

Original Research Article

The activity of some clinically used biocides to commonly isolated bacterial pathogens from tertiary hospitals in Nigeria

Variable efficacy of clinically important biocides against common bacterial pathogens isolated from tertiary hospitals in Nigeria.

ABSTRACT

Aim: There has not been any critical assessment of the efficacy and choice of biocides/disinfectants used in various tertiary hospitals of Nigeria despite several reports of high-level bacterial resistance to the antibiotics. The aim of the study was to determine the activity of some commonly used biocides against bacterial isolates from tertiary hospitals of Nigeria.

Study Design: This following study was a cross-sectional study.

Methods: Fifty-three non-duplicate bacterial isolates were obtained from different tertiary hospitals. Susceptibility to the selected antibiotics was determined using the disc diffusion Method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a range of biocides been also determined using microtitre broth testing followed by inoculation onto agar.

Results: A high prevalence of resistance to critical antibiotics were observed for Gram-negative bacteria (>85% of isolates were resistant to cephalosporins and >75% were resistant to fluoroquinolones). Imipenem remained active against >90% of Gram-

negative isolates. Over half (52.2%) of the isolates of *S. aureus* were found to be resistant to cefoxitin, indicating methicillin resistance which is common.

S. aureus showed the greatest tolerance to all the biocides that are used & compared to other species although all species exhibited tolerance to some agents. Whilst sodium hypochlorite remained effective against all the strains, (at 0.5 – 2%), like ethanol and triclosan which were less effective even at the common 70% formulation of ethanol. None of the *S. aureus* isolates was inhibited by any alcohol concentration tested and various other species had high levels of tolerance. Chloroxylenol was active against some strains but some were fully tolerant of the concentrations tested.

All the test of biocides were bactericidal with MBC/MIC ratios of >4. There was no statistical difference between diffusion and dilution techniques in determining the susceptibility of the isolates to biocides except for *P. aeruginosa*.

Conclusion: Sodium hypochlorite and chloroxylenol are the biocides of choice for hospital disinfection in Nigeria according to this study. Ethanol is unreliable at lower concentrations and should be used at high concentrations in order to achieve the requisite efficacy.

Keywords: Biocides, Bacteria, Susceptibility, Resistance, Nigeria

1. INTRODUCTION

The issue of bacterial resistance to biocides has been considered to have a very low profile and priority unlike antibiotic resistance [1]. Biocides have been far less studied than antibiotics in terms of efficacy, mechanisms of action and epidemiology of bacterial resistance. Despite this paucity of information on many biocides, current knowledge still indicates that bacteria possess a number of mechanisms to survive exposure to a biocide. Some of these mechanisms include; efflux pumps, permeability changes and biofilm formation which also confer resistance to antibiotics [2, 3]. Other mechanisms have also been described, such as mutation and by-pass of metabolism [4]. It has also been recognized that some environmental factors contribute to a reduced susceptibility to biocides including new factors such as bacterial swarming and motility which are emerging as contributing to cross-resistance [5].

The concentration of a biocide is the most important factor that affects its efficacy. In many cases bacterial survival of biocide exposure occurs following the improper storage or use of the formulations, resulting in a decrease in the effective concentration [6]. To improve hygiene measures or reduce nosocomial infections to the barest minimum in hospitals and its environs there is need to increase the use of biocides for effective control of these organisms and biocides are crucial for infection prevention. Recently, most chemical based disinfectants are now also incorporated into many personal care products that are used at home or hospitals; these include cosmetic, hand sanitizers, lotions [7]. In the health care setting, bacterial resistance to biocides has long been reported for compounds such as chlorhexidine [8], quaternary ammonium compounds; bisphenol, and triclosan [9, 10].

There has been an increase in bacterial resistance to biocides and antibiotics and this has led to the development of multidrug-resistant strains that are the major causes of hospital-acquired infections [11]. Biocides, unlike antibiotics, lack selective toxicity, and most do not have a distinct bacterial cell target upon which to act (except for triclosan and chlorhexidine) [12]. In Nigeria, there have been several reports of high-level bacterial resistance to antibiotics including Gram-negative bacteria to carbapenems; the last resort antibiotics [13] and methicillin resistance *Staphylococcus aureus* (MRSA) [14]. Therefore, it is imperative to study the activities of commonly available biocides against some of these multidrug-resistant bacterial isolates in order to have background information for the use of these biocides in our hospitals and various homes since routine biocide susceptibility testing is not popular or practicable. Moreover, there is little or no current information on the activities of available biocides against these isolates in Nigeria. The aim of this study was to determine the activities of some commonly available biocides against bacterial isolates from tertiary hospitals in Nigeria.

MATERIALS AND METHODS

2.1 Bacterial strains

A total of 53 bacterial strains were collected from varying clinical specimens from 4 tertiary hospitals in Nigeria. These strains included 23 *Staphylococcus aureus*; 15 *Klebsiella pneumonia*; 6 *Pseudomonas* species and 9 *Escherichia coli* obtained from varying clinical specimens including urine, blood, vaginal swab, wound swab, etc

submitted to the diagnostic laboratory of Medical Microbiology departments of these hospitals (Table 1).

2.2 Chemicals

Antibiotics and biocides, sodium hypochlorite (NaClO) solution; chlorhexidine (CHX) were all purchased from Sigma-Aldrich, UK. Disinfectants; ethanol was purchased from VWR Chemicals, France; chloroxylonol (C₈H₉ClO) was purchased from Reckitt Benckiser, UK and triclosan from CIBA, Germany.

Determination of susceptibility to conventional antibiotics

The antibiotic susceptibility testing was determined using disc diffusion method on Muller Hinton (MH) agar, Oxoid UK as described by Clinical Laboratory Standards Institute, CLSI [15]. Gram- positive and negative antibiotic discs from Oxoid, UK were used for the study, these included erythromycin (15 µg); cefoxitin (30 µg); mupirocin (200 µg); vancomycin (30 µg); tetracycline (30 µg); linezolid (30 µg); teicoplanin (30 µg); clindamycin (2 µg) and gentamicin (30 µg) as well as Gram negative antibiotic discs, aztreonam (30 µg); tigecycline (15 µg); amoxycillin (20 µg); ceftriazone (30 µg); ceftazidime (30 µg); imipenem (10 µg); meropenem (10 µg); ciprofloxacin (5 µg). Bacteria inoculum was prepared to match 0.5 McFarland standards and was tested against all the antibiotics. Sterile swab stick was used to streak the surface of Muller Hinton agar plate, after which the antibiotic discs were placed on the surface of Muller Hinton plate and incubated at 37°C overnight. The zones of inhibition were measured and interpreted as sensitive and resistant based on the guidelines of CLSI [15].

2.3 Determination of minimum inhibitory concentrations

2.3.1 Diffusion technique

Punch hole diffusion method was employed to determine the minimum inhibitory concentrations of the isolates against the biocides. Several punch holes were made in agar plates that have been previously seeded with the test organisms. These punched holes were then filled with varying concentrations of biocides to be tested. The working stock of the various biocides used included ethanol (100%), sodium hypochlorite (6%), chlorhexidine (4%), chloroxylenol (2.4%) and triclosan (4%). These stocks were prepared using sterile distilled water except for triclosan where 1 M sodium hydroxide was used. The plates were incubated at 37°C overnight, and the zones of inhibition were measured in millimetres using a transparent metric ruler. Various controls were set up; control organisms were run alongside the tests using *E. coli* ATCC 25922 for *Enterobacteriaceae*, *P. aeruginosa* ATCC 27853 for *P. aeruginosa* and ATCC 25923 for *S. aureus*, while sterility control was set up with lowest concentrations of the various biocides without organisms, viability control contained the test strains in the broth without biocide. Experiments were repeated three times in independent experiments.

2.3.2 Dilution techniques

The minimum inhibitory concentrations of a range of biocides and antibiotics were determined, defined as the lowest biocide or antibiotic concentration that inhibit bacterial growth or no visible growth using the microtitre broth dilutions as recommended by CLSI [15]. Using a multi-stopper pipette, 50 µl of Mueller- Hinton broth was added to

microtiter plate wells 2-12, 50 µl of biocide diluted in broth at twice the required final concentration was added to column A1-H1 and A2-H2 using the working stocks previously described above. 50 µl of biocide from column 2-11 was double diluted while 50 µl was removed from column 11. 50 µl of an overnight broth culture of the organisms already diluted to 10^4 orgs/ml was added. The microtitre plates were covered with sterile lids, incubated at 37°C and examined for growth after overnight incubation. All MIC determinations were also repeated three times in independent experiments.

2.4 Determination of minimum bactericidal concentrations

Minimum bactericidal concentrations were determined by inoculating a 50 µl aliquot of all suspensions where no visible bacterial growth were observed on LB agar, (Sigma-Aldrich UK) and incubating at 37°C overnight. MBC was recorded as the lowest dilution where there was no bacterial growth.

2.5 Statistics analysis

Data were analysed using statistical package within the Microsoft Excel and Epi-info software from Centre for Disease control and prevention, USA. Students *t*-test was used to determine the statistical significance. A *p* value less than 0.05 was considered to be significant.

3.0 Results

3.1 Antibiotics susceptibility

The susceptibility patterns of all the strains to conventional antibiotics are shown in Table 2; panel I is for Gram-negative bacteria while panel II shows *S. aureus*. Several differences in antimicrobial susceptibility patterns were observed, high-level resistance to third-generation cephalosporin antibiotics were obtained for Gram-negative bacteria; 100% and 87% resistance to ceftriaxone and ceftazidime respectively. Fluoroquinolone, ciprofloxacin also showed a similar pattern with 76.7% resistance and for tigecycline 70% of isolates were resistant. Sensitivity was found to be highest to imipenem, with 6.7% of isolates being resistant. For *S. aureus*, 52.2% of isolates were found to be resistant to ceftazidime, an indication of the presence of methicillin resistance. Overall, these patterns of susceptibility depict a common pattern of multidrug resistance amongst these strains.

3.2 Biocide susceptibility

The number of strains inhibited with different concentrations of various biocides (sodium hypochlorite, chloroxylenol, triclosan, chlorhexidine, and ethanol) using agar diffusion and broth microtiter dilution techniques are shown in Table 3. Sodium hypochlorite was more effective against all the strains compared to the other biocides, with 0.5 – 2% of active agent inhibiting all strains. Ethanol and triclosan were less effective, even at the popular 70% for ethanol normally used for antiseptics. *S. aureus* showed 100% resistance to ethanol at 100% concentration while some levels of activities was observed at 80 – 90% ethanol for other bacteria. *P. aeruginosa* was 100% resistant to triclosan at all concentrations. The minimum bactericidal concentration for all the

167 biocides was determined. In most cases, all test compounds appeared to be bactericidal
168 with MBC/MIC ratios of >4 (Table 4).

169 The disk diffusion technique appeared to be superior to broth micro dilution in terms of
170 sensitivity and reproducibility in determining the MICs of biocides in this study, however
171 this was not statistically significant except for *Pseudomonas aeruginosa* for certain
172 biocides such as sodium hypochlorite; chlorhexidine; chloroxylenol and *S. aureus* with
173 sodium hypochlorite ($P < 0.05$).

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4.0 Discussion

In Nigeria, there are several reports of high-level bacterial resistance to antibiotics in Gram-negative bacteria including last resort antibiotics such as carbapenem [13] and MRSA is common with a prevalence of 41.4% of all *Staphylococcus aureus* isolates [14]. To the best of our knowledge, there has not been any critical assessment of the efficacy and choice of biocides/disinfectants used in our various tertiary hospitals. A great number of disinfectants are used in the health care setting including sodium hypochlorite, triclosan, chloroxylenol, ethanol, and chlorhexidine. These agents are chemicals that are capable of rendering harmless or exerting a controlling effect on any harmful microorganism by chemical means meanwhile, the goal of disinfection is to reduce the risk of endemic and epidemic nosocomial infections in hospital patients and limiting its spread [16, 17]. The majority of the isolates were resistant to more than three different types of antibiotics including carbapenem for the Gram-negative bacteria depicting multidrug resistance isolates, cefoxitin and teicoplanin for *S. aureus* confirming the common prevalence of MRSA.

All the bacterial isolates were resistant to chlorhexidine with a MIC >4%, similarly no activity was obtained for ethanol at 70%, once the concentration was increased to 80% and above efficacy began to appear against some isolates. It is pertinent that 70% ethanol has been the mainstay for ensuring antisepsis on the skin or in clinical settings for swabbing animate or inanimate objects. The 70% formulation came into being essentially due to the fact that 100% alcohol cannot be used as a disinfectant because of its volatility and denaturation of external membrane proteins only. In order to prevent

breeding biocide-resistant strains we need to increase the concentration of ethanol as disinfectant/biocide to 80 – 85% as a matter of emergency with little or no economic cost in view of the global high level bacteria resistance to antimicrobial agents rather than adhering strictly to the conventional 70% which is still the current practice worldwide. Already the use of biocides in higher concentrations is inevitable due to the decrease in susceptibility as observed in this study and elsewhere [18]. Likewise, there have been cases where combined antibiotic therapy or increased concentrations are recommended solely to avoid the emergence of resistant strains and this can be emulated for biocides [19].

A MIC range of 0.5 - 1% sodium hypochlorite was required to inhibit Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and *P. aeruginosa*, while MICs in the range of 1 - 3% was effective to inhibit the growth of *S. aureus*. Concentrations of 3 - 6% were required for chlorhexidine to work effectively; most Gram-negative bacteria were inhibited at 5% while *S. aureus* strains were essentially prevented from growing by 3%, while 0.15 - 1.2% was required for chloroxylonol to exert an inhibitory effect. The minimum bactericidal concentrations for most of the biocides were greater than four times the corresponding MIC, another demonstration that strains have a high level of tolerance because an agent is usually regarded as bactericidal if the MBC is no more than four times the MIC.

Because there are no standardized susceptibility testing techniques and/or critical concentrations for disinfectants to classify bacterial susceptibility to biocides like in antibiotics, we included two methods for susceptibility testing in order to determine

which method best suits biocide(s). We discovered that there was no difference between disc diffusion and broth microdilution except for *P. aeruginosa* but we do not understand why this is so because the results were products of repeated independent experiments.

Conclusion

The more appropriate disinfectants for hospital disinfection are sodium hypochlorite and chloroxylonol. Ethanol should be used in higher concentration more than the conventional 70% in current usage in order to achieve the requisite efficacy in routine surface disinfection particularly in hospitals and environs.

Consent

It is not applicable

Ethical Approval

It is not applicable

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295 **Table 1. Sources and distribution of bacterial strains**

Source	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	Total
Wound swab	2	4	3	7	16
Eye swab	1	0	0	4	5
HVS	1	0	0	2	3
Blood culture	3	3	0	4	10
Urine	4	2	3	2	11
Sputum	2	0	0	0	2
Catheter tip	1	0	0	0	1
Aspirate	1	0	0	4	5
Total	15	9	6	23	53

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297 **Table 2. Antibiotic susceptibility pattern of 53 bacterial strains**

Antibiotics (µg/ml)	No. sensitive	% Sensitive	% Resistance
Panel I: Gram negative: 30 isolates			
Ceftazidime (30)	4	13.3	86.7
Ceftriazone (30)	0	0.0	100.0
Amoxyclav (30)	6	20.0	80.0
Ciprofloxacin (5)	7	23.3	76.7
Gentamicin (10)	19	35.9	64.2
Tigecycline (15)	9	30.0	70.0
Imipenem (10)	28	93.3	6.7
Aztreonam (30)	19	63.3	36.7
Panel II: Gram positive: 23 isolates			
Tetracycline (30)	12	52.2	47.8
Cefoxitin (30)	11	47.8	52.2
Linezolid (30)	17	73.9	26.1
Clindamycin (2)	15	62.2	34.8
Teicoplanin (30)	22	95.7	4.4
Erythromycin (15)	17	73.9	26.1

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299 Table 3. Minimum inhibitory concentrations of bacterial isolates to biocides

MICs, %; wt/vol or vol/vol (Range)					
Isolate, n	SH (0.12 – 6)	CHX (0.02 – 2.4)	CHD (0.5 – 10)	EtOH (10 – 100)	TRIC (0.01 – 2)
Panel I: Diffusion					
<i>E. coli</i> , 9	0.25(3) 0.5(4) 1(1) 2(1)	0.02(3) 0.04(1) 0.6(3) 1.2(1) 2.4(1)	5(2) 6(5) >10(2)	75(2) 80(2) 85(1) 95(1) 100(1) >100(2)	0.02(3) 0.25(1) 1(1) >2(4)
<i>K. pneumoniae</i> , 15	0.25(3) 0.5(7) 1(5)	0.08(1) 0.15(3) 0.3(6) 0.6(2) >2.4(3)	5(14) >10(1)	75(3) 80(2) 85(3) 90(1) 100(2) >100(4)	0.02(1) >2 (14)
<i>P. aeruginosa</i> , 6	0.12(2) 0.25(4)	1.2(2) 2.4(3) >2.4(1)	5(3) 6(1) >10(2)	75(3) 80(1) >100(2)	>2(6)
<i>S. aureus</i> , 23	0.12(1) 0.5(3) 1(5) 2(14)	0.02(5) 0.04(4) 0.08(2) 0.15(2) 0.3(2) 0.6(3) 1.2(2) >2.4(3)	3(17) 6(2) 7(3) >10(1)	>100(23)	0.02(6) 0.04(1) 0.08(1) 0.5(2) 1(1) >2(12)
Panel II: Dilution					
<i>E. coli</i> , 9	0.5(2) 1(5) 2(2)	0.3(1) 0.6(8)	5(1) 6(2) 8(3) 9(2) 10(1)	85(1) 90(3) 95(1) 100(4)	0.02(3) 0.25(1) 1(1) >2(4)
<i>K. pneumoniae</i> , 15	0.5(9) 1(6)	0.15(1) 0.3(11) 0.6(3)	5(1) 6(2) 8(3) 9(2) 10(1)	85(4) 90(2) 95(4) 100(5)	0.02(1) 2(1) >2(13)
<i>P. aeruginosa</i> , 6	0.5(5) 1(1)	0.3(1) 0.6(5)	8(4) 9(1) 10(1)	90(3) 95(1) 100(2)	>2(6)
<i>S. aureus</i> , 23	1(4) 2(10) 3(9)	0.15(5) 0.3(10) 0.6(4) 1.2(4)	6(12) 7(3) 8(5) 9(2) 10(1)	95(5) 100(6) >100(12)	0.02(6) 0.04(1) 0.08(1) 0.5(2) 1(1) >2(12)

300 n - number of strains, () - numbers in parentheses are the number or frequency of strains with the MICs by their sides,
301 SH – sodium hypochlorite, CHX – chlorxylenol, CHD – chlorhexidine, EtOH – ethanol, TRIC – triclosan.

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303 **Table 4. Minimum bactericidal concentrations of bacterial isolates to biocides**

Isolate, n	MBC, % (Range)				
	SH (0.12 – 6)	CHX (0.02 – 2.4)	CHD (0.5 – 10)	EtOH (10 – 100)	TRIC (0.01 – 2)
<i>E. coli</i> , 9	4(2) 6(4) >6(3)	2.4(6) >2.4(3)	10(5) >10(4)	95(2) 100(7)	0.25(3) 1(1) >2(5)
<i>K. pneumoniae</i> , 15	6(6) >6(9)	0.3(2) 1.2(6) 2.4(6) >2.4(1)	10(10) >10(5)	95(4) 100(7)	0.25(1) >2 (14)
<i>P. aeruginosa</i> , 6	6(4) >6(2)	0.6(1) 2.4(2) >2.4(3)	10(2) >10(4)	100(6)	>2(6)
<i>S. aureus</i> , 23	3(6) 4(5) 6(12)	0.3(4) 0.6(10) 1.2(9)	7(9) 8(11) 10(3)	>100(23)	0.25(6) 2(2) >2(15)

304 () - numbers in parentheses are the number or frequency of strains with the MBCs by their sides, SH – sodium hypochlorite, CHX – chlorxylenol, CHD –
 305 chlorhexidine, EtOH – ethanol, TRIC – triclosan.