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# Comparative Study of Disinfectant Efficacy of Bleach (JIK) and Ethanol against Staphylococcus Aureus and Pseudomonas Aeruginosa

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**Abstract:** A disinfectant is one of the diverse groups of chemicals which reduce the number of microorganisms present on an inanimate object. To determine the comparative study of disinfectant efficacy of Bleach (Jik) and Ethanol against Staphylococcus aureus and Pseudomonas aeruginosa, pure bacteria isolates of S. aureus and P. aeruginosa were obtained from Microbiology Laboratory, Federal University of Agriculture Makurdi. These isolates were further confirmed by subjecting them to series of biochemical tests. Results from these confirmatory tests were compared with standard identification keys. Comparative experiment of these two disinfectants (ethanol and bleach) efficacy was conducted against Staphylococcus aureus and Pseudomonas aeruginosa using agar well diffusion method. Different concentrations 100% (v/v), 75% (v/v), 50% (v/v) and 25% (v/v) of bleach and ethanol were tested on both test organisms. The results showed that all the disinfectants inhibited the growth of the test organisms in their concentrated forms. The diameters of zone of inhibitions showed that 100% (v/v) concentration bleach (jik) had the highest zone of inhibition on Staphylococcus aureus and Pseudomonas aeruginosa which was measured to be 26.33±1.53 and 20.67±0.58 respectively with 25% (v/v) of bleach showing the least zone of inhibition on the test organisms. For ethanol the highest zone of inhibition was shown at 100% (v/v) concentration with 12.00±2.65 and 10.00±1.00 on Pseudomonas aeruginosa and Staphylococcus aureus respectively while at 25% (v/v) both test organisms were resistant to ethanol. The minimum inhibitory concentration of the bleach disinfectant on Staphylococcus aureus and Pseudomonas aeruginosa is determined at 50%, while the test organisms were resistant to the ethanol at all concentrations. Bleach had a better effect on Staphylococcus aureus than Pseudomonas aeruginosa whereas; ethanol had a better effect on Pseudomonas aeruginosa than Staphylococcus aureus. Generally Ethanol showed least sensitivity on both test organisms as compared to Bleach.

**Keywords:** Disinfectant, Staphylococcus aureus, Pseudomonas aeruginosa, Ethanol, Bleach.

## INTRODUCTION

### 1.1 Background of Study

Disinfectants are important chemical agents used for variety of purposes. In the mid 1800s, the Hungarian physician Ignaz Semmeliveis and English physician Joseph Lister used these thoughts to develop some of the first microbial control practice for medical procedures. These practices include hand washing with microbes killing chloride of lime and use of techniques of aseptic surgery to prevent microbial contamination of surgical wounds (Hamamah, 2004). Over the last century, scientists have continued to develop a variety of physical methods and chemical agents to control microbial growth. Control directed at destroying harmful microorganisms is called disinfection. It usually refers to the destruction of vegetative (non-endospore forming) pathogens example bacteria by using a disinfectant to treat an inert surface or substances (Bhatia and Icchpujani, 2008).Ethanol and bleach are believed to have immediate effect against most organisms (Carly *et al.*, 2006).

Disinfectants are groups of chemicals which reduces the total number of microorganisms present (normally on an inanimate object). There are different definitions of the process of disinfection and disinfectant agents. Some defined as a chemical that inactivates vegetative cells of microorganisms but not necessarily high resistant spores. Cleaning and disinfection of surfaces are essential steps for maintaining the cleanliness of pharmaceutical industries, hospitals and environments (Rollins, 2000). Disinfectant as effective agents that kill or eliminates bacteria is widely used in various ways; especially in microbial laboratory. Disinfectant can be mainly divided into five agents; alkylating, sulfhydryl combining, oxidizing, dehydrating and permeable. The most commonly used disinfectants in laboratories are ethanol, bleach and Isol (Larson and Morton, 1991). Bleach also known as sodium hypochlorite is a broad spectrum disinfectant, nonspecific in their action, only action biological material that is present on any surface. Bleach oxidizes the cell of microorganism and attacking essential cell components including lipid, protein and DNA (Ho-HyukJang *et al.*, 2008). Ethanol, as a dehydrating agent, lies between the highly specific and broadly based categories. It is effective against actively growing bacteria and viruses with lipids based outer surfaces, but are not effective against bacterial spores or viruses that prefer watery environment. They cause cell membrane damages, rapid denaturalization of proteins with subsequent metabolism interference on cell lyses (Larson and Morton, 1991).

The aim of this research is to compare disinfectants efficacy of ethanol and bleach against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## 3.0 Materials and Method

### 3.0.1 Method

Agar well diffusion method was used to test for antibacterial susceptibility, using Kirby Bauer assay.

### 3.1 Sample Collection

Disinfectants (Ethanol, Bleach) and pure stocked bacteria isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Microbiology Laboratory Federal University of Agriculture Makurdi.

### 3.2 Preparation of test organisms

Pure colonies of the Isolates were obtained from the microbiological laboratory, Department of Microbiology, Federal University of Agriculture Makurdi.

Viability test of each organism was carried out by reviving the organisms in buffered peptone broth which was further sub-cultured in nutrient agar medium and incubated at $37^0$C for 24 hours. The likely identity of the clinically sourced isolates was further confirmed by subjecting the cultures to series of biochemical tests which includes; coagulase, catalase, indole, citrate and oxidase as described by (Sharma, 2009). Results from these biochemical tests were compared with standard identification keys as described by (Sharma, 2009).

1. **Catalase test:** Two (2) drops of hydrogen peroxide solution was added on a sterile grease free slide. A colony of the *Staphylococcus aureus* was collected using a sterile wire loop and then placed on the solution; bubbles of gas were examined within 10 seconds. This indicated a catalase positive test; control was also set up using a known colony of *Staphylococcus aureus* and examined together with the test. The results were read and recorded. Method used was described by (Cheesbrough, 2000).
2. **Coagulase test:** Slide Coagulase test is used to identify *Staphylococcus aureus* which produces the enzyme coagulase. A drop of physiological saline was made on two separate grease free slides. A drop of fresh human plasma was added to one of the suspensor (test) and they were mixed. The other suspension was used as negative control. A visible clumping of the test organism within 10 seconds was examined and result recorded.
3. **Citrate test:** Simmons citrate agar was inoculated lightly on the slant by touching the tip of the needle to the culture colony of 18-24 hours old. This was then incubated for 24 hours, after which development of blue color denoting alkalization was observed.

4. **Oxidase test:** This test is used to differentiate *Pseudomonas aeruginosa* from other enteric organisms, which are oxidase negative. Oxidase test strip (oxistrips) was placed on a sterile Petri dish; area to be tested was moistened with distilled water ensuring vividly that the strip wasn't saturated. Using a sterile inoculating loop a colony of the test organism suspected to be *Pseudomonas aeruginosa* was collected and smeared on the moistened area. Appearance of blue/purple color within 30 seconds indicated a positive result. The result was read and recorded. Method applied here was described by (Cheesbough, 2000)
5. **Indole test:** This test demonstrate the ability of certain bacteria to decompose the amino acid tryptophanto **indole.** This test was carried out by inducing 4ml of tryptophan broth into sterile test tubes after which the tube was inoculated aseptically by taking the growth from the culture. After which the tube was incubated at $37^0$C for 24 hours. 0.5 ml of Kovac's reagent was then added to the broth culture. Thereafter presence or absence of ring was observed.

### 3.3 Preparation of Disinfectants Concentrations

One milliliter (1ml) each of the Ethanol and Bleach (Jik) was added to 1ml of distilled water to give a concentration of 100% (v/v), Other concentration of 75% (v/v), 50% (v/v), and 25% (v/v)were prepared by double broth dilution method as described by (Udochukwu *et al.*, 2015).

For each disinfectant, four different disposable tubes were used with disinfectant name, tube number and concentration.

### 3.4 Susceptibility Testing (Agar Well Diffusion Method) Using Kirby Bauer Assay.

The inocula were prepared by inoculating the test organism in nutrient broth and incubated at for 24 hours. The turbidity of the overnight culture was compared to $10^5$McFarland Standard. After which 0.5ml of the cultured organisms each was pipette into the Petri dish and prepared Mueller Hinton Agar was pour plated and allowed to gel.

Wells were bored on solidified culture plates on the surface of the agar plates using 4mm cork borer. 0.2ml of different concentrations of each disinfectant was transferred into the wells using Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. The experiment was performed for both disinfectant on each organisms and the resulting zone of inhibition measured the diameter of the well using a meter rule in millimeters (mm).

### 3.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the disinfectants were determined according to themacro broth dilution technique as described by Baron and Finegold (1990). Standardized suspension of the test organisms was inoculated into series of five sterile test tubes of nutrient broth containing two fold dilution of the disinfectant and incubated at $37^0$C for 24 hours. After which the test tubes were observed for growth. The lowest concentration of the disinfectant in the broth which shows no growth of test organisms was recorded as the minimum inhibitory concentration.

### 3.6 Statistical Analysis

Data were analyzed for mean and standard deviation. Difference in parameter was tested for statistical differences at $P < 0.05$ using student ANOVA. All the analysis was done using statistical package service solution (SPSS) version 21.

## 4.0 RESULTS

### 4.1 Results for Susceptibility Testing

Table 1 shows zone of inhibition of bleach on both test organisms. *Staphylococcus aureus* had the highest zone of inhibition when the highest concentration of 100% (v/v) was used. All test organisms were susceptible to bleach at different concentration ranging from 100% (v/v) to 25% (v/v)

Table 2 shows zone of inhibition of ethanol on both test organisms. *Pseudomonas aeruginosa* had the highest zone of inhibition when the highest concentration of 100% (v/v) was used. All test

organisms were susceptible to ethanol at different concentration ranging from 100% (v/v) to 50% (v/v) but at 25% (v/v) both test organisms were resistance.

Table 3 displays the bactericidal test result of bleach and ethanol on the test organisms. Bleach and ethanol had no bactericidal effect at all concentrations. However, the Minimum Inhibitory Concentration (MIC) of bleach was observed at 50% concentration for both test organisms while the test organisms where resistance to ethanol at all concentrations.

**Table1: Zone of Inhibition of Bleach (Jik) on *pseudomonas aeruginosa* and *Staphylococcus aureus***

| **Organisms** | | **Concentrations** | | |
|---|---|---|---|---|
| | **100% (v/v)** | **75% (v/v)** | **50% (v/v)** | **25% (v/v)** |
| *Pseudomonas aeruginosa* | 20.67 ± 0.58 | 18.00 ± 0.00 | 15.67 ± 0.58 | 11.33 ± 1.16 |
| *Staphylococcus aureus* | 26.33 ± 1.53 | 22.67 ± 0.58 | 18.33 ± 1.53 | 14.00 ± 1.00 |

**DF=3 P=0.15**

**Table 2: Zone of Inhibition of Ethanol on *Pseudomonas aeruginosa* and *Staphylococcus aureus***

| **Organisms** | | **Concentrations** | | |
|---|---|---|---|---|
| | **100% (v/v)** | **75% (v/v)** | **50% (v/v)** | **25% (v/v)** |
| *Pseudomonas aeruginosa* | 12.00 ± 2.65 | 10.67 ± 0.58 | 5.67 ± 1.53 | 0.00 ± 0.00 |
| *Staphylococcus aureus* | 10.00 ± 1.00 | 5.67 ± 0.58 | 2.33 ± 0.58 | 0.00 ± 0.00 |

**DF=3 P= 0.17**

**Table3: Minimum Inhibitory Concentration of Bleach (Jik) and Ethanol against *Pseudomonas aeruginosa* and *Staphylococcus aureus***

| **Disinfectants** | **Organisms** | **Concentrations** | | | |
|---|---|---|---|---|---|
| | | 50% | 25% | 12.5% | 6.25% |
| Bleach | *Pseudomonas aeruginosa* | S | R | R | R |
| Bleach | *Staphylococcusaureus* | S | R | R | R |
| Ethanol | *Pseudomonas aeruginosa* | R | R | R | R |
| Ethanol | *Staphylococcus aureus* | R | R | R | R |

**KEY:**

**R** =Resistance

**S** = Susceptible

## 5.0 Discussion

The result obtained in this research shows the efficacy of bleach and ethanol against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* which supports former studies (Gaonkar *et al.*, 2006).The distribution of the activities of bleach is greater than ethanol because oxidation reactions will occur when bleach is dissolved in water, which can destroy organisms fold structure leading to sterilization this is supported by former study BarindraSena *et al.*, (2006) and ethanol sterilization is mainly due to dehydration of protein and the enzymes to deactivate and prevent bacteria growth (James *et al*., 1999). It is reasonable to explain that most protein have generally similar chemical characters for bleach to oxidize and deconstruct, but different protein has different biological characters, which causes selectivity for ethanol to deactivate. Ethanol bactericidal activities drop sharply when diluted below 50% concentration and optimum bactericidal concentration in the range of 60% - 90% solution in water this is because proteins are denatured more quickly in the presence of water (Moorer, 2009). Ethanol is used in the laboratories for disinfection because it evaporates at slow rate and less harmful to the hands .According to Moorer (2009) 70% ethanol had been found to be most effective to denature protein thereby killing bacteria, because of its diffusion rate and

transportation into the cells organism. Below 70% does not denature protein, while 85%-absolute ethanol evaporates fast and leave the protein untouched.

The susceptibility test is credibly significant because the significant differences of the analyzed data are higher than the probability value ($P<0.05$). According to Yi Hsing *et al.*, (2002) research work, some kinds of bacteria have resistance characteristics on ethanol. Its sterilization is mainly due to dehydration of protein enzyme deactivation and inhibits bacteria growth. Different proteins have different biological characters which cause selectivity in ethanol deactivation of organisms. This explains why *Pseudomonas aeruginosa* is observed to be significantly more resistant to ethanol disinfectant in this research work as compared to bleach. However, this conforms to Yi Hsing *et al.*, (*2002*). In addition, both disinfectants are effective for sterilization against the test organisms however; bleach (jik) has the highest inhibitory effect on *Staphylococcus aureus* while ethanol has more effect on *Pseudomonas aeruginosa.*

Overall, bleach and ethanol are both effective disinfectants for sterilization against kinds of bacteria, but bleach is slightly better in general cases.

### 5.1 Conclusion

Bleach has more effect on the test organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) at concentrations of 25% (v/v) (minimum concentration), 50% (v/v), 75% (v/v) and 100% (v/v) as compared to ethanol which presented inhibition effect at the concentration of 50% (v/v)(minimum concentration), 75% (v/v)and 100% (v/v).

*Staphylococcus aureus* shows higher degree of susceptibility to bleach as compared to *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is significantly more susceptible to ethanol disinfectant compared to *Staphylococcus aureus*, it is confirmed that bleach has more effect on the test organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) compared to ethanol.

The minimum inhibitory concentration (MIC) of the bleach disinfectant on *Staphylococcus aureus* and *Pseudomonas aeruginosa* is determined at 50%, while the test organisms were resistant to the ethanol at all concentration.

### 5.2 Recommendations

1. It is recommended that disinfectants be used at right concentrations to retain adequate activities of these disinfectants thereby improving health for all.
2. It is recommended that further research be carried out on disinfectant efficacy of bleach (Jik) and ethanol, to determine the minimum bactericidal concentration (MBC) of bleach and ethanol on the test organisms.

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