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ISSN: 2348-1927

RESEARCH ARTICLE

Annals of Biological Sciences
2015, 3 (4): 8-14

Study of antimicrobial effects of some disinfectants on bacteria isolated from the operating theatre of Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria

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ABSTRACT

Study of antimicrobial effects of some disinfectants on bacteria isolated from the operating theatre of Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria was evaluated. Bacterial species were isolated by settling plate method, using Nutrient and MacConkey agar as the isolating medium. The isolates were fully characterized by standard methods. The isolated bacteria includes; *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium, Bacillus cereus, Streptococcus sp* and *Bacillus subtilis.* The in used povidone iodine and chlorhexidine solution in the operating theatre were collected from theatre management and evaluated using Kirby-Bauer ditch dilution method. The agar was inoculated with standardized isolates and allowed to dry; holes were then bore on the agar by using 8mm cork borer. 0.1ml of the graded concentrations of each disinfectant were added into each hole, allowed to diffuse and then incubated at 37^0C for 24hrs. Zones of inhibitions developed after incubation was measured. Povidone iodine obtained from the theatre showed no zones of inhibition in all the isolates tested, chlorhexidine solution obtained from the theatre showed appreciable zones of inhibition. Also Povidone iodine purchased from a pharmacy store in town produced zones of inhibitions ranging from 10.50mm to 23.00mm. Killing rate on the most resistant isolates showed that *B. megaterium*, *S. aureus* and *B. cereus* required 25 to 30 minutes of exposure time of the disinfectant to bring about total killing of resistant isolates. We conclude that degradation of povidone iodine is responsible for the failure of povidone iodine obtained from the theatre.

Keywords: Povidone iodine Solution; Chlohexidine Solution; Hospital Disinfectants; Bacterial isolates from operating theatre.

INTRODUCTION

Microbial contamination of the operating theatre and other specialized units had continued to increase prevalence of nosocomial infection in our hospital environment [1]. With resultant effect of high morbidity and mortality rate among patient admitted for post-operative surgery, patients in intensive care units with multi-drug resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) has shown difficulty in infection control [21]. In our hospital setting especially in the operating theatre, reduction of microbial contamination depends primarily on improved cleaning and proper disinfection of the hospital environment, especially high risk areas as these measures are crucial in stemming down dissemination of these microbial infection [12].

Sources of microbial contamination in the operating theatre are diverse these include frequent movement of surgical and medical team, movement within the operating theatre, high presence of human population, especially the theatre staff and medical students, theatre gown, foot wares, drainage of the wounds, and transportation of patients [9]. All these factors play a role in contaminating the operating theatre and subsequently cause post operative infection [10].

The clinical implication of bacterial contamination in operating theatre and other specialized care units and the overall effect on infection control in hospital setting is enormous on both the patient and the caring medical team [19]. Based on this observation, this research work was aimed at studying antimicrobial effect of povidone iodine and chlorhexidine solution on bacteria isolated from the operating theatre of Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria.

MATERIALS AND METHODS

Sampling Procedure

Different theatres (6) were sampled, by exposing the agar plates (Nutrient agar and MacConkey agar) for a period of 30 minutes. These theatres includes: Theatre 1 (T_1, Orthopaedics surgery) (05), Theatre 2 (T_2, Ear, nose and throat and neurosurgery) (12), Theatre 3 (T_3, Cardiothoracic surgery, neurosurgery plastic surgery and maxillofacial) (09), Theatre 4 (T_4, Ophthalmic surgery) (07), Theatre 5 (T_5, Obstetrics and gynaecology and Emergency) (08) and Theatre 6 (T_6, Obstetrics and gynaecology) (25). The plates were then taken to the laboratory and incubated at 37°C for 24hours. After 24hours of incubation all the plates were observed for growth, the colonies were counted, using electronic colony counter and recorded. All isolates were subjected to biochemical test, with a view of identifying the various bacterial isolated. This study was conducted at Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria. A total of sixty-six samples (66) were collected in all the above mentioned theatres.

Determination of Susceptibility of Isolates to the Disinfectant

Disinfectants used include povidone iodine and chlorhexidine solution. Effect of different concentrations of the disinfectants were measured on all isolated bacteria by preparing different concentrations of the disinfectant; the following concentrations used in the operating theatre were prepared, for povidone iodine 2%, 3%, 4%, 5% chlorhexidine 0.10%, 0.07%, 0.06%, 0.05%. All isolated and identified bacterial species were standardized to 10^6cfu/ml [15]. Agar ditch diffusion method was used to test the effectiveness of each disinfectant. Plates containing Mueller Hinton agar were inoculated with standardized inoculums of the isolates as described by [17]. Using sterile cork borer of 8mm, holes were created and the bottom partly covered with molten agar to prevent the disinfectant solution from draining away. Using sterile 1ml syringe, 0.1ml of the desired concentration of each disinfectant was added into each hole created, allowed to diffuse for 1hour and thereafter incubated at 37^0C for 24hours. After 24hours all the plates were observed for the presence and absence of zones of inhibition. The inhibition zone diameters formed were measured and recorded using a millimeter rule (mm).

Comparative Disinfectant Activity

As a result of the failure of povidone iodine obtained from the operating theatre, a new povidone iodine was purchased from a pharmacy store in town, from the same manufacture as of the one provided in the theatre. Using the same concentration used in the operating theatre, the purchased povidone iodine was used against all isolated bacteria. This was done to compare the activity of povidone iodine kept in the operating theatre and the povidone iodine purchased from a pharmacy store in town, to find out the reason for the failure. The results obtained were measured and recorded.

Determination of Rate of Kill by Disinfectants on Resistant Bacteria Isolates

Rate of kill was carried out on *S. aureus, B. megaterium* and *B. cereus* (being the resistant isolates found in this work) using [13] method. 1ml of 10^6cfu/ml of the test isolates was mixed with 9ml of different concentrations of each disinfectant solution. Using a sterile syringe, 0.1ml from the mixture was taken and plated on Mueller-Hintin agar at an interval of 0, 5, 10, 15, 20, 25 and 30 minutes. The plates were then incubated at 37^0C for 24hours. Surviving bacteria were counted and logarithms of the survivors was taken and plotted against time.

RESULTS

The total colony count from the six operating theatres is presented in Table 1; Theatre 1 has the lowest colony count of 7cfu/m^3 while Theatre 6 has the highest count of 81cfu/m^3. A total of 256 colony count where isolated from all the six operating theatre with a mean of 42.6cm.

Table 2, shows the percentage occurrence of each bacteria isolated, *B. cereus* accounted for 32.6% (n=73), *S. epidermidis* accounted for 32.6% (n=73), they both has the highest percentage of occurrence, closely followed by *B. megaterium* which accounted for 16.1% (n=36), *S. aureus* accounted for 8.9%, (n=20), *B. subtilis* accounted for 5.8% (n=13) and *Streptococcus* sp accounted for 4.0 % (n=9).

Table 3, shows the comparative sensitivity test of isolated bacteria to different disinfectant, from the table none of the tested isolates was sensitive to all the concentrations of povidone iodine collected from the operating theatre. Similar results were obtained from a repeated test carried out on the same isolates using the same concentrations of povidone iodine, hence no zones of inhibition were observed from all the isolates. But from the povidone iodine purchased in town, all the isolates showed appreciable zones of inhibitions using the same concentrations. From the Table, *S. aureus* is most sensitive with 18.00mm zone of inhibition and *B. megaterium* with the least zone of inhibition of 7.75mm.

Table 4, shows the average sensitivity pattern of chlorhexidine solution to all isolated bacteria species. From the Table the isolates were sensitive to all the concentration used, *B. megaterium was* most sensitive with the highest zone of inhibition of 23.00mm while *B. cereus* has the least zone of inhibition of 10.50mm.

The killing rates of chlorhexidine on *S. aureus*, is shown in Figure 1, the figure shows that there was reduction in the population of *S. aureus* by log of 2 in the first 5 minutes, but no observable reduction was observed from then till the 30th minute when concentration of chlorhexidine was 0.05%, but when the concentration was increased to 0.06%, there was a noticeable decrease in the 20th minutes before the population suddenly reduced to zero within 30 minutes.

The killing rates of chlorhixidine on *B. cereus*, is shown in Figure 2. From the figure there was log of 2 reduction in the population of *B. cereus* within the first 5 minute, but reduction in population of *B. cereus* was less than log of 1 in the following 20 minutes, but the population suddenly came down to zero at the 30th minutes.

Table 1: Total colony counts from the six operating theatres

Sampled areas in and around the theatres	Bacterial count cfu/m^3	Mean	Total
Theatre 1	07	42.60	256
Theatre 2 *	48	42.60	256
Theatre 3	36	42.60	256
Theatre 4	57	42.60	256
Theatre 5 *	27	42.60	256
Theatre 6	81	42.60	256
Total	**256**	42.60	256

**Indicating that the plates were exposed when surgery was going on.*

Table 2: Bacterial pathogens isolated and frequency of occurrence

Organism	Frequency	Percentage (%)
Bacillus cereus	73	32.6
Bacillus megaterium	36	16.1
Bacillus subtilis	13	5.8
Staphylococcus aureus	20	8.9
Staphylococcus epidermidis	73	32.6
Streptococcus sp.	09	4.0
Total	**224**	**100**

Figure 3, shows the rate of killing of *B. megaterium* when exposed to 0.05% dilution of chlorhexidine solution. The figure shows that there was log of 2 reductions in the population of *B. megaterium* within the first 5 minute, the

reduction was less than log of 1 in subsequent 20 minutes, but suddenly the population was reduced to zero in the 25th and 30th minutes.

Table 3: Comparative sensitivity test of the isolated bacteria to the different disinfectants

Bacterial Species	Zones of inhibitions (mm)				Concentration of two sets of disinfectants			
	Povidone iodine from UDUTH				Povidone iodine from Town			
	2%	3%	4%	5%	2%	3%	4%	5%
Bacillus megaterium	0.00	0.00	0.00	0.00	7.75	9.80	11.50	13.50
S. epidermidis	0.00	0.00	0.00	0.00	8.50	12.25	15.00	17.50
S. aureus	0.00	0.00	0.00	0.00	8.00	8.00	12.00	18.00
Bacillus subtilis	0.00	0.00	0.00	0.00	8.30	9.16	9.83	11.00
Bacillus cereus	0.00	0.00	0.00	0.00	8.35	8.40	9.00	10.00
Streptococcus sp	0.00	0.00	0.00	0.00	8.40	9.00	9,20	11.50

Table 4: Average sensitivity test result of isolated bacterial species to chlorhexidine solution at different concentration

Bacterial species	Zones of Inhibition at Different Concentration (mm)			
	0.10%	0.075%	0.06%	0.05%
Bacillus megaterium	23.00	21.70	17.50	13.50
Bacillus cereus	17.60	14.30	12.00	10.50
Staphylococcus aureus	16.70	13.50	12.07	11.06
Staphylococcus epidermidis	17.00	15.10	13.05	11.01
Bacillus subtilis	15.60	14.00	13.00	12.10

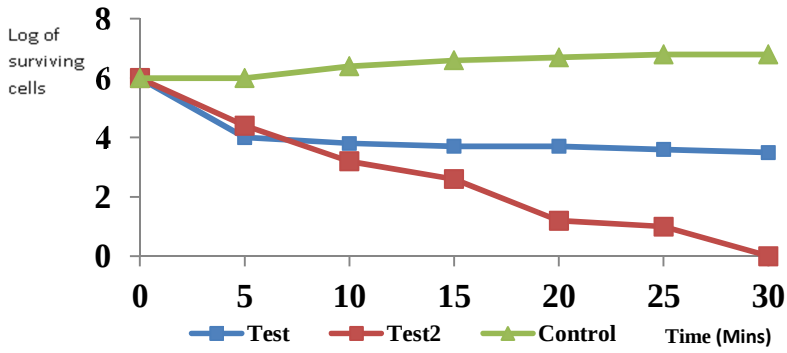

Figure 1: Killing rate of *S. aureus* when exposed to 0.06% and 0.05% dilution of chlorhexidine for 30 minutes.

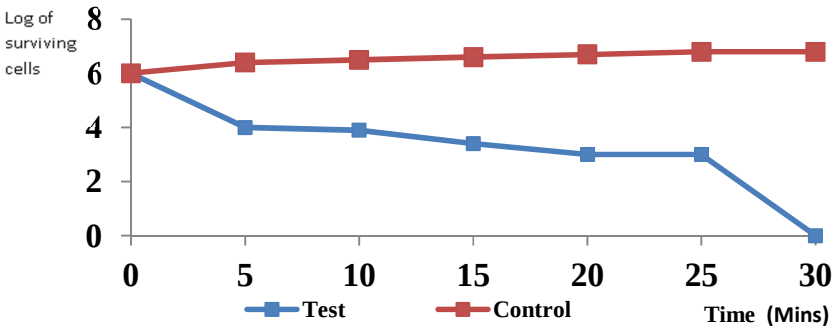

Figure 2: Killing rate of *B. cereus* when exposed to 0.05% dilution of chlorhexidine for 30 minutes

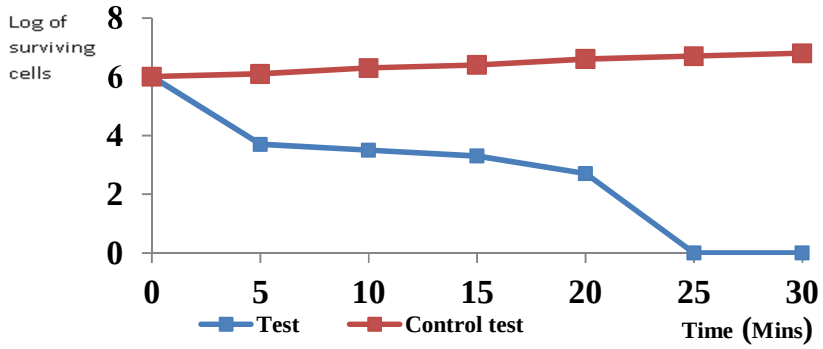

Figure 3: Killing rate of *B. megaterium* when exposed to 0.05% dilution of chlorhexidine for 30 minutes

DISCUSSION AND CONCLUSION

Study of antimicrobial effect of povidone iodine and chlorhexidine solution on bacteria isolated from the operating theatres of Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria was evaluated. The results of the bacterial colony count revealed bacterial load ranging from 7cfu/m^3 to 81cfu/m^3 (Table 1).

Sources of bacteria in the operating theatres are diverse, which may include frequent movement of surgical and medical team within the operating theatre, high presence of human population especially theatre staff and medical students. Movement within these units might be responsible for bacterial contamination [5]. All these factors play a role in the bacteria load found in this research work, thereby contaminating the operating theatre and subsequently may cause post operative infection [20].

The clinical implication of bacterial contamination in the operating theatre had enormous effect on both the patient and the caring medical team [21]. *S. epidermidis* and *S. aureus* are the most frequently isolated species and are mostly responsible for nosocomial infection. These organisms are responsible for 50-70% of nosocomial infection among hospitalized patients [8]. And this has a great clinical impact on the patient. *B. cereus* can produce biofilms [3], which play a major role in attachment to catheters thereby prolonging healing process and leading to injurious infection.

The following bacterial species were isolated from the operating theatres; *B. cereus* accounted for 29.7% (n=76), *S. epidermidis* accounted for 29.7% (n=76), they both had the highest percentage of occurrence. *S. epidermidis* is always associated with humans. It is a normal flora of the slain of man. The results of this work corroborated with the work of [18], where presence of *S. aureus*, *S. epidermidis* and *Bacillus* species in operating theatre were documented. The clinical implication of these organisms, it can affect immune-compromised patients, as entry into their systemic environment could initiates infection. Intra-hospital transmission of these bacterial pathogens can also occur from transportation of patient either from the wards to the operating theatre and the specialized units [8].

The results of the effect of the disinfectants collected from the operating theatre and tested on isolated bacteria showed that, povidone iodine formulated at 5% was not effective. Lack of sensitivity of the isolate to the disinfectant could be attributed to three main factors;
i. Degradation of the disinfectant during storage
ii. Storage temperature of the disinfectant
iii. Faking of the disinfectant by the producer

According to [2], povidone iodine is not always effective at killing common bacteria when stored for a prolonged period of time. It can also be contaminated by some bacteria known to have the ability to degrade chemical compounds like *Pseudomonas aeruginosa* have been reported to have this ability [2].
Storage temperature is another critical factor that could accelerate degradation of chemical disinfectant. At temperature above 40^0C, povidone iodine loses its potency. This result corroborates with the work of [16].

According to [16] raise in temperature above 35^0C, causes povidone iodine to degrade and weakens its germicidal activity. And most times temperature in Sokoto State is above 40^0C, this would have affected the potency of the disinfectant adversely.

Faking of the disinfectant is another important factor that can lead to the failure of the disinfectant. Sometime the percentage potency of the disinfectants is far below the claimed standard by the manufacturers [6]. The end users rely on the information provided by the manufacturers to make dilution of the product thereby over diluting the products which rendered it ineffective.

Results of the test carried out on the povidone iodine purchased from pharmacy store in town showed that the povidone iodine is effective at the formulated concentration (5% w/v) against all isolated bacteria from the theatre. Similar work by [4] found that povidone-iodine diluted to concentrations of 2% to 5% were more effective in killing common wound contaminants than was the 10% stock solution. This implied that the failure observed in the result of povidone iodine collected from the operating theatre may be as a result of one or three factors discussed above.

Chlorhexidine solution is one of the commonly used disinfectants in all the theatres of the hospital, the results of the test showed that the agent is effective Judging from the size of zones of inhibition against all the bacteria isolated from the theatre. Similar findings were reviewed by [7] the study shows that chlorhexidine is more effective within the concentration of 0.05% to 0.04% dilutions. [14] and [11] review that chlorhexidine can reduced microbial population to zero within 25 minutes to 30 minute of exposure.

In conclusion, this research work showed that *B. cereus* accounted for 32.6% (n=73), *S. epidermidis* accounted for 32.6% (n=73), they both had the highest percentage of occurrence, closely followed by *B. megaterium* which accounted for 16.1% (n=36), *S. aureus* accounted for 8.9%, (n=20), *B. subtilis* accounted for 5.8% (n=13) and *Streptococcus* sp accounted for 4.0 % (n=9). These were isolated from the operating theatre of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Study of antimicrobial effect of some disinfectants in the operating theatre shows that povidone iodine collected from the operating theatre was not effective, when tested against isolated bacterial species. However, the result of povidone iodine purchased from a chemist store in town showed appreciable zones of inhibition. Results of chlorhexidine solution collected from the operating theater shows appreciable level of effectiveness judging by zones of inhibition it produced 23.00mm. The killing rate on resistant isolates shows that it requires 25 to 30 minutes of exposure to the disinfectants. However, the level of hospital hygiene can be achieved if hospital hygiene can be adhered to by health care worker. The type of ventilation used in operation theatre also helped to reduce the level of bacterial contamination.

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