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Susceptibility Pattern of Some Clinical Bacterial Isolates to Selected Antibiotics and Disinfectants

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Abstract

The antibacterial activities of five antibiotics, three brands of Ofloxacins (Obenasin, Floxavid and Drovid) and two brands of Ciprofloxacins (Uroxin and Siprosan), and five commonly used disinfectants (Lysol, Dettol, Purit, Roberts and Wex-cide) against *Staphylococcus aureus*, *Escherichia coli, Proteus* spp., *Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus* spp. were investigated. The growth inhibitory effect of both the antibiotics and disinfectants were determined using paper disk diffusion method and well-in-agar technique respectively. The highest mean zone of growth inhibition (19.3 mm) was given by Drovid on *Streptococcus* spp., while the smallest (7.0 mm) was by Floxavid on *P. aeruginosa*. Lysol had the highest mean zone of growth inhibition (18.0 mm) on *Streptococcus* spp. while *P. aeruginosa* and *Bacillus* spp. had no zone of growth inhibition with Roberts at 100-fold dilution. All the isolates were also resistant to Wex-cide. The test organisms were found to be significantly susceptible to the routinely used antimicrobials tested. However, there is the need for continuous surveillance for the detection of emerging resistance pattern.

K e y w o r d s: antimicrobials, clinical bacterial isolates, disinfectants, growth inhibition, susceptibility pattern

Introduction

Antiseptics and disinfectants are used extensively in hospitals and healthcare settings for a variety of topical and hard surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (Larson and Morton, 1991). The selection, use and control of the effectiveness of disinfectants have been emphasized, since environmental surfaces and medical and surgical instruments can serve as vehicles for infectious agents in susceptible hosts associated with the hospital setting (Rutala, 1997).

Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public. A wide variety of active chemical agents (or biocides) are found in these products, many of which have been used for hundred of years for antisepsis, disinfection and preservation (Block, 1991). Despite this, little is known about the mode of action of these active agents than about antibiotics. In general biocides have a broader spectrum of activity than antibiotics, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets.

The widespread use of antiseptics and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross-resistance to antibiotics (McDonnell and Russell, 1999). Disinfectant-resistant strains have arisen as a result of the lack in standardization of some factors, such as criteria for use of chemical agents, specifications in the labels of available products and scarcity of well-trained personnel (Pannutti and Grinbaum, 1995). Considering the importance of disinfection in the prevention and control of nosocomial infections, the aims of this study were to evaluate the bactericidal activity of five commonly used disinfectants against some clinical bacterial isolates, to evaluate the susceptibility pattern of the hospital isolates to two brands of new antibiotics (*i.e.* Ofloxacins and Ciprofloxacins) and the determination of a possible correlation between antibiotic-resistance and the resistance to disinfectants in isolated strains.

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Experimental

Materials and Methods

Collection of samples. The test samples (urine, wound swabs, nasal swabs, urethra and high vaginal swabs) were collected from patients at the Microbiology laboratory unit of the Imo State University Teaching Hospital Orlu, Imo State using sterile swab sticks for the swabs. Mid-stream urine samples were collected in sterile universal bottles. The samples were all transported to the laboratory and cultured within four hours of collection. A total of five hundred and forty (540) samples were collected.

Test organisms. A total of three hundred and thirty three isolates were recovered and used for the study. These are clinical isolates commonly encountered in Nigerian hospitals. They included eighty one strains of *Staphylococcus aureus*, fifty one strains of *Escherichia coli*, fifty strains of *Pseudomonas aeruginosa,* fifty one strains each of *Proteus* and *Bacillus* genera and forty-nine strains of *Streptococcus.*

Antimicrobial agents and nutrient media. The antimicrobial agents used for this study were six antibiotics, three brands of Ofloxacins (Obenasin 5 µg, Drovid 5 µg, Floxavid 5 µg) and two brands of Ciprofloxacins (Uroxin 5 µg, Siprosan 5 µg) and five commonly used disinfectants namely Lysol (Reckitt benckiser, Lagos) Dettol (Reckitt benckiser, Lagos), Purit (Chemical and allied products, Lagos), Roberts (Roberts Pharmaceuticals, Lagos) and Wex-cide (Wexford labs Inc.). Nutrient agar, MacConkey agar, and Chocolate agar (Antec Diagnostic products, UK) were the media used.

Isolation of test organisms. The test organisms were isolated using the streak plate technique as in Cruickshank *et al*. (1986) and Cheesbrough (1984). The plates were incubated at 37°C for 24 hours and examined for bacterial growth. The isolates were identified using their growth morphology, Gram-stain, motility and other biochemical tests as in Cheesbrough (1984). Each identified pure isolate was subcultured onto nutrient agar slants and stored in the refrigerator for further use in the study.

Collection and processing of antimicrobial agents. Commercially prepared antibiotic disks (Uroxin, Floxavid, Obenasin, Drovid, Siprosan) were obtained from pharmaceutical representatives of the respective drug manufacturers who make supplies to the teaching hospital. The disks were collected directly from the suppliers. They were stored according to the manufacturer's instruction before use. A bottle each of Lysol, Dettol, Purit, Roberts, and Wex-cide were purchased from the marketers at Owerri. Their batch numbers and expiring dates were noted. They were carried to the Laboratory for analysis. Appropriate dilutions of each selected disinfectant were made with distilled water. The dilutions were used to test for antibacterial activity of the disinfectants.

Testing for antibacterial properties. The susceptibility pattern of the test organisms to the selected antibiotics and disinfectants was tested using the disk paper method for antibiotics and well-in-agar diffusion technique for the disinfectant. The same test organisms were used for both antibiotics and disinfectants selected.

Each test organism was subcultured on nutrient agar medium by streak plate technique from the slants and incubated for 24 hours at 37°C to obtain young pure culture of the isolates. Discrete colonies of each test organism was collected and used to inoculate nutrient agar plate for susceptibility testing. A sterile forceps was used to collect each antibiotic disk and placed over the surface of the inoculated plate. A total of five disks representing the five selected antibiotics were placed on each inoculated plate. The plates were incubated at 37°C for 24 hours and examined for growth inhibitory effects.

The test organisms were inoculated in six duplicate nutrient agar plates for susceptibility pattern of the organisms to the selected disinfectants. A sterile cork borer was used to make standard wells (of about 2 mm diameter) on the surface of each inoculated plates. A total of six wells were made on each plate, one for each disinfectant and a control (distilled water). The six duplicate plates were used for six dilutions of the selected disinfectants. Micropipettes were used to deliver the disinfectants to the respective wells. The plates were incubated at 37°C for 24 hours and zones of growth inhibition were measured in millimeters using transparent metric rule. The mean zones of growth inhibition were recorded.

Results

Out of the one hundred and twenty samples from high vaginal and urethra swabs examined, only sixty samples yielded significant bacterial growth, namely 31 (9.3%) were *Staphylococcus aureus* and 29 (8.7%) *Escherichia coli* strains. From the one hundred and fifty wound swap samples examined, 132 yielded positive significant bacterial growth and identification test revealed that *Streptococcus* and *Pseudomonas* spp. were obtained from 40 (12.0%) samples each whereas, *Proteus* spp. and *Bacillus* spp. were isolated from 31 (9.3%) and 21 (6.3%) samples respectively. One hundred and twenty nasal swab samples were examined, out of which 31 (9.3%) samples contained *Staphylococcus aureus*, 30 (9.0%) samples *Bacillus* spp. while 9 (2.7%) samples β-haemolytic *Streptococcus* spp. respectively. On the other hand, of the 150 urine

Table I
Test samples and their percentage (%) isolates*

Samples	No. examined	Total No. of isolates	*Staphylococcus* spp.	*Streptococcus* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*E. coli*	*Proteus* spp.
U/V or HVS	120	60	31 (9.3%)	–	–	–	29 (8.7%)	–
Wound swab	150	132	–	40 (12%)	21 (6.3%)	40 (12.0%)	–	31 (9.3%)
Nasal Swab	120	70	31 (9.3%)	9 (2.7%)	30 (9.0%)	–	–	–
Urine	150	71	19 (5.7%)	–	–	10 (3.0%)	22 (6.6%)	20 (6.0%)
TOTAL	540	333	24.3%	14.7%	15.3%	15.0%	15.3%	15.3%

* The percentages refer to fraction of total number of strains = 330

samples examined, 22 (6.6%) samples yielded *Escherichia coli*, 20 (6.0%) samples *Proteus* spp., 19 (5.7%) samples *Staphylococcus aureus respectively*, while 10 (3.0%) samples contained *Pseudomonas* spp. (Table I).

The occurrence of the isolates in the samples studied revealed that *Staphylococcus aureus* (24.3%) was most prevalent with an even (15.2%) preponderance of all other isolates compared to *Bacillus*, *Pseudomonas*, *Streptococcus*, *Escherichia coli* and *Proteus* spp. The result of the susceptibility of the isolates to antibiotics revealed *Streptococcus* spp. to be the most susceptible to the selected antibiotics. It was followed by *Staphylococcus aureus* and *Bacillus* spp*.,* while *Pseudomonas* spp. are less susceptible isolates, followed by *Proteus* spp*.,* and *Escherichia coli.*

Generally, the response of the clinical isolates to test antibiotics used for the study reveals *Streptococcus* spp. to be most susceptible to Drovid with a mean zone of growth inhibition of 19.3 mm and least susceptible to Floxavid with a least mean zone of inhibition of 15.2 mm. *Staphylococcus aureus* was most susceptible to Siprosan and least susceptible to Floxavid with mean zones of inhibition of 18.0 mm and 10.8 mm respectively. While *Escherichia coli* was most susceptible to Drovid and least susceptible to Siprosan with mean zones of growth inhibition of 17.0 mm and 11.2 mm respectively. *Pseudomonas* genus representative. were found to be most susceptible to Siprosan with a mean zone of growth inhibition of 12.0 mm and least susceptible to Floxavid with 7.0 mm mean zone of growth inhibition. On the other hand, *Proteus* isolates showed the highest and least susceptibility to Drovid (17.2 mm) and Siprosan (8.6 mm). While *Bacillus* spp. showed the highest and least susceptibility to Obenasin (17.0 mm) and Floxavid (11.0 mm) respectively.

Table II
Mean zone of growth inhibition (mm) and average (%) susceptibility of the isolates to different antibiotics

Antibiotics	DROVID	OBENASIN	FLOXAVID	UROXIN	SIPROXIN	Average % susceptibility to antibiotics
Min zone of growth inhibition for *Streptococcus* spp.	11	13	11	9	10	90%
Max zone of growth inhibition for *Streptococcus* spp.	25	25	18	21	25	
Mean zone of growth inhibition	19.3	18.2	15.2	15.3	17.8	
Min zone of growth inhibition for *Staphylococcus* spp.	6	11	0	13	13	87.5%
Max zone of growth inhibition for *Staphylococcus* spp.	22	27	15	25	28	
Mean zone of growth inhibition	13.8	14.9	10.8	17.4	18.0	
Min zone of growth inhibition for *E. coli*	13	7	9	7	5	76%
Max zone of growth inhibition for *E. coli*	23	21	19	29	15	
Mean zone of growth inhibition	17	13.4	13.4	15.2	11.2	
Min zone of growth inhibition for *Pseudomonas* spp.	4	3	0	0	8	44%
Max zone of growth inhibition for *Pseudomonas* spp.	15	18	19	20	17	
Mean zone of growth inhibition	9.2	10.8	7.0	10.8	12.0	
Min zone of growth inhibition for *Proteus* spp.	11	5	5	5	5	48%
Max zone of growth inhibition for *Proteus* spp.	21	16	15	19	17	
Mean zone of growth inhibition	17.2	10.8	9.6	10.6	8.6	
Min zone of growth inhibition for *Bacillus* spp.	5	15	5	5	15	84%
Max zone of growth inhibition for *Bacillus* spp.	25	25	15	25	15	
Mean zone of growth inhibition	13	17	11	15	15	
% Isolates susceptible to tested antibiotics	77.1%	86.7%	65.8%	73.9%	76.7%	

Table III
Mean zone of growth inhibition (mm) of clinical isolates on different dilutions of the selected disinfectants

Disinfectant	Dilution factor	*Streptococcus* spp.	*Staphylococcus* spp.	*E. coli*	*Pseudomonas* spp.	*Proteus* spp.	*Bacillus* spp.
LYSOL	1:10	33.6	25.4	32.6	21.2	29.0	36.0
	$1:10^2$	18.0	12.6	15.0	11.0	13.0	8.8
	$1:10^3$	0.0	0.0	9.60	0.0	0.0	7.4
	$1:10^4$	0.0	0.0	0.0	0.0	0.0	0.0
DETTOL	1:10	15.4	22.2	18.6	19.0	17.80	22.4
	$1:10^2$	11.8	11.4	10.6	10.0	12.80	9.2
	$1:10^3$	0.0	0.0	0.0	0.0	0.0	0.0
	$1:10^4$	0.0	0.0	0.0	0.0	0.0	0.0
PURIT	1:10	17.4	21.8	18.4	16.6	15.0	29.2
	$1:10^2$	12.8	17.4	10.8	11.8	11.6	13.0
	$1:10^3$	8.8	4.0	0.0	0.0	0.0	0.0
	$1:10^4$	0.0	0.0	0.0	0.0	0.0	0.0
ROBERTS	1:10	16.8	15.6	18.8	0.0	17.8	32.2
	$1:10^2$	11.4	8.0	10.6	0.0	12.6	0.0
	$1:10^3$	0.0	0.0	0.0	0.0	0.0	0.0
	$1:10^4$	–	–	–	–	–	–
WEXCIDE	1:10	0.0	0.0	0.0	0.0	0.0	0.0
	$1:10^2$	0.0	0.0	0.0	0.0	0.0	0.0
	$1:10^3$	–	–	–	–	–	–
	$1:10^4$	–	–	–	–	–	–

Comparative analysis of the activities of Ofloxacins and Ciprofloxacins revealed that Ofloxacins showed greater inhibitory effect against *Streptococcus*, *Proteus*, *Bacillus* species and *E. coli* than the ciprofloxacins while the ciprofloxacins exhibited a greater growth inhibitory effect on *Staphylococcus aureus* and *Pseudomonas* spp. than the Ofloxacins. It was also shown that Drovid elicited the highest mean zone of growth inhibition (19.3 mm) on *Streptococcus* spp. while Floxavid exhibited the least mean zone of growth inhibition (7.0 mm) on *Pseudomonas* species (Table II).

Evaluation of the test disinfectants revealed that Lysol, Dettol and Purit exhibited growth inhibitory effect on all the test organisms in 10 to 100-fold dilution. However, the test organisms exhibited variable susceptibility pattern to the disinfectants in 1000-fold dilution and none of the test isolates was inhibited at dilution greater than 1000-fold. A minimal inhibitory concentration (MIC) of 1 in 10^2 was determined for Lysol against *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas* and *Proteus* spp., while the MIC against *Bacillus* spp. was a 1000-fold dilution. Dettol has a MIC of a 100-fold dilution for all the test organisms while Purit showed variable efficacy against the test organisms in both 100-fold and 1000-fold dilution. The MIC of Roberts against *Staphylococcus aureus, Escherichia coli*, *Streptococcus* and *Proteus* spp was a 100-fold dilution. However, the compound had no inhibitory effect against *Pseudomonas* spp. in dilution 100-fold or greater. Wex-cide did not exert any inhibitory effect on any of the test organisms (Table III).

Discussion

One of the goals of disinfection in hospitals is to reduce the risk of nosocomial infection in patients. A great number of disinfectants are used in health-care settings, including glutaraldehyde, formaldehyde and chlorine releasing agents and compounds. These agents are considered germicidal when recommended and used in appropriate concentrations for cleaning patient-care items and instruments (Rutala, 1997).

Although bacterial resistance to antibiotics has been extensively studied, only a few reports are available on the action of disinfectants action against microorganisms particularly in Nigeria. In this study, it was verified that most of these selected disinfectants commonly used were effective when tested against clinical bacterial strains. The susceptibility pattern of the isolates to selected antibiotics showed that *Streptococcus* spp. were the most susceptible isolates with 90% susceptibility to the tested antibiotics (Table II) while *P. aeruginosa* is the least susceptible isolate with 44% susceptibility to the tested antibiotics. Obenasin proved the most effective of the antibiotics tested with 86.7% of all isolates susceptible to it, while 65.8% were susceptible to Floxavid, which is the least effective. The highest observed mean zone of growth inhibition (19.3 mm) was for Drovid against *Streptococcus* spp., while the least mean zone of growth inhibition (7.0 mm) was for Floxavid against *P. aeruginosa*. The isolates were the most susceptible to Lysol in 1:100 dilution with a mean zone of growth inhibition of 13.1 mm, while they were not suscep-

Table IV
Percentage susceptibility of clinical isolates to selected disinfectants

Disinfectant	Dilution	*Streptococcus* spp.	*Staphylococcus* spp.	*E. coli*	*Pseudomonas* spp.	*Proteus* spp.	*Bacillus* spp.	Average
Lysol	1:100	18.0	12.6	15.0	11.0	13.0	8.8	13.1
Dettol	1:100	11.8	11.4	10.6	10.0	12.8	9.2	10.9
Purit	1:100	12.8	17.4	10.8	11.8	11.6	13.0	12.9
Roberts	1:100	11.4	8.0	10.6	0.0	12.6	0.0	7.1
Wexcide	1:100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean		10.8	9.8	9.4	6.5	10.0	6.2	
% Susceptible		80%	60%	80%	60%	80%	20%	

tible to Wex-cide at the same dilution – the mean zone of growth inhibition being 0.0 mm (Table III). *Streptococcus* spp., *E. coli*, and *Proteus* spp. were the most susceptible to the disinfectants, being susceptible to 80% of the disinfectants in 1:100 dilution, while *Bacillus* spp. was the least susceptible, being susceptible to only 20% of the disinfectants at the same dilution.

Very few studies demonstrate the correlation between antibiotics and disinfectants. Anderson *et al*., 1997, testing hospital isolates did not find evident correlation between susceptibility to antibiotics and to disinfectants. Gram-negative bacteria are generally less susceptible to biocides than Gram-positive species. Such resistance is likely to be intrinsic rather than plasmid-mediated, due to outer membrane that act as a protective barrier. This was also observed in this study. *Pseudomonas aeruginosa*, *Proteus* spp. and *E. coli* were 44%, 48%, and 76% susceptible respectively to all the antibiotics tested, while the Gram-positive bacteria *Streptococcus* spp., *S. aureus*, and *Bacillus* spp., were 90%, 87.5%, and 84% susceptible, respectively. The above trend was not observed in the disinfectants, were *Streptococcus* spp., *E. coli*, and *Proteus* spp. showed susceptibility to 80% of the various disinfectants tested (Table IV).

Due to the capacity of surviving in unfavorable environmental conditions and its high resistance to antibiotic agents, antiseptics and disinfectants, *Pseudomonas aeruginosa* continues to be an important pathogen in hospital acquired infections, mainly respiratory and urinary infections (Olowe *et al*., 2004). Fernandez-Astorga *et al.* (1995) reported that the high resistance of *Pseudomonas* spp. to cationic agents seems to be associated with the chemical composition of their external membrane. This study also demonstrated that *Pseudomonas aeruginosa* was a problem to the antibiotics, as well as the disinfectants tested (Tables II and III).

It is clear that microorganisms can adapt to a variety of environmental, physical and chemical conditions, and therefore not surprising that resistance to extensively used antiseptics and disinfectants has been reported. Many of these reports of resistance has arisen due to inadequate cleaning, incorrect product use and ineffective infection control practices which cannot be underestimated. With growing concerns about the development of biocidal resistance and cross-resistance with antibiotics, clinical isolates should be under continual surveillance and other possible mechanisms of resistance should be investigated. Also, antiseptic and disinfectant products can vary significantly despite containing similar levels of biocides, which underlies the need for close inspection of efficacy claims. In addition, a particular antiseptic or disinfectant product may be better selected (as part of infection control practices) based on particular circumstances or nosocomial outbreaks; for example, certain active agents are clearly more efficacious against Gram-positive than Gram-negative bacteria.

In conclusion, a great deal remains to be learned about the mode of action of antiseptics and disinfectants. Although significant progress has been made with bacterial investigations, a great understanding of these mechanisms of action will help prevent their microbial resistance. It will also make for more efficient use of these agents clinically with the potential for design of newer, more effective compounds and products.

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