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Comparative Study of Disinfectants and Conventional Antibiotics Efficacy on Selected ESKAPE Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Disinfectants and conventional antibiotics are used daily in Nigeria's households and hospitals, in various approaches and at exceptional concentrations with inside to combat infectious diseases. The prevalence of antimicrobial resistance globally has made most chemotherapeutic agents less efficient to target pathogens.
Aim: This research was done to determine the efficacy of some disinfectants and conventional antibiotics used against ESKAPE pathogens.
Methodology: The *in vitro* efficacy of the disinfectants and antibiotics were compared using the disc diffusion (Kirby-Bauer) method.
Results: Inhibition zone diameters were observed in all of the disinfectants and conventional antibiotics at concentration-dependent for the tested pathogenic isolates. Chloroxylenol was effective at higher concentrations and showed a progressive decrease in zones of inhibition as the concentration decreases. Ethanol was effective at 70% and 35% concentrations against *Staphylococcus aureus.* Hypochlorite was effective against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli* at a 100% - 25% concentration and effective against *Staphylococcus aureus* at 100% and 50% concentrations. Therefore, the efficacy of disinfectants and antibiotics arise to be crucial however concentration-dependent.

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Conclusion: The results obtained from this study may be used as an alternative for medical applications. However, inappropriate disinfectant and conventional antibiotic use resulted in emergence of resistant microorganisms; hence these therapeutic agents should be used properly at a sufficient concentration to prevent diseases caused by these pathogenic bacteria. Nevertheless, the need to compare the efficacy of these disinfectants and conventional antibiotics against ESKAPE pathogens *in vivo* is very important.

Keywords: Antimicrobial resistance; ESKAPE pathogens; disinfectants; conventional antibiotics.

1. INTRODUCTION

The gradual decrease in research output towards the development of new antibiotics has brought about the rise of multidrug-resistant (MDR) bacteria [1]. However, these bacteria have continued to develop resistance to many conventional antibiotics that were thought to be effective; as a result, hospital-acquired infections have become the leading factor in the high death rate, expenses, and prolonged hospitalization in healthcare settings [2]. The ESKAPE pathogens are medically significant because of their resistance, mode of transmission, and ability to cause serious infections [3]. Subsequently, the availability of novel compounds has lessened the burden of rising resistance and is widely approved as the established response [4] in fighting these pathogens. According to the Infectious Diseases Society of America (IDSA), the decrease in the availability of new therapeutic agents has led to an increase in drug-resistant pathogens [5]. The ESKAPE pathogens include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp.* due to the high resistant rate among these pathogens, they cause hospital-acquired severe infections, especially in immunocompromised and critically ill patients [6].

Many disinfectants have been used daily to fight these pathogenic bacteria. Disinfectants have various modes of action against bacteria. For example, Chloroxylenol has been known to denature protein components of the bacterial cell. It has since been used in healthcare settings, and due to its toxicity, it has been restricted to domestic use [7]. On the other hand, cresol is a derivative of phenol that damages bacterial cell membranes, inhibits the virulence activity of bacterial enzymes and toxins, and suppresses biofilm formation [8]. Also, the sodium hypochlorite efficacy depends on the chlorine concentration and its pH. Moreover, its germicidal action depends solely on -OCl which is the key factor for its disinfecting efficacy [9]. Lastly, Ethanol (70%) has a wide range of activity for its germicidal action compared with isopropyl [10]. It denatures protein and also it is bactericidal, tuberculocidal, virucidal, and fungicidal. It does not destroy spores formed by bacteria but has been found to destroy enzyme activity in *Escherichia coli* [11].

It is generally believed that inappropriate antibiotic use increases the survival rate of resistant strains in healthcare settings [12]. It has also been observed that the antibacterial efficacy may be reduced when antibiotics are used in synergy with vitamins and other supplements [13].

Studies have shown that the use of disinfectants and antibiotics play a crucial role in hospitals, microbiological laboratories, human and animal care settings [14]. Al-dabbagh *et al.* showed that the disinfectants and some antibiotics were effective against the tested bacteria [14]. However, the resistance by bacterial pathogens calls for intense research. This is important so as to mitigate the spread of resistant pathogens. The present study aimed to investigate the *in vitro* effects of disinfectants and antibiotics at different concentrations on selected ESKAPE pathogens causing infections in hospitals and homes.

2. MATERIALS AND METHODS

2.1 The Test Organisms

Organisms used in this study were pure clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* collected from the Diagnostic Microbiology Laboratory, Department of Microbiology, University of Nigeria, Nsukka.

2.2 Disinfectants and Antibiotics

Disinfectants used were Chloroxylenol, Cresol, 70% Ethanol, and Hypochlorite. 1:2 dilution was carried out on each disinfectant to give varying concentrations (50%, 25%, 12.5%, 6.3%, 3.1%, 1.6%, and 0.8%) and 70% ethanol concentrations (35%, 17.5%, 8.8%, 4.4%, 2.2%, 1.1%, and 0.6%). Abtek antibiotic discs were used in this study. The Mueller-Hinton agar (MHA) (*OXOID LTD, Basingstoke, Hampshire, England*) was augmented with disinfectant working solutions and antibiotic discs.

2.3 Evaluation of the Efficacy of Disinfectants and Antibiotics

The efficacy of the disinfectants and antibiotics was determined using the disc diffusion method (Kirby Bauer) according to the guidelines outlined by the Clinical Laboratory Standard Institute [15]. Sets of freshly prepared Mueller-Hinton agar Petri dishes (24) were inoculated with an 18h standardized inoculum (10^8 CFU/ml) using a sterile swab stick and allowed to dry. A pair of sterile forceps was used to pick filter paper discs containing disinfectants at different concentrations and the antibiotic discs were gently placed on the surface of the Petri dishes. The plates were inverted and incubated under aerobic conditions at 35°C overnight. The plates were then examined and the diameters of the inhibition zones were measured to the nearest millimeters and recorded.

2.4 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software version 23.0 (SPSS, 2018) was used to analyze the data. Results with $P<0.05$ was considered significant.

3. RESULTS

The efficacy of the disinfectants is shown in Fig. 1. The disinfectants were effective against the ESKAPE pathogens and various inhibition zone diameters were observed. Chloroxylenol was effective at higher concentrations and showed a progressive decrease in zones of inhibition as the concentration decreases. At its concentration 0.8%, no inhibition zone diameter was observed. Ethanol was effective at 70% and 35% concentrations against *Staphylococcus aureus* with inhibition zone diameters of 10mm and 12mm respectively. Other organisms showed no inhibition zone diameter against Ethanol. Cresol was effective against *Staphylococcus aureus, Klebsiella pneumoniae* and *E. coli.* It was only effective against *Pseudomonaas aeruginosa* at 100% concentration with an inhibition zone diameter of 12mm. Hypochlorite on the other end was effective against *Staphylococcus aureus* but at concentrations of 100% and 50% which showed zones of inhibition of 13mm and 11mm respectively. It was effective against *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *E. coli* at a 100% - 25% concentration.

Fig. 2 shows the efficacy of conventional antibiotics against the test isolates. Streptomycin was the most effective against *Staphylococcus aureus* with an inhibition zone diameter of 40mm. Other antibiotics were effective except Amoxicillin, Septrin, Chloramphenicol, Sparfloxacin and Augmentin showed no inhibition zone diameter against the tested organisms. Rocephin, Zinnacef, Gentamycin, Ampliclox and Augmentin were not effective against *Klebsiella pneumoniae*. Other antibiotics showed different zones of inhibitions, Pefloxacin the most effective, with an inhibition zone diameter of 34mm against *Klebsiella pneumoniae*. *P. aeruginosa* was resistant to Rocephin, Zinnacef, Erythromycin, Gentamycin and Amplicox. Pefloxacin was the most effective with an inhibition zone diameter of 30mm against *P. aeruginosa* and *E. coli*. Amoxicillin and Streptomycin have identical inhibition zones of 28mm against *P. aeruginosa* while Chloramphenicol and Sparfloxacin also have the same inhibition zones of 22mm against *P. aeruginosa*. Rocephin, Zinnacef, Streptomycin, Erythromycin, Gentamycin, Ampiclox and Augmentin were ineffective against *E. coli*. Furthermore, Pefloxacin and Septrin have the same inhibition zone diameter of 30mm and are the most effective against the organism.

Fig. 3 also illustrates the efficacy of conventional antibiotics against ESKAPE pathogens. All the antibiotics were effective against *S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli* except Cefixim, Augmentin, Ceftazidime and Cefuroxime. Only *E . coli* was resistant to Gentamycin. Oflaxin and Nitrofurantoin were the most effective against *S. aureus* and *E. coli* and had inhibition zone diameter of 28mm and 30mm respectively. Ofloxacin and Ciprofloxacin were the most effective against *K. pneumoniae* with an inhibition zone diameter of 30mm in this study.

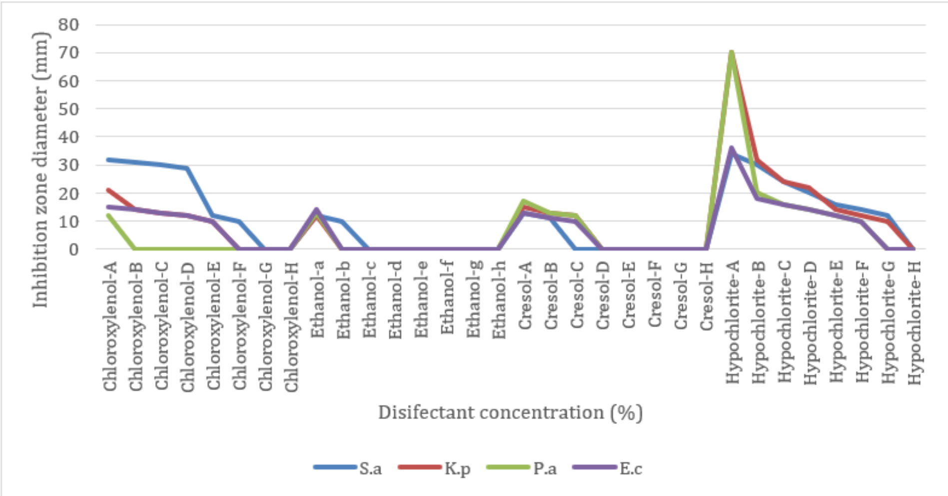

Fig. 1. Activities of disinfectants concentrations against ESKAPE pathogens

Key: S.a (*Staphylococcus aureus),* K.p (*Klebsiella pneumoniae),* P.a (*Pseudomonas aeruginosa),* E.c (*Escherichia coli*)

A- 100%, B- 50%, C- 25%, D- 12.5%, E- 6.3%, F- 3.1%, G- 1.6%, H- 0.8%

a- 70%, b- 35%, c- 17.5%, d- 8.8%, e- 4.4%, f- 2.2%, g- 1.1%, h- 0.5%

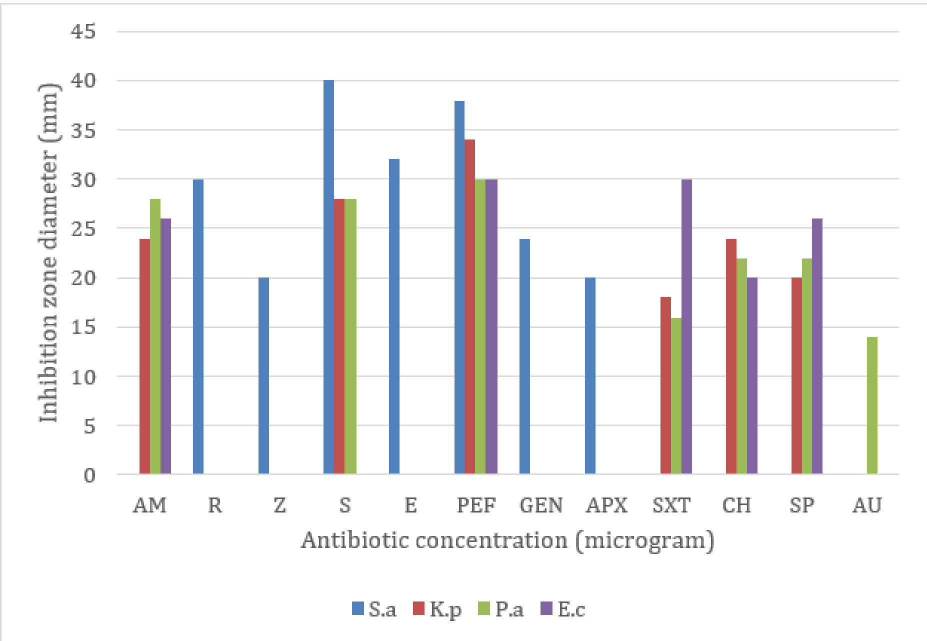

Fig. 2. Activities of conventional antibiotics concentrations against ESKAPE pathogens

Key: S.a (Staphylococcus aureus), K.p (Klebsiella pneumoniae), P.a (Pseudomonas aeruginosa), E.c (Escherichia coli)

AM-Amoxicillin (30microgram), R- Rocephin (25 microgram), Z- Zinnacef (20 microgram), S- Streptomycin (30 microgram), E- Erythromycin (10 microgram), PEF- Pefloxacin (10 microgram), GEN- Gentamycin (10 microgram), APX- Ampiclox (30 microgram), SXT- Septrin (30 microgram), CH- Chloramphenicol (30 microgram), SP- Sparfloxacin (10 microgram), AU- Augmentin (25 microgram).

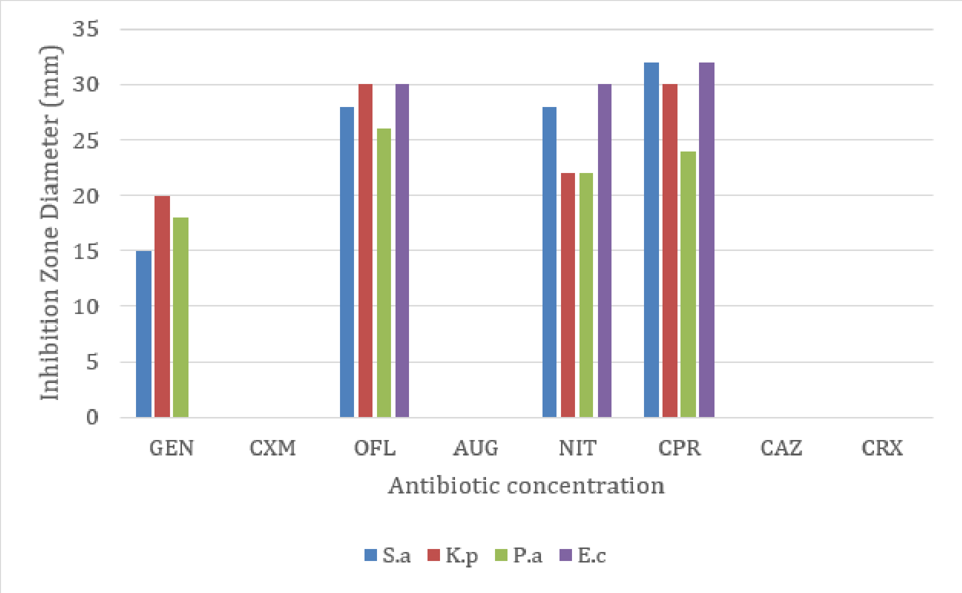

Fig. 3. Activities of conventional antibiotics concentrations against ESKAPE pathogens

Key: S.a (Staphylococcus aureus), K.p (Klebsiella pneumoniae), P.a (Pseudomonas aeruginosa), E.c (Escherichia coli)

GEN- Gentamycin (20 microgram), CXM- Cefixime (5 microgram) , OFL- Ofloxacin (5microgram), AUG- Augmentin (25microgram), NIT- Nitrofurantoin (300microgram), CPR- Ciprofloxacin (5microgram), CAZ- Ceftazidime (30microgram), CRX- Cefuroxime (30microgram)

4. DISCUSSION

Pathogens are increasingly acquiring resistance to most conventional antibiotics [4]. Antimicrobial resistance will continue to be the center of research interest because these pathogens are continually causing diseases globally that are presently difficult to treat. The results from this research demonstrated substantial impacts of various concentrations of disinfectants and conventional antibiotics against ESKAPE pathogens. The concentrations of the disinfectants and antibiotics, which showed no inhibition zones, imply that the test organisms were resistant.

Previous studies have documented the effects of disinfectants and conventionally used antibiotics against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans* [16]. *Corynebacterium renale* [14] and *E. coli* [17]. Similarly, this research is based on the efficacy of commonly used disinfectants and conventional antibiotics on *Klebsiella pneumoniae, E. coli, S. aureus,* and *Pseudomonas aeruginosa.* The study of El Mahmood and Doughari ascertained the impact of Dettol (chloroxylenol) on the viability of *Staphylococcus aureus, E. coli,* and *Candida albicans* [17], while Al-Dabbagh *et al.* also ascertain the impact of Dettol (chloroxylenol), bleach (sodium hypochlorite), Ethanol (70%) and Hibitine (chlorhexidine gluconate) 6% on *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Corynebacterium renale.* Ogunsola *et al.* ascertained the effect of chloroxylenol against Methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* while Al-Dabbagh *et al.* obtained a different result against *S. aureus* which showed a discrepancy in the efficacy of chloroxylenol owing to different concentrations used in their respective study. According to Al-Dabbagh *et al.*, Bleach (Hypochlorite), Ethanol (70%) Hibitine (chlorhexidine gluconate) 6% follow the order of corresponding activity against the test organisms used in their study [14]. Gomes et al. showed the effect of sodium hypochlorite on *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia,* where *Stenotrophomonas maltophilia* showed resistance against the antimicrobial [18]. The susceptibility of the Gram-postive bacteria owe to its single thick peptidoglycan cell wall and the resistance exhibited by the Gram-negative bacteria is as a result of its freeze-fractured outer and inner cell membrane [19] which contribute to this effect.

In this *in vitro* effect, various concentrations of disinfectants and conventional antibiotics were used against the ESKAPE pathogens. Chloroxylenol was the most effective against the test isolates. It showed a progressive decrease in zones of inhibition as the concentration decreased. Chloroxylenol was highly inhibitory in its undiluted (100%) form against *S. aureus, P. aeruginosa, K. pneumoniae,* and *E. coli* (Fig. 1). A similar result was obtained from the study of Saha *et al. w*hich showed that chloroxylenol was effective against the tested isolates used in their study except for *P. aeruginosa* [20]. Ethanol was also effective but only at a concentration of 70% against the test organisms which is similar to the result obtained from the study of Al-Dabbagh *et al*. Hypochlorite was also effective at its diluted concentrations, showing more inhibition zones against *Klebsiella pneumoniae, P. aeruginosa,* and *E. coli.* Cresol showed more inhibitions against *S. aureus, K. pneumoniae,* and *E. coli. P. aeruginosa* was resistant to cresol at its diluted concentrations which were similar to the findings of Alabi and Sanusi [21]. Among the conventional antibiotics used, Pefloxaxin was effective against the test organisms while Streptomycin also was effective except for *E. coli* which was resistant. Amoxicillin, Septrin, Chloramphenicol, and Sparfloxacin were effective against *Klebsiella pneumoniae, P. aeruginosa*, and *E. coli.* Augmentin was only effective against *P. aeruginosa,* while Rocephin, Zinnacef, Erythromycin, Gentamycin, and Ampiclox were only effective against *S. aureus.* It can be ascertained that Gentamycin was effective against *S. aureus*, *K. Pneumoniae,* and *P. aeruginosa* except for *E. coli* which was resistant. Ofloxacin, Nitrofurantoin, and Ciprofloxacin were effective against the test organisms. The discrepancy in the effectiveness of Gentamycin is a result of the difference in the concentration used in this study.

From this study, it can be deduced that the cell structure of both the Gram-negative and Gram-positive bacteria plays a crucial role in both their susceptibility and resistance to antimicrobial agents [19]. However, it has been shown that these ESKAPE pathogens acquire some of these resistance genes from the environment [22]. Also, inappropriate use of these antimicrobial agents can result in resistance [23].

5. CONCLUSION

It has been shown in this study that concentrations of disinfectants and conventional antibiotics play an impact on their effectiveness. Notwithstanding, the difference in concentration implies different effects of these antimicrobial agents and so, efforts should be made to use the appropriate concentration in fighting these pathogenic bacteria. Moreover, the right choice of disinfectants and antibiotics should also be put into consideration when treating and preventing infections caused by these pathogens and as such it will help to prevent them. It is worth knowing that any of these disinfectants and antibiotics used in this study can eradicate these organisms but this is when used appropriately. Antimicrobial agents which will be more effective on drug-resistant bacterial pathogens are needed and so the *in vivo* effects should be studied.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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