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Effect of Hydrogen Peroxide on *E. coli* and *S. aureus*

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Hydrogen peroxide is used as an antiseptic due to its ability to produce oxidants such as singlet oxygen, superoxide radicals and hydroxyl radicals. It is also effective in rapidly stopping capillary bleeding. H_2O_2 inhibits *E.coli* and *S.aureus* by formation of highly toxic hydroxyl radicals. These organisms are the most common pathogens having an unlimited reservoir and they can be eliminated by using hydrogen peroxide. This study aims to study the broad range effect of H_2O_2 in eliminating the common pathogens. The oxidative stress showed reduction in number of organisms at a concentration of 0.3 mM. These oxidative stressed organisms had reduced serum resistance and surface hydrophobicity.

Key words: Hydrogen peroxide, *E. coli* ,*S. aureus*, Serum resistance and Surface hydrophobicity.

Hydrogen peroxide (H_2O_2) is an antiseptic (compared to a preservative) since it quickly acts to kill microorganisms and has no long-term or preserving effect[1]. This short-lived action is due to hydrogen peroxide's rapid decomposition to oxygen and water upon contact with organic material. The antimicrobial action of hydrogen peroxide is not due to its oxidative properties as a molecule, but primarily in the production of other powerful oxidants such as singlet oxygen, superoxide radicals, and the hydroxyl radical[2]. These reactive oxygen species cause irreversible damage to a host of cell components such as enzymes, membrane constituents, and DNA. In fact, aqueous solutions of H_2O_2 alone will not cause protein, lipid, or nucleic acid modification without the presence of radical formation catalysts[3]. H_2O_2 is naturally produced by enzymatic systems, and is notably utilized by phagocytes in the destruction of bacteria within the phagolysozome[4].Of those radicals mentioned above, hydroxyl radical (HO.) production likely plays the largest role in the toxicity of hydrogen peroxide[5]. When produced adjacent to DNA, hydroxyl radicals are unique in that they can "both add to DNA bases and abstract H-atoms from the DNA helix"[6]. Hydroxylradicals may also damage cell membranes.

Escherichia coli is a gram-negative, rod shaped, facultative anaerobe[7]. As a part of the normal intestinal flora of humans and warm-blooded animals, it is a good indicator of fecal contamination. Only a few *E. coli* strains are pathogenic to humans. Pathogenic strains can cause infections of the urinary tract, lungs (pneumonia), blood (bacteremia), and intestines. Intestinal infections are caused by five types of *E.coli*: enterotoxigenic (ETEC), enteroaggregative (EAggEC), enteropathogenic (EPEC),

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enteroinvasive (EIEC), and enterohemorrhagic (EHEC) or verotoxin producing (VTEC).The EHEC strains produce two different cytotoxins that acts like the Shiga toxin produced by *Shigella dysenteriae*. These toxins cause both hemorrhagic colitis (inflammation of the large intestine) and hemolytic-uremic syndrome. Hemorrhagic colitis is characterized by abdominal cramps, diarrhea, and bloody discharge. Hemolyticuremic syndrome causes anemia, kidney damage, and possibly kidney failure[8].

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus and is the most common cause of staph infections. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population are long-term carriers of *S. aureus*[9] The carotenoid pigment staphyloxanthin is responsible for *S. aureus'* characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staph organisms which lack the pigment are more easily killed by host defenses. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections.

MATERIAL AND METHODS

The standard laboratory cultures of *E. coli* and *S.aureus* were used for all the experiments. Each experiment was carried out in triplicates. The culture were grown on sterile nutrient agar slants at 37°C for 24 hours. Fresh cultures were used for every experiment. The suspensions were prepared in fresh sterile physiological saline and cell density was maintained as 0.1 OD_{600}(approx $1x10^8$cells/ml).

Determination of Minimum inhibitory concentration (MIC) of test organisms against H_2O_2

MIC helps to find out the minimum concentration at which the growth of the organisms is completely inhibited. Concentrations of H_2O_2 ranging from 0.05mM to 0.5 mM were prepared using stock solution of 0.5mM standard (GRAS) H_2O_2 .The diluent used was sterile nutrient broth.the amount of inoculum was maintained as 0.1 ml/tube in a total volume of 10 ml.

Effect of oxidative stress

Hydrogen peroxide is a weak acid having strong oxidizing properties and acting as a powerful bleaching agent. The oxidizing capacity of hydrogen peroxide is so strong that it is considered a highly reactive oxygen species. The increasing concentrations of H_2O_2 in medium results in degradation of microorganisms by production of free radicals that cause oxidative stress and are therefore bactericidal.

10ml of sterile nutrient broths were prepared containing 0.1mM, 0.2mMand 0.3mM H_2O_2 respectively in 100ml capacity Erlenmeyer flasks. Each of these flask was inoculated with the test cultures to obtain a cell density of 0.1 OD600. These flasks were incubated on shaker at 37° C for 24 hours. The cultures thus obtained were centrifuged at 3000xg for 10 minutes. The supernatant was discarded and the packed cells were used for surface hydrophobicity and serum resistance.

Effect of stress on surface hydrophobicity

The effect of oxidative stress on the surface hydrophobicity of *E. coli* was studied by quantitative surface hydrophobicity assay[10]. Samples of cultures prepared as described above were washed with sterile phosphate buffered saline (PBS) and then suspended in PBS to get a density of 0.3 at OD_{600} (OD Initial). Three millilitres of bacterial suspension was mixed with 0.3 mL of p-xylene and vortexed for 1 minute and left for 30 minutes at room temperature. The hydrophobicity index was calculated by applying the following formula:

$$HI = \frac{ODI - ODF}{ODI} \times 100$$

Effect of stress on serum resistance

Effect of oxidative stress on the serum resistance of bacteria was evaluated by quantitative serum bactericidal assay[11]. Serum was obtained from healthy donors on the day of each test. Bacterial suspension (0.5 mL) was mixed with 1.5 mL of fresh undiluted serum and incubated at 37 °C. The viable count was determined at the beginning of incubation (0 h) and after 1, 2, 3 h of incubation by surface plating.

The number of bacteria killed was calculated by applying the following formula:

$$\% \text{ Death} = \frac{\text{Initial count} - \text{Final count}}{\text{Initial count}} \times 100$$

RESULTS

The minimum inhibitory concentration for *E.coli* was found to be 0.35mm of H_2O_2 whereas for *S. aureus* it was 0.4mMof H_2O_2. The oxidative stress had reduced the number of organisms as observed by surface spread plating.

Oxidative stress had a significant effect on the surface hydrophobicity of *S.aureus* as compared to *E.coli* (Table 1). There was almost 50% decrease in the cell surface hydrophobicity of *E. coli* and *S.aureus* when exposed to 0.3 mM of H_2O_2.

Oxidative stress caused a decrease in serum resistance of both the *E. coli* and *S.aureus* (Table 2). The decrease in serum resistance was significant in *E. coli* on exposure to 0.02 and 0.03 mm H_2O_2 . Exposure to 0.1 mm H_2O_2 did not cause a significant change in serum resistance of this strain. On the other hand, *S.aureus* showed a significant decrease in serum resistance when exposed to 0.1, 0.2 and 0.3 mm H_2O_2 . It is clear from the results that exposure to oxidative stress caused a significant decrease in serum resistance of *E. coli*.

Table 1. Effect of oxidative stress on surface hydrophobicity

Organism	Concentration of H_2O_2(mM)	Initial O.D $_{600nm}$	Final O.D $_{600nm}$
E.coli	0.1	0.3	0.21
	0.2	0.3	0.20
	0.3	0.3	0.19
S.aureus	0.1	0.3	0.19
	0.2	0.3	0.17
	0.3	0.3	0.14

Table 2: Effect of stress on serum resistance

Organism	Concentration of H_2O_2(mM)	Initial count (0hour)	Final count (3hour)
E.coli	0.1	117cfu/ml	85cfu/ml
	0.2	102cfu/ml	57cfu/ml
	0.3	82cfu/ml	44cfu/ml
S.aureus	0.1	144cfu/ml	57cfu/ml
	0.2	129cfu/ml	34cfu/ml
	0.3	105cfu/ml	07cfu/ml

DISCUSSION

During infection microorganisms are subjected to different kinds of stress. The oxidative stress affects the virulence of the microorganisms since surface hydrophobicity is required to adhere to the epithelial lining of the organ. The pathogen also needs to escape the phagocytic attack by showing serum resistance which is adversely affected by oxidative stress.

Hydrogen peroxide has been used as an antimicrobial agent since the early 1800's and is well known for its use as a topical skin application in 3% concentrations. Hydrogen peroxide has GRAS status and is approved by the FDA for packaging and surface sterilization in the food industry. The use of hydrogen peroxide to extend the shelf life of the minimally processed fruits and vegetables[12] is also reported.

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