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Assessment of the Efficacy of Selected Disinfectant Formulations against Clinical Isolates of *Staphyloccocus Aureus* from a Tertiary Hospital in Ibadan, Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *S. aureus* is one of the most common causes of nosocomial infections and can cause a range of illnesses, from minor skin infections to life threatening diseases.
Objective: This study was done to assess the efficacy of some commercially available disinfectants and to determine the antimicrobial susceptibility profile of clinical *S. aureus* isolates.
Material and Methods: Fifty *S. aureus* isolates were obtained from the Microbiology unit of the University College Hospital, Ibadan and characterized by standard biochemical tests. The efficacy of the test disinfectant formulations was assessed using standard method. Antimicrobial susceptibility profile of the clinical isolates to commonly prescribed antibiotics was determined by modified Kirby-Bauer method.
Results: Salvon ® was most effective with 100% efficacy both at the manufacturer's in-use concentration and double. Dettol® showed 76% efficacy at the manufacturer's in-use concentration and 100% efficacy when concentration was doubled. Jik® showed activity only when the manufacturer's in-use concentration was doubled with 94% efficacy. All the isolates were resistant to Germicide® both at the manufacturer's in-use concentration and double. The isolates (68%) were observed to be most susceptible to Ofloxacin, 44% susceptibility to Gentamicin, 10% susceptibility to Cefuroxime and Amoxycillin/Clavulanate respectively. Only 4% susceptibility was observed to cloxacillin, and 100% resistance was observed to Erythromycin.
Conclusion: Savlon® was the most effective disinfectant, and Germicide® the least (even at double manufacturer's in-use concentration). Majority of the isolates (96%) were found to be multidrug resistant.

Keywords: *S. aureus*, Chemical disinfectants, Manufacturers' concentration, Microbial resistance.

INTRODUCTION

The control of microorganisms is critical for the prevention and treatment of disease. *S. aureus* is a widely recognized human pathogen that continues to represent a significant public health challenge globally. Owing to its broad spectrum of virulence factors, it has been shown to cause a variety of infectious diseases such as superficial skin infection, septicaemia, and toxin mediated diseases, which are often difficult to treat (Furuno, 2004).
One of the ways of controlling spread of infections within hospital and domestic environments is the rational use of chemical disinfectant formulations. These chemical disinfectants are made use of in cleaning hospital wards, cleaning skin surface before injecting patients, mopping floors, cleansing equipment, clothings and are therefore an essential part of infection control practice and in the prevention of nosocomial infections (Rutala, 1995).
Various disinfectant preparations are available commercially, hence the need to assess their efficacy against pathogens such as *S. aureus*. The optimum concentration required to produce a standardized microbial effect is described as the in-use concentration (Greenwood *et al*, 2002). In preparing an accurate in-use concentration, care must be taken to prevent over-dilution which may result in disinfection failure (Greenwood *et al.*, 2002) but in some few cases, the in-use manufactures' concentration show no effect on the organisms, therefore it is important to evaluate the efficacy of the disinfectant both at the

manufacturers' concentration and when the manufacturers' recommendation is not adhered to by users. This work is aimed at assessing the efficacy of some commercially available disinfectant formulations and determining the antimicrobial susceptibility profile of *S. aureus* isolates to commonly prescribed antibiotics.

MATERIALS AND METHODS

Collection of clinical isolates

A total of 50 *S. aureus* isolates were obtained as pure cultures on agar slants from the medical microbiology department, University College Hospital (UCH) Ibadan, Oyo State from different clinical sources. Seventeen (34%) from wounds, 19(38%) from boils, 3(6%) from pus, 10(20%) from vagina and 1(2%) from blood sample. A standard strain of *S. aureus* (ATCC 25923) was used as control.

Authentication of Clinical Isolates

The isolates obtained were streaked on Mannitol salt agar (the differential and selective medium for *S. aureus*) and cultured at 37^0C for 24hours. Morphology of colonies was observed (Cheesbrough, 1985). Appropriate biochemical tests were performed (Collee and Miles, 1985; Cowan, 1974). The isolates were screened for catalase production (Chelikani *et al*, 2004). Two drops of hydrogen peroxide were placed on a clean grease free microscope slide with a flamed and cooled inoculating loop. A speck from the colony of the microorganism in a plate was taken and rubbed in the hydrogen peroxide. Immediate evolution of gas bubble indicates the presence of catalase enzyme in the isolate. Confirmatory coagulase test was done on all the fifty *S. aureus* isolates (Cheesbrough, 1985).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profile of the various isolates to eight (8) commonly prescribed antibiotics were determined according to Bauer *et al*. (1966), and Wolf (1975) with modifications by Clinical Laboratory Standards Institute (2006). The antibiotics used in this study are ceftazidime, cefuroxime, gentamicin, ceftriaxone, erythromycin, cloxacillin, ofloxacin, amoxycillin/clavulanate (Rapid Labs® United Kingdom, CM-12-8PR100).

Screening of isolates with test disinfectant formulations

The agar diffusion method was used (Alabi and Sanusi, 2012). For each of the different class of disinfectant formulation employed, two test concentrations were prepared: the manufacturer's recommended in-use concentration, and double strength of manufacturer's in-use concentration. These test concentrations were freshly prepared before each use by diluting the disinfectant formulations with sterile distilled water. The concentrations used are as follows: Dettol®(2.7%v/v and 5.4%v/v), Savlon® (6.0%v/v and 12.0%v/v), Jik®(2.5%v/v and 5.0%v/v), Germicide® (0.022%v/v and 0.044%v/v).

Twenty milliliters (20ml) of melted and cooled agar was seeded with 0.2ml of 10^{-2} dilution of an overnight broth culture of the isolate, rolled between palms and poured into a sterile petri-dish to set. The surface was then dried in a drying oven and with the aid of a flamed and cooled 8mm cup borer; four wells were bored into the agar plates. The wells were filled with each of the two concentrations (manufacturer's and double) of the disinfectant aseptically.

After about an hour of pre-diffusion, the plate was then incubated at 37^oC for 24hours in an upright position. The zones of growth inhibition were measured for each well and recorded. This was carried out for each of the four disinfectant formulations. This process was repeated for the fifty isolates of *S. aureus*.

RESULTS

Thirty four (68%) of the isolates were susceptible to Ofloxacin, 22(44%) were susceptible to Gentamicin, 12(24%) to Ceftazidime, 5(10%) to Cefuroxime, Ceftriaxone and Augumentin (Amoxycillin/Clavulanate), 2(4%) to Cloxacillin and none of the isolates was susceptible to Erythromycin. Multidrug resistance was observed in 48 (96%) of the *S. aureus* isolates.

Thirty-eight (76%) of the isolates were susceptible to Dettol® at the recommended manufacturer's in-use concentration (2.7%v/v), while 12 were resistant. The minimum and maximum zones of inhibition were 10mm and 24mm respectively. At double manufacturer's in-use concentration (5.4%v/v), all isolates were susceptible to Dettol®. The minimum and maximum zones of inhibition were 10mm and 36mm respectively. The control organism (*S. aureus* ATCC 25923) showed zone of inhibition of 18mm at manufacturer's concentration and 20mm when doubled.

For Savlon®, at the manufacturer's in-use concentration (6.0%v/v), the isolates were all susceptible (100%). The minimum and maximum zones of inhibition were 10mm and 26mm respectively. At double manufacturer's in-use concentration (12%v/v), all the isolates were susceptible (100%), with a minimum zone of inhibition of 14mm and maximum zone of inhibition of 35mm. The control organism showed a zone of inhibition of 14mm at the manufacturer's concentration and 20mm when doubled.

All the isolates and the control organism were resistant to Germicide® both at the manufacturer's in-use concentration (0.022%v/v) and double (0.044%v/v).

All the isolates were resistant to Jik® at the manufacturer's in-use concentration (2.5%v/v), but when doubled (5.0%), forty-seven (94%) were susceptible to the disinfectant while 3 were resistant. The control organism (*S. aureus* ATCC 25923) was resistant to Jik® at the manufacturer's concentration but had a zone of inhibition of 18mm when doubled.

Table 1: Antimicrobial screening of 50 *S. aureus* isolates against disinfectant formulations at manufacturer's recommended in-use concentration and double

S. aureus isolates Lab No	DETTOL® 2.7(5.4)%v/v	SAVLON® 6.0(12.0)%v/v	GERMICIDE® 0.022(0.044)%v/v	JIK® 2.5(5.0)%v/v
	Zones of growth inhibition (mm)			
1	18(22)	24(26)	-	- (13)
2	10(14)	14(16)	-	- (16)
3	10(32)	20(30)	-	- (18)
4	10(14)	12(14)	-	- (10)
5	- (16)	14(16)	-	- (12)
6	12(14)	14(18)	-	- (12)
7	- (20)	10(19)	-	- (10)
8	- (14)	14(20)	-	- (14)
9	18(21)	22(27)	-	- (14)
10	18(20)	24(32)	-	- (12)
11	- (16)	12(16)	-	- (18)
12	16(20)	20(34)	-	- (12)
13	12(14)	19(24)	-	- (14)
14	14(34)	14(34)	-	- (16)
15	13(16)	15(19)	-	- (15)
16	10(18)	14(18)	-	- (10)
17	23(30)	24(32)	-	- (16)
18	- (10)	12(18)	-	- (12)
19	12(14)	24(26)	-	- (18)
20	16(22)	20(35)	-	- (14)
21	14(16)	16(20)	-	- (15)
22	22(26)	24(26)	-	- (14)
23	20(24)	22(26)	-	- (14)
24	20(32)	26(30)	-	- (16)
25	24(30)	26(28)	-	- (14)
26	10(16)	14(18)	-	- (20)
27	12(12)	12(28)	-	- (18)
28	- (29)	16(32)	-	- (14)
29	20(22)	24(26)	-	- (16)
30	- (16)	14(20)	-	- (18)
31	10(16)	14(18)	-	- (16)
32	10(16)	18(22)	-	- (14)
33	20(36)	22(26)	-	- (12)
34	18(32)	26(28)	-	- (14)
35	- (14)	14(16)	-	- (18)
36	22(26)	26(28)	-	- (16)
37	12(18)	14(18)	-	- (16)
38	18(19)	24(26)	-	-(10)
39	- (16)	14(24)	-	- (18)
40	10(18)	24(28)	-	- (20)
41	24(26)	24(26)	-	- (16)
42	14(18)	10(20)	-	- (20)
43	20(21)	20(28)	-	- (18)
44	10(14)	14(16)	-	- (14)
45	- (16)	22(21)	-	- (10)
46	14(22)	22(26)	-	- (18)
47	16(18)	24(28)	-	- (16)
48	10(14)	14(16)	-	- (10)
49	- (10)	18(20)	-	- (14)
50	- (29)	20(30)	-	- (14)
Control	18(20)	14(20)	-	- (18)

Control: *S. aureus* ATCC 25923
- : No zone/growth

Table 2: Efficacy of different disinfectant formulations against 50 *S. aureus* isolates showing the zones of growth inhibition at the manufacturer's recommended in-use concentration and double

Disinfectant/ Concentration (%v/v)		Number of isolates	Resistant	Susceptible	Zones of growth Inhibition of susceptible *S. aureus* (mm)		Mean zones of inhibition of 50 *S. aureus* (mm)
					Minimum	Maximum	
Dettol®	2.7	50	12	38	10	24	11.6
	5.4	50	X	50	10	36	20.3
Savlon®	6.0	50	X	50	10	26	18.4
	12.0	50	X	50	14	35	23.9
Jik®	2.5	50	50	Y	-	-	-
	5.0	50	X	50	10	20	14.8
Germicide®	0.022	50	50	X	-	-	-
	0.044	50	50	X	-	-	-

Manufacturer's in-use concentration: 2.7%v/v
Double manufacturer's in-use concentration: 5.4%v/v
No Resistance observed: X
No susceptibility observed: Y
None: -

Table 3: Interpretation of antimicrobial screening (CLSI 2012) and antimicrobial resistance category of 50 *S. aureus* isolates

S. aureus isolates Lab No	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	% of Antibiotics Resistance	Resistance Category
1	I	R	I	R	R	R	R	R	75.0	MDR
2	R	R	S	R	R	R	S	R	75.0	MDR
3	I	R	S	R	R	R	S	R	62.5	MDR
4	R	R	S	R	R	R	I	R	75.0	MDR
5	I	R	R	R	R	R	R	R	87.5	MDR
6	R	R	S	R	R	R	S	R	75.0	MDR
7	R	R	S	R	R	R	S	R	75.0	MDR
8	R	R	R	R	R	R	S	R	75.0	MDR
9	I	R	R	I	R	R	R	R	75.0	MDR
10	S	R	S	R	R	R	I	R	62.5	MDR
11	R	R	S	I	R	R	S	R	62.5	MDR
12	I	R	R	R	R	R	I	R	62.5	MDR
13	R	R	I	I	R	R	S	R	75.0	MDR
14	R	R	S	R	R	R	S	R	62.5	MDR
15	S	R	R	R	R	R	S	R	75.0	MDR
16	R	R	I	R	R	R	I	R	75.0	MDR
17	R	R	S	I	R	R	S	R	62.5	MDR
18	R	R	S	R	R	R	S	R	75.0	MDR
19	R	R	R	I	R	R	R	R	87.5	MDR
20	R	S	R	R	R	R	S	R	75.0	MDR
21	R	R	R	I	R	R	S	R	75.0	MDR
22	S	S	R	R	R	R	R	R	75.0	MDR
23	R	R	S	I	R	R	S	R	62.5	MDR
24	S	R	I	R	R	R	S	R	62.5	MDR
25	R	R	I	I	R	R	S	R	62.5	MDR
26	R	R	S	R	R	R	S	R	75.0	MDR
27	R	S	S	I	R	S	S	S	25.0	NMDR
28	R	R	R	I	R	R	S	S	62.5	MDR
29	R	R	S	S	R	R	R	S	62.5	MDR
30	S	R	S	I	R	R	R	R	62.5	MDR
31	I	R	R	R	I	R	S	R	62.5	MDR
32	R	S	S	S	I	S	S	S	12.5	NMDR
33	R	R	R	R	R	R	R	R	100	MDR
34	R	R	R	R	R	R	S	R	87.5	MDR
35	S	R	I	I	R	R	R	R	62.5	MDR
36	R	R	I	I	R	R	S	R	62.5	MDR
37	I	R	I	R	R	R	S	R	62.5	MDR
38	R	R	R	I	R	R	R	R	87.5	MDR
39	S	R	I	R	R	R	S	R	62.5	MDR
40	I	R	S	I	R	R	S	S	37.5	MDR
41	I	R	R	I	R	R	S	R	62.5	MDR
42	I	R	S	R	R	R	S	R	62.5	MDR
43	R	R	S	R	R	R	S	R	75.0	MDR
44	S	R	S	R	R	R	S	R	62.5	MDR
45	S	R	S	R	R	R	S	R	62.5	MDR
46	S	R	I	R	R	R	R	R	75.0	MDR
47	S	R	R	S	R	R	S	R	62.5	MDR
48	S	R	S	I	R	R	R	R	62.5	MDR
49	R	R	R	R	R	R	S	R	87.5	MDR
50	I	R	I	R	R	R	S	R	62.5	MDR
Control	S	R	S	S	R	R	S	R	50.0	MDR

MDR: multidrug resistant, NMDR: Not multidrug resistant CAZ: Ceftazidime CRX:Cefuroxime GEN: Gentamicin CTR: Ceftriaxone ERY:Erythromycin CXC:Cloxacillin OFL:Ofloxacin AUG:Amoxycillin/Clavulanate S: Sensitive R; Resistant I: Intermeditae Control: *S. aureus* ATCC 25923

Table 4: Percentage sensitivity of 50 *S. aureus* isolates to some commonly prescribed antibiotics

	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
%Sensitivity	24.0	10.0	44.0	10.0	0.0	4.0	68.0	10.0
%Intermediate	20.0	0.0	22.0	34.0	4.0	0.0	8.0	0.0
%Resistance	54.0	92.0	34.0	58.0	96.0	96.0	22.0	92.0

CAZ: Ceftazidime CRX: Cefuroxime GEN: Gentamicin CTR: Ceftriaxone ERY:Erythromycin CXC:Cloxacillin OFL:Ofloxacin AUG:Amoxycillin/Clavulanate

This study allowed for the comparison of the efficacy of some commercially available disinfectants in Nigeria. Savlon® was found to be the most effective among the four different classes of disinfectants with 100% sensitivity both at the manufacturer's in-use concentration and double. Dettol® showed 76% sensitivity, and at double the manufacturer's in-use concentration, it exhibited better activity. Jik® and Germicide® were not effective against *S. aureus* isolates at the manufacturer's in-use concentrations. Jik® was more effective when the manufacturer's in-use concentration is doubled. This is in agreement with a study reported in Ibadan, Southwest Nigeria (Alabi and Sanusi, 2012). At double the manufacturer's in-use concentration 3 out of the 4 disinfectants exhibited activity against the isolates, 100% sensitivity in Dettol®and Salvon,® 94% in Jik®, while Germicide® showed no activity which makes it the least effective disinfectant formulation. The efficacy of the disinfectant formulations in descending order is: Savlon ® > Dettol® > Jik® > Germicide®Sixty eight (68%) of the isolates were susceptible to Ofloxacin, 44% were sensitive to Gentamicin, 24% to Ceftazidime, 10% to Cefuroxime, Ceftriaxone and (Amoxycillin/Clavulanate), 4% to Cloxacillin and none of the isolates were susceptible to Erythromycin.

The prevalence of multidrug resistant bacterial isolates as seen in this study was 96%, this is similar to the 100% prevalence reported in a study by Alabi and Sanusi, 2012. The emergence of multidrug resistant hospital acquired bacteria (nosocomial agents) has been reported throughout the world and the mechanisms to which they resist the antimicrobial activity of the various antimicrobial agents particularly antibiotics have also been studied extensively (Mc Donell and Russell, 1999). The development of resistance to the phenolic class of disinfectants has long been reported (Mc Donell and Russell, 1999). Some microorganisms, for example, *Pseudomonas aeruginosa*, have been found to utilize some phenolic compounds as their carbon source (Hugo and Russell, 2004; Mc Donell and Russell, 1999).

CONCLUSION

It can be concluded from the study that Savlon ® is the most effective disinfectant against *S. aureus* isolates, while Germicide® is the least even at double the manufacturer's in-use concentration. This calls for further study. Ofloxacin was the most effective antibiotic, while Erythromycin is the least. Majority of the clinical *S. aureus* isolates are multidrug resistant, and this calls for rational use of antibiotics.

RECOMMENDATIONS

It can be recommended that manufacturers of some of these disinfectant formulations should pay close attention to their recommended in-use concentrations. Users of these disinfectant formulations should adhere strictly to manufacturer's recommended in-use concentrations and avoid over dilution which is often practiced. Continuous rational use of antibiotics should be encouraged to combat the emergence of antibiotic drug resistance.

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