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RESEARCH ARTICLE

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Effectiveness of Povidone iodine solution as a preoperative scrubbing agent in operating theatre

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ABSTRACT

Effectiveness of Povidone iodine solution as a scrubbing agent in Operating Theatre was evaluated. The test bacteria were isolated from the theatres by settling plate method, using Nutrient Agar as the isolating medium. The isolates were fully characterized by standard method. The isolated bacteria include; Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium, Bacillus cereus, Streptococcus sp and Bacillus subtilis. Effectiveness of Povidone iodine obtained from the Theatre and the other purchased from a Chemist store was tested and compared by using Kirby-Bauer diffusion method. Mueller Hinton agar was used for the test. The agar was inoculated with standardized isolated bacteria and allowed to dry; holes were then bore in the agar by using 8mm cork borer. A 0.1ml of the graded concentrations of each solution were added into each hole, allowed to diffuse and then incubated for 24hrs. Zones of inhibitions developed after incubation were measured and recorded. Povidone iodine obtained from the theatre showed no zones of inhibition in all isolates tested, while the Povidone iodine purchased from a Chemist store produced appreciable zones of inhibitions ranging from 03mm to 19mm. We conclude that Povidone iodine obtained from the Theatre failed as an antimicrobial agent, using it as a scrubbing agent to control microbial contamination during operation in the theatre will not produce desired effect. We recommend frequent evaluation of all disinfectants in the operating theatres before use.

INTRODUCTION

Microbial contamination of the operating theatre and other specialized units had continued to increase prevalence of nosocomial infection in our hospital environment [3], [4]. 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 [17]. In our hospital setting especially in the operating theatre, reduction of microbial contamination impact 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 contaminations [9].

Povidone Iodine is a highly effective topical antimicrobial agent that has been used clinically in the treatment of wounds for more than 170 years. It has a broad spectrum of antimicrobial activity with efficacy against bacteria, mycobacteria, fungi, protozoa and viruses and can be used to treat both acute and chronic wounds [7]. It is also relatively inexpensive and easy to use but is often under used as a topical antiseptic due to its perceived toxicity.

Povidone Iodine remains one of the cornerstones for proper maintenance of low level of microbial contamination of hospital environment and reduction of nosocomial pathogenic infections. There is however a large body of evidence which suggest that when disinfection is poorly carried out, it can serves as a veritable focus for dissemination of microorganisms throughout the hospital environment, which besides been economically wasteful is extremely dangerous to patients health and hospital staff [5].

MATERIALS AND METHODS

Study Location

The study was carried out in Sokoto metropolis, the capital city of Sokoto State.Sokoto State was created as a State on 3rd Feb, 1976 by the then Military Government, from the former North-Western State. Sokoto is geographically located in the extreme north-western part of Nigeria, between latitude 10° 14' North and 3° 7'' East; [15]. The State covers a total area of about 266,484.8 square kilometres and it shares borders with Niger republic in the north, Zamfara State to the east and Kebbi State in the South-west [16]. In 2010, Sokoto State had 23 local government areas and a population of 3.69 million people, with an annual growth rate of 3.0% [11]. The topography of the State is dominated by plain and with the vast Fadama land, with networks of River Rima and River Sokoto dissecting the State [8].

Study Design

This study was divided into two parts.

The first part involves isolation and identification of bacteria species isolated from the operating theatre and the theatres environments such as; theatre seminar room, theatre sterile room, theatre pharmacy room, theatre corridor i, theatre corridor ii, theatre reception room, theatre instrument room, theatre recovery room and theatre toilets. And this was carried out over a period of three months from February to April, 2014, to determine the effectiveness of Povidone-iodine solution as a pre-operative scrubbing agent in the operating theatre. Pharmaceutical Microbiology laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology of Usmanu Danfodiyo University, Sokoto was used for this purpose.

In the second part, Povidone-iodine solution commonly used in the operating theatres were collected and the efficacy of these disinfectants were tested on all the isolated bacteria and where there is a lack of efficacy in any of the hospital used Povidone-iodine solution collected, a similar Povidone-iodine solution was purchased from a chemist store in town, the efficacy of the Povidone-iodine solution purchased in town was measured and compared with the failed one to adduce reasons for lack of efficacy on the isolate.

Sample collection

All the six operating theatres were sampled; the theatres were numbered as theatre 1 (T_1) (Orthopaedics surgery), theatre 2 (T_2) (Ear, nose and throat and neurosurgery), theatre 3 (Cardiothoracic surgery, neurosurgery plastic surgery and maxillofacial), theatre 4 (Ophthalmic surgery), theatre 5 (Obstetrics and gynaecology and Emergency) and theatre 6 (Obstetrics and gynaecology). The theatre environment sampled include the following; theatre seminar room, theatre sterile room, theatre pharmacy room, theatre corridor i, theatre corridor ii, theatre reception room, theatre instrument room, theatre recovery room and theatre toilets.

Sampling Procedure

The agar plates of Nutrient agar were exposed in all the areas mentioned for a period of 30minutes. The plates were then taken to the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology of Usmanu Danfodiyo University, Sokoto and incubated at 37°C for 24hours. After 24hours of incubation all the plates were observed for growth, the colonies were counted by using electronic colony counter and recorded.

Gram's Staining

All the isolated bacteria species were gram stained and allowed to air dry thoroughly before it was examined under a microscope using oil immersion objective lens (X100) and observations were recorded as results. The gram stained bacteria was then viewed under oil immersion lens (X100) microscopy, with the view of identifying the morphological characteristics of each organism for example the rod shape, cocci shape or clustered shape.

Collection of Disinfectants

The disinfectant commonly used in the Usmanu Danfodiyo University Teaching Hospital's Operating Theatres is Povidone iodine solution. A liter of Povidone iodine solution was collected in a sterile container as produced from the factory.

Determination of Susceptibilities of Isolates to Different Disinfectants

Different concentrations of disinfectant were prepared based on initial information obtained from the chief theatre operator who provided information on the in-use concentrations of the disinfectants in the theatres and the theatres environments. Based on that the following concentrations of the disinfectant were prepared, Povidone iodine solution: 2%, 3%, 4% and 5%.

Testing Procedure

All isolated and identified bacterial species stored in slant nutrient agar were standardized for the disinfectant for susceptibility tests. The isolates were sub-cultured into nutrient broth and incubated at 37^0C for 24hours. The turbidity produced was adjusted by using sterile physiological saline to match 0.5 McFarland standards (ca 10^8 $cfuml^{-1}$) [12]. This was further diluted to produce cell concentration of $10^6 cfuml^{-1}$.

Plates containing Mueller Hinton agar were dried and flooded with standardized inoculums of the isolates as described by [14]. Using sterile cork borer of 8mm (8mm bore size), holes were created and the bottom partly covered with molten agar to prevent the disinfectant solution from draining away. With sterile 1ml syringe, 0.1ml of the desired concentration of different disinfectant was added, allowed to diffuse for 1 hour and thereafter incubated at 37^0C for 18 to 24hours. After 24hours all the plates were observed for the presence and absence of zones of inhibition.

Determination of Rate of Killing by Disinfectants on the Most Resistant Isolates

Rate of killing was carried out on *Staphylococcus aureus* (been the most resistant isolates found in this research work) by using [10] method. 1ml of different concentrations of 10^6cfu/ml of the test isolates was mixed with 9ml of different concentrations of disinfectant solution. Using a sterile syringe, 0.1ml from the mixture was then taken and plated on the dried Mueller-Hinton agar at a time 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

Occurrence and frequencies of the bacteria species in the operating theatre and theatres environment of the Usmanu Danfodiyo University Teaching Hospital Sokoto

Bacteria species	Frequency	Percentage frequency
Bacillus cereus	26	24.5
Bacillus megaterium	24	22.7
Bacillus subtilis	10	9.4
Staphylococcus aureus	18	17.0
Staphylococcus epidermidis	26	24.5
Streptococcus sp.	2	1.9
Total	**106**	**100**

Table 1: Sensitivity of the isolated bacterial species to Povidone Iodine solution collected from the operating theatre at different concentrations

Location	Isolated bacterial species	Zones of Inhibition at Different Concentration (mm)			
		2%	3%	4%	5%
T_1	*Bacillus megaterium*	NI	NI	NI	NI
T_2	*Staphylococcus epidermidis*	NI	NI	NI	NI
C_{11}	*Bacillus megaterium*	NI	NI	NI	NI
C_I	*Bacillus subtilis*	NI	NI	NI	NI
T_4	*Bacillus megaterium*	NI	NI	NI	NI
T_3	*Staphylococcus aureus*	NI	NI	NI	NI
T_4	*Bacillus megaterium*	NI	NI	NI	NI
T_2	*Staphylococcus aureus*	NI	NI	NI	NI
T_5	*Bacillus megaterium*	NI	NI	NI	NI
T_3	*Staphylococcus aureus*	NI	NI	NI	NI
T_5	*Bacillus megaterium*	NI	NI	NI	NI
T_3	*Staphylococcus epidermidis*	NI	NI	NI	NI
T_5*	*Bacillus megaterium*	NI	NI	NI	NI
T_4	*Staphylococcus aureus*	NI	NI	NI	NI
C_I	*Bacillus megaterium*	NI	NI	NI	NI
DSR_2	*Bacillus subtilis*	NI	NI	NI	NI
T_2	*Staphylococcus epidermidis*	NI	NI	NI	NI
T_2	*Staphylococcus aureus*	NI	NI	NI	NI
T_3	*Staphylococcus epidermidis*	NI	NI	NI	NI
T_3	*Staphylococcus aureus*	NI	NI	NI	NI
T_4	*Bacillus megaterium*	NI	NI	NI	NI

*NI=No Inhibition, T_1 = Theatre 1, T_2 =Theatre 2, T_3 = Theatre 3, T_4 = Theatre 4, T_5 = Theatre 5, C_I= Corridor 1, C_{11}= Corridor ii, DSR_1 =Doctor's Seminar Room. *Indicating that the plates were exposed when surgery was going on.*

Table 2: Sensitivity of the isolated bacterial species to Povidone Iodine solution bought from a chemist store at different concentrations

Location	Isolated bacterial species	Zones of Inhibition at Different Concentration (mm)			
		2%	3%	4%	5%
T_1	*Bacillus megaterium*	10	11	12	13
T_3	*Staphylococcus epidermidis*	03	12	15	20
T_4	*Bacillus megaterium*	NI	NI	NIL	NI
T_4	*Bacillus subtilis*	10	10	13	15
T_5	*Bacillus megaterium*	11	13	13	16
T_5	*Staphylococcus aureus*	14	14	15	19
T_5*	*Bacillus megaterium*	10	11	13	13
C_I	*Staphylococcus aureus*	11	14	14	16
C_I	*Bacillus megaterium*	09	10	13	14
DSR_2	*Staphylococcus aureus*	13	14	16	19
T_2	*Bacillus megaterium*	11	14	14	16
T_2	*Staphylococcus epidermidis*	11	13	14	20
T_2	*Bacillus megaterium*	13	13	15	22
T_2	*Staphylococcus aureus*	12	13	14	20
T_3	*Bacillus megaterium*	06	07	12	14
T_3	*Bacillus subtilis*	06	06	11	17
T_3	*Staphylococcus epidermidis*	10	13	17	19
T_3	*Staphylococcus aureus*	10	14	14	15
T_4	*Staphylococcus epidermidis*	10	11	14	15
T_4	*Staphylococcus aureus*	10	12	15	19

*T_1 = Theatre 1, T_2 =Theatre 2, T_3 = Theatre 3, T_4 = Theatre 4, T_5 = Theatre 5, C_I= Corridor 1, DSR_2 =Doctor's Seminar Room 2. *Indicating that the plates were exposed when surgery was going on.*

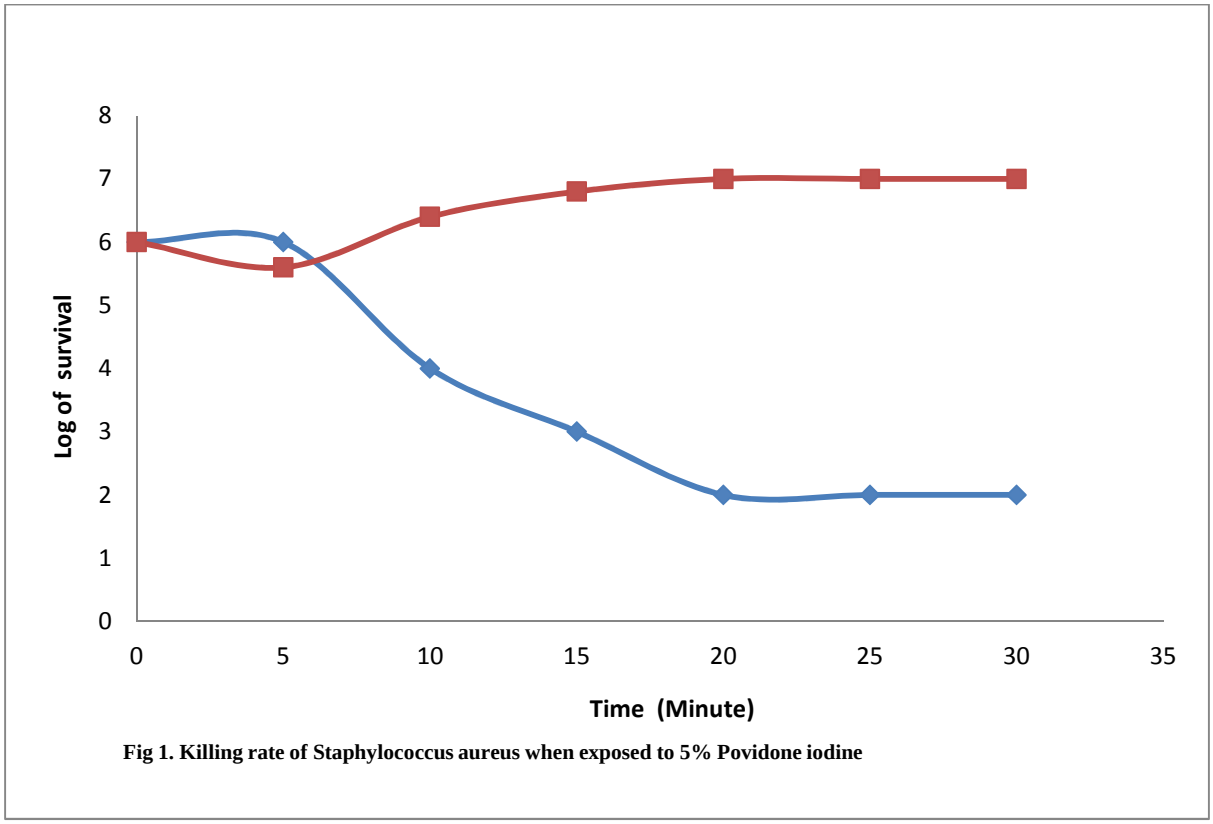

Fig 1. Killing rate of Staphylococcus aureus when exposed to 5% Povidone iodine

DISCUSSION

The result in Table 1 shows that Povidone iodine solution collected from the theatre was not effective, none of the isolates produced zones of inhibition against the solution at the highest concentration (5%). But when compared with, Povidone iodine solution purchased from a chemist store in town it showed appreciable zones of inhibitions, ranging from 3mm to 22mm, but lack of sensitivity of the disinfectant on the isolates could be attributed to two main factors;

i. Degradation of Povidone iodine solution during storage
ii. Faking of the solution by the producer.

According to [1], Povidone iodine is not always effective at killing common bacteria when Povidone iodine is stored for a prolonged period of time. Because it can be contaminated by some bacteria known to have the ability to degrade chemical compound for instance *Pseudomonas aeruginosa* and other species have been reported to have this ability, thereby rendering it useless as an active agent [1]. Storage temperature is another critical factor that could accelerate degradation of Povidone iodine solution, at temperature above 40^0C Povidone iodine solution loses its potency, this result work corroborates with the work of [13] increase in temperature causes the disinfectant to degrade and weakens its germicidal activity. Temperature in Sokoto State most times 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 Povidone iodine solution. Sometimes the percentage potency of the disinfectants is far below the claimed 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. Because the end users relied on the information provided by the producers on the packaging, the chance is high that the disinfectants may be over diluted since the actual concentration of the disinfectant is lower than the claimed concentration by the producers, and hence ineffectiveness of disinfectant.

But the results of the test carried out on the Povidone iodine solution purchased from chemist showed that the Povidone iodine solution is effective at the formulated concentration (5% w/v) against all isolated bacteria from the theatres. Similar work by [2] found that Povidone-iodine solutions 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 solution collected from the theatre may be as a result of one or the two factors discussed above.

The result in figure 1 shows that the killing rate of the isolate showed that the population of the organism was reduced from 10^6cfu/ml to less than 10^2cfu/ml within 30 minutes of exposure to 5% Povidone iodine. But the isolates were not reduced to zero, with 5% Povidone iodine is an indication that 5% Povidone iodine solution may not effectively reduce the population to zero, at that concentration. It is possible that the isolate may rise up again after 30 minutes.

CONCLUSION

From the above research work, we conclude that Povidone iodine solution at 5% is effective to some extent, since it inhibited growth in all the isolates, but its effectiveness become doubtful since it could not kill all the population of the *S. aureus* exposed to it.

It is therefore recommended that;

- From time to time potency of Povidone iodine solution must be evaluated in order to keep pace with degradation of the disinfectant which normally occurs with time as seen in Povidone iodine.
- Povidone iodine should be re-evaluated after each months of storage.

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