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RESISTANCE PATTERNS OF STAPHYLOCOCCUS AUREUS 700698 TO VARIOUS STRESS CONDITIONS AND PATHOGENICITY PROFILES ON HEP-2 CELL LINES

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ABSTRACT Staphylococcus aureus (S.aureus) is a major pathogen causing life threatening diseases worldwide. In vitro, it has been shown to acquire resistance towards various stresses and adhere and invade various host cells. This study examined the resistance patterns of S. aureus to various stresses and their pathogenicity profiles in absence of fibronectin in host cells. S. aureus 700698 was subjected to various stresses (heat, cold, acid, hydrogen peroxide and sodium chloride). Stress resistance was analyzed by quantifying the population reduction and protein expression profiles by 2-D SDS-PAGE. HEp-2 cells were used for bacterial adhesion and internalization. Percentage of HEp-2 cell lysis was determined using released lactate dehydrogenase, and ability to induce apoptosis was analyzed by DNA fragmentation. Statistical analysis was performed on adhesion and internalization assays. Stress resistance was highest for heat (log10 0.14) and lowest for H2O2 (log10 2.6). Approximately 292 proteins were expressed by 2-D SDS-PAGE, having acidic proteins in higher ratio. Adhesion and invasion was significantly ($p \leq 0.05$) increased under H2O2 (log10 6.49), acid (log10 5.46) and heat (log10 5.32) stresses, but numerically higher under Cold and NaCl stresses. Varying LDH profiles were observed in stress treatments, but after 24h, NaCl stress had the highest LDH release (34.8%) and lowest (18%) was observed in heat stressed bacteria. No apoptosis was observed in HEp-2 cells by DNA fragmentation and morphological changes. Further research on identifying proteins is required to understand host-microbe interactions of S. aureus.

KEYWORDS : Staphylococcus aureus, Stress, Pathogenicity, HEp-2 cell Lines

Introduction

Staphylococcus aureus is a major pathogen attributing to foodborne diseases (Akineden et al., 2008). Many strains of S. aureus produce toxins that potentially cause health hazard from food poisoning (Bania et al., 2006; Akineden et al., 2008). Metabolites of S. aureus are chief causes of gastroenteritis and staphylococcal food poisoning (SFP), and staphylococcal enterotoxins (SEs) pose the greatest risk in consumer health (Agsaw and Addis, 2015). This organism, originating from raw materials, environment or equipment, can contaminate and grow well in various foods (meats, salads, sandwich fillings, milk and dairy products) (Loir et al., 2003). Outbreaks occur due to cross contamination of food by bacteria from an unfavorable food to a growth permitting food or due to temperature abuse of the food.

Food processing methods (analogous to environmental stresses) create (Erickson and Doyle, 2007) deleterious conditions, that are conducive to the development of increased bacteria resistance and survivability to subsequent stress, (Considine et al., 2008). Fermentation, preservatives, and acid washes used to decontaminate carcasses can lead to acid stress (Considine et al., 2008). Starvation stress occurs in low-nutrient environments, (e.g., animal carcasses, food, equipment surfaces and water) (Frank, 2001). Rapid rehydration of foods and exposure to high salt concentrations can cause osmotic injury (Bremer and Krämer, 2000) and oxidative stress results from exposure to certain sanitizers (Yousef and Courtney, 2000). Microorganisms inhabiting refrigerated foods may undergo cold stress, and cold shock occurs when growing microbes are subjected to sudden temperature reductions (15°C or more) (Wesche et al., 2009). Conversely, heat shock results when an organism is shifted from lower to higher temperatures within or above their normal growth range.

The ability of S. aureus to cause endovascular diseases and high frequency of relapses after chemotherapy suggests that this is an intracellular organism (Garzoni et al., 2009). Different models proposed, that this organism can internalize host cells by binding; invading cells at damage sites; binding to host cells via adherent serum or cellular proteins, that act as bridging ligands between cells and bacteria (Garzoni et al., 2009).

S. aureus' ability to produce various factors, to different types of treatment stresses at processing, storage or other environments, contribute to its survival unassociated with pathogenesis (Loir et al., 2003). The stress response of S. aureus has been well studied however mechanisms behind these stress response factors specifically in human laryngeal epithelial (HEp-2) cells remain unclear. Also, mode of action of this bacterium on host cells (e.g., alteration of adhesion and invasion) or cytotoxic effects under stressful conditions requires further studies to be elucidated. This study endeavored to understand the bacterial virulence properties under various stresses, that may permit discovering new technologies to avert this bacterium and new targets for chemotherapy.

Material and Methods

Bacterial strains and growth conditions

S. aureus 700698, acquired from American Type Culture Collection (ATCC), was grown in Brain Heart Infusion (BHI, Sigma, MO, USA) broth to stationary phase (OD600 - 1.6, corresponding to approximately 1×1010 CFU/ml) at 37° C in a shaker incubator. For virulence assays, bacteria were given the stress treatments, centrifuged and resuspended in Dulbecco's Modified Eagle Medium (DMEM, Gibco, CA, USA) without serum and antibiotics (invasion medium) and immediately used for successive testing.

Stress resistance Assays

Stationary phase cultures were used for all stress treatments. The temperature (heat and cold) stress treatments were performed as described by Kalchayanand et al. (1992) with minor alterations. For heat stress, bacteria were centrifuged, resuspended in preheated BHI broth to 55°C, incubated for 10 min and cooled in an ice cold water bath or on ice. The cold stress was given by incubating bacteria at -20°C for 2 h, then thawing to room temperature.

Acid and sodium chloride stresses were performed according to Chan et al. (1998) with minor modifications. Acid stress was determined by resuspending bacteria in BHI acidified to pH 4 with HCL for 1 h. For oxidative stress, bacteria were centrifuged and resuspended in PBS containing 10mM H2O2 for 10 min. For NaCl stress, cultures were grown to mid-exponential phase (OD600=0.5-0.6), harvested by centrifugation, resuspended in equal volume of BHI containing 2M NaCl and incubated until stationary phase. Bacteria grown in BHI without treatments were used as controls. Viability of bacteria was assessed immediately after treatments by plating on BHI agar.

Protein extraction

Proteins from S. aureus were extracted using 50 µg/ml of lysostaphin (Sigma, St. Louis, MO). Five ml of stationary phase cultures of untreated and treated S. aureus were centrifuged separately and incubated in 400µl of Tris-HCl buffer (50 mM Tris, 150 mM HCl, pH 8.0) containing lysostaphin (100µg/ml), lysozyme (1mg/ml) (Sigma) and protease inhibitors (1mM phenylmethylsulfonyl fluoride, Sigma) and 1mM EDTA (Sigma), final concentrations) for 2h at 37°C.

Extracts were then added to 3% trichloro-acetic acid (TCA) and sulfosalicylic acid mixture to concentrate. The proteins were centrifuged and the precipitate was washed with absolute ethanol and dried. The dried pellet was dissolved in buffer containing 8M urea, 4% CHAPS, 1% DTT and bromophenol blue. Total protein concentration was determined by Bradford method and proteins were separated using polyacrylamide gel strips (ReadyStrip™, Bio-Rad, CA, USA) with the range of 3-10, on isoelectric focusing unit (IEF) (Bio-Rad). Briefly, gel strips were rehydrated in the presence of proteins along with ampholytes (Biolytes™, Bio-Rad) overnight, placed in a Protean IEF cell (Bio-Rad, Hercules, CA) and ran for at least 7h at 250V and 20°C. After IEF, gel strips were equilibrated in buffer containing 6M urea, 4% SDS, 1% DTT and 4.8% iodoactamide. Total proteins were then separated by 13% SDS-PAGE gel and fixed for 45min. The gel was washed with washing buffer for 15 min. and stained with Coomassie Brilliant Blue R-50 (Sigma). Destaining was performed by methyl alcohol and acetic acid buffer and documented by photography using Alpha Imager Imager 2200 (v5.5, Alpha Innotech Corporation, San Leandro, CA).

HEp-2 cell culture

Human laryngeal epithelial cells, HEp-2 (ATCC-CCL-23, Manassas, VA), were seeded at 2×105 cells in 24-well plates with Dulbecco's modified Eagle medium (DMEM; Gibco, CA) supplemented with 10 % fetal bovine serum (FBS; Gibco, USA) in 7 % CO2 at 37°C.

Invasion and Adhesion assay

HEp-2 cells were grown to confluence in 24-well plates and utilized for invasion assays. Bacterial invasion and adhesion assays were performed according to Dziewanowska et al., (1999) with minor modifications. After washing, HEp-2 cells (2×105 cells/ml) were inoculated with bacterial suspension containing (107 CFU/ml) at a multiplicity of infection (MOI) of 1:100 in 1xPBS. After 2 h incubation, cultures were washed with PBS and 1 ml of invasion medium containing 100µg of gentamicin (Sigma) per milliliter, added to each well to kill the extracellular bacteria. After 1 h incubation, HEp-2 cell monolayers were washed three times with sterile PBS, lysed with 100µl of 0.25% trypsin-EDTA in Hanks balanced salt solution (Gibco), and further lysed with 0.9 ml of 2% Triton X-100 in sterile distilled water to release the intracellular bacteria. Cell lysates were serially diluted in PBS and viability was determined by plating on BHI agar and incubated at 37° C overnight. All experiments were conducted in triplicates Adhesion assay was performed as described above except gentamicin was omitted to count the extracellular bacteria. Percent invasion values were determined based on the proportion of attached S. aureus which had invaded the HEp-2 cell, as follows:

Percent invasion = (no. of attached S. aureus which had invaded the HEp-2 cell / initial bacterial population) x 100

Cytotoxicity assay

Cytotoxic potential of unstressed (untreated) and stressed (sub-lethally injured) S. aureus bacteria was investigated for the release of lactate dehydrogenase (LDH) enzyme from the cytosol of HEp-2 cells into the culture supernatant using a cytotoxicity assay kit (Roche, IN). LDH activity was quantified using a colorimetric cytotoxicity detection kit (LDH) (Roche, IN) per manufacturer's instructions. Percentage cell lysis of HEp-2 cells induced by S. aureus was measured per manufacturer's instructions. Assay was performed in triplicate for 24h and percent cytotoxicity was determined based on the amount of LDH released from 12-min readings for both the negative and positive controls in each experiment, as follows:

Percent cytotoxicity = (Aexp-APBS)/ (ATriton x-APBS) x 100

Where Aexp is the absorbance of the sample, APBS is the absorbance of phosphate buffered saline, and ATriton X is the absorbance of triton x-100 at a one-percent concentration.

DNA fragmentation

Occurrence of apoptosis in infected HEp-2 cells was assessed by DNA fragmentation (Matsuo et al., 2013). HEp-2 cells were infected with untreated and treated bacteria. After 2h incubation, cells were washed with PBS and DMEM without serum containing gentamicin (10µg/ml) was added. DNA from infected HEp-2 cells was extracted at 6h and 22h after addition of gentamicin with Wizard genomic DNA purification kit® (Promega Corp. Madison, WI), per manufacturer's protocols. Samples were analyzed by gel electrophoresis (1.8% agarose), for 90min with 100V and stained with ethidium bromide and photographed under UV light.

Statistical analysis

All viable bacterial counts were converted to their log values prior to statistical analysis. Results of stress treatments and virulence assays on HEp-2 cells were determined on an average of three individual experiments. Statistical analysis was conducted on adhesion and internalization assays using Analysis of Variance (ANOVA) and standardized student's t test at a significance level of $p \leq 0.05$).

Results

Stress resistance assays

Stationary cultures of S. aureus 700698 were subjected to heat, cold, acid and H2O2 stresses and analyzed for population reduction (Figure 1). Compared to the untreated bacteria, all treatments reduced viability of S. aureus. Hydrogen peroxide (log10 2.6) and acid (log10 2.4) treated bacteria showed significantly ($p \leq 0.05$) higher population reduction than treatment with heat and cold. Acid treatment resulted in a significant ($p \leq 0.05$) reduction in growth (log10 2.45) compared to untreated bacteria. Hydrogen peroxide treatment exhibited the highest bactericidal effect, which significantly ($p \leq 0.05$) reduced the bacterial population by log10 2.6. The lowest population reduction was observed in heat treated cells (log10 0.04). At stationary phase, NaCl treated bacteria showed more growth than the untreated bacteria (Figures 4 and 5)

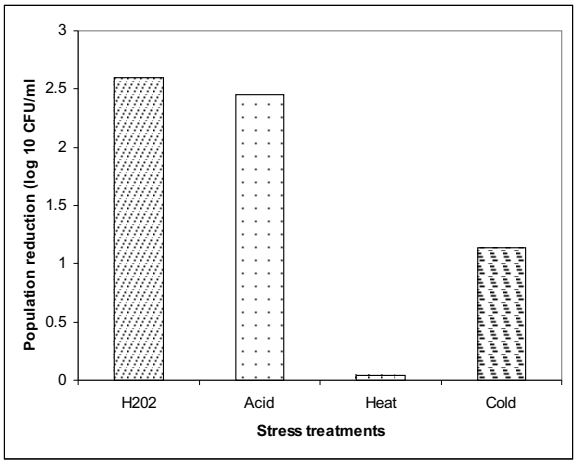

Figure 1. Population reduction of S. aureus 700698 due to H2O2, acid, heat and cold treatments Letters a, b, c, d indicates significant difference

Protein expression profiles of S. aureus

Relative molecular mass (Mr) values of proteins and their isoelectric points were determined based upon molecular markers on the gel strips (Figures 3A, 3B, 3C). Approximately, 343 protein spots were expressed in all the gels (Table 1). All the treatments and untreated S. aureus showed a higher ratio of acidic proteins than the basic proteins.

Table 1 shows similarities between proteins of treated and untreated S. aureus. In sodium chloride stress, 31 proteins were either newly induced or over expressed, of which 18 were acidic and 13 were alkaline in nature. Total number of proteins counted in NaCl treated was less than the untreated organism. Only 21 proteins (40.38%) appeared to have similarity with untreated bacterial proteins. In acid treated S. aureus, 39.28% of counted proteins were identical to untreated S. aureus. A total of 34 proteins were counted as induced or over-expressed, of which 21 were acidic and 13 were alkaline.

Hydrogen peroxide treatments had highest percentage (72%) of similarity to untreated bacterial proteins. Surprisingly, no alkaline proteins were visible in hydrogen peroxide treated bacterial proteins. Approximately 50% of heat treated proteins were similar in Mr and pI ranges. Among all the proteins counted, 28 proteins were either induced or over expressed, of which 18 were acidic and 10 were alkaline. Cold stress yielded a total of 47 induced or over expressed countable proteins, 36 proteins of acidic nature. Interestingly, more proteins were identified in cold stress than the untreated and caused induction or over expression of more proteins than other treatments.

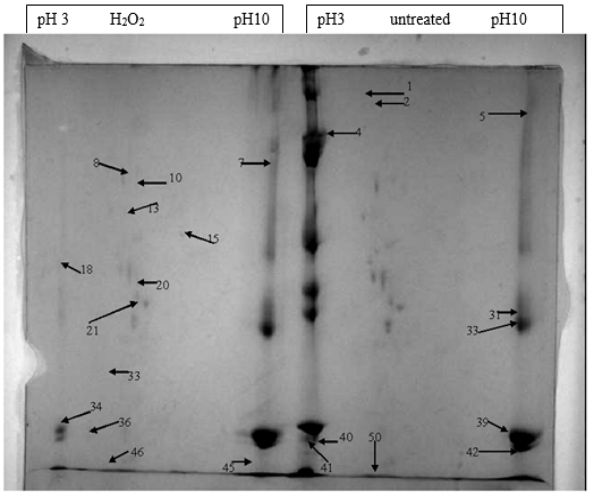

Figure 2A. 2-D SDS-PAGE (pH 3-10) analysis of hydrogen peroxide and untreated S. aureus 700698. Arrows in hydrogen peroxide gel represent induced protein spots different from untreated S. aureus and arrows in untreated S. aureus gel showing common proteins found in all treated treatments

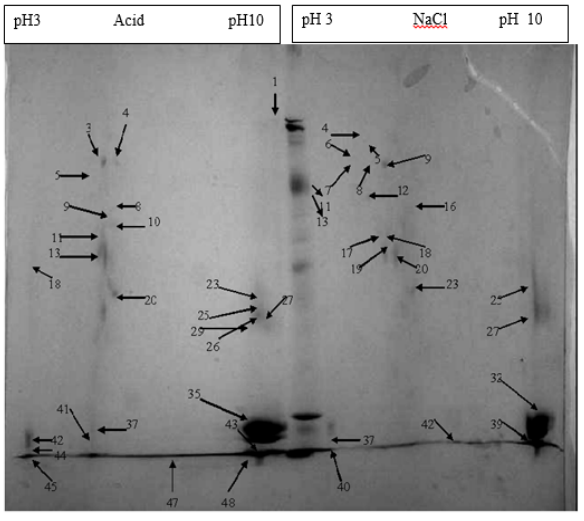

Figure 2B. 2-D SDS-PAGE (pH 3-10) analysis of Acid and sodium chloride treated S. aureus 700698. Arrows represent induced protein spots different from untreated S. aureus.

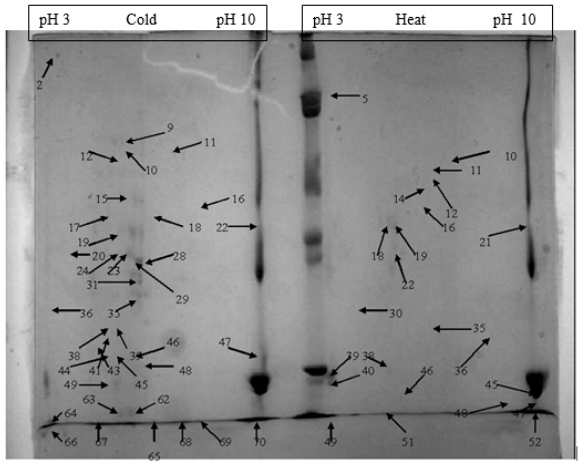

Figure 2C. 2-D SDS-PAGE (pH 3-10) analysis of Cold and heat-treated of S. aureus 700698.

Arrows represent induced protein spots different from untreated S. aureus.

Table 1. Selected relative molecular weight (Mr) and iso electric point (pI) of protein spots in stressed and unstressed S. aureus 700698.

	unstressed		NaCl		Acid		H_2O_2		Heat		Cold	
	Mr	pI	Mr	pI	Mr	pI	Mr	pI	Mr	pI	Mr	pI
1	~195	4.8	~195	4.6	~190	9.60	~190	3.5	~190	4.0	~190	4.2
2	~190	5.0	~190	**4.8**	~175	9.80	~187	3.7	~188	4.2	~188	3.9
3	~175	10.0	~175	9.4	~140	5.40	~160	10.0	~145	10.0	~185	4.0
4	~120	3.1	~175	4.8	~135	5.80	~120	10.0	~120	3.2	~145	10.0
5	~110	10.0	~140	4.9	~115	5.20	~100	10.0	~110	3.7	~115	10.0
6	~100	8.9	~140	5.2	~100	9.80	~90	5.8	~100	10.0	~95	10.0
7	~95	8.0	~138	4.5	~90	5.00	~90	10.0	~85	5.1	~85	4.9
8	~95	10.0	~137	4.6	~75	5.60	~85	5.5	~80	7.1	~80	5.6
9	~90	4.9	~136	4.9	~74	5.20	~80	5.2	~78	7.2	~80	5.8
10	~88	7.7	~116	3.1	~74	5.40	~80	5.5	~60	6.7	~78	6.8
11	~85	5.0	~110	3.1	~65	5.20	~76-78	10.0	~50	6.0	~75	7.4
12	~80	10.0	~110	4.5	~65	9.80	~75	10.0	~46-50	5.9	~70	5.5
13	~73	4.3	~95	3.1	~60	5.10	~70	5.2	~45	3.1	~70	8.1
14	~70	7.8	~95	4.7	~60	5.20	~66	3.3	~40-44	6.0	~50	10.0
15	~70	10.0	~85	9.1	~60	5.40	~66	5.7	~40-44	10.0	~48	6.2
16	~68	7.8	~70	5.8	~44	9.70	~66	7.0	~38	5.7	~45	8.0
17	~55	3.0	~66	5.0	~44	5.40	~50	10.0	~35	5.0	~43	5.9
18	~50	5.2	~66	5.4	~44	3.40	~40	3.1	~35	5.1	~43	7.0
19	~50	10.0	~60	5.3	~42	5.50	~36	5.5	~35	5.3	~40	6.4
20	~44	4.9	~50	5.5	~40	5.60	~36	5.6	~34	3.1	~38	4.8
21	~42	4.5	~50	5.2	~40	5.40	~33	5.7	~34	10.0	~38	6.8
22	~36	4.8	~45	9.3	~35	3.30	~30	3.3	~32	5.5	~35	10.0
23	~35	5.0	~40	6.1	~35	9.10	~30	5.8	~28	5.6	~32	5.8
24	~32	3.1	~40	9.3	~33	5.20	~30	10.0	~25	5.8	~31	4.9
25	~28	5.7	~33	9.4	~32	9.50	~25	5.8	~20	3.3	~31	6.0
26	~26	5.3	~32	5.8	~31	9.50	~25	6.0	~20	5.2	~29	3.5
27	~26	7.1	~32	9.4	~30	9.50	~23	10.0	~20	10.0	~28	5.0
28	~25	10.0	~31	9.5	~30	5.30	~20	5.7	~19	5.0	~28	6.1
29	~22	5.7	~20	9.5	~27	9.10	~19-20	3.3	~19	10.0	~25	6.0
30	~21	3.0	~18	5.7	~27	9.80	~19-20	5.5	~18	3.9	~24	5.8
31	~21	10.0	~18	9.5	~25	5.50	~18	10.0	~18	10.0	~22	6.1
32	~20-20.5	5.2	~15	9.3	~25	5.30	~17-18	5.5	~17	4.9	~21	10.0
33	~20-20.5	10.0	~12	3.9	~20	9.10	~16-17	3.9	~16	5.9	~20	4.8
34	~19-20	5.2	~12	9.3	~18	5.20	~14.5-15	3.2	~16	6.3	~20	5.9
35	~18-18.5	4.9	~10	3.9	~14	9.60	~14	3.2	~15-16	8.1	~19	6.0

Adhesion of S. aureus to Hep-2 cells

Adhesion ability of untreated and treated S. aureus on HEp-2 cells was determined and the results were presented in (Figure 3). Hydrogen peroxide, acid and heat stressed bacteria showed significantly ($p \leq 0.05$) higher adherence than untreated bacteria. The bacteria treated with cold (5.01 log10) and NaCl (4.98 log10) stress had significantly ($p \leq 0.05$) lower adhesion than H2O2 treated bacteria. The highest (6.49 log10) adhesion of S. aureus 700698 to Hep-2 cells occurred in H2O2 treated S. aureus and lowest adhesion was recorded in untreated S. aureus (4.52 log10).

Cell Invasion assay

Invasion of treated and untreated bacteria in HEp-2 cells (Figure 4) showed similar trend as adhesion except acid treated (2.21 log10) bacterial invasion was significantly ($p \leq 0.05$) more than the cold (1.7 log10) and NaCl (1.57 log10) treatments. Hydrogen peroxide (2.75 log10) treated bacteria showed significantly ($p \leq 0.05$) higher invasion ability than all other treatments. Invasion was significantly ($p \leq 0.05$) lower in untreated bacteria (log10 1.35). Among treatments, NaCl treated bacteria (21.7%) showed the lowest cell invasion compared to hydrogen peroxide, acid, heat (25.7 %) and cold (23.9%) treated bacteria (Figure 4).

All treatments showed a higher percentage of adhesion and internalization than untreated S. aureus (61.2 and 18.3%,

respectively), with hydrogen peroxide treatment (91.5% and 38.7%, respectively) being highest, followed by acid stress (79% and 31.2 %). Among treatments, the lowest adhesion and internalization was observed in NaCl treatment (Figure 4).

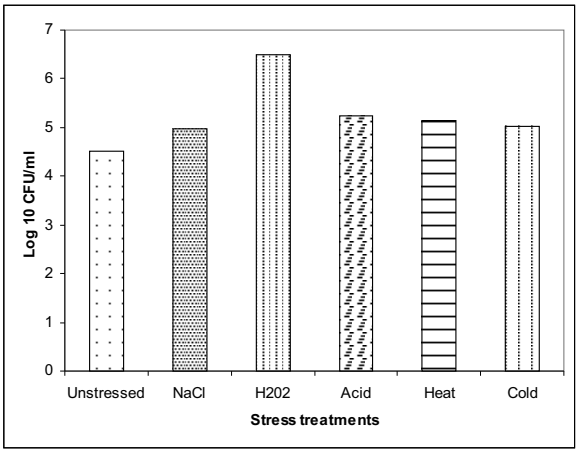

Figure 3. Adhesion of untreated and treated S. aureus 700698 onto HEp-2 cells. Letters a, b, c, d indicates significant difference.

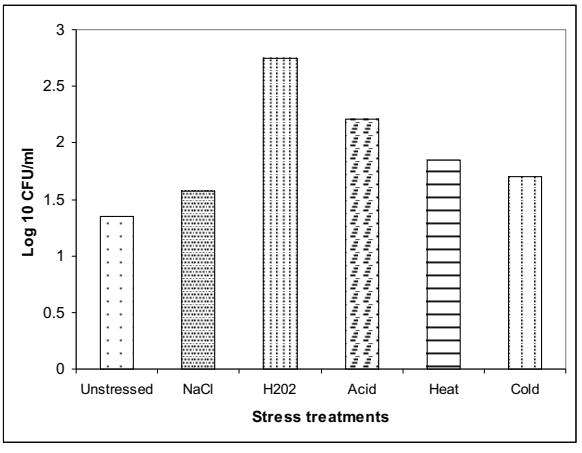

Figure 4. Invasion of untreated and treated S. aureus 700698 in HEp-2 cells. Letters a, b, c, d indicates significant difference.

Cytotoxicity Testing

Cell cytotoxicity delineated by the amount of LDH released from the cell, was categorized (low = 1-20%), (medium = 21%-49%) and (high = >50%). The cytotoxic potential of S. aureus tested against multiple stress conditions resulted in low percent cytotoxicity, (2 to 17%), compared to 8% for untreated cells (Figure 6). After 8h, heat and acid treatments resulted in medium to high LDH release (44% and 58%, respectively). Sodium chloride treatment had the lowest LDH release compared to untreated cells (9%), (Figure 5). After 16 and 24h treatment of Staphylococcus aureus under various stress conditions, all treatments generated low to medium release of LDH. Acid, cold and sodium chloride treated cells exhibited similar cytotoxicity profiles, with low LDH release (16 to 20%). Hydrogen peroxide treated cells exhibited medium (24 and 21%) cytotoxicity at 16 and 24h. Sodium chloride treated cells at 24h, exhibited a 12% increased (18% to 30%) release of LDH compared to untreated cells (Figure 5).

Apoptosis and DNA laddering

To confirm the occurrence of cell death, DNA extracted from HEp-2 cells at 6h and 22h was subjected to agarose gel electrophoresis. Demonstration of internucleosomal DNA fragmentation is often used to indicate cells undergoing apoptosis. After 22h, DNA from untreated and treated bacteria was observed as intact bands, suggesting that there was no apoptosis. No treatments showed evidence of chromatin cleavage, confirming that these bacteria were not able to induce apoptosis in HEp-2 cells (figure not shown).

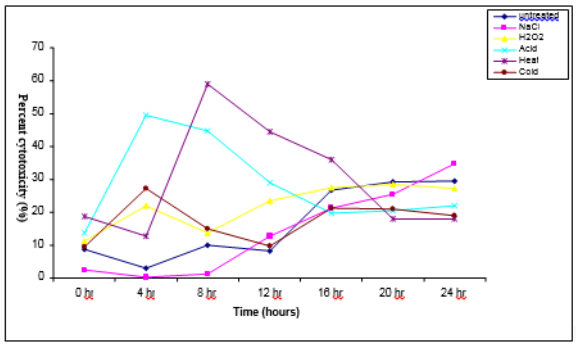

Figure 5. Cytotoxicity Percentage of HEp-2 cells infected with untreated and treated S. aureus 700698 by the release of lactate dehydrogenase enzyme.

Discussion

In this study, S. aureus 700698 grown to stationary phase were exposed to temperature (heat and cold), acid, H2O2 and sodium chloride stress treatments. Among all the stress treatments, hydrogen peroxide showed higher lethality, reducing the population of bacteria by log10 2.6. This might be due to the defective regulator, rsbU, which controls the expression of sigB gene (Horsburgh et al., 2002). Horsburgh et al. (2002) reported an increase in resistance of an rsbU cloned S. aureus 8325-4 to H2O2 than the parental 8325-4 strain. The sigB components were not expressed very well in S. aureus 8325-4 due to absence of an 11-base pair sequence in promoter region of rsbU gene, which resulted in poor expression of sigB and other stress response proteins that are controlled by sigB.

Protein expression of S. aureus in response to stress

All treatments showed different protein patterns at altered levels and produced higher numbers of acidic proteins than alkaline ones. Stress treatments may change the pI values of proteins and these proteins might appear in more than one spot (Godoy et al., 2013). Degradation of proteins due to stress conditions is also an important factor for appraising Mr of proteins. Cold stress altered the expression of 40 proteins, but had closely related spots with nearly equal pI values, which might represent similar proteins.

In this study, untreated S. aureus showed 61% adhesion and 19% invasion in the HEp-2 cells, perhaps due to decreased expression of cell surface proteins in stationary phase. The cell surface proteins of S. aureus are expressed maximally during the exponential phase of growth and are down regulated as cells enter stationary phase (Crossley et al., 2009).

Adhesion and invasion activity of S. aureus

The results obtained from adhesion and invasion assays for untreated S. aureus suggest that proteins other than FnBP are compensating for the internalization, which perchance contributed poorly to internalization (19%) (Foster et al., 2014) or other proteins performing internalizing functions, up to a certain extent. Another possibility is that internalization might be an FnBP-independent process, which may include binding of clumping factor of S. aureus to other host proteins such as cytokeratins resulting in a lesser amount of expression, leading to poor invasion. The significant reduction of staphylococcal protein expression in NaCl treated could be due to survival mechanism of S. aureus with NaCl treatment. Treatment with hydrogen peroxide caused highest population reduction but showed significantly higher adhesion and invasion than untreated S. aureus, supporting expression of binding proteins in higher amounts by the bacterium under stressful conditions.

Cytotoxicity activity of unstressed and stressed S. aureus

The initial increase in LDH in heat and acid treatments, followed by a subsequent decrease is an indication of a potential sudden increase in expression of specific proteins as in adhesion and invasion. But in NaCl treated cells LDH release was lower than any other treatment and unstressed bacteria up to 8h, suggesting possibly a lack of protein expression required for pathogenicity. Genesitier et al. (2005) showed that S. aureus can also induce apoptosis as early as 4h of infection. Kahl et al. (2000) reported that apoptosis may occur after 24h and replication of S. aureus inside host cell is essential for inducing apoptosis. Results of DNA fragmentation and morphological changes

of HEp-2 cells infected by untreated and treated S. aureus 700698 suggest that no apoptosis was observed until 24h, or due to inability of bacteria to replicate in HEp-2 cells. Da Silva et al. (2004) reported that airway epithelial cells infected with high concentration of S. aureus died after 24h and were more permeable to propidium iodide, a characteristic of necrosis than apoptosis. These results also indicated that stress treatments could not induce the expression of proteins, which are required for replication.

Conclusion

In summary, almost all treatments showed higher invasion ability than normal bacteria. S. aureus 700698 exposed to various stress conditions could result in change in LDH and protein expression which ultimately has an effect on pathogenicity. Additionally, there was no apoptosis in HEp-2 cells infected by untreated and treated S. aureus 700698. Further research is required to identify any protein complexes or pathways that play vital role in invasion process.

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Disclosure Statement

Authors disclosed no conflict of interest.

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