usher protein            |
| STM1460   | ydgK          | 1.4↑      | inner membrane protein ydgK                                 |
| STM1571   | yddG          | 2.4↑      | inner membrane protein YddG                                 |
| STM2202   | yeaH          | 2.4↑      | putative inner membrane protein                             |
| STM3608   | yhjD          | 1.4↑      | inner membrane protein YhjD                                 |
| STM2815   | emrB          | 1.4↑      | Inner membrane component of tripartite multidrug resistance system |
| STM1154   | yceE          | 1.2↑      | multidrug efflux MFS transporter MdtG                      |
| STM1425   | yadE          | 1.4↑      | multidrug efflux MATE transporter MdtK                     |
| STM1428   | yadC          | 2.6↑      | Bcr/CIA-family multidrug efflux MFS transporter             |
| STM0196   | stfC          | 2.0↑      | fimbral biogenesis outer membrane usher protein            |
| STM3000   | saaB          | 1.2↑      | putative fimbral assembly chaperone                         |
| STM3337   | stbD          | 1.2↑      | fimbral usher protein StbD                                  |
| STM3619   | yhjO          | 1.1↑      | UDP-forming cellulose synthase catalytic subunit            |
| STM3623   | yhfT          | 1.9↑      | cellulose biosynthesis protein BcsF                         |
| STM3624   | yhfU          | 2.6↑      | cellulose biosynthesis protein BcsG                         |
| STM2066   | kdpC          | 1.9↓      | potassium-transporting ATPase subunit C                     |
| STM2075   | kdpB          | 3.6↓      | potassium-transporting ATPase subunit KdpB                  |
| STM2074   | kdpA          | 3.7↓      | potassium-transporting ATPase subunit A                     |
| STM1505   | aroP          | 3.5↑      | aromatic amino acid transporter AroP                        |
| STM3999   | bmrQ          | 1.4↑      | branched-chain amino acid transporter carrier protein BmrQ  |
| STM3560   | livF          | 1.7↑      | branched-chain amino acid ABC transporter ATP-binding protein LivF |
| STM0736   | sucA          | -1.9↓     | 2-oxoglutarate dehydrogenase E1 component                   |
| STM0737   | sucB          | -1.4↓     | 2-oxoglutarate dehydrogenase                               |
| STM0730   | gilA          | -2.5↓     | type II citrate synthase                                    |
| STM1238   | icdA          | -1.9↓     | NADP-dependent isocitrate dehydrogenase                     |
| STM1468   | tnaA          | -1.8↓     | fumarate hydratase                                          |
| STM0738   | sucC          | -1.4↓     | ADP-forming succinate-CoA ligase subunit beta               |
| STM0739   | sucD          | -1.2↓     | succinate-CoA ligase subunit alpha                           |
| STM0740   | argC          | -1.5↓     | argininosuccinate synthetase                                |
| STM0734   | sdiA          | -3.2↓     | succinate dehydrogenase flavoprotein subunit                |
| STM0733   | sdiD          | -3.0↓     | succinate dehydrogenase membrane anchor subunit             |
| STM0735   | sdiB          | -2.8↓     | succinate dehydrogenase iron-sulfur protein                |
| STM0732   | sdiC          | -3.4↓     | succinate dehydrogenase cytochrome b556 subunit            |
| STM3599   | mdh           | -2.1↓     | malate dehydrogenase                                        |
| STM0112   | leuB          | -1.8↓     | 3-isopropylmalate dehydrogenase                            |
| STM0158   | acnB          | -2.6↓     | bifunctional aconitase hydratase 2,2'-methyleneisocitrate dehydratase |
| STM1712   | acnA          | -1.2↓     | aconitase hydratase AcnA                                    |
| STM0965   | dmsB          | -1.8↓     | dimethylsulfoxide reductase subunit B                       |
| STM0609   | ahpF          | 1.5↑      | Alkyl hydroperoxide reductase protein F, partial            |
| STM4266   | sosR          | 1.2↑      | redox-sensitive transcriptional activator SoxR             |
| STM2841   | ygbD          | 1.5↑      | NADH:flavobacteroid reductase NorW                          |
| STM0075   | fvaA          | 5.4↑      | putative electron transfer flavoprotein FvA                 |
| STM0936   | hcr           | 2.0↑      | NADH oxidoreductase Hcr                                     |
| STM0735   | sdiH          | 2.8↑      | succinate dehydrogenase iron-sulfur protein                |
| STM2541   | yhiF          | 2.3↑      | iron-sulfur cluster assembly protein IscA                   |
| STM4399   | yfeE          | 2.7↑      | iron-sulfur cluster repair protein Yfe                      |
| STM0831   | dps           | 1.2↑      | DNA starvation/stationary phase protection protein Dps      |
| DNA repair | recO          | 1.3↑      | DNA repair protein RecO                                     |
| STM1875   | holE          | 2.4↑      | DNA polymerase III subunit theta                            |
| STM3837   | dnaN          | 1.1↑      | DNA polymerase III subunit beta                             |
| STM2414   | yfeO          | 1.1↑      | putative DNA-binding transcriptional regulator              |
| STM0682   | nagC          | 1.7↑      | DNA-binding transcriptional regulator NagC                  |
| STM0374   | yaiW          | 2.5↑      | DNA-binding transcriptional regulator                      |

↓, downregulated; ↑, upregulated.
DNA, allowing chlorine-damaged conditionally pathogenic bacteria to be transferred from non-antibiotic resistant bacteria to antibiotic resistant bacteria by natural transformation during chlorination, thereby increasing the likelihood of bacterial survival (Thomas et al., 2005).

The upregulation of exocytosis pump-related genes (emrB, yceE, ydhE, and ydhC) may also be related to the stability of cell membranes, which are capable of transporting structurally distinct molecules (including antibiotics) out of bacterial cells. Increased expression of the triple resistance system (emrB) genes were able to reduce disinfectant entry and express NaClO resistance of Salmonella by causing conformational changes in membrane properties and overproduction of EPS molecules (da Cruz Nizer et al., 2020). This efflux lowers the intracellular antibiotic concentration, allowing bacteria to survive at higher antibiotic concentrations (Blair et al., 2014).

In addition, upregulation of genes for important components of extracellular polymersubstances (EPS) such as curly hairs (stfc, safB, stbD) and cellulose synthesis (yjhO, yjhT, yjhU) may increase cell membrane stability. NaClO solution are HCIO, ClO- and OH- ions, and when the pH tends to be neutral, NaClO generally plays a bactericidal role in the form of HCIO (Fukuzaki, 2006). On the one hand, HCIO can interact with a variety of biomolecules, such as lipids, nucleic acids and membrane components, causing severe cellular damage (da Cruz Nizer et al., 2020). On the other hand, HCIO increases the resistance of Salmonella to NaClO by causing conformational changes in membrane properties and overproduction of EPS substrates (da Cruz Nizer et al., 2020).

Membrane Transport Function

In our study DEGs encoding the Kdp-ATPase transporter system (kdpABC) and the amino acid transporter system (aroP, bmnQ and livF) were down-regulated in expression following NaClO treatment, which may be a protective effect of Salmonella against NaClO stress. Any object that enters or leaves a cell must penetrate one or more enclosing membranes (Rees et al., 2009). Channel proteins are essential for the passage of molecules (including disinfectant molecules) through the cell membrane and are essential channel components. The ATP-binding cassette (ABC) transporters are a superfamily of membrane-associated bacterial proteins that are responsible for the ATP-powered translocation of numerous types of substrates (Li et al., 2021). The kdpABC encodes a potassium transport channel that is required for ATP homeostasis. K⁺ is essential for many cellular functions, including maintenance of intracellular pH and transmembrane potential (Liu et al., 2020). Downregulation of the Kdp-ATPase system genes may go so far as to inhibit active microbial transport, inhibit microbial metabolism, and thus affect bacterial energy metabolism. In addition, the downregulation of genes related to amino acid metabolism is able to maintain low concentrations of these biomolecules in the cell. The main target of HCIO action is the amino acid side chain, so the low yield of these molecules could reduce the damage caused by this disinfectant (da Cruz Nizer et al., 2020). Finally, due to the rate of reaction of HOCl with proteins, membrane transport proteins may be affected, affecting cellular homeostasis. Therefore, the downregulation of these proteins may be a protective strategy against HOCl damage.

Carbohydrate and Energy Metabolism

We found that numerous the tricarboxylic acid (TCA) cycle DEGs were downregulated with NaClO exposure. These included genes encoding citrate synthase (gltA), aconitase (aconA, aconB), isocitrate dehydrogenase (idcA), α-ketoglutarate dehydrogenase complex (succA, succB), succinyl-CoA synthetase (succC, succD, argG), succinate dehydrogenase (sdhABCD), fumarase (fumA) and malate de-hydrogenase (mdh, leuB). TCA cycle produces the reductive equivalents for the electron transport chain and the carbon backbone for various amino acids, making it an important hub for efficient bacterial metabolism in changing environments (Noster et al., 2019). Citrate synthase is in a key position as the first enzyme of the TCA cycle and citrate is a component of biosynthetic intermediates and reduced purine nucleotides that are used for energy generation through phosphorylation reactions linked by electron transport (Park et al., 1994). The reaction catalyzed by IDH is a regulatory checkpoint and α-ketoglutaric acid produced by this reaction contributes to the synthesis of glutamic acid, a key amino acid precursor (Kobayashi et al., 2014a). The TCA cycle converts NAD⁺ into NADH and FAD into FADH₂ and these components feed electrons into the oxidative phosphorylation pathway (Gel’Man et al., 1967; Li et al., 2021). Therefore, decreasing the overall yields of the TCA cycle would most likely lead to an overall decrease in cellular energy charge. Limited energy contributes to the quiescence of growth and metabolism, which may subsequently lead to the formation of the viable but non-culturable (VBNC) state (Zhu et al., 2021). Moreover, VBNC state cells will have greater resistance to chlorine (Dong et al., 2020). In addition, the dimethylsulfoxide reductase subunit B encoded by dmsB is able to transfer electrons, and it is significantly downregulated by downregulating 1.8 log2(FC) may everywhere lead to a decrease in the energy generated (Molinas et al., 2011). If this is a functional adaptation of this bacterium, the mechanism is currently unclear. In contrast, the overall adaptation in other metabolic pathways that we identified may be directly related to the overall decrease in cellular energy charge.

Oxidative Stress Response

Oxidative stress and production of reactive oxygen species (ROS) is a natural consequence of aerobic metabolism (Chiang et al., 2012). Under aerobic conditions, the disinfection effect is largely caused by the damage caused by elevated ROS levels (Brynildsen et al., 2013). Our results indicated that DEGs related to oxidative stress (ahpF, soxR, ygbD, hcr) were upregulated. Generally, there are two major stress response pathways required in Salmonella when coping with oxidative stress; peroxide stress-response and superoxide stress-response systems (Bai et al., 2021). In living cells, elimination of alkyl hydroperoxides is particularly important since they can initiate lipid peroxidation and consequently propagate free radicals leading to DNA and membrane damage (Rocha et al., 1999). Alkyl hydroperoxide
reductase encoded by ahpF significantly reduces the frequency of oxygen-dependent mutations caused by oxidative DNA damage. In enteric bacteria this is regulated via soxR that upregulates superoxide dismutase (SOD), the outer-membrane drug efflux protein TolC and DNA repair-related endonuclease IV (Kobayashi et al., 2014b). Antioxidant enzymes may be overexpressed to protect the receptor from damage caused by elevated ROS levels (Bae et al., 2011). ROS are mainly generated by the autoxidation of reduced flavoproteins that are not involved in the respiratory chain, and in our study fixA was significantly downregulated by 5.4 log2(FC) may also be a strategy to cope with ROS (Imlay et al., 2013). In addition, the upregulation of the NADH oxidoreductase hcr is another gene that responds to oxidative stress. NADH is a primary cofactor involved in detoxification and elevated NADH/NAD⁺ ratios are associated with oxidative stress (Ying, 2008). These reactions greatly increase the resistance of cells to oxidants.

On the one hand, HClO also reacts with the iron centres in microbial enzymes, leading to enzyme inactivation (Fukuzaki, 2006). On the other hand, HClO can also cause damage to DNA (Storz et al., 1999). In our study, the gene (sdhB, yfhF, yfFE) associated with the iron-sulfur cluster reaction was downregulated. The unscheduled transfer of electrons from oxidoreductase to oxygen produced a mixture of +O2 and H₂O₂ (Imlay, 2019). These oxidized the solvent-exposed iron centres of the mononuclear Fe²⁺ enzyme and [4Fe-4S] dehydratase, causing iron dissociation and loss of activity (Imlay, 2019). As iron is required for the Fenton reaction, higher levels of unincorporated cellular iron may increase DNA damage (Touati et al., 1995). Therefore, downregulated iron-sulfur cluster genes may be critical for cells to cope with this damage. In addition, upregulated dps is a miniature ferritin that keeps free iron levels low enough to inhibit damage to DNA by sequestering loose iron from the cell (Altuvia, 1994).

DNA Repair
DNA repair system can repair damage caused by oxidative stress (Buchmeier et al., 1995). In the current study, we identified significantly upregulated DEGs associated with DNA repair and replication, including recO, holE and dnaN, which were upregulated by 1.3 log2(FC), 2.4 log2(FC) and 1.1 log2(FC), respectively. Slight upregulation of nucleoside and nucleotide biosynthesis when Salmonella is exposed to disinfectants such as NaClO and peroxyacetic acid (PAA), which triggers DNA repair mechanisms (Dunn et al., 2020). DNA polymerase encoded by dnaN is able to repair damage caused by H₂O₂ (Burton et al., 2007). Therefore, it is critically important for a cell to have the capacity to properly respond to and repair DNA damage as it occur (Minten et al., 2019). Interestingly, we also identified DEGs involved in transcriptional regulation that are key elements in environmental adaptation (Li et al., 2021) including yaiV, nagC and yfeD. Under NaClO stress, ribosome synthesis is enhanced, stimulating DNA repair functions and possibly promoting translation to some extent, which may contribute to increased resistance of Salmonella to NaClO.

CONCLUSIONS
Transcriptional upregulation of genes associated with key cellular processes such as membrane damage oxidative stress and DNA repair was observed under NaClO stress. On the one hand, altered cell membrane permeability and increased pore space accelerated the frequency of DNA transfer from non-drug-resistant to drug-resistant bacteria through natural transformation. On the other hand, upregulation of efflux pump genes enables the exclusion of disinfectants from the bacterial cell body. Salmonella can reduce DNA damage by eliminating alkyl hydroperoxides, while upregulation of DNA repair-related genes can repair DNA damage induced by oxidative stress, thereby reducing damage to Salmonella by NaClO. However, we also identified downregulation of genes related to membrane transport function and energy metabolism, which may reduce active transport in bacteria and force them into a VBNC state to resist NaClO attack by reducing their own metabolism. Therefore, this study suggests that Salmonella enterica has a genomic mechanism to adapt to NaClO stress, which may provide a reference for the application of NaClO in industrial production facilities.

DATA AVAILABILITY STATEMENT
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS
YD and WenW: writing-review and editing. SW and XX investigation and writing-original draft preparation. MQ and WensiW data curation. YX and HY: resources. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING
This research was supported by State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products (2010DS700124-ZZ2002), Walmart Foundation (UA2020-152, UA2021-247) and Ministry of Agriculture and Rural Affairs (14215033).
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