

The Influence of Antimicrobial Disinfectants on the Survival of Food Borne Pathogens

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Abstract

These was carried out to evaluate the effectiveness of a selection of domestic products marketed as household disinfectants such as Sodium hypochlorite (NaOCl); 0.25%, 0.5%, 1%, 2%, 12% v/v & Chlorhexidine (CHX); 0.25%, 0.5%, 1%, 2% w/v], against some potential bacterial contaminants *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The research evaluated the effectiveness in relation to microbial susceptibility of target microbes and the effects of ionic strength and organic load. Disinfectant effectiveness was assessed by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), using Broth Dilution Method. It was found that 1% CHX was effective on both the organisms, 1% NaOCl did not inhibit the growth, but repeated experiments with increased concentration (12% NaOCl) inhibited the growth. The *S. aureus* appeared with high MIC of 0.03125%, was more resistant than *E. coli* (low MIC of 0.01563%). Manipulation of ionic strength [using sodium chloride (NaCl) w/v concentrations of varying percentages) and organic load [by addition of Bovine Serum Albumin (BSA) 1%, 2%, 4%, 5% and 10% w/v] was evaluated using Agar Diffusion tests in duplicate. The resistance ability of the organisms against the disinfectants had increased when grown in NaCl, where *E. coli* became more resistant than *S.*

aureus. However, Agar Diffusion Method, by using zones of inhibition; the results showed that 0.25% CHX was effective against both test organisms, while NaOCl was only effective against *E. coli* at the concentration of 0.5-1%. But, contrary to the Broth Dilution Method, *S. aureus* was more sensitive to the disinfectants than *E. coli*. When the disinfectants were used with BSA/NaCl, the antimicrobial activities were reduced. The BSA had more influence on the disinfectants than NaCl, and *E. coli* was more resistant than *S. aureus*.

Key words: Disinfectant, *E.coli*, *S.aureus*, Chlorhexidine, Minimum inhibitory concentration, Minimum bactericidal concentration

1.1 INTRODUCTION

The consumption of food contaminated with pathogenic bacteria and their toxins has resulted in food borne illnesses that have been of serious concern to public health [44]. In 1983-1987, an outbreak was reported in the United States in which the etiology was determined, and bacterial pathogens caused the largest number of outbreaks (66%) and cases (92%) [6]. Therefore, controlling the pathogens could reduce the incidence of food borne outbreaks and ensure foods supplied to the consumers would be wholesome, safe and nutritious [44].

Antimicrobial agents have been used in the control and to inhibit food borne bacteria and prolong the shelf life of processed food [44]. Food contaminated by pathogens results into food borne complications constituting major public health impacts in the Africa, United States, Europe and around the world. The United States Centre for Disease Control and prevention (CDC) reported data for food borne illnesses which accounted for approximately 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in U. S alone, each year [45]. It was reported that six pathogens are responsible for over 90% of estimated food-related deaths: Salmonella (31%), Listeria (28%), Toxoplasma (21%), Campylobacter (5%), Norwalk-like viruses (7%) and *Escherichia coli* 0157:H7 (3%) [44], [2].

However, some authors argued that *E. coli* is considered to be responsible for most of food borne illnesses [44], [20]. There are more than one million cases of food poisoning in the United Kingdom, with Campylobacter remaining as the most common bacterial food borne pathogen. In 2013, the European Union has reported a total of 5,196 food borne outbreaks, resulting in 43,183 infected humans, 5,946 hospitalizations and 11 deaths [18].

1.2 Antimicrobials

This is a term used to describe substances which demonstrate the ability to kill microorganisms or stop their actions or growth [44]. According to [24] antimicrobials are agents or drugs that prevent pathogenic action of microbes. They differ in chemical, physical, pharmacological properties. They differ in antibacterial spectrum of activity as well as in their mechanism of action [4].

The food industry has been making efforts using variety of non-antibiotic based antimicrobials which includes food additives and disinfectants to control the spread of food borne spoilage and pathogenic microorganisms [44]. Some of the additives like garlic, salts, sulphites and nitrites have been used for over 100 years. Later, information was discovered about the development of microbial resistance to some of these antimicrobials [17]. Hence an alarm was raised by the food industry for the development of more strategies and non-reliance on only non-antibiotic based antimicrobials for the control.

1.3 Disinfectants

Disinfectants are the substances that play a major role in maintaining acceptable health standards by considerably reducing microbial load and/or eliminating pathogens [21], [11],[4]. Disinfectants are the chemicals used to prevent infection, they kill

or inactivate microbes but not necessarily the spore forms. They are mostly used on inanimate objects like surfaces in food industry, toilets and kitchens to destroy or inhibit the growth of harmful microbes [4], [8]. There are different types of disinfectants, such as; alcoholic solutions, hypochloric solutions like sodium hypochlorite, peracetic acid, Quaternary Ammonium Compounds(QACs) such as Benzalkonium Chloride (BAC) [9].

Food borne pathogens have resulted into many serious health and economic problems of public health concern worldwide [44]. Globally, two-thirds of foods borne illnesses are considered to be caused by bacteria [26], [43]. The consumption of food contaminated by these bacterial species like *E. coli* and *S. aureus* may result into gastrointestinal diarrheagenic infections [7], [6] or gastroenteritis, skin infections, pneumonia [13],[26]. Due to the aforementioned, one of the greatest challenges faced mostly by food industries are the cleanliness and disinfection of utensils/other surfaces [43]. Therefore, knowledge of the best antimicrobial agents to be applied, the demonstration of effectiveness with regards to common commercial disinfectants under practical conditions, may play an important role in addressing the issues of food borne losses and illnesses [26].

2.1 MATERIALS AND METHODS

Reagents and chemicals used were of analytical grade. All glassware were cleaned with detergent and water and raised with distilled water, acetone and autoclaved at temperature 121oc before use.

The test solutions were prepared at the time of the experiment. The original solution of NaOCl (12%), CHX, BSA and NaCl (powder form) were obtained from Sigma Alderich Chemical Company Ltd, UK. the weighing balance used (Muttler P.E 1600), and appropriate grams of each chemical were weighed and dissolved accordingly. NaOCl was diluted to 0.25%, 0.5%, 1%, 2% and 12% (v/v). CHX was then dissolved in distilled water to produce 0.25%, 0.5%, 1% and 2% (w/v), BSA: 1%, 2%, 4%, 5% and 10% (w/v), NaCl: 5%, 10%, 20%, 30%, 40% and 60% (w/v). Each concentration was applied to its appropriate test. The strains of microorganisms used in this experiment were obtained from the National Collection of Industrial and Marine Bacteria (NCIMB).

The stock cultures were first grown on Tryptic Soy Agar (TSA) plates, and then transferred to fresh Tryptic Soy Broth (TSB) before use as 24 hrs

cultures. The TSA and TSB were obtained from Lab M Chemical Company Ltd UK.

2.2 Preparation of culture media

Tryptic Soy Agar (TSA) was prepared according method [1] and slightly modified.

2.3 Determination of MIC and MBC

The MIC of the test compounds was determined using the broth dilution method. The effectiveness of NaOCl and CHX was first analyzed against *E. coli* and *S. aureus* using broth dilution method [24].

In the second part of the determination of MIC and MBC, the disinfectants were mixed with the appropriate BSA and NaCl solutions in varying percentages, to determine their influence on the effectiveness of the disinfectants. That is, in each 1% CHX and 1% NaOCl, 12% NaOCl, for each organism; 500 µl of 30% NaCl and 20% BSA were added into TSB tubes (separately), inoculated with 100 µl of the appropriate organism and incubated at 37°C for 24hrs. Also, after the incubation, MBCs were determined from the clear tubes.

2.4 Antimicrobial Assay Using Disk Diffusion Method

The effectiveness of NaOCl and CHX disinfectants were tested for the growth and survival of *E. coli* and *S. aureus* using a zone of inhibition assay on TSA. Appropriate concentrations of NaOCl, CHX, BSA and NaCl were prepared. That is, 0.25%, 0.5%, 1% & 2% CHX (w/v); 0.25%, 0.5%, 1%, 2% and 12% NaOCl (v/v); 1%, 2%, 4%, 5% and 10% BSA (w/v); 5%, 10%, 20%, 30%, 40 and 60% NaCl (w/v). CHX, NaOCl and BSA solutions were filter sterilized with

0.45µm micro filters. While NaCl solutions were sterilized using the autoclave. 100 µl aliquot of 24 hrs culture was evenly spread on TSA plate, using plate spreader. Then, wells were dug on the inoculated agar plate surface using a 6 mm cork borer sterilized with 70% ethanol and flamed in Bunsen burner flame.

The first experiment was set up by adding 50 µl of the appropriate concentration of disinfectant only in each well. That is, 4 wells were dug on each plate agar surface; 0.25%, 0.5%, 1% solutions of the disinfectant and then one with sterile distilled water as control. Each treatment was in triplicate, and incubated at 37°C for 24 hrs.

The second experiment was carried out with combination of organic load or ionic strength to test for their influence on the effectiveness of the disinfectants in varying percentages.

The third experiment was set up with an increase in the concentrations of the disinfectants, organic load and ionic strength. For the disinfectants 2% solutions were used, and for NaCl; 10%, 20%, 40% and 60% solutions were used, while BSA; 2%, 4% and 10% solutions were used.

Therefore, at the end of each disk diffusion assay, the mean average of the radii of the zones of inhibition of each experiment, and for each concentration were measured using metric ruler in millimeter, recorded and analyzed statistically using one factor and two factor ANOVA

3.1 RESULT AND DISCUSSION

Table 1: Result of MIC and MBC of CHX and NaOCl on *E. coli* and *S. aureus*, with and without 20% BSA and 30% NaCl

stock organism	test	1%CHX			1% NaOcl			12% NaOcl		
		CHX	BSA + CHX	NaOCl Only	NaOCl	BSA + NaOCl	NaOCl	NaOCl	BSA + NaOCl	NaCl + NaOCl
E.Coli	MIC	0.0156	0.125	0.125	ND	0.25	0.125	0.75	0.75	0.375
	MBC	0.0625	0.5	0.125	ND	0.5	0.25	1.5	0.75	0.375
S.aureus	MIC	0.03125	0.0625	0.03125	ND	0.5	0.25	3.0	0.375	0.2875
	MBC	0.03125	0.25	0.125	ND	>0.5	>0.5	3.0	1.5	0.75

ND =not detected, CHX = Chlorhexidine, E.coli = Escherichia coli, S.aureus = staphylococcus, MIC = minimum inhibitory concentration, MBS =Minimum bactericidal concentration, BSA = Bovine serum albumi

Table 2: Result of MIC and MBC for the *E. coli* and *S. aureus* grown with 2.7% NaCl in TSB

organism	Test	1% CHX	1% NaOC	12% NaOcl
E.coli	MIC	0.0625	0.25	0.375
	MBC	0.25	0.25	0.375
S.aureus	MIC	0.0312	0.25	0.1875
	MBC	0.0625	0.5	0.750

E.coli = *Escherichia coli*, *S.aureus* = *staphylococcus*, MIC = minimum inhibitory concentration, MBS =Minimum bactericidal concentration, CHX = Chlorhexidine

Table 3: Result MIC and MBCfor the *E. coli* and *S. aureus* grown with 18% BSA in TSB

Organism	Test	1% CHX	1% NaOcl	12% NaOcl
E.coli	MIC	0.0625	0.25	0.375
	MBC	0.25	0.25	0.75
S.aureus	MIC	0.0313	0.125	0.01875
	MBC	0.0625	0.25	0.75

E.coli = *Escherichia coli*, *S.aureus* = *staphylococcus*, MIC = minimum inhibitory concentration, MBS =Minimum bactericidal concentration, CHX = Chlorhexidine

4.1 DISCUSSION

This present studied evaluated the antimicrobial activity of CHX and NaOCl against *E. coli* and *S. aureus* as food borne pathogens, using Broth Dilution and Agar Diffusion Methods. In the assessment method, ionic strength and organic load were added to evaluate the effectiveness of the disinfectant compounds [21]

The research data for this study were collected using two main methods; Broth Dilution and Agar Diffusion methods, where MICs/MBCs of the disinfectant agents were obtained. Also, zones of inhibitions of the test organisms by the disinfectants were determined. Therefore, both qualitative and quantitative methods were used in this investigation. In this study, CHX and NaOCl were found to have such relationship as common similarities and differences. They are common household disinfectants, effective against wide range of microorganisms, such as bacteria and fungi, but do not deactivate spore forms. The MIC/MBC results of this study, using Broth Dilution Method, indicate that, using the disinfectants only, 1% NaOCl did not inhibit the growth of the test organisms, which is in line with result reported in [42]. Another similar important finding was that, 0.25% NaOCl was not effective on *E. coli*, showing no zone of inhibition on TSA plates, but using 0.5-1% was effective (p=0). It is interesting to note that in almost all the results obtained, *S. aureus*, a Gram-positive bacterium

demonstrated to be more resistant to almost all the disinfectants, with low MICs and MBCs (Table 1). This result is consistent with the findings of [35] confirming that, *S. aureus* has an increased resistance to antimicrobial agents. In this current study, comparing the effectiveness of CHX with NaOCl showed that CHX appeared to be more effective against all the test organisms. Because 1% stock solution of CHX was observed to have inhibited the two organisms with low MICs of 0.01563% and 0.03125% for *E. coli* and *S. aureus* respectively (Table 1). These low MICs further indicated that *S. aureus* was more resistant to CHX than *E. coli*. However, MBCs for CHX were observed to be 0.0625% and 0.03125% for *E. coli* and *S. aureus* respectively, which were in contrary with the previous statement. This was consistent with [38] that low concentrations of CHX have bacteriostatic effect while high concentrations results in membrane disruption of the cells. Based on results of this work, 1% stock solution did not inhibit growth and thus effectiveness of NaOCl was observed to be less. Therefore, there was an increase in the concentration and the experiment was repeated with 12%. The 12% concentration yielded 0.75% and 3% MICs for *E. coli* and *S. aureus* respectively, while MBCs were also observed to be 1.5% and 3% for *E. coli* and *S. aureus*. This was in support of the initial statement, showing more resistant nature of *S. aureus* [11]. On the CHX and NaOCl than *E. coli*. These results corroborate the evidence documented in [35]

about the effectiveness of the two disinfectants used. The effectiveness of the CHX and NaOCl was also evaluated using BSA and NaCl. As shown in Table 2 BSA with CHX reduced the MICs of CHX to 0.125% and 0.0625% for *E. coli* and *S. aureus*, while MBCs were 0.5% and 0.25%. It was also been observed that NaCl with CHX have reduced the MICs to 0.125% and 0.03125% for *E. coli* and *S. aureus*, while MBCs were observed to be 0.125% for both organisms. This outcome is supported by the findings [8],[23]. However, NaOCl + BSA and NaCl, has a decrease in the effectiveness as observed. In respect to NaOCl with BSA, MICs were observed to be 0.75% and 0.375% for *E. coli* and *S. aureus*, while MBCs were 0.75% and 1.5%. But, NaOCl with NaCl experienced more effectiveness, with increased MICs and MBCs. The increased MICs were 0.375% and 0.18756% and MBCs; 0.375% and 0.75% for *E. coli* and *S. aureus* respectively (Table 2). These results were in line with those of previous studies [3],[7], [22]. Also base on the above outcome, as stated by [11] careful addition of ingredients to the disinfectants should be maintained for best efficacy. Therefore, the reduction in the effectiveness of the disinfectants by BSA is likely because it is an organic substance that could interact with the disinfectants. Thereby, reducing their oxidizing power, and lower their effectiveness against the test organisms, while NaCl is already known to be antimicrobial and antiseptic agent [42]. Different growing condition was studied for the disinfectants susceptibility of microorganisms grown with BSA/NaCl in TSB. It has shown that *E. coli* and *S. aureus* grown in 2.7% NaCl experienced certain changes in their MICs and

MBCs. As shown in Table 2, the MICs were observed to be 0.0625% and 0.03125% in CHX on *E. coli* and *S. aureus* respectively, while in Table 2, for those organisms grown without NaCl, the MICs were 0.01563% and 0.03125%. This shows that growing the *E. coli* in 2.7% NaCl rendered it to become more resistant to CHX because of the high MIC, while *S. aureus* was not affected, since it is among the normal flora of the skin usually associated with the NaCl excretion. Moreover, it was mentioned in the literatures [22], [38] that *S. aureus* can withstand up to 15% concentration of NaCl. Exactly, 12% NaOCl in TSB was also used to analyze the effect of the growth in NaCl. Data in Table 2 indicated 0.375% and 0.18756% MICs for *E. coli* and *S. aureus*, but in Table 3 (without NaCl), MICs for the two organisms were 0.75% and 3%, which further suggested the influence of NaCl on the resistivity of *E. coli*, while *S. aureus* was less affected. In the result section, Table 14 shows the effect of growth in BSA on *E. coli* and *S. aureus*. The MICs of CHX on *E. coli* and *S. aureus* were; 0.0625% and 0.03125% respectively, while in Table 1; it is shown to be 0.01563% and 0.03125% for the MICs. Also, the MICs of 12% NaOCl on the *E. coli* and *S. aureus* grown with BSA were; 0.375% and 0.08756% shown in Table 2, while MICs of 0.75% and 3% were obtained as indicated in Table 2 (those grown without BSA). Therefore, based on these MICs and MBCs from Tables 2 and 3 to those obtained in Table 1; it could be stated that growth of *E. coli* in NaCl/BSA is influenced and also affected the disinfectant sensitivity more than the *S. aureus*.

It seemed that, growing the organisms in NaCl or BSA might have led to the change in their physiology, increasing their resistance against the disinfectant agents, and subsequently, gaining the tendency to acquire resistance to the antimicrobials [31]. As shown in the result section, the test antimicrobial compounds (CHX and NaOCl) in most cases, were observed to be significantly different ($p < 0.05$) from each other and between different concentrations. However, the results of 1% disinfectant with BSA against the two test organisms were not significantly different at $p \geq 0.05$. However, CHX was very effective, as explained by [10], that 0.5-1% CHX can be used for disinfecting hands by health care providers. The CHX demonstrated clear zones of inhibition and a significant increase in concentration ($p = 0.021$). A significant difference exists between the two disinfectants as shown by two way ANOVA ($p = 0.008$). Significant differences between different concentrations in NaOCl and CHX, with p-values of $p = 0.0009$ and $p = 0$ respectively. However, there was no significant difference between the two disinfectants ($p = 0.869$). As showed that *S. aureus* was more susceptible than *E. coli*. This finding is in line with outcomes of [29] that concentration of ≥ 1 $\mu\text{g/ml}$ of CHX is effective against Gram-positive, while higher concentrations of 10-73 $\mu\text{g/ml}$ are the effective concentrations for Gram-negative bacteria like *E. coli*. Therefore, based on the results of this study it could be agreed that two assessment methods used are reliable and have

best determined the relationships between the two disinfectant agents and the test organisms. This assertion was earlier upheld by [38],[12] [23]

5.1 CONCLUSION AND RECOMMENDATION

The purpose of the current study was to evaluate the effectiveness of NaOCl and CHX against *E. coli* and *S. aureus*. Also, the influences of BSA/NaCl were tested on the antimicrobial activities of NaOCl and CHX against *E. coli* and *S. aureus*. Likewise the effectiveness of the disinfectants was evaluated in relation to microbial susceptibility of the target microbes (*E. coli* and *S. aureus*) and environmental factors (BSA/NaCl). The evaluation process was assessed using MIC, MBC and inhibitory zones by Broth Dilution and Agar Diffusion Methods.

It was found that using the Broth Dilution Method (MICs and MBCs), 1% CHX was more effective than 1% NaOCl, against the test organisms. Moreover, *S. aureus* was more resistant to the disinfectants than *E. coli*, having high MIC. The BSA/NaCl combination with disinfectants increased the MICs and reduced their effectiveness.

The study further showed that; using the Agar Diffusion Method, inhibitory zones observed on TSA plates demonstrated that, 0.25% CHX emerged to be effective on all test organisms, while 0.5-1% concentration of NaOCl formed the zones of inhibition than BSA/NaCl reduced the effectiveness of the disinfectants, with the reduced zones of inhibitions

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