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EFFICACY OF THREE DISINFECTANT FORMULATIONS AGAINST MULTIDRUG RESISTANT NOSOCOMIAL AGENTS

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RUNNING TITLE: DISINFECTANT FORMULATIONS AGAINST MULTIDRUG RESISTANT NOSOCOMIAL AGENTS

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ABSTRACT

The current increase in the prevalence of nosocomial infections within the hospital environment despite adequate cleaning and disinfection can be said to be due to the following: (i) ineffectiveness of the various disinfectant formulations used in infection control on the various hospital equipments and wards; (ii) development of resistance to the various chemical disinfectant formulations been used in the hospitals by the various microorganisms.

Ten bacteria isolates from different clinical specimens of hospitalized patients identified using standard bacteriological methods and found after screening to be resistant to two or more classes of the antibiotics: cephalosporins, quinolones, Betalactams, nitrofuran, macrolide and an aminoglycoside using the Kirby-Bauer method of disc diffusion test were used in this study. These were subjected to susceptibility testing against three selected disinfectant formulations (Izal, Dettol and Jik) at the manufacturer's dilutions and half the dilutions prescribed on their labels by using agar diffusion method. Out of these clinical isolates used in this study, 5 (50%) and 2 (20%) were resistant to manufacturer's dilution and half the dilution of Dettol respectively, 9 (90%) and 6 (60%) to manufacturer's dilution and half the dilution of Izal respectively while 1 (10%) and 0 (0%) were resistant to manufacturer's dilution and half the dilution of Jik respectively.

The resistance demonstrated by some of the nosocomial agents in this study against the selected disinfectant formulations at their manufacturer's dilution and half the prescribed dilutions showed the probability of the nosocomial agents developing some mechanisms of resistance against the various disinfectant formulations rather than ineffectiveness of the disinfectant formulations. However, the effectiveness of Jik formulation at half the manufacturer's prescribed dilution shows that Jik is still an important disinfectant formulation in the control of nosocomial agent most especially the resistant strains.

INTRODUCTION

The term disinfectant is generally used for chemical agents employed to disinfect inanimate objects. They are use to reduce or eliminate pathogenic microbes in or on materials so that they are no longer a hazard. The process of using chemical disinfectants in inhibiting or killing pathogenic microorganisms is known as disinfection. Disinfection is however not an absolute term, this implies that some living microbes may persist but the possibility of sterilization resulting from disinfection cannot be ruled out (1, 22). Disinfectants used to treat skin and other external body membranes and cavities are termed Antiseptics (1, 22).

There are various classes of chemical disinfectants, these are; Acids and their esters, Alcohols, Aldehydes, Biguanides, Halogens, Heavy metals, Oxidizing compounds, Phenols and Phenolic compounds, Surface active agents, Quinoline and Isoquinoline derivatives and Dyes (1, 22). The choice of the most appropriate disinfectant formulation for a particular purpose depends on some factors like (1):

- Properties of the chemical agent in terms of activity and solubility
- Microbiological challenge in terms of types and level of microbial contamination
- Intended application either for antiseptic or disinfection
- Environmental factors such as presence of organic matter or specificity of microorganism to a particular environment
- Toxicity of the agent i.e disinfectant toxicity effect.

In the hospitals, disinfectants have been found to play an important role in the prevention and control of hospital acquired infections (Nosocomial infections). Nosocomial infections are those infections acquired in hospital or healthcare service unit that first appear 48 hours or more

after hospital admission or within 30 days after discharge following in-patient care (2).
They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission (2, 3). Nosocomial agents could be bacteria, fungi, viruses and protozoan in nature (4). There are several reasons why nosocomial infections are more alarming than the community acquired infections; firstly, many medical procedures that bypass the body's natural protective barriers could result into infecting the patients. Secondly, medical staffs move from patient to patient thus providing a way for pathogens to spread. Thirdly, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing with disinfectant and other preventive measures that may either be unheeded by hospital personnel or too late to sufficiently isolate patients from infectious agents and lastly the routine use of antimicrobial agents especially the broad-spectrum antibiotics in hospitals creates selection pressure for the emergence of the resistant strains of microorganisms [(2)].

The significance of nosocomial infection lies not only in its ability to substantially alter morbidity and mortality statistics, but also in its economic implications [(5,6)]. Nosocomial infection prolongs duration of hospitalization, increases the cost of health care, emergence of multiple antibiotic resistance microorganisms and reduces the chances of treatment for others (7, 8, 9).

In various hospitals, one of the ways among others, of controlling spread of infections within the hospital environment is the use of chemical disinfectant formulations in disinfecting the hospital environment and equipment, washing of hands when moving from patient to patient and after and disinfection of patient's skin before injection, catheterization and operation is perform on them (19). However, failures in the antimicrobial activity of some of the disinfectants have been reported (1, 22). The utilization of phenolic constituent of some phenolic disinfectant as carbon source by some bacteria such as *Pseudomonas aeruginosa* and complete resistance of some microorganisms to some of the classes of disinfectants e.g. *Staphylococcus aureus*, are some of these reports (1, 22). The possibility of more microorganisms particularly the nosocomial bacteria further developing resistance to more of these disinfectant formulations, just like they do with antibiotics, underscore the need to constantly evaluate the antimicrobial activities of various chemical disinfectant formulations against the nosocomial agents.

This study therefore, assesses the efficacy of some disinfectant formulations in Nigerian markets at their prescribed dilutions/concentrations of use by their manufacturers against some multidrug resistant bacteria of nosocomial origin.

MATERIALS AND METHODOLOGY

BACTERIOLOGY

The microorganisms used in this study were obtained on slants as pure culture from the microbiology unit of the University College Hospital (UCH) Ibadan, Oyo State from hospitalized patients who develop infection diagnosed to be acquired from the hospital environment. Ten microorganisms mainly bacteria were collected and used in this study. They include: Two strains of *Pseudomonas aeruginosa* (P1 and P2), Two strains of *Staphylococcus aureus* (S1 and S2) Two strains of *Klebsiella* species (K1 and K2), Two strains of *Proteus* species (Pr1 and Pr2) and Two strains of *Escherichia coli* (E1 and E2).

DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PROFILES OF THE TEST CLINICAL ISOLATES USING STANDARD ANTIBIOTIC DISCS

The standard disk diffusion method recommended by the National Committee for clinical laboratory standards (NCCLS, 2003) was used in determining bacterial susceptibility to antimicrobials as described by Qin et al in 2004. The antibiotics used are Gentamicin, Cefixime, Ofloxacin, Ceftazidime, Augmentin, Nitrofurantoin, Ciprofloxacin, Cefuroxime, Erythromycin, Cloxacillin and Ceftriaxone. The standard antibiotic discs were placed at equal distance in a circular pattern on the surface of the Mueller Hinton agar with the aid of a sterile forcep. The antibiotic discs were designated differently for Gram positive and Gram negative organisms. The plates were then incubated at $37^{o}C$ for 24hrs in an upside down position. The zones of growth inhibition were then recorded.

PREPARATION OF THE TEST CONCENTRATIONS OF DISINFECTANT FORMULATIONS

Two test dilutions each; of the three disinfectant formulations were prepared at a dilution a little below the dilutions prescribed for use by the manufacturers (e.i. higher concentrations). They were diluted to the test concentrations with sterile distilled water as stated below.

- DETTOL (3%v/v and 6%v/v)
- IZAL (0.5%v/v and 1%v/v)
- JIK(SODIUM HYPOCHLORITE) (2.5%v/v and 5%v/v)

ANTIMICROBIAL SCREENING OF THE DISINFECTANT FORMULATIONS

Using agar-cup diffusion method, two dilutions each, of the three test disinfectant formulations was used in this screening. Twenty millilitres of melted and cool Mueller Hinton agar was seeded with 0.2ml of 10^{-2} dilution from an overnight broth culture of the multidrug resistant strains of the test clinical isolates, rolled between palms and poured into sterile petri-dishes and allowed to set. The surface was then dried in a sterile drier and with the aid of a sterile 8mm cup borer; five wells were bored into the agar plates. The first three wells were filled with two drops of the manufacturer's dilutions for the three disinfectant formulations (3%v/v Dettol, 0.5%v/v Izal and 2.5%v/v Jik) and 10µg of Gentamicin was introduced into the fourth well as positive control while sterile distilled water was introduced into the fifth well as negative control. This procedure was also carried out for the corresponding half dilutions of the test disinfectant formulations (6%v/v Dettol, 1%v/v Izal and 5%v/v).

This whole process was done in duplicates. The plates after about one hour of pre-diffusion were then incubated at 37^{o}C for 24hrs in an upright position. The averages of the corresponding zones of growth inhibition were then recorded for both dilutions.

RESULTS

The clinical isolates screened with some antibiotics namely Gentamicin, Cefixime, Ofloxacin, Ceftazidime, Augmentin, Nitrofurantoin, Ciprofloxacin, Cefuroxime, Erythromycin, Cloxacillin and Ceftriaxone shows that they were resistant to more than one class of the antibiotics used, making them multidrug resistant clinical strains. *Proteus* specie Pr1 was resistant to all the antibiotics except nitrofurantoin, *Klebsiella* specie K2, *Pseudomonas aeruginosa* P2, *Escherichia coli* E1 and *Staphylococcus aureus* S1 are resistant to all the antibiotics. *Proteus* specie Pr2 was susceptible only to ofloxacin, ciprofloxacin, nitrofurantion and cefixime, *Klebsiella* specie K1 to nitrofurantoin and gentamicin, *Pseudomonas aeruginosa* P1 to ofloxacin and ciprofloxacin while *Streptococcus aureus* S2 was susceptible to ofloxacin, ceftazidime, cefuroxime, gentamicin and ceftriaxone (Table 1).

The susceptibility test for the three disinfectant formulations against the test clinical isolates shows that few of the test clinical isolates are resistant to some of the disinfectant formulations at a dilution below (i.e. higher concentration) the manufacturers prescribed dilutions as used in this study. *Proteus* specie Pr1, *Pseudomonas aeruginosa* P1 and *Streptococcus aureus* S2 were resistant to 3%v/v Dettol, 0.5%v/v and 1%v/v Izal. *Streptococcus aureus* S1 was resistant to 2.5%v/v Jik, 3%v/v Dettol and 0.5%v/v Izal while *Klebsiella* specie K1 was resistant to the two dilutions of Dettol and Izal used in this study (Table 2).

TABLE 1: ANTIBIOTIC SUSCEPTIBILITY PROFILE OF THE TEST CLINICAL ISOLATES

Gram negative organisms								
Clinical isolates	**Antibiotics zones of growth inhibition (mm)**							
	CXM	**OFL**	**AUG**	**NIT**	**CPR**	**CAZ**	**CRX**	**GEN**
Pr1	**R**	**R**	**R**	**15**	**R**	**R**	**R**	**R**
Pr2	**17**	**15**	**R**	**12**	**20**	**R**	**R**	**R**
K1	**R**	**R**	**R**	**23**	**R**	**R**	**R**	**14**
K2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
E1	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
E2	**R**	**R**	**R**	**24**	**R**	**R**	**R**	**15**
P1	**R**	**25**	**R**	**R**	**39**	**R**	**R**	**R**
P2	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Gram positive organisms								
	ERY	**CXC**	**OFL**	**AUG**	**CAZ**	**CRX**	**GEN**	**CTR**
S1	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
S2	**R**	**R**	**30**	**R**	**20**	**15**	**15**	**25**

Key:
CXM- CEFIXIME (5µg); OFL- OFLOXACIN (5µg); AUG- AUGMENTIN (30µg); NIT- NITROFURANTOIN (300µg); CPR- CIPROFLOXACIN (5 µg); CAZ- CEFTAZIDIME (30 µg); CRX- CEFUROXIME (30 µg)-; GEN- GENTAMICIN (10 µg); ERY- ERYTHROMYCIN (5 µg); CXC-CLOXACILLIN (5 µg) ; CTR- CEFTRIAXONE (30 µg), R – Resistant.

TABLE 2: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE TEST DISINFECTANT FORMULATIONS AGAINST THE TEST CLINICAL ISOLATES.

Clinical isolates	Detol 1 (3%V/V)	Detol 2 (6%V/V)	Izal 1 (0.5%V/V)	Izal 2 (1%V/V)	Jik 1(2.5%V/V)	Jik 2 (5%V/V)	Controls	
							+ve	-ve
							G (10µg/ml)	W
Test Disinfectants zones of growth inhibition (mm)								
Pr1	**R**	**13**	**R**	**R**	**20**	**24**	**16**	**-**
Pr2	**18**	**20**	**R**	**R**	**25**	**30**	**16**	**-**
K1	**R**	**R**	**R**	**R**	**15**	**17**	**17**	**-**
K2	**13**	**13**	**R**	**13**	**14**	**17**	**17**	**-**
E1	**19**	**21**	**12**	**15**	**14**	**16**	**15**	**-**
E2	**16**	**16**	**R**	**12**	**14**	**15**	**15**	**-**
P1	**R**	**12**	**R**	**R**	**13**	**15**	**R**	**-**
P2	**14**	**15**	**R**	**R**	**12**	**14**	**16**	**-**
S1	**R**	**15**	**R**	**12**	**R**	**15**	**25**	**-**
S2	**R**	**R**	**R**	**R**	**13**	**14**	**13**	**-**

Keys: E- *E. Coli*; K- *Klebsiella* spp; S- *Staphylococcus aureus*; P- *Pseudomonas aeruginosa*; Pr- *Proteus* spp; G – Genticin; W – Water - No activvity

DISCUSSION

All over the world, nosocomial infection is a recognized public health problem; Surveillance programmes estimate the rate of infection at 5-10% of hospital admissions (10, 11, 12, 13). In Nigeria, nosocomial infection at a rate of 2.7% was reported in Ife (15), while 3.8 % [(14)] was reported in Lagos and 4.2 % in Ilorin [(16)]. These continue to increase yearly. Nosocomial infection rates vary substantially by body site, by type of hospital and by the infection control capabilities of the institution [(17)]. Although viruses, fungi, bacteria and parasites are recognized as sources of nosocomial infections, bacterial agents remain the most commonly recognized cause (18).

The emergent of multidrug resistant hospital acquired bacteria have been reported throughout the world and the mechanisms to which they resist the antimicrobial activity of the various antimicrobial agents particularly antibiotics have also been studied extensively (22). However, such studies have not been adequately done for most of the classes of disinfectants in Nigeria. The development of resistance to the Phenolic class has long been reported (22). Some microorganisms, for example, *Pseudomonas aeruginosa*, have been found to utilize some phenolic compounds as their carbon source (1, 22).

In this study, the presence of multidrug resistance bacteria nosocomial agents was observed as all the clinical isolates used in this study were found to be multidrug resistant (100% prevalence). Resistance was observed with some of the clinical isolates being resistant to the disinfectant formulations at the dilution prescribed by their manufacturers. *Proteus* specie (Pr1), *Klebsiella* specie (K1), *Pseudomonas aeruginosa* (P1), and *Staphylococcus aureus* (S1 and S2) were resistant to 3%v/v Dettol and 0.5%v/v Izal while *Staphylococcus aureus* (S1) was resistant to 2.5%v/v Jik. *Proteus* specie (Pr2), *Klebsiella* specie (K2), *Escherichia coli* (E2) and *Pseudomonas aeruginosa* (P2) were resistant to 0.5%v/v Izal. However, some became susceptible when the disinfectants were diluted at half the prescribed dilution i.e higher

concentration than the prescribed concentrations. *Proteus* specie (Pr1 and Pr2), *Klebsiella* specie (K1), *Pseudomonas aeruginosa* (P1 and P2) and *Staphylococcus aureus* (S2) remained resistant to the Izal formulation when the dilution was lowered to half the prescribed dilution (1%v/v) while *Klebsiella* specie (K1) and *Staphylococcus aureus* (S2) remained resistant to 6%v/v of Dettol formulation.

All the clinical isolates were susceptible to Jik formulation when the dilution was lowered to half that of the prescribed dilution i.e higher concentration of 5%v/v. This shows that Jik disinfectant formulation stand as an effective disinfectant formulation against nosocomial agent particularly the multidrug resistant strains of bacteria when used at a much lower dilution (i.e. higher concentration) to that prescribed by the manufacturer.

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