



The Effect of pH and Temperature on Phenol Coefficients of Two Common Disinfectants Using Clinical Isolates of *Escherichia coli* and *Staphylococcus aureus*

Chukwuebuka M. Ononugbo¹, Eleazar E. Reward¹ and Anthony C. Ike^{1,2*}

¹Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Biological Sciences, Benue State University, Makurdi, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors CMO and ACI designed the study and wrote the protocol. Authors CMO and EER managed the literature searches and wrote the first draft of the manuscript. Author ACI managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to evaluate the efficacy of two disinfectants, Jik and Roberts, under use-conditions against some hospital isolates using their phenol coefficient. The effects of pH and temperature on the phenol coefficients were also tested. Phenol coefficient still remains a valuable means of determining the effectiveness of disinfectants, even though phenol is no longer commonly used for disinfection.

Materials and Methods: Bacteria were isolated and identified using standard microbiological procedures from samples collected from the skin of patients and hospital environments like beddings, floors and trawlers. A 5% (w/v) solution of phenol and 5% (v/v) solution of disinfectants were used for determination of their phenol coefficients on standardized organisms containing about 1.5×10^8 cfu/ml. The effect of temperature was determined at 4°C and 45°C, while that of pH was

*Corresponding author: E-mail: anthonyc.ike@unn.edu.ng;

determined at pH 1 and 13.

Results: The results showed that *Staphylococcus aureus* was more susceptible to both disinfectants. Jik had a higher phenol coefficient for the test organisms (16 and 8) compared to Roberts (4 and 2) for *S. aureus* and *Escherichia coli* respectively. Both temperature and pH had a direct effect on the antibacterial activities of the disinfectants. The phenol coefficient was higher for both organisms at 45°C than at 4°C for Roberts. In the case of Jik, the phenol coefficient reduced as the temperature was increased to 45°C. At pH 13, Jik gave a higher phenol coefficient, while Roberts gave a higher phenol coefficient at pH 1.

Conclusions: Temperature enhances the performance of Roberts but has a negative effect on that of Jik. Roberts performs better at acidic pH while Jik performs better at alkaline pH. For disinfection purposes, it is recommended that different types of disinfectants be employed in the rotation to help prevent the development of resistant strains of microorganisms.

Keywords: Phenol coefficient; disinfectants; pH; temperature; *Escherichia coli*; *Staphylococcus aureus*.

1. INTRODUCTION

Since the identification of microorganisms as agents of infection, various methods to either totally eliminate them or just reduce the number of viable cells, have been described. A great variety of microorganisms, both pathogenic and commensals, potentially contaminate inanimate surfaces, water, wound surfaces, etc. Contamination is even highest in the hospital environment and particularly in the microbiology laboratory. The sources of the contamination may be air, skin, hair, clothing, working surfaces, among others. Environmental surfaces are an epidemiological important reservoir of nosocomial bacterial species, which causes nosocomial infections [1].

Nosocomial infection is an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission [2]. Members of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) are the primary cause of nosocomial infections around the world [3]. The most frequent infections are those of surgical sites as well as skin and soft tissue sites, blood, urinary tract, upper and lower respiratory tracts. Most of these infections are associated with invasive medical devices or invasive surgical procedures [4]. Factors facilitating the spread of nosocomial infections are impaired immunity, extremities of age, severe illnesses, treatments with broad-spectrum antibiotics, the ever-increasing variety of medical procedures and invasive techniques. These create potential routes of infection and transmission of drug-resistant microorganisms

among crowded hospital populations where poor infection control practices may facilitate transmission [5].

Disinfection is defined as the selective elimination of certain undesirable organisms in order to prevent their transmission [6]. This is achieved by the use of chemical substances called disinfectants. Disinfectants may be defined as agents that kill or inhibit the growth and development of microorganisms [7]. Disinfectants are used in hospitals as pre-operative and surgical scrubs, general disinfection of surfaces and for disinfecting equipment. Given that only a few numbers of disinfectants are available in developing countries because of limited resources or cost restrictions, the surveillance of nosocomial pathogens and proper use of whatever disinfectants and other antimicrobial agents available cannot be over emphasized. There are three main purposes for which disinfectants are used. These are a decontamination of objects before disposal or re-use, reduction of microbial contamination of the inanimate environment and lastly disinfection of the skin or hands. The use of disinfectant at a concentration lower than the recommended concentration has been identified as dangerous practices [8].

Roberts and Jik are liquid disinfectants frequently used in hospitals and other health care settings in Nigeria and are constituted in various dilutions with varying degrees of effectiveness against microorganisms. This study is aimed at evaluating the efficacy of the two disinfectants under use-conditions against some hospital isolates notably, *Staphylococcus aureus* and *Escherichia coli* using the phenol coefficient test and determining the effect of pH and

temperature on the phenol coefficients of these disinfectants.

2. MATERIALS AND METHODS

2.1 Samples Collection

Sterile swab sticks were used for collection of samples. Samples were collected from the University of Nigeria, Nsukka (UNN) Medical Center. Hospital beddings, floors, trawlers, and the skin of some patients were swabbed and taken to the Department of Microbiology laboratory UNN for analysis.

2.2 Isolation and Identification of Test Organisms

Two selective media notably Manitol Salt Agar (M. S. A.) and Eosin Methylene Blue (E. M. B.) were used for the isolation of *S. aureus* and *E. coli* respectively. The samples were cultured using the streak plate method and plates were incubated at 37°C for 24-48 h. Suspected colonies showing typical morphology of *S. aureus* and *E. coli* on their respective selective media were purified on nutrient agar plates and were further identified using standard microbiological techniques, which included: Gram stain, motility test, methyl red test, urease test, indole test, citrate test, catalase test and coagulase test [9]. Identified isolates were stored in a nutrient agar slants at 4°C until used.

2.3 Preparation of Test Disinfectants

A 5% (w/v) solution of phenol was prepared by dissolving 5 g of phenol crystals in 95 ml of sterile distilled water. A 5% (v/v) solution of the disinfectants was prepared by adding 15 ml and 7.5 ml of the test disinfectants into 300 ml and 150 ml of sterile distilled water for Roberts and Jik respectively. Doubling dilutions was then carried out on each of the 5% solution (1:20) of the test disinfectants by adding 50 ml of 5% solution into 50 ml of sterile distilled water to give 1:40. The same procedure was carried out up to the last dilution. The control was sterile distilled water.

2.4 Standardization of Test Organisms

Three fold serial dilutions of test organisms were carried out by standing six test tubes containing 2 ml of sterile normal saline. Serial dilutions were carried out by taking 1 ml of 24 h nutrient broth

culture of the test organism using a micropipette and adding it into the first test tube, this was mixed and 1 ml taken and inoculated into the second test tube. This was done until the sixth test tube. Then, the dilution of test organism that corresponds to the freshly prepared 0.5% McFarland standard (1.5×10^8 cfu/ml) was used.

2.5 Phenol Coefficient Test

Serial dilutions of both disinfectants and phenol were made in distilled water, starting with 1:20 (5%) dilution. A 0.5 ml of a 24 h standardized culture was dispensed into each dilution of the disinfectants and phenol and incubated. At exactly 5 min after the culture was added to the first dilution, one loopful from each tube was streaked on already prepared nutrient agar plates. This was done for all dilutions and incubated at 37°C for 24 - 48 h. The same was done at 10 min and 15 min of incubation. The results were expressed as growth or no growth. The phenol coefficient was determined as the minimum dilution of phenol and test disinfectant that killed the test organism at 10 min but not at 5 min.

Phenol coefficient =

$$\frac{\text{The concentration of test disinfectant killing at min but not at 5 min}}{\text{The concentration of phenol killing at 10 min but not at 5 min}}$$

Disinfectants with a phenol coefficient greater than 1 were more effective than phenol. The higher the phenol coefficient value, the more the efficacy of the disinfectants was compared to phenol.

2.6 Effect of Temperature on the Phenol Coefficient

The effect of temperature on the phenol coefficient of the test disinfectants was determined. This was done by standing the different dilutions of the test disinfectants in a water bath at 45°C for 40 min and in the refrigerator at 4°C for 40 min. Then the phenol coefficient test was carried out for each disinfectant, using the test organisms one at a time. The phenol coefficients were then calculated.

2.7 Effect of Ph on the Phenol Coefficient

The pH of the different dilutions of the test disinfectants was adjusted to 1 and 13 by adding drops of hydrochloric acid or sodium hydroxide.

Then the phenol coefficient test was done, using one of the test organisms for each disinfectant at a time. The phenol coefficients were then calculated.

2.8 Data Analysis

The data were organized and analyzed with simple descriptive statistical methods and presented in graphs and table.

3. RESULTS

Staphylococcus aureus was identified as golden yellowish colonies on M. S. A., Gram-positive cocci, catalase and coagulase positive organism. *E. coli* were identified as greenish colonies with a metallic sheen on E. M. B., Gram-negative short rods, motile, indole positive, urease negative, citrate negative and positive to methyl red test.

The results of phenol coefficients of the two disinfectants used against the test organisms are presented in Table 1. Both test disinfectants had a phenol coefficient greater than 1, with Jik recording higher phenol coefficient for the two test organisms.

The results on the effect of temperature on the phenol coefficients show that for *S. aureus*, Jik had the highest phenol coefficients of 16 at 4°C, while Roberts had the lowest phenol coefficient of 4 at the same temperature. At a temperature of 45°C, both disinfectants showed an equal level of activity, with the same phenol coefficient of 8 (Fig. 1).

For *E. coli*, Roberts showed higher activity at a higher temperature, with phenol coefficient of 16 at 45°C and a phenol coefficient of 4 at 4°C, while temperature did not affect the activity of Jik on the organism, which had the same phenol coefficient at both temperatures tested (Fig. 2).

As shown in Figs. 3 and 4, pH had a limited effect on the activity of Roberts, with a moderate activity at acidic pH and a low activity at alkaline pH on both test organisms. However, pH had a drastic effect on the activity of Jik, which had low phenol coefficient at acidic pH and very high phenol coefficient at alkaline pH on both organisms. The highest phenol coefficient of 32 was recorded with Jik on *S. aureus* at pH 13 (Fig. 3).

Table 1. The phenol coefficient of the disinfectants against the test organisms

Disinfectant	Test organism	Phenol coefficient
Roberts	<i>S. aureus</i>	$320/80 = 4$
	<i>E. coli</i>	$160/80 = 2$
Jik	<i>S. aureus</i>	$1280/80 = 16$
	<i>E. coli</i>	$640/80 = 8$

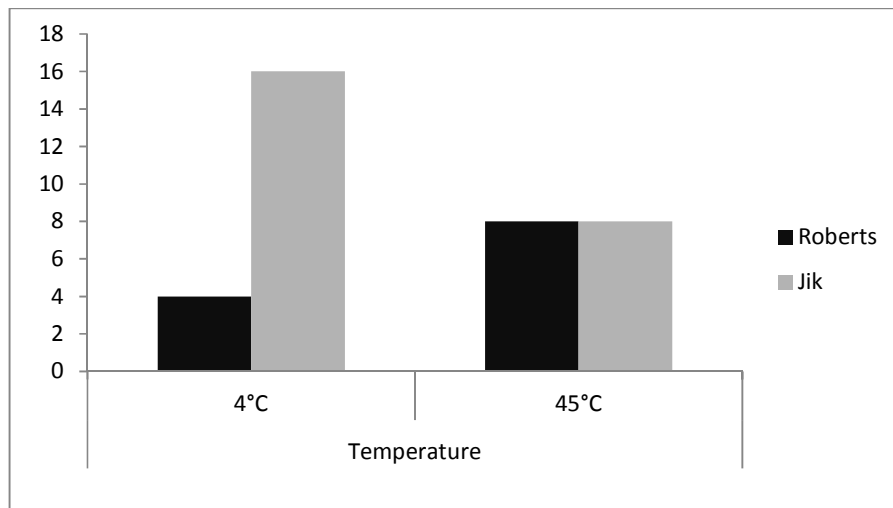


Fig. 1. Effect of Temperature on the phenol coefficient of Roberts and Jik on *Staphylococcus aureus*

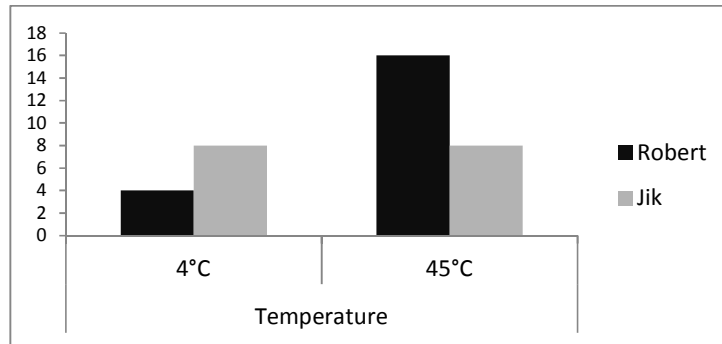


Fig. 2. Effect of Temperature on the phenol coefficient of Roberts and Jik on *Escherichia coli*

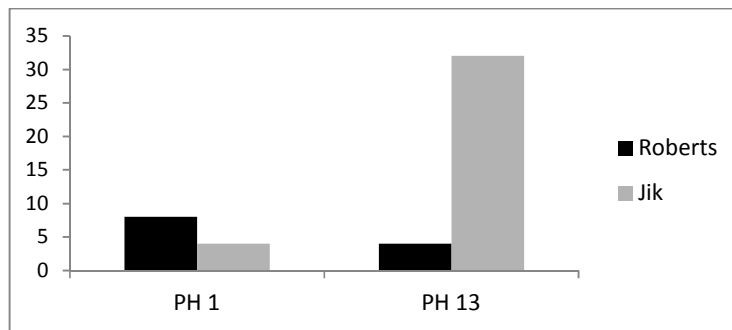


Fig. 3. Effect of pH on the phenol coefficient of Roberts and Jik on *Staphylococcus aureus*

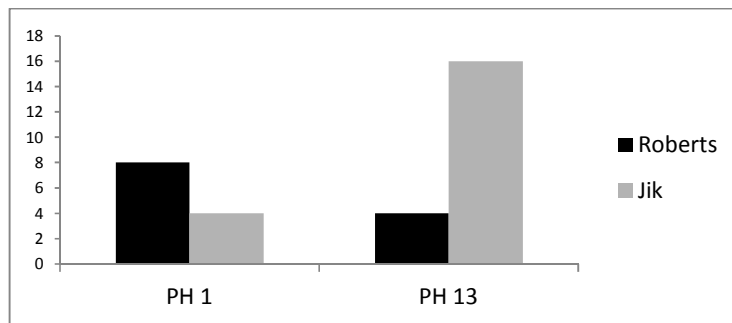


Fig. 4. Effect of pH on the phenol coefficient of Roberts and Jik on *Escherichia coli*

4. DISCUSSION

A good-quality disinfectant should be affordable, less toxic, non-irritating, non-corrosive and have a broad spectrum of antimicrobial activity. It is important for healthcare facilities to carry out periodic checks on the efficacy of disinfectants prior to first use and while in-use in order to forestall large-scale increase in microbial load due to disinfectant failure.

The antimicrobial efficacy of the Jik (*active ingredient* - hypochlorite) and Roberts (*active ingredient* - Dichlorometaxyleneol) against *Escherichia coli* and *Staphylococcus aureus* was

investigated in this study. These two organisms are members of the ESKAPE pathogens, which are the foremost cause of nosocomial infections throughout the world [3]. The findings of this work correlate with that of another study, in which it was reported that due to the higher degree of complexity in cell wall structure, Gram-negative organisms are more resistant to the effects of disinfectants compared to Gram-positive organisms [10]. However, the findings of this study differ from the findings of another study that reported Gram-positive test organism (*S. aureus*) to be more resistance than Gram-negative test organism (*E. coli*) [11]. The differences in the findings could be attributed to

several factors such as differences in the active components, mode of action and the activity of the disinfectants studied. The two disinfectants analyzed had a broad spectrum of activity, showing activity against both Gram positive and Gram negative bacteria which is in agreement with what was reported in another study [12].

Jik was effective against both *S. aureus* and *E. coli* in this study contrary to the findings of another study that reported Jik to be ineffective against *Salmonella* test isolates [13], which is also a Gram-negative organism. This disparity can be attributed to the decreased efficacy of hypochlorite against pathogens in the presence of fat. However, our findings correlate with that of a study carried out recently that reported the effectiveness of sodium hypochlorite against *S. aureus* biofilms [14], which is an important factor to consider in the efficacy of disinfectants as the formation of biofilms confers up to 1,000 times more resistance to pathogens [15]. Also, in tandem with the findings in this study, other studies have reported the sensitivity of other important agents of nosocomial infections such as *Enterococcus faecalis* [16], *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *E. coli* [17], to disinfectants containing dichlorometaxyleneol as the major active ingredient.

Disinfectants that have a phenol coefficient greater than 1 are more effective than phenol and vice versa [11]. Both Jik and Roberts are more effective than phenol from the results of this study. The phenol coefficient of Roberts increased with increase in temperature. This shows that Roberts is more active at high temperature than low temperature. In the case of Jik, the phenol coefficient reduced as the temperature was increased. This is due to the fact that high temperature favours the formation of sodium chlorate at the expense of hypochlorite. However, this observation is in variance to 100-fold increase in antimicrobial efficacy of sodium hypochlorite reported in another study [18]. The disparity may be attributed to the concentration of the active components present in the test disinfectants analyzed. However, our findings indicate that Roberts is a better disinfectant than Jik at high temperature. Some workers have also reported the lower efficacy of sodium hypochlorite compared to other agents like Hyperclean and Chlor-Xtra at different temperatures [19]. At low temperature, phenol coefficients of the two disinfectants were not affected; an indication that

cold conditions have minimal effects on the activities of the disinfectants.

The effect of pH on the phenol coefficient of Roberts indicates that Roberts is more active at acidic conditions than at alkaline conditions. High phenol coefficients were obtained at low pH than at high pH for the two test organisms. In the case of Jik, it performed better at alkaline pH, since it gave high phenol coefficient at this pH as compared with acidic pH, for the two test organisms. This finding though differs from that of another study, which reported that the antimicrobial efficacy of hypochlorite increased with decreasing pH [20]. This may be attributed to the disparity in methodology between the two studies.

5. CONCLUSION

This study has shown that the two widely used disinfectants, Jik and Roberts are effective against both Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*). These two organisms are well-known agents of nosocomial infections. However, the two disinfectants showed differing activities under different conditions. It is therefore recommended that different types of disinfectants be used in rotation to cover the different prevailing environmental conditions in hospitals, laboratories and clinics. This will also help to prevent the development of resistant strains of microorganisms. Ethical approval and consent are not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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