

Full Length Research Paper

Evaluation of activity of dichloroxylenol (1.2% w/v) on *Staphylococcus aureus* Oxoid 701/1 Lot 610254 and clinical isolates of *Escherichia coli* and *Salmonella typhi*

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The activity of dichloroxylenol (1.2% w/v), a commercial disinfectant formulation called Tiscol, on *Staphylococcus aureus* Oxoid 701/1 Lot 610254 and clinical isolates of *Escherichia coli* and *Salmonella typhi* was carried out using the kill kinetics and the Kesley – Sykes procedures. The study demonstrates that the effectiveness of dichloroxylenol (1.2% w/v) against the test bacteria was time and concentration dependent and markedly affected by the presence of organic matter. Dichloroxylenol (1.2% w/v) also showed slightly greater activity against *S. aureus* Oxoid 701/1 Lot 610254 than against *Escherichia coli* and *Salmonella typhi*, indicating a probable role for cell wall in the susceptibility of bacteria to dichloroxylenol (1.2% w/v). The Kesley – Sykes results suggest that in conditions of high contamination or presence of organic matter such as food, blood or tissue particles, dichloroxylenol (1.2% w/v) at or above 6% v/v can be appropriate in disinfection.

Key words: Dichloroxylenol (1.2% w/v), Kill kinetics, Kesley – Sykes method.

INTRODUCTION

Antiseptics and disinfectants are essential part of infection control practices and are useful in prevention of nosocomial infections (Rutala, 1996) as well as prevention of microbial contamination and infection risks in foods. Despite the widespread use of disinfectants, however, food poisoning outbreaks and nosocomial infection cases are still being reported with the attendant economic and health implications on the general public (Johnson et al., 2002). It has been stated that 5 to 10% of the annual population of hospitalized patients in the United States acquired nosocomial infection during hospitalization (Sagripanti and Bonifacino, 1999). Between January and June, 2001 alone, a total of 4 348 cases of food poisoning were reported with 87 deaths in Nigeria

(Federal Epidemiology Division, 2001). Mead et al. (1999) reported that an average of 76 million cases of food poisoning was recorded in the United States alone each year.

The increasing reports of food borne diseases and nosocomial infections have not only suggested an exaggerated belief in the effectiveness of disinfection and sterilization procedures (Sagripanti and Bonifacino, 1999) but more importantly have increased interest in the evaluation of effectiveness of disinfectants in destroying pathogens and microbial contaminants (Gronholm et al., 1999). While several chemical agents are commercially available as disinfectants, dichloroxylenol (1.2% w/v) with the brand name Tiscol is a popular disinfectant formula-

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lation widely applied in hospitals and laboratories in Nigeria. It has also found wide applications in domestic sanitation in homes and restaurants as a cleaning and disinfecting formulation. Dichloroxylenol (4 – chloro – 3, 5 – dimethylphenol, p – chloro – m – xylenol), a chlorinated phenol is a colourless, crystalline substance. It is the key halophenol used in many disinfectant formulations and has a widespread use over many years (Bruch, 1996; McDonnell and Russell, 1999).

Notable obligate and opportunistic pathogens of importance in infection control include *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. *Staphylococcus aureus* which is persistently present on carrier individuals was considered by Johnson et al. (2002) as a transient skin microorganism due to its ability to cause skin infections. It is one of the leading pathogens that cause hospital - acquired infections, such as tissue abscess, endocarditis, and bacteraemia and it has also been implicated in food poisoning (Felmingham et al., 1998; Rattanachaiakunsopon and Phumkhachorn, 2009). *Salmonella typhi* is a predominant etiologic agent of enteric fever and salmonella food poisoning. In the United States, 40 000 cases of salmonellosis are reported annually, with approximately 1000 deaths from acute salmonellosis (Ramesh et al., 2002). Salmonellosis continues to be one of the most common bacterial gastroenteritis in many countries of the world, including Nigeria, Singapore, and many of the developing countries (Tay et al., 1994; Gautam et al., 2002). Pathogenic strains of *E. coli* have been implicated in urinary tract infections (UTIs), dysentery, cholera – like diseases, and diarrheas that may be associated with the hemolytic uremic syndrome (Nester et al., 2004). The objective of this study was to determine the effectiveness of dichloroxylenol (1.2% w/v), as formulated in Tiscol, in reducing or eliminating populations of *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* in the presence or absence of organic materials.

MATERIALS AND METHODS

Sample

Dichloroxylenol (1.2% w/v) with the brand name Tiscol was purchased from a chemical manufacturing company in Lagos, Nigeria.

Test organisms

Staphylococcus aureus Oxoid 701/1 Lot 610254 was obtained from the Lagos State Drug Quality Control Laboratory, Ikeja. It was confirmed by the decolourisation of mannitol salt agar upon inoculation and incubation at 37°C for 24 h. *Escherichia coli* and *Salmonella typhi* were clinical isolates from Lagos State University Teaching Hospital (LASUTH), Ikeja. *Escherichia coli* was confirmed by inoculation on eosin methylene blue (EMB) agar to produce a greenish metallic sheen upon incubation at 37°C for 24 – 48 h.

Salmonella typhi was confirmed by inoculation onto deoxycholate citrate agar (DCA) with the development of pale non – lactose fermenting, urease negative colonies that failed to produce gas with sugar substrates.

Kill kinetics test

The kill kinetics test was carried out as previously described by Boswell et al. (1997). An overnight culture of each test organism in the logarithmic phase of growth was washed and diluted using normal saline and standardized to obtain transmittance of 25% at wavelength of 580 nm using a colorimeter. From the standardized bacterial suspension, 0.2 ml was transferred into different tubes containing the disinfectant dilutions of 1:10, 1:20, 1:30, 1:40 and 1:50, while another 0.2 ml was plated on nutrient agar (NA) to determine the initial population of each bacterial suspension. At intervals of 5 min, 1 ml from each bacterial – disinfectant suspension was inoculated onto NA and incubated at 37°C for 24 h. The colony forming unit per milliliter (cfu/ml) was determined for each interval, from which the log cfu/ml was calculated.

Kesley – Sykes test

The Kesley – Sykes test was carried out as previously described by Callegaro et al. (1992). An overnight culture of *S. aureus* and *E. coli* were used. The test materials were prepared in two sets; one for the performance of the test under clean condition and the other for the performance under dirty condition. The dirty condition was simulated by the addition of autoclaved cells of *Candida albicans*.

An overnight slant culture of test organism was washed with sterilized hard water (342 hardness) and made up to 10 ml suspension with the hard water in two test tubes. One suspension (suspension A) was for the test under clean condition. To the other suspension (suspension B; for test under dirty condition) was added autoclaved cells of *Candida albicans*, amounting to a final concentration of about 0.5%.

From both suspensions, 1 ml was separately plated on NA to determine the initial population of the bacterial cells. One milliliter of suspension A and B was separately added, at times 0 min, 10 min and 20 min, to different test tubes containing 3 ml of dichloroxylenol 1.2 % (w/v) at the three required concentrations; the concentration anticipated for positive outcome of the test, concentration at 50% higher and the concentration at 50% lower (Kelsey and Maurer, 1974). An aliquot, 0.2 ml of the microorganism – disinfectant mixture was inoculated into each of 3 sets of five replicate test tubes containing 10 ml thioglycolate broth at time 8 min after each addition of inoculum. In all, the microorganism – disinfectant mixture was inoculated into 3 sets of five replicate culture tubes. The entire sets of tubes were then incubated at 32°C for 24 to 48 h and observed for growth. Absence of growth in at least two of the broth cultures in the first two sets of test tubes was indicative of positive outcome of the test (Callegaro et al., 1992).

RESULTS

Kill kinetics of dichloroxylenol (1.2% w/v) on the test bacteria

The activity of different dilutions of dichloroxylenol (1.2% w/v) with time of exposure against *Staphylococcus aureus* Oxoid 701/1 610254 (Figure 1), *Escherichia coli*

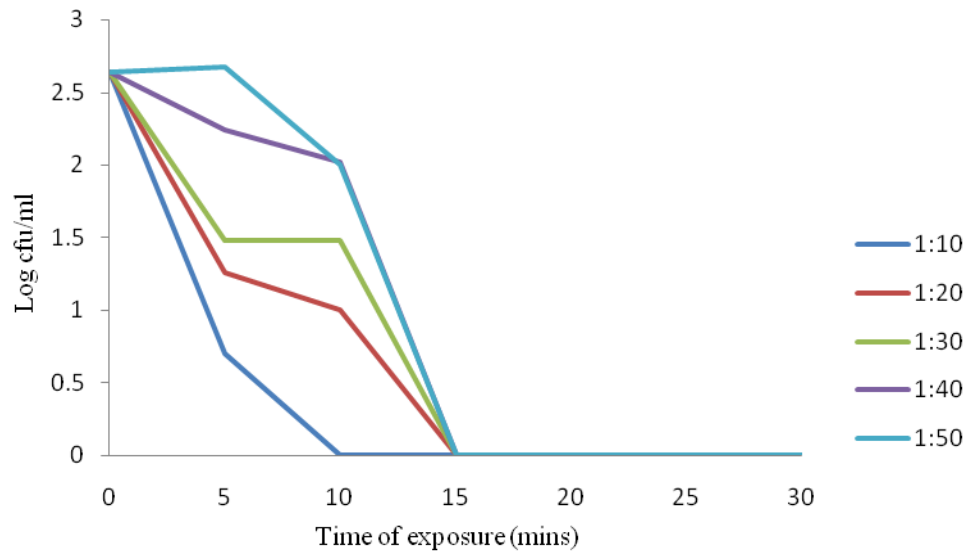


Figure 1. Kill kinetics of dichloroxylenol (1.2% w/v) against *S. aureus* Oxoid 701/1 Lot610254. Values are in cfu ml^{-1} , inoculum concentration was $4.35 \times 10^2 \text{ cfu ml}^{-1}$.

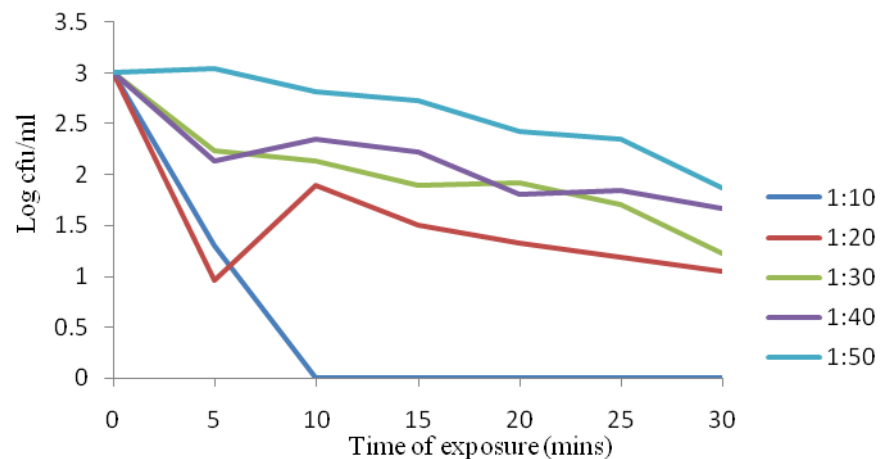


Figure 2. Kill kinetics of dichloroxylenol (1.2% w/v) against *E. coli*. Values are in cfu/ml , Inoculum concentration was 1.0×10^3 .

(Figure 2) and *Salmonella typhi* (Figure 3) was demonstrated in terms of log value of colony forming units per milliliter (log cfu/ml) at 5 min intervals for 30 min exposure. The results in Figures 1, 2 and 3 revealed that at the least dilution (1:10) used in this study, dichloroxylenol (1.2% w/v) reduced the populations of *S. aureus* Oxoid 701/1 610254, *E. coli* and *S. typhi* approximately by 99, 98 and 96% respectively at 5 min exposure time. At 10 min exposure, the same dilution completely destroyed *S. aureus* Oxoid 701/1 610254 and *E. coli* while achieving the complete destruction of *S. typhi* at 15 min exposure. At 30 min., all the dilutions of dichloroxylenol (1.2% w/v) except 1:50, achieved

complete destruction of cells of *S. aureus* Oxoid 701/1 610254 and *S. typhi*. For the *E. coli* strain, however, only the 1:10 dilution achieved total destruction of the cells at 30 min maximum exposure time.

Kesley – Sykes test

The capacity test for dichloroxylenol (1.2% w/v) was carried out using autoclaved yeast cells to simulate presence of organic matter in dirty condition. In clean condition (Tables 1 and 2), dichloroxylenol (1.2% w/v) failed against the two test organisms; *S. aureus* Oxoid

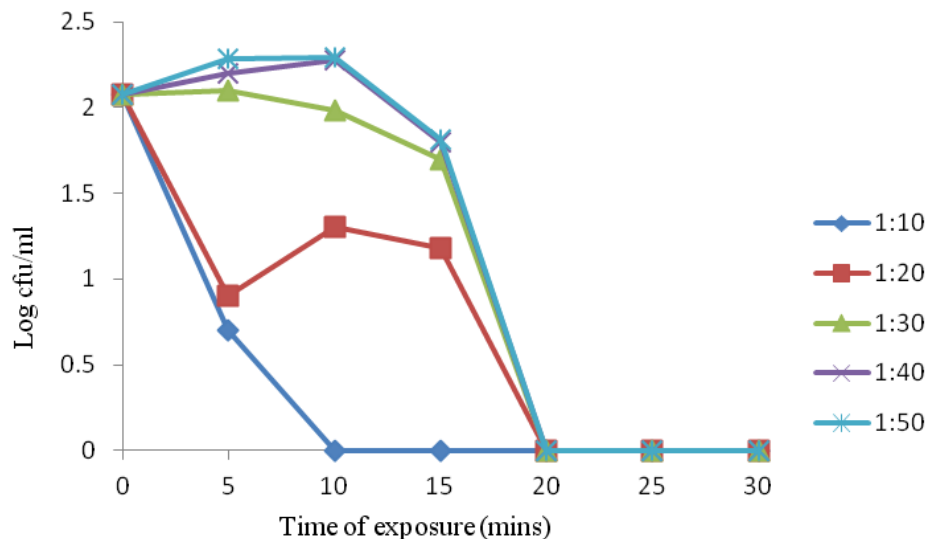


Fig 3: Kill kinetics of dichloroxylenol(1.2% w/v) against *Salmonella typhi*

Figure 3. Kill kinetics of dichloroxylenol (1.2% w/v) against *S. typhi*.

Table 1. Kesley – sykes test result of dichloroxylenol (1.2% w/v) with *Staphylococcus aureus* (clean condition)

Concentration (% v/v)	Inoculum size	Challenge number			Result
		1 (0 min)	2 (10 min)	3 (20 min)	
2	1.4×10^3	+++++	+++++	+++++	Fail
4	1.4×10^3	-----	-----	+++++	Pass
6	1.4×10^3	-----	-----+	---++	Pass

+++++ Shows growth in all set of 5 tubes; - - - + + Shows growth in 2 of a set of 5 tubes; - - - - + Shows growth in 1 of a set of 5 tubes; - - - - Shows no growth in all tubes of a set of 5 tubes.

Table 2. Kesley – sykes test result of dichloroxylenol (1.2% w/v) with *Escherichia coli* (clean condition)

Concentration (% v/v)	Inoculum size	Challenge number			Result
		1 (0 min)	2 (10 min)	3 (20 min)	
2	1.6×10^3	+++++	+++++	+++++	Fail
4	1.6×10^3	-----	-----	- +++++	Pass
6	1.6×10^3	-----	-----	---++	Pass

+++++ Shows growth in all tubes of a set of 5 tubes; - +++++ Shows growth in 4 of a set of 5 tubes; - - - + + Shows growth in 2 of a set of 5 tubes; - - - - Shows no growth in all tubes of a set of 5 tubes.

701/1 610254 and *E. coli* at 2%, a concentration below the manufacturer's recommendation, as there was growth in all the fifteen tubes. At 4 and 6% (manufacturer and above manufacturer's recommended concentrations, respectively), dichloroxylenol (1.2% w/v) passed against test organisms. In dirty condition (Tables 3 and 4), dichloroxylenol (1.2% w/v) passed against the test bacteria only

at 6% concentration.

DISCUSSION

This study demonstrates the effectiveness of different dilutions of dichloroxylenol (1.2% w/v) against *Staphylococcus aureus* Oxoid 701/1 610254, *Escherichia*

Table 3. Kesley – sykes test result of dichloroxylenol (1.2% w/v) with *Staphylococcus aureus* in the presence of autoclaved yeast (dirty condition).

Concentration (% v/v)	Inoculum size	Challenge number			Result
		1 (0 min)	2 (10 min)	3 (20 min)	
2	1.4 x 10 ³	+++++	+++++	+++++	Fail
4	1.4 x 10 ³	+++++	+++++	+++++	Fail
6	1.4 x 10 ³	----+	--+++	+++++	Pass

+++++ Shows growth in all set of 5 tubes; --+++ Shows growth in 3 of 5 set of tubes; ----+ Shows growth in 1 of 5 set of tubes.

Table 4. Kesley – sykes test result of dichloroxylenol (1.2% w/v) with *Escherichia coli* in the presence of autoclaved yeast (dirty condition).

Concentration (% v/v)	Inoculum size	Challenge number			Result
		1 (0 min)	2 (10 min)	3 (20 min)	
2	1.6 x 10 ³	+++++	+++++	+++++	Fail
4	1.6 x 10 ³	+++++	+++++	-++++	Fail
6	1.6 x 10 ³	-----	-----	--+++	Pass

+++++ Shows growth in all set of 5 tubes; --+++ Shows growth in 3 of 5 set of tubes; -++++ Shows growth in 4 of a set of 5 tubes.

coli and *Salmonella typhi* with time of exposure and in clean and soiled situations. The results indicate that the effectiveness of dichloroxylenol (1.2% w/v) increased with time of exposure and decreased with dilution. This is in agreement with the report of Sagripanti and Bonifacino (1999) that changes in incubation time influenced the sporicidal activity of different sterilant formulations. They reported a relatively large increase in the sporicidal activity of Wavicide 01 (a sterilant formulation) following the extension of treatment from 10 min to 10 h. Cidexplus (another sterilant formulation) was, however, reported to have a slightly higher sporicidal effect after 20 min than after 10 h. Goeres et al. (2004) also reported that a 6 h (or overnight) super-chlorination treatment against biofilm and suspended bacteria in a laboratory swimming pool model was as effective as a 64 h super-chlorination treatment. These different antimicrobial responses may be understood from the fact that antimicrobial agents have different activity – exposure time relationship. Sagripanti and Bonifacino (1999), therefore, have highlighted the importance of precise adherence to the exposure times specified by the particular product's label.

In practice, however, it should be expected that concentrations greater than 1:10 (or others used in this study) may be required to achieve similar results within the same time of exposure. This position is supported by several reports on the greater effectiveness of disinfectants and cleaning agents in suspension tests than in surface tests (Gronholm et al., 1999; Johnson et al., 2002; Ramesh et al., 2002). Some of the probable reasons for this fact are that on surfaces, the disinfectants make con-

tact with the microorganisms from only one direction, while in suspensions the microorganisms are surrounded by the disinfectants and are more easily killed. The attachment of microorganisms to surfaces also tends to shield them from disinfectants. The surface tests, however, mirror more closely the practical situations.

Dichloroxylenol (1.2% w/v) was slightly more effective against *S. aureus* Oxoid 701/1 Lot 610254 than against the *E. coli* and *S. typhi* strains, while the *E. coli* strain was the least susceptible. This, probably, is due to the fact that bacteria differ in their susceptibility to biocides, with Gram – negative bacteria generally less susceptible to biocides than Gram – positive bacteria. Gram – negative bacteria can possess high cell impermeability, thereby preventing or reducing access of biocide to its target sites (Russell, 1999).

The in – use capacity of dichloroxylenol (1.2% w/v) was determined, using the Kesley – Sykes test, for *S. aureus* Oxoid 701/1 Lot 610254 and the *E. coli* strain, as representatives of Gram – positive and Gram – negative organisms. Under clean condition, dichloroxylenol (1.2% w/v) failed at 2% (that is a concentration below manufacturer's recommendation) and passed at 4% (manufacturer's recommended concentration) and 6% (above recommended concentration) against both bacteria. These results further highlighted strict adherence to the product's label concentration.

In dirty conditions, simulated by the presence of autoclaved yeast cells, dichloroxylenol (1.2% w/v) passed the test against *S. aureus* Oxoid 701/1 Lot 610254 and the *E. coli* strain only at 6% v/v concentration. The result clearly

showed that dichloroxylenol (1.2% w/v) activity was affected by the presence of organic matter (dead yeast cells). One of the possible implications of this result is that in situations of heavy contaminations or in the presence of organic matter, such as blood or tissue particles, concentrations greater than the product's label may be required for effective disinfection. Gronholm et al. (1999) reported that organic soil reduced the antimicrobial activities of disinfectants and cleaning agents. The dead yeast cells, probably, acted as a protective coat which reduced the access of dichloroxylenol molecules to the bacterial cells.

Conclusion

An important implication of the results of this study is that high level of hygienic practices is necessary in reducing contamination to a minimal level that will enhance the success of disinfection and in situations of heavy contamination or presence of organic matter such as food particles (on surfaces of food processing equipment), blood or tissue particles, disinfectant concentrations greater than the label stipulation may be useful.

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