Original Research Article

Comparative Studies on Effectiveness of Branded and Unbranded Disinfectants on *E. coli* and *Staphylococcus* species.

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Aims: To compare the antimicrobial potential of branded and unbranded disinfectants on clinical bacterial isolates.
Study design:the agar-well diffusion and micro broth dilution were adopted for the study. Ten disinfectants of which five were branded (industrial prepared) and five unbranded (indigenous prepared) were used against *E. coli* and *Staphylococcus aureus*.
Place and Duration of Study: Department of Microbiology, Rivers State University. the study was for a period of two months (June-July, 2018).
Methodology: Faecal samples were collected from the University Medical centre and was analyzed in the Microbiology Laboratory for the isolation of *Escherichia coli* and *Staphylococcus aureus* using standard microbiological method. The antimicrobial potential of both branded and unbranded disinfectants on the clinical isolates were evaluated using the micro dilution technique and the well in agar technique
Results: The result in this study showed that both branded and unbranded disinfectants were effective on the *E. coli* and *Staphylococcus* isolates. However, the unbranded were only effective at high concentrations. *E. coli had* zone of inhibitionranging from 0 to 22mm when tested with the unbranded disinfectant, while 0 to 17mm was recorded for*Staphylococcus aureus*.The zones of inhibition of the branded disinfectant on*E. coli* ranged from 0 to 28mm, while zone diameter of *Staphylococcus aureus* ranged from 0 to 21mm. Among the unbranded disinfectants, Lysol produced the highest zone of inhibition While among the branded disinfectants, Savlon produced the highest zone of inhibition. Thepositive control was effective against all tested organisms with zones of inhibition ranging from 17-26 mm. On the other hand, as expected, the negative control (sterile distilled water) did not show any zone of inhibition.
Conclusion: The study showed that branded disinfectants were more effective on the clinical isolates than the unbranded disinfectants.

Keywords: Escherichia coli, Staphylococcus aureus, branded disinfectants, unbranded disinfectants, microdilution, well-in-agar diffusion.

1. INTRODUCTION

Antimicrobials are substances that have the ability to kill or inhibit the growth or proliferation of microorganisms [17]. This implies that these substances when introduced in objects or other materials or consumed could either be bacteriostatic or bactericidal in action. According to Douglas and Braide [6], antimicrobial substances that when introduced on inanimate objects kills or inhibits the growth of microbes. Thus, a good disinfectant should be able to offer complete and full microbiological sterilization, without harming humans and useful form of life, be inexpensive and noncorrosive. However, most disinfectants are also, by nature, potentially harmful to humans and animals. The choice of disinfectant to be used may depend on the demanding situation. According to Van et al [15], the idea of using disinfectants and antiseptics is to control or reduce the presence of microorganisms. In order to prevent infections as it regards injury, the most vital measure is to kill or inhibit the growth of microorganisms on the skin, wounds and in human body cavity [3]. The antimicrobial potentials of these disinfectants could be influenced by their formulation properties, concentration of organic components, temperature, synergy, rate of dilution and experimental procedures, mode of application, water solubility and pH[5 and 6]. Application factors include the type of surface to be applied, the type of (organic) soil, the temperature and contact time as well as humidity and the method of application (with or without mechanical action) [8]. A disinfectant could be branded or unbranded (Maillard, 2005). These unbranded disinfectants are hawked from place to place and also sold in the local markets [9]. They could be good alternative disinfecting agents if their effectiveness against some clinical isolates is known [10]. Unbranded disinfectants are produced locally by people that are taught how to make different household washing, cleaning and disinfecting agents. When these disinfectants are made by these persons, they are normally packaged in containers (usually liable plastic bottles). There are two different ways by which disinfectants can act on microorganisms: growth inhibition (bacteriostasis and fungistasis) or lethal action (bactericidal, fungicidal or viricidal effects) [2]. Thus, this study is aimed at comparing the antimicrobial potential of branded and unbranded disinfectants on clinical bacterial isolates.

2. METHODOLOGY

2.1 Collection of clinical Samples

Faecal samples were collected from the Rivers State University Medical Center, Port Harcourt in specimen bottle and transported to the Microbiology laboratory of Rivers State University, Port Harcourt.

2.2 Collection of Disinfectant Samples

The branded disinfectants used were; Purit, Dettol, Ivy's, Savlon and Robert. While the unbranded disinfectants were; Lysol, Pine oil, Morigade, Nigertol, Chlonoxynol. The disinfectants were purchased from different markets within Port Harcourt Metropolis, Rivers State.

2.3 Isolation of Test Organisms

Isolation of the test organisms was carried out as described by Cheesbrough [4]. A thick suspension of the faecal sample was emulsified in 1ml sterile peptone water. Afterwards a loop full of the emulsified sample was inoculated on Mannitol salt agar plates (MSA) and Eosin methylene blue agar plates (EMB). Plates were then incubated at 37^{o}C for 24 hours.

2.3.1 Confirmation of Test Organisms

Ensuing colonies on the MSA and EMB plates were carefully picked using a sterile wire loop and subcutured on fresh plates of MSA and EMB agar. Pure isolates were then stored in nutrient agar slants and stored in the refrigerator for further use.

The respective pure isolates were identified using conventional methods as described by Cheesbrough [4]. Further confirmation of isolates was done by comparing their biochemical results with those presented in Bergey's manual of determinative bacteriology [18]. The conventional methods include; microscopy, motility, coagulase, catalase, oxidase, indole production, methyl red, citrate utilization, Voges–Proskauertest and sugar fermentation [4].

2.4 Standardization of Test Inoculum

Test isolates were standardized using the 0.5 McFarland. The test isolates were placed in sterile test tubes containing 4ml distilled water. The turbidity was ascertained using the already prepared McFarland standard [4].The standardized isolates were carefully spread on prepared sterile Mueller-Hinton agar plates as described by Wemedo and Robinson [16]. Plates were allowed to dry before 4 wells using a 6mm well borer were made on the dried seeded plates.

2.4.1 Antimicrobial Assay (Well-in-agar method)

The antimicrobial activity of each disinfectants with different concentration was tested in vitro against *E. coli* and *Staphylococcus aureus*. Aliquots (0.1ml) of 10%, 25%, 50% and 100% concentration of the different disinfectants were transferred using sterile Pasteur pipette in to the four wells. The plates were then incubated at 37℃ for 18 to 24hours in an upright position. Autoclaved distilled water was used as negative control while ofloxacin was used as a positive control. After incubation, the plates were observed and the zones of inhibition that developed were read and interpreted [16].

2.4.2 Broth Dilution Method

The minimum inhibitory concentrations of the different disinfectants were carried out using the broth dilution method as described by Prescott *et al* (2011). Different concentrations of the disinfectants were prepared (10, 25, 50, 75 and 100) mg/ml [1]. One milliliter (1ml) of the standardized inoculum and the various concentrations of the disinfectants were put into the sterile tubes of nutrient broth respectively. Tubes containing nutrient broth and organisms without the disinfectant served as negative control while the tube containing only the broth and disinfectant without organism served as positive control. These tubes were incubated at 37 ^{o}C for 18 to 24 hours. Thereafter, the tubes were examined for visible growth or turbidity and recorded. The MIC is the concentration at which no visible growth was observed when compared with the control [9].

3. RESULTS AND DISCUSSION

The result in Table1 showed the characteristics of the two bacterial isolates to some biochemical tests as well as their morphology. The result showed that the isolates were *Escherichia coli* and *Staphylococcus aureus.* In this current study, the antimicrobial activities of both the branded disinfectants and unbranded disinfectants on *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion showed some level of inhibition. In Table2, the effect of the unbranded disinfectants on *Escherichia coli* showed that the effectiveness of the unbranded disinfectants occurred at the 50% and 100% concentration and at 50% concentration only Lysol, morigade and pine oil were able to produce a clear zone of inhibition while Chlonoxynol and Nigertol showed no antimicrobial effect. Furthermore, all the unbranded disinfectants were able to exert some antimicrobial properties thereby leading to the formation of zones of inhibition at 100% concentration (Table2.). Lyaol and Morigade showed the highest zones of inhibition of 22mm and 20 mm respectively thereby making them the most effective unbraded disinfectants on *E. coli*.

The antimicrobial activities of the unbranded disinfectant on *Staphylococcus aureus* showed that the unbranded disinfectants were not effective at 10 and 25 % concentrations. Also, at 50% concentration only pine oil was able to inhibit the staphylococcal isolates at a zone of

10mm. whereas at 100% concentration, all unbranded disinfectants except Pine oil exerted some level of antimicrobial activities showing visible zones (Table 3).

The result of the antimicrobial activities of the branded disinfectant on *Escherichia coli* is presented in Table 4. The result showed that only Robert and Savlon were able to inhibit the isolates of *E. coli* at 10% with zones of inhibition observed to be 12mm and 7mm respectively. While at 25, 50 and 100%, all branded disinfectants produced visible zones of inhibition on the isolates. At 25% concentration, Robert was the most effective having zone of 15mm while Ivy's and Savlon were the most effective disinfectants at the 50% concentration with zones observed around 18mm and 20mm respectively. At 100% only Purit produced the least zone of inhibition of 18mm on the isolates while other disinfectants had greater zones of inhibitions.

Table 1: Colonial morphology and biochemical characteristic of the bacterial isolates

Isolate	Morphology	microscopy	G	L	S	M	F	Cat	Coa.	Ind.	MR.	Cit.	Mot	Identity
A	Metallic-silver small round flat	-ve bacilli	+	+	+	-	-	+	-	+	+	-	+	*Escherichia coli*
B	Golden yellow round smooth	+ve clustered cocci	+	+	+	+	+	+	+	-	+	+	-	*Staphylococcus aureus*

Key: *G; glucose, L; Lactose, S; Sucrose, M; Maltose, Cat. ;Catalase, Coa.; Coagulase, Ind.;Indole; MR; Methyl red, Mot.; Motility, Oxi.; Oxidase, SSA; Salmonella and Shigella Agar, MSA; Manitol salt Agar*

Table2:Effect of unbranded disinfectants on *Escherichia coli*

	Control				**Disinfectants**									
Con.	**Positive (Ofloxacin)**		**Negative (Sterile water)**		**Chlonoxynol**		**Lysol**		**Morigade**		**Nigertol**		**Pine oil**	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10%	**8**	**10**	00	00	00	00	00	00	00	00	00	00	00	00
25%	**12**	**11**	00	00	00	00	00	00	00	00	00	00	00	00
50%	**16**	**18**	00	00	00	00	12	12	10	10	00	00	6	6
100%	**10**	**22**	00	00	00	00	22	22	20	20	12	10	10	14

Table 3: Effect of unbranded disinfectants on *Staphylococcus aureus*

	Control				Disinfectants									
Con.	**Positive (Ofloxacin)**		**Negative (Sterile water)**		**Chlonoxynol**		**Lysol**		**Morigade**		**Nigertol**		**Pine oil**	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10%	**12**	**10**	00	00	00	00	00	00	00	00	00	00	00	00
25%	**17**	**16**	00	00	00	00	00	00	00	00	00	00	00	00
50%	**21**	**20**	00	00	00	00	00	00	00	00	00	00	10	10
100%	**28**	**28**	00	00	00	00	17	19	12	10	14	14	00	00

Table 4:Effect of branded disinfectants on *Escherichia coli*

	Control				Disinfectants									
Con.	**Positive (Ofloxacin)**		**Negative (Sterile water)**		Dettol		Ivy's		Purit		Robert		Savlon	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10%	**8**	**10**	00	00	00	00	00	00	00	00	12	10	5	7
25%	**12**	**11**	00	00	12	10	12	14	11	9	13	15	12	14
50%	**16**	**18**	00	00	14	14	18	16	12	14	15	17	20	18
100%	**10**	**22**	00	00	22	20	26	25	16	18	24	26	26	28

Table 5:Effect of branded disinfectants on *Staphylococcus aureus*

	Control				Disinfectants									
Con.	**Positive (Ofloxacin)**		**Negative (Sterile water)**		**Dettol**		**Ivy's**		**Purit**		**Robert**		**Savlon**	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
10%	**12**	**10**	00	00	12	10	00	00	9	11	00	00	8	8
25%	**17**	**16**	00	00	16	18	00	00	21	17	16	14	16	14
50%	**21**	**20**	00	00	20	22	10	8	23	21	17	18	22	20
100%	**28**	**28**	00	00	22	24	14	16	24	26	21	23	22	24

Table6: Concentration of Activity of Branded Disinfectants (MIC)

Organisms	**Branded Disinfectants**	**Concentration (%)**
Staphylococcus aureus	**Purit**	**75**
	Dettol	**50**
	Ivy's	**75**
	Salvon	**75**
	Robert	**75**
Escherichia coli	**Purit**	**50**
	Dettol	**75**
	Ivy's	**75**
	Salvon	**50**
	Robert	**75**

MIC: minimal inhibitory concentration

Table 7: Concentration of Activity of Unbranded Disinfectants (MIC)

Organisms	Unbranded Disinfectants	Concentration (%)
Staphylococcus aureus	**Lysol**	**75**
	Pine oil	**75**
	Morigade	**75**
	Nigertol	**75**
	Chlonoxynol	**75**
Escherichia coli	**Lysol**	**50**
	Pine oil	**75**
	Morigade	**50**
	Nigertol	**75**
	Chlonoxynol	**75**

MIC: minimal inhibitory concentration

The bacterial isolates in this current study have shown some level of resistance and susceptibility to the various form of disinfectants.
Staphylococcusaureus is a known cause of various form of infections ranging minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, gastrointestinal diseases, bacteremia and sepsis [13 and 20]. While some strains of *Escherichiacoli* are virulent and are responsible for diarrheal infections worldwide as well as neonatal meningitis, septicemia, and urinary tract infections (UTIs) [14].

The result in this study showed that the branded disinfectants are very much effective than the unbranded disinfectants. There is also a dearth of information on the effectiveness as well as the composition of unbranded disinfectants. However, Douglas and Braide (2015) in a study of the effectiveness of Locally Formulated Unbranded disinfectants on clinical bacterial isolates reported that unbranded (locally formulated) disinfectants are more potent when not diluted and that the differences in the activities of the unbranded and branded disinfectants may be due to the different substances used in formulations, as well as the structure and nature of the cell wall of the microbes. The disinfectants in this current study showed some level of activity on both Gram negative and positive bacterial isolates indicating that they have broad spectrum of activity. This is in agreement with Douglas and Braide [6] who had earlier reported that disinfectants show a broad spectrum of activity against different bacterial isolates.
Effectiveness of Dettol and Savlon has been reported by [9] who carried out a study on the efficacy of some disinfectants on clinical isolates including *Escherichia coli* and *Staphylococcus aureus*.in their study, Dettol was more active against the isolates compared with Savlon and other tested disinfectants. Other studies carried out by Olowe [12] and Olasehinde*et al* [11] also reported Dettol to be a strong disinfectant. Furthermore, El-Mahmood and Doughari[7] in a study of Bacteriological examination of some diluted disinfectants routinely used in the specialist hospital Yola, Nigeria reported that Purit has a higher activity on *E. coli* than *S. aureus* whereas in this study Purit was more effective against *S. aureus* than *E. coli*.

4. CONCLUSION

The antimicrobial effectiveness of five unbranded disinfectants and five branded disinfectants *Staphylococcus aureus*, and*Escherichia coli*was evaluated. Despite some level

of antimicrobial actions observed in the unbranded disinfectants, the findings in this study have shown that the branded disinfectants are more effective than the unbranded disinfectants. Also, since the unbranded disinfectants have shown some level of antimicrobial actions, increasing the formulation or the quantity for disinfection would be necessary.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Competing interests

Authors have declared that no competing interests exist.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

The permission to undertake this study was obtained from the Rivers State Health Research Ethical Committee.

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