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A Study of the Antimicrobial Effectiveness of Diluted Antiseptics in Nigeria

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Authors' contributions

Author EOA designed, coordinated the study and wrote the manuscript. Author EOA carried out the experimental analysis. Author OOA was involved in some microbiological studies with author EOA. Authors EOA and OOO were involved in ESβL analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The antimicrobial effectiveness of diluted antiseptics and the health risks that may be associated with any surviving pathogens were investigated.
Study Design: Experimental Study.
Place and Duration of Study: Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, between March 2013 to December 2014.
Methodology: Six of the commonly used antiseptics selected for the study contain phenolics as main active agents while the seventh contains chlorhexidine gluconate and cetrimide. Dilutions of the antiseptics in Mueller Hinton Agar were done with water from the tap and with sterile distilled water inoculated with 10^8cfu/ml multidrug resistant *Escherichia coli* and *Staphylococcus aureus* clinical isolates. The identity, antibiotic resistance characteristics and production of Extended Spectrum β-lactamase (ESβL) by microbes that grew on antiseptics-agar plates at dilutions higher than manufacturers stated in-use concentrations were determined using morphological and

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biochemical characteristics as well as disc-diffusion methods.
Results: The tap water samples were found to contain heterotrophic bacteria, coliforms and staphylococci as contaminants. Four of the antiseptic product samples could not inhibit growth at dilutions higher than the in-use concentration while the effects of dilutions on the remaining were inconclusive since the concentrations after dilution could not be determined in all cases from the instruction given by the manufacturer. Nineteen (70.4%) of the 27 surviving organisms on the diluted antiseptics were *Klebsiella pneumonia* strains. Other isolates were 3 *Streptococcus* spp; 2 *Pseudomonas aeruginosa*; 2 *S. aureus* and 1 *S. epidermidis* strain. All the isolates were multidrug resistant and four of the *K. pneumonia* isolates produced ESβL. There was no growth in the antiseptics diluted with sterile distilled water.
Conclusion: It is concluded that instructions for dilutions of antiseptics should consider the effects of dilutions on the antimicrobial activities of the antiseptics in order to prevent failure of antisepsis which might have been happening all along with these commonly used antiseptics.

Keywords: Antiseptics; extended specrum β-lactamase; Klebsiella pneumonia; antibiotic resistance; dilutions.

1. INTRODUCTION

Antiseptics are chemical agents of disinfection that are mild enough to be used on human skin or tissues [1]. They are crucial in the prevention of wound infections, colonization of medical devices as well as nosocomial and community transmission of microorganisms [2,3]. Because of these crucial roles, they are expected to be of optimal efficacy an absence of which normally results in substantial infectious morbidity, mortality and increased health care cost [4,5].

For antiseptics to function optimally however, several factors have to be taken into consideration. One major factor is the concentration of the antiseptic. It is known that there is an exponential relationship between potency and concentration of an antimicrobial agent (6,7]. This means that the more concentrated an agent, the greater its efficacy, and the shorter the time necessary to destroy the microorganisms. Because of the possibility of toxicity, however, the concentration of antiseptics must be strictly controlled [4]. Concentration exponent is the numerical value that relates concentration to the antimicrobial effectiveness of an antimicrobial agent [6].

There are various classes of antiseptics and agents which constitute members of these classes have similar concentration exponents. Thus, peroxides have values of 0.5 to 1.0, aldehydes, 1.0, quaternary ammonium compounds, 0.8 to 2.5, phenolic compounds, 4 to 10.0 and aliphatic alcohols, 6.0 to 12.7 [6,7]. Other classes include: acids and their esters, alcohols, biguanides, halogens, heavy metals, surface active agents, quinoline and isoquinoline derivatives and dyes [6,7]. Antiseptics that are examples of each of these classes normally constitute the active ingredients, either singly or in multiple, in the antiseptics available under various trade names all over the world [8].

The antimicrobial activity of agents with a high concentration exponent is easily extinguished on simple dilution. For example, mercuric chloride with a concentration exponent of 1 will be reduced by the power of 1 on dilution, and a threefold dilution means the antimicrobial activity will be reduced by the value 3^1, or to a third of its original activity. Phenol, on the other hand, has a concentration exponent of 6, so a threefold dilution in this case will mean a decrease in activity of 3^6 or 729 times less active than the original. With this in-view, the effects of dilutions as seen in the manufacturers' instructions in the product package of many antiseptics need to be investigated.

This study aims to investigate the effects of dilution on commonly used antiseptics following the instruction given by the manufacturers. This is done by determining the antimicrobial effectiveness of diluted antiseptics exposed to clinically significant microbes. The identity, antibiotic resistance characteristics as well as production of extended spectrum β-lactamase (ESβL) by the surviving microbes, where they exist, were also determined in order to be able to provide useful information on the public health importance of such.

2. MATERIALS AND METHODS

2.1 Collection of Antiseptic Products

Antiseptics commercially available in the Nigerian markets were surveyed and seven of

the most commonly used ones, as discovered in an earlier pilot study, were selected for this study. Two samples of each antiseptic were screened. The main basis for selection was the inclusion of instruction for dilution, for various uses of the antiseptic by the manufacturers, as part of the packaging. The antiseptics employed in this study were Savlon®, Izal®, Robert antiseptic®, ncp®, Dettol®, Septol® and Z germicide®. The properties of these antiseptics are shown in Tables 1 and 2. All the antiseptics used were certified by the Nigerian National Agency for Food Drug Administration and Control (NAFDAC) and are listed in EMDEX [8].

2.2 Sources and Preparation of Water Samples Used for Dilutions

Water samples used for the dilution were from domestic and hospital sources. Domestic water samples were obtained from the tap in two different residential areas in Ile-Ife and the hospital water samples were also obtained from the tap in two health care facilities in the town. All water samples were collected following standard protocols [7].

2.3 Bacteriological Analysis of the Water Samples: Total Heterotrophic Bacteria Plate Counts, Faecal Coliform Bacteria Counts, and Faecal Staphylococci Counts

Total heterotrophic bacteria, faecal coliform bacteria and faecal staphylococci were isolated and quantified from all water samples by means of membrane filteration techniques [9]. One hundred ml water samples were poured through individual sterile 0.45 µm pore size cellulose acetate membrane filters (Corning, England). The membrane filters were then placed aseptically, with the grid side up, on Petri dishes of Nutrient Agar (NA), MacConckey Agar (MAC), Mannitol salt Agar (MSA) and Eosin Methylene Blue agar (EMB) (Fluka, USA). The NA plates were incubated at 37ºC for 24 h, MSA at 37ºC for 48 hr, while MAC and EMB were each incubated at 37ºC and 44ºC for 24 h.

Purple or violet colonies on MAC at 37ºC were counted as presumptive faecal coliform bacteria; colonies with similar properties on MAC at 44ºC were counted as *Escherichia coli* bacteria. Colonies with green metallic sheen on EMB at 44ºC were also regarded as *E. coli* bacteria. Also, yellow colonies surrounded by bright yellow zones on MSA were counted as presumptive faecal pathogenic staphylococci, while colonies grown on NA were counted as total heterotrophic bacteria.

2.4 Dilution of the Antiseptics and Inoculation into Agar

The raw water samples found to contain microbes were used for the dilutions of the antiseptics. Ten ml of dilutions of the agents were added to ten ml melted Double strength Mueller Hinton Agar (already cooled to about 45°C) to get a final concentration of 10.0%, 5.0%, 1.0%, 0.1% and 0.05%v/v of the antiseptics in the agar plate. These dilutions were used in view of the calculated dilutions as stated by the manufacturers of the antiseptics (Table 2). Sterile distilled water was also innoculated with clinical isolates of *S. aureus* and *E. coli* to an innoculum size of 10^8cfu/ml and the resulting bacterial suspension was then used to prepare Muellller Hinton Agar plates with final concentrations of each of the antiseptics as above. Finally, sterile distilled water was used to prepare similar concentrations. The plates were then incubated at 37°C for 7 days and observed daily for growth of bacterial colonies.

2.5 Identification of Organism that Grew in the Antiseptic Dilutions

Morphological characteristics on media such as Nutrient agar, Mannitol Salt agar, MacConkey agar and Eosine Methylene Blue agar was determined for bacteria colonies that grew on the antiseptics plates at concentrations above and around the manufacturers' stated in-use dilutions. Biochemical characterizations included gram staining, catalase test, triple sugar iron test, oxidase test, fermentation of sucrose, mannitol, glucose, lactose, arabinose and starch, citrate utilization test, indole productions as well as methyl red and Voges Proskauer tests.

2.6 Antimicrobial Susceptibility Test

The agar disc diffusion method [10] was employed for the antibiotic susceptibility test following an earlier protocol [11]. The antibiotic discs employed were: Amoxicllin (25 µg), Nalidixic acid (30 µ), Nitrofurantoin (200 µg), Augmentin (30 µg), Ofloxacin (5 µg), Tetracycline (25 µg), Cotrimoxazole (25 µg) Tobramycin (30 µg), Trimethoprim (5 µg) Gentamicin (10 µg) and Penicillin V (10 µg).

Table 1. Properties of antiseptic products used in this study

Serial no	Antiseptics	Ingredient(s) [as indicated on product package]	Class of main active ingredients of product	Places of manufacture	Volume
1.	Dettol®	Chloroxylenol BPC 4.8%w/v; oleum pini aromaticum, 8.38%w/w, Isopropyl alcohol 9.43%w/w, Sapo vegetable oil 5.60%w/w, saccharum ustum qs, aqual ad 100 ml	Phenolics	Lagos, Nigeria	50ml
2.	Izal®	Saponated cresol	Phenolics	Bangalore-560 033 India, London, England	150ml
3.	ncp®	Each ml contains: Phenol (1.75 mg), Halogenated phenol (6.8 mg), sodium salicylate (0.5 mg)	Phenolics	Lagos, Nigeria.	150ml
4.	Roberts antiseptic disinfectant®	Dichloroxylenol (2%w/v); methylated spirit, terpineol, d-chloro-m-xylenol, pine oil, castor oil, sodium hydroxide, caramel.	Phenolics	Lagos, Nigeria, Tema, Ghana, Nairobi, Kenya	150ml
5.	Savlon®	Chlorhexidine gluconate (0.3 g) Cetrimide (3.0 g), n-propyl alcohol as preservative (2.84% m/v)	Biguanide plus quarternary ammonium compound	East London, England, South Africa	125ml
6.	Septol®	2.3% pine oils and 1.1% 5-chloro-2-hdroxy diphenyl methane	Phenolics	Kano, Nigeria.	500ml
7.	Z germicide®	7% Tar acid phenol; 2% cresylic creseote	Phenolics	Kano, Nigeria	150ml

Table 2. Manufacturers' instructions on the antiseptic products as stated on product container

Antiseptics	Instructions for use [Calculated resultant %v/v dilution]
Dettol®	First Aid; Use 13.5 ml to 250ml of water [5.4%]. Bathing; Use 27 ml added to bath water [Not specific]. Domestic cleansing; Use 27ml to 1 liter of water [2.7%]. Laundry; Napkins, Undergarments e.t.c. Use 27ml to 1 liter of water [2.7%].
Izal®	How to make an izal solution For toilet purposes-: add 3 or 4 drops of izal to a large cup of water [Not specific]. For general disinfection, when a larger quantity is required: Add 1 table spoonful of izal to half a bucket of water [Not specific].

Antiseptics	Instructions for use [Calculated resultant %v/v dilution]
	Directions for use for cuts, sores and insect bites: 10 drops in a large cup full of water [Not specific].
	GENERAL PURPOSE: Sink, drains and lavatories. Wash or scrub down with hot 1;200 solution [0.5%].
	INFECTED LINEN: Soak in 1 to 200 solution for 1hour, or in 1 to 600 solution for 12hrs [0.5% or 0.17%]. Add Izal® to the water and mix well before immersing linen.
	FOR PERSONAL PROTECTION: Add little Izal® to your wash bowl. This is a good protection against infection after contact with crowds [Not specific].
ncp®	Directions
	Colds, sore throats: At first sign, gargle with the liquid diluted with 5parts water (20%) or spray the throat with slightly diluted ncp® using an all glass throat spray [Not specific].
	Mouthwash: Use daily, diluted with 5 parts water after meals [20%]. Additionally, for gum troubles and mouth ulcers, apply undiluted three times daily and consult your dentist.
	Bruises, sprains: Apply lint dressing soaked with ncp® liquid antiseptic diluted with 3 parts water. Dab again at intervals. For severe sprains, also see your doctor. [33.3%].
Roberts	Recommended Dosage (1 cap=15ml)
Antiseptic	Washing and bathing: add 2-3 capfuls to bath water [Not specific].
Disinfectant®	Cuts, stings, bites and wounds: 1 capful to 300ml of water [5.33%], wash unaffected area and cover with a clean bandage
	Laundry: for washing napkins, undergarments e.t.c add 2 capfuls per 1litre of water [3.2%].
	Floors/Surfaces: 2 capfuls to 4.5L of water [0.7%].
	Toilet and drains: apply directly, do not dilute [100%].
Savlon®	Dilute before use:
	First aid: Dilute 5ml in 100ml water [5.0%].
	General antiseptic cleansing; 60-90 ml in 1-1,5 litres of water [4.0%].
Septol®	Dilute Septol with water as follows:
	For dressing wounds, abrasions, skin infections and midwifery- Use 1capful in 300ml water [5.0%].
	For bathing- Use 1-2 capfuls in bath water. This kills germs in the water, on the skin and soothes cuts and insect bites. [Not specific].
	For laundry and baby nappies- use 2 caps- full for every 2 liters of soapy water [1.5%].
	For washing walls and floors – use 1 cap- full for every 2 liters of soapy water [0.75%].
	For drains, sinks and lavatories –Use 1 cap-full in 500ml water [3.0%].
	{NB: Capacity of cap: 1 cap=15 ml}
Z Germicide®	Shake well before use, make a Z solution by adding 4 drops of Z to 1litre of warm water [Not specific].
	For personal hygiene: add 1 drop of Z to a large cup of warm water [Not specific].

The diameters of inhibition zones were measured with a transparent ruler after the incubation period, and interpretation was in accordance with manufacturer instructions, AB-Biodiscs laboratory Manual.

2.7 Detection of ESβL in *K. pneumonia* Isolates

ESβL production was detected using the disc diffusion technique as earlier described [12]. The test plates were inoculated as for the standard disc diffusion test. Discs containing extended spectrum cephalosporins (ceftazidime-30µg and cefpodoxime-10 µg) [Mast group, Merseyside, UK] and their respective combinations with clavulanic acid (5 µg) were applied 30 mm apart (centre to centre). After an overnight incubation, ESβL positive strains were identified by an initial resistance pattern (≤22 mm for ceftazidime and ≤17 mm for cefpodoxime) which is offset by the presence of clavulanic acid in the combination discs by a standard of ≥5mm difference in the inhibition zones.

3. RESULTS

3.1 Microbiological Analysis of Water Samples

All the water samples were found to be free of *E. coli* while they contain different ranges of coliforms (315-366.67 cfu/100 ml), staphylococci (122.0-316.67 cfu/100 ml) and total heterotrophic bacteria [THB] (3533.33-12500 cfu/100 ml) (Table 3).

3.2 Identification of Organisms that Grew in the Antiseptics Dilutions

On incubation for seven days some organisms were observed to grow on some of these dilutions as shown in Table 4. Nineteen (70.4%) of the 27 organisms that grew on antiseptic-water-Mueller Hinton Agar plates were multidrug resistant *K. pneumonia* strains. Other isolates were 3 *Streptococcus* spp, 2 *Pseudomonas aeruginosa,* 2 *S. aureus* and 1 *S. epidermidis* (Table 5). There was no growth in the antiseptics diluted with sterile distilled water.

3.3 Antimicrobial Susceptibility Properties of the Isolates

The organisms showed varying resistance characteristics but most were observed to be multidrug resistant with resistant ranging from 5 to 11 of the 11 antimicrobial agents screened (Table 5). The *S. aureus* and *E. coli* strains used as inoculums were also multidrug resistant (Table 6). The *K. pneumonia* strains expressed up to 8 different resistance phenotypes while the others express one phenotype each (Table 6). More than 80% isolates were resistant to amoxicillin, augmentin, cotrimoxazole, nitrofurantoin, penicillin and trimetoprim. Gentamicin and tobramycin had the least resistance (21.4%) rate of the isolates (Table 7).

3.4 ESβL Production by the *K. pneumonia* Isolates

Four out of the nineteen isolates studied were found to be ESβL producers (Table 8).

4. DISCUSSION

It was observed that some multidrug resistant vegetative bacterial pathogens were not inhibited or killed by concentrations higher than the manufacturers stated in-use concentrations of the antiseptics. Though the antiseptics were bought in Ile-Ife, Nigeria, the same brands could be found available throughout the country and could be among the ones being used in hospitals and sold in community pharmacies and other sale outlets in other countries beyond Nigeria because package labels indicated that they were manufactured and distributed by local and foreign companies. One basic assumption in this study is that the antiseptics used are of appropriate pharmacopoeia quality.

It is noted that the active ingredients in these antiseptics are known antimicrobial agents. These agents include: chlorhexidine gluconate and cetrimide which are biguanides and quartenary ammonium compounds respectively. Others are tar acid phenol**,** dichloroxylenol, saponated cresol, phenol, halogenated phenol, pine oil and chloroxylenol which are all phenolics. Since phenolics, known to have high concentration exponents of 5.5 to 10.0, are the active antimicrobial agents in most of these antiseptics, there is the need to pay special attention to the means and degree of dilutions of these agents as far as their use as antiseptics is concerned [7,13].

Table 3. Range and mean values of bacterial counts from water samples

Microbiological parameters	Range values (mean) of three counts				Standards
	Hospital 1 tap	Domestic 1 tap	Hospital 2 tap	Domestic 2 tap	British*
Total heterotrophic bacteria (cfu/100 ml)	3500-3600 (3533.33)	4300-6400 (5233.33)	12000-13000 (12500)	5700-10000 (7800)	<1000
Faecal coliform (cfu/100 ml)	250 -500 (366.67)	210-450 (330.0)	200-430 (315.0)	250-460 (355.0)	≤10
E. coli (MPN/100 ml)	0	0	0	0	Not detectable
Faecal *Staphylococci* (cfu/100 ml)	146-300 (206.33)	55-300 (175.67)	300-350 (316.67)	82-172 (122.0)	-

** British standards PAS39:2003 (Hlavsa et al., 2014)* [38]

Table 4. The minimum inhibitory concentration of the antiseptic products against water bacteria as well as against *S. aureus* and *E. coli* innoculum

Product code	Resultant concentrations after dilutions as instructed by the manufacturer	The maximum concentrations of disinfectants in MHA plates that support bacteria growth after dilution with the water sample from sources indicated*						Remark
		Hospital water 1	Hospital water 2	Domestic water 1	Domestic water 2	*S. aureus* suspension (10^8cfu/ml)	*E. coli* suspension (10^8cfu/ml)	
Dettol®	2.7% and not specific	1.0%	1.0%	2.5%	2.5%	-	-	Inconclusive
Izal®	0.17%, 0.5% and not specific	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	Fail
ncp®	20%, 33.3% and not specific	10.0%	10.0%	10.0%	10.0%	>10.0%	5.0%	Inconclusive
Roberts Antiseptic Disinfectant®	0.7%, 3.2% and 5.33%	10.0%	5.0%	5.0%	5.0%	2.5%	10.0%	Fail
Savlon®	4.0% and 5.0%	5.0%	1.0%	5.0%	5.0%	1.0%	10.0%	Fail
Septol®	0.75%, 1.5%, 3.0%, 5.0% and Not specific	2.5%	2.5%	10.0%	10.0%	2.5%	2.5%	Fail
Z germicide®	Not specific	1.0%	2.5%	2.5%	2.5%	>10.0%	2.5%	Inconclusive

**These were the results after 7 days of incubation at 37°C*

Table 5. Identity of isolates that grew on specific dilutions of the antiseptic products and the antibiotic resistance phenotypes of the isolates

Serial no	Mueller Hinton agar plate	Organism code	Identity of the isolate	Resistance phenotype of the isolate	No of resistance
1	2.5% Dettol prepared with domestic water 1	D2.5FAJ	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nal,Nit,Ofl,Tet,Tr,Tm	11
2	2.5% Dettol prepared with domestic water 2	D2.5SPT	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Ofl,Tet,Tr	9
3	1.0% Izal prepared with hospital water 1	I1.0SDA	*Staphylococcus aureus*	Pv,Amo,Aug,Cot,Nal,Nit,Tet,Tr	8
4	1.0% Izal prepared with hospital water 2	I1.0OMP	*K. pneumonia*	Pv,Amo,Aug,Nit,Tr	5
5	1.0% Izal prepared with domestic water 1	I1.0FAJ	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nal,Nit,Ofl,Tet,Tr,Tm	11
6	1.0% Izal prepared with domestic water 2	I1.0SPT	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Ofl,Tet,Tr	9
7	10.0% ncp prepared with hospital water 1	N10.0SDA	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nit,Tr	7
8.	10.0% ncp prepared with hospital water 2	N10OMP1	*S. epidermidis*	Pv,Amo,Aug,Cot,Nit,Ofl,Tet,Tr,Tm	8
9.	10.0% ncp prepared with hospital water 2	N10OMP2	*K. pneumonia*	Pv,Amo,Aug,Nit,Tr	5
10.	10.0% ncp prepared with domestic water 1	N10FAJ1	*K. pneumonia*	Amo,Cot,Nit,Ofl,Tet	5
11.	10.0% ncp prepared with domestic water 2	N10SPT	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Tr	7
12.	10.0% Robert prepared with hospital water 1	R10SDA	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nit,Tr	7
13.	5.0% Robert prepared with hospital water 2	R5OMP	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Tet,Tr	8
14.	5.0% Robert prepared with domestic water 1	R5FAJ	*K. pneumonia*	Pv,Amo,Aug,Cot,Nit,Tr	6
15.	5.0% Roberts prepared with domestic water2	R5SPT	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Tr	7
16.	5.0% Savlon prepared with hospital water 1	S5SDA	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nit,Tr	7
17.	1.0% Savlon prepared with hospital water 2	S1OMP	*Streptococci*	Pv,Amo,Aug,Cot,Nit,Ofl,Tet,Tr,Tm	9
18.	5.0% Savlon prepared with domestic water 1	S5FAJ	*K. pneumonia*	Amo,Cot,Nit,Ofl,Tet	5
19.	5.0% Savlon prepared with domestic water 2	S5SPT	*P. aeruginosa*	Pv,Amo,Cot,Nal,Nit,Ofl	6
20.	2.5% Septol prepared with hospital water 1	Se2.5SDA	*K. pneumonia*	Pv,Amo,Aug,Cot,Gen,Nit,Tr	7
21.	2.5% Septol prepared with hospital water 2	Se2.5OMP	*Streptococci*	Pv, Amo,Aug,Cot,Nit,Ofl,Tet,Tr,Tm	9
22.	10.0% Septol prepared with domestic water 1	Se10FAJ	*K. pneumonia*	Pv,Amo,Aug,Cot,Nit,Tr	6
23.	10.0% Septol prepared with domestic water 2	Se10SPT	*P. aeruginosa*	Pv,Amo,Cot,Nal,Nit,Ofl	6
24.	1.0% Z germicide prepared with hospital water 1	Z1SDA	*S. aureus*	Pv,Amo,Aug,Cot,Nal,Nit,Tet,Tr	8
25.	1.0% Z germicide prepared with hospital water 2	Z1OMP	*Streptococci*	Pv,Amo,Aug,Cot,Nit,Ofl,Tet,Tr,Tm	9
26.	2.5% Z germicide prepared with domestic water 1	Z2.5FAJ	*K. pneumonia*	Pv,Amo,Aug,Cot,Nit,Tr	6
27.	2.5% Z germicide prepared with domestic water 2	Z2.5SPT	*K. pneumonia*	Pv,Amo,Aug,Cot,Nal,Nit,Tr	7

Key: Amo: Amoxillin; Aug: Augmentin; Cot: Cotrimoxazole; Gen: Gentamicin; Nal: Nalidixic Acid; Nit: Nitrofurantoin; Ofl: Ofloxacin; Tet: Tetracycline; Tm: Tobramycin; Tr: Trimetoprim

Phenolics are known to have strong antiseptics properties possessing both microbicidal or microbistatic activities depending on the concentrations used. Phenolics have demonstrated activities against most vegetative bacteria, fungi and viruses with the exception of bacteria spore and prions [6,7]. They act by combining with and denaturing proteins, as well as disrupting cell membranes [6,7]. Halogenated phenols have been reported to have wider spectrums of activity and improved water solubility than ordinary phenols [6,7]. For these reasons they are widely found as main active ingredients in many antiseptics, as is the case in the antiseptics used for this study. The observed complete inhibition of growth at higher concentrations of these antiseptics corroborates the wide spectrum of activities of these phenolics. It also indicates that the growth of bacteria observed at lower concentrations, which were nevertheless higher than those prescribed by the manufacturers for specific antiseptic purposes, was due to lack of effectiveness of those dilutions used rather than the ineffectiveness of the antiseptics. This observation is noted in difference to the fact that development of resistance to the phenolic class has been reported earlier, because some microorganisms, for example *Pseudomonas aeruginosa*, were reported to utilize some phenolic compounds as their carbon source [14].

Table 6. Resistance phenotypes of the clinical strains used in preparing the innoculum used in diluting the antiseptics

Serial no	Organism	Resistance phenotype
1.	*S. aureus*	Pv,Amo,Aug,Cot,Nit,Tet ,Tr
2.	*E. coli*	Pv,Amo,Aug,Cot,Nal,Nit ,Ofl,Tet,Tr

The microbiologic quality of the water samples used in this study may indicate the poor quality of the water used in the hospital and domestic settings in the study area [15]. Tap water, in addition to being a possible source of microbial contamination, may include substances that may interfere with the microbicidal activities of antiseptics and disinfectants [14]. It has been observed that it is important to maintain a disinfectant residual in treated water while it is in transit so as to be able to limit the growth of microorganisms in the distribution system and to inactivate any pathogens that may enter the distribution system through cross-connections, leakage, seepage or backflow [16]. However, it has also been shown that conventional levels of disinfectant residuals may be ineffective against massive contamination influx [16].

Table 7. Antibiotic resistance rates of the isolates from antiseptic-water combinations

Serial number	Antibiotics	Number resistant (%)
1	Amoxicillin	28 (100.0)
2	Augmentin®	24 (85.7)
3	Cotrimoxazole®	26 (92.9)
4	Gentamicin	6 (21.4)
5	Nalidixic acid	12 (42.9)
6	Nitrofurantoin	28 (100.0)
7	Ofloxacin	12 (42.9)
8	Penicillin	26 (92.9)
9	Tetracycline	13 (46.4)
10	Tobramycin	6 (21.4)
11	Trimetoprim	24 (85.7)

Although all the water samples passed the test for *E. coli* they were found to contain a high content of coliforms, staphylococci and total heterotrophic bacteria. The presence of heterotrophic bacteria in drinking water is not typically considered a human health concern [17,18] but the potential for such heterotrophs harboring resistance genes to spread via tap water has been well documented [19,20]. It is to be noted that, if these antibiotic resistance genes are carried on mobile genetic elements, they may be transferable to bacteria of the same or different species [19,21,22]. Some strains of the organisms present in these water samples were those found to survive in diluted antiseptics which the waters were used to prepare. Several workers had reported the isolation of multiply antibiotic resistant heterotrophic bacteria from rural water supplies in some areas in Nigeria [23,24]. Furthermore, the microbial contamination of antiseptics by water used for dilution has been widely reported in literature in order parts of the world [25]. Further researches are critically needed to investigate the potential roles of the surviving pathogens in community-acquired infections.

In view of the results obtained in this study, it is recommended that all these waters may need to be treated either by boiling, distilling, filtering, disinfection etc. before they are used for the dilution of the antiseptic products [18]. However, the implications of these results go beyond the quality of the water used for the dilution. Further implication is that the growth of these pathogens

in the presence of the various dilutions of the antiseptics can be inferred to mean that these dilutions would not be able to destroy these organisms wherever they are found, either on inanimate surfaces or even on living tissues.

As noted above, results show that the minimum concentrations of use as indicated by the manufacturers of some of these antiseptics could not inhibit some commonly encountered bacterial pathogens. For example, in the case of Izal®, it should inhibit the growth of pathogens when used at a dilution as low as 0.17%v/v which was the concentration calculated for general purpose use of the antiseptics. The results however indicate that even at a concentration of 1.0%v/v the antiseptics still supported the growth of these multidrug resistant bacteria pathogens. In view of the fact that the chemical disinfectant in this antiseptic is a phenolic, the loss in activity due to this concentration difference is expected to be very great (as much as 6^{10}). The same can be said of Robert Antiseptics® with a minimum manufacturers stated in-use concentration of 0.7%v/v but which at same time was able to support growth of the multidrug resistant *E. coli* prepared in sterile water suspension at a concentration greater than 10%v/v.

It is observed that while the resultant concentration of the antiseptics after dilution can be determined for some antiseptics the same cannot be said of some others where some of the dilutions appear to be *ad-infinitum*. These cases of *ad-infinitum* dilutions were observed in five out of the seven antiseptics studied viz; Dettol®, Izal®, ncp®, Septol^R and Z germicide^R. These give an idea of the prevalence of these cases in antisepsis. These cases of *ad-infinitum* dilutions for antiseptics with high concentration exponents is an indication of lack of appreciation of disinfection kinetics and could account for the failure in antisepsis reported in some studies [14]. This is an area to which the regulatory agencies need to pay close attention as it appears little investigated.

The identity and characteristics of the surviving organisms are a cause for concern. It was observed that most of these organisms (70.4%) were multidrug resistant *K. pneumonia* strains with varied resistance phenotypes. Others were multidrug resistant *P. aeruginosa, Streptococcus* spp*, S. epidermidis* and *S. aureus* strains. It is important to note that even though *S. aureus*, and especially the methicillin resistant *Staphylococcus aureus* (MRSA) strains, has attracted the most attention the world over, recent reports appear to be indicating that multi drug resistant gram negative rods are more menacing [26]. Results of this study readily corroborate these reports.

All the isolates were found to be resistant to more than four of the eleven antibiotics screened. The high resistant rate obtained which was as high as 100% to amoxicillin and nitrofurantoin, 96.9% to cotrimoxazole and penicilin V as well as 85.4% to augmentin and trimethoprim underline the public health importance of these strains of the organisms which probably were already circulating in the hospital and the community. Results indicate, however, that gentamicin and tobramycin had lower resistance rate of 21.4%, making these two agents of potential in treating possible infections caused by these pathogens.

The isolation of multiple drug resistant *K. pneumonia* strains as the most predominant surviving bacteria pathogens on these antiseptics corroborates recent observations by various workers. *K. pneumoniae* is a member of the family *Enterobacteriacea* and is one of the most common pathogens causing pneumonia, abscess, bacteremia, and urinary tract infections [27,28]. Hence its infections often result in significant morbidity, mortality and socio-economic impact. The infections are also often difficult to treat due to the innate and acquired resistance mediated by the organisms' genome and other transferable genetic elements [27,28].

Results indicate that four of the *K. pneumonia* isolates studied were found to be ESβL producers. Preferential cephalosporin hydrolysis was observed in three of the isolates: D2.5SPT, N10FAJ1 and R5OMP whereas both cephalosporins were hydrolysed in R5FAJ. This is an observation that underscores the principle of ESβLs conferring resistance to one or more cephalosporins, sensitivity varying from substrate to substrate [29]. The zone diameters obtained for isolates N10FAJ1, N10SPT and S5FAJ suggest that the resistance to cephalosporin observed may be due to the presence of either an inhibitor resistant β-lactamase as a result of mutation or another class of β-lactamase that is unresponsive to the effects of clavulanic acid such as the AmpC category of β-lactamases [30,31].

Table 8. ESβL production by the *K. pneumonia* isolates

Serial number	Organism code	Zone of inhibition (mm)				Remark
		Ceftazidime	Ceftazidime plus clavulanic acid	Cefpodoxime	Cefpodoxime plus clavulanic acid	
1	D2.5FAJ	--	--	--	--	-
2	D2.5SPT	--	10	--	--	+
3	I1.0OMP	--	--	--	--	-
4	I1.0FAJ	--	--	--	--	-
5	I1.0SPT	22	25	30	30	-
6	N10.0SDA	--	--	--	--	-
7	N10OMP2	--	--	--	--	-
8	N10FAJ1	18	20	11	16	+
9	N10SPT	18	20	20	21	-
10	R10SDA	22	25	30	30	-
11	R5OMP	--	12	--	13	+
12	R5FAJ	--	--	--	11	+
13	R5SPT	30	30	30	27	-
14	S5SDA	--	--	--	--	-
15	S5FAJ	20	20	11	11	-
16	Se2.5SDA	--	--	--	--	-
17	Se10FAJ	--	--	--	--	-
18	Z2.5FAJ	27	28	30	35	-
19	Z2.5SPT	26	26	20	20	-

-- = No inhibition; + = ESβL production; - = No ESβL production

An earlier study had reported that strains of *Klebsiella* has taken lead among uropathogens in the University of Benin Teaching Hospital, Benin City, a tertiary hospital in the South Eastern part of Nigeria [32]. Other studies had reported multiple indices of antibiotic resistance in *Klebsiella* spp and *Pseudomonas* spp in other tertiary institutions in South Western Nigeria [5,33]. Other workers reported the increasing prevalence and global spread of carbapenem-resistant *K. pneumoniae* infections as well as the growing resistance of *K. pneumonia* to many antibiotics in hospitals [34]. Another group of researchers actually reported a hospital outbreak involving a *K. pneumonia* strain which survived on a ventilator that had been cleaned three times with two different disinfectants [35]. In view of these reports, the need to have a comprehensive review of the treatments strategy of *K. pneumonia* infections, as well as other gram negative opportunistic pathogens such as *P. aeruginosa*, as earlier proposed by some workers, is worth giving full support [36,37].

5. CONCLUSION

In conclusion, it has been shown that some multidrug resistant pathogenic microbes such as *K. pneumonia*, *P. aeruginosa, Streptococci* spp, *S. aureus* and *S. epidermidis*, including ESβL producing strains, circulating in the community can survive at manufacturers' stated in–use dilutions of some commonly used antiseptics. One of the implications of this is that the pathogens can be selected and gradually displace sensitive strains. Instructions for dilutions of antiseptics should therefore consider the effects of dilutions on the antimicrobial activities of the antiseptics in order to prevent failure of antisepsis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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