

EVALUATION OF SOME COMMERCIAL ANTIMICROBIAL OINTMENTS ON SELECTED BACTERIAL AND FUNGAL STRAINS OF CLINICAL IMPORTANCE

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ABSTRACT

Background: Antimicrobial ointments are topical products used for the treatment of common skin infections. Potency superiority between certain ointments and creams used in the treatment of skin infections has been a controversial subject among clinicians.

Objective: This study was carried out to investigate the activities of some antimicrobial ointments on selected bacteria and fungi of clinical importance that caused skin infections.

Methods: Three brands of antibacterial; gentamicin, chloramphenicol, bactroban and two brands of antifungal ointments; nystatin and Whitefield's were evaluated by agar-cup diffusion method for their antimicrobial activity. Minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of the ointments were determined. Kinetic study of bactroban on all the clinical isolates was evaluated to determine their efficacy within a specific time lag.

Results: All the isolates of *Pseudomonas aeruginosa* were susceptible to bactroban while 4 of the 5 isolates of *Streptococcus pyogenes* were resistant. Three of the five isolates of *Staphylococcus aureus* were susceptible to bactroban. Gentamicin had no activity on *Pseudomonas aeruginosa* while 4 of the 5 isolates of *Streptococcus pyogenes* were susceptible to gentamicin. Three of the 5 isolates of *Staphylococcus aureus* were susceptible to gentamicin and varied resistance were recorded for chloramphenicol and antifungal agents. The MIC's values recorded for the antimicrobial ointments examined varied with respect to concentrations and composition. Bactroban and gentamicin gave the MIC's 20µg/mL - 2000µg/mL and 50µg/mL - 400µg/mL while the MIC's range 160µg/mL - 400µg/mL, 100µg/mL - 160µg/mL and 180µg/mL - 200µg/mL were also recorded for chloramphenicol, nystatin and Whitefield's against the isolates concerned respectively. The MBC's and MFC's values recorded against the isolates doubled the values obtained from the MIC's. Kinetic studies showed various population reduction to zero at varied contact time for clinical isolates of bacterial and fungi exposed to bactroban.

Conclusion: These findings elicited potency differences among the ointments tested on the selected clinical microbial isolates examined, this could be useful in the selection of antimicrobial ointments for the management of skin infections caused by the microorganisms tested and their closely related strains.

Keywords: Antimicrobial ointments, Skin infection, Microbial agents.

INTRODUCTION

The skin is a relatively inhospitable environment for the growth of most pathogenic microorganisms. The hostility of the skin environment is attributed to relative dryness of cutaneous surfaces that provides insufficient amount of moisture that could support significant growth of pathogens and colonization with resident microflora that produces metabolites that are inhibitory to the growth of invading/competing pathogens¹. Eradication of resident flora greatly enhances the survival of *Staphylococcus aureus* and the subsequent development of infection.

Skin infections may be either primary or secondary. Primary infections have characteristic morphologies and

courses, and are initiated by single organisms, and usually occur in normal skin. They are most frequently caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, and *coryneform* bacteria. Impetigo, folliculitis, boils, and erythrasma are common examples. Secondary infections originate in diseased skin as a superimposed condition, when the skin is damaged by inflammation, burn or exfoliation, absorption is further increased².

Pseudomonas aeruginosa is an opportunistic pathogen that can cause skin infection especially in burns and wounds, sores and ulcers often as secondary invaders. Skin lesions have been reported to accompany *Pseudomonas aeruginosa* sepsis in 13-39 percent of patients studied

over the years³. The dermatological manifestations of *Pseudomonas aeruginosa* include *erythema gangrenosum* which was however reported in 25% of patients with bacteraemia. *Staphylococcus aureus* is responsible for variety of infection syndromes that may produce local or diffuse skin lesions by producing toxins (Staphylococcal scalded skin syndrome and toxic shock syndrome due to vascular invasion often in association with carditis⁴. Other spectrum skin mediated infections that *Staphylococcus aureus* can cause include; impetigo, folliculitis, furuncles, and ecthyma. Fungi and yeasts are capable of causing many different forms of skin infections broadly referred to as dermatomycoses. Dermatomycoses are a group of common infections generally caused by *Trichophyton species*. These infections include tinea capitis, tinea barbae, tinea corporis, tinea pedis and tinea cruris^{5,6}.

Candida albicans that causes vaginitis is manifested by vaginal itching and discharge, often accompanied by dysuria and pain. Pelvic examination may reveal a creamy to cheesy whitish discharge, redness of the vagina wall and external genitalia may also be present⁷. Antibiotic ointments are topical preparation that are used for the treatment of common skin infections. They contain a medicament dissolved, suspended or emulsified in a base and in their pharmaceutical dosage forms, they are anti-infective and protective on host skin⁸. Antimicrobial ointments are used topically for several purposes; as protectants, antiseptics, emollients, antipruritis, keratolytics and astringents. The use of ointments can be an effective part of wound care, along with regularly cleansing the wound in ensuring speedy healing. Ointments are made up of bases which may be hydrocarbon (oleaginous), absorption, water removable and water soluble type⁹. In general, ointments are intended to speed healing and prevent infection.

Some people also choose to use antibiotic ointment on a new tattoo, though some tattoo artists advise against this for various reasons. Antimicrobial ointments are designed to wipe off bacterial, fungal and yeast skin infection depending on their formulations. The entry of these ointments and creams into the skin is determined by the rate of diffusion of drug from the vehicle to the surface of the skin, the partitioning of the drug from the vehicle and the stratum, the degree of hydration of the stratum and variation in the extent to which they increase the hydration of the stratum^{10,11}.

Bactroban (mupirocin) is an antibiotic that prevents bacteria from growing on skin when applied topically. It is effective against impetigo and other spectrum of Staphylococcal infection of the skin. Bactroban exerts

its bacteriostatic effect at low concentration and its bactericidal effect at high concentration when applied locally, it is very active against Gram negative bacteria. Nystatin Ointment USP is virtually non-toxic and non-sensitizing, and is well tolerated by all age groups including debilitated infants, even on prolonged administration. Nystatin Ointment USP is indicated in the treatment of cutaneous or mucocutaneous mycotic infections caused by *Candida albicans* [Monilia] and other *Candida spp*. The use of certain topical ointments has also been suggested in the treatment of superficial fungal infections. Nystatin Ointment USP is contraindicated in patients with a history of hypersensitivity to any of its components.

Topical antimicrobial agents can be used for some infections, but systemic therapy may be necessary for patients with extensive disease¹².

Chloramphenicol ointment is an antibiotic formulation that is used to treat bacterial eye infections of the eyelid, pink eye infection, bacterial inflammation of the cornea and covering of eye. It works by stopping the growth of bacteria¹³. Gentamicin ointment is used to treat minor skin infection and infection related some skin condition such as impetigo, folliculitis, eczema, psoriasis and minor cuts/burns or wounds. It is an aminoglycoside and has no effect on fungi¹⁴. This study was carried out therefore to determine the activity of some antimicrobial ointments on selected bacterial and fungal isolates from clinical sources.

MATERIALS AND METHODS

Sample Collection

Test organisms

The clinical isolates used for this findings was collected from dermatological unit of the Olabisi Onabanjo University Teaching Hospital and were confirmed by conventional biochemical characterization tests. The organisms collected were; *Pseudomonas aeruginosa*, *Streptococcus pyogene*, *Staphylococcus aureus*, typed strains of *Staphylococcus aureus* ATCC29213 and *Candida albicans*.

Antimicrobial ointments

The antimicrobial ointments used were: Bactroban (Beecham pharmaceuticals), Gentamicin ointment (Drugfield, Nigeria) Chloramphenicol ophthalmic ointment (Hamburg Germany), Nystatin ointment (Squibb/USA) and Whitefield's ointment (Nigeria).

Antimicrobial assay

The antimicrobial activity of the ointments was carried out using the agar diffusion technique. The molten Mueller Hinton agar medium was seeded with 0.2ml of 1:100 overnight culture of each bacteria isolates

while fungi isolates were surface spread with the same dilution of isolates respectively. Using a sterile cork borer, 8mm wells were bored into the agar medium. Then varied concentrations of ointments was introduced to the wells and plates were allowed to stand on the bench for 1 hour for pre-diffusion before incubation at 25°C and 37°C for 72 hours and 24 hours for *Candida albicans* and bacteria respectively. The inhibitory zones (mm) were taken as a measure of antimicrobial activity and interpreted according to CLSI standard.

Determination of Minimum Inhibitory Concentration

Broth dilution method

A volume of 0.1mL from 10⁻² dilution of an overnight broth culture equivalent to 10⁶ cell/mL of each of the test organisms was pipetted into DMSO which was prepared in 1% concentration using Tryptone soya broth as diluent containing the respective antimicrobial ointments prepared in the 1% DMSO in concentrations ranging from 1000µg/mL to 25µg/mL. The tubes were incubated at 37°C for 48 hours and 25°C for 72 hours for the bacterial and *Candida albicans* isolates respectively. The least concentration of each ointment that showed no growth was recorded as MIC and MFC of such ointment against the test organisms.

Determination of Minimum Bactericidal Concentration by Spread Plate Technique

A volume of 0.2mL of samples were removed from the tubes used in the determination of MIC with no discernible growth and was spread plated on the surface of dry nutrient agar (bacteria) medium and Saboraud Dextrose Agar (*Candida albicans*) plates.

The culture plates were incubated at 37°C for 48 hrs. and 28°C for 72 hrs. for bacteria and *Candida albicans* respectively. The lowest concentration of the aliquot which showed no trace of growth on recovery plates was taken as the minimum bactericidal concentration.

Kinetics of Bactericidal Activity

A volume of 0.1mL of broth culture of each of every test organism was added to a test tube containing 9.9ml of 0.1%w/v peptone water to produce a bacterial and fungal suspension of 10⁶ cells per mL. Appropriate concentration of the selected ointment: bactroban only that was equivalent to the ½ MIC, MBC and 2MBC, 1/2 MFC, MFC and 2MFC of the ointment was added to the suspension of the test organism respectively. A volume of 0.1mL of each of the suspensions was seeded into nutrient agar medium and poured aseptically at intervals of 0, 0.5, 1, 2,3, 4, 5, 6 and 8 hours and thereafter incubated at 37°C for 48 hours and 25°C for 72 hours for bacterial and fungal respectively. The numbers of surviving cells per mL were calculated and the kinetics of bactericidal activity of the cream was determined by plotting the percentage survivors against time.

RESULTS

The three antibacterial ointments (bactroban, gentamicin and chloramphenicol) and two antifungal ointments (nystatin and Whitefield's) were assessed for antimicrobial activity in this study and they showed varied antimicrobial activity on the selected test organism. Bactroban showed antipseudomonas activity against all the test isolates of *Pseudomonas aeruginosa* were susceptible to bactroban while four out

Table 1: Sensitivity of selected test organisms to antimicrobial ointments.

ORGANISMS	BACT	GENT	CHLO	NYST	WF
<i>Pseudomonas aeruginosa 1</i>	20(S)	14(R)	14(R)	ND	ND
<i>Pseudomonas aeruginosa 2</i>	25(S)	12(R)	12(R)	ND	ND
<i>Pseudomonas aeruginosa 3</i>	25(S)	12(R)	12(R)	ND	ND
<i>Pseudomonas aeruginosa 4</i>	25(S)	14(R)	12(R)	ND	ND
<i>Pseudomonas aeruginosa 5</i>	25(S)	14(R)	12(R)	ND	ND
<i>Streptococcus pyogene 1</i>	12(R)	12(S)	28(S)	ND	ND
<i>Streptococcus pyogene2</i>	14(R)	25(S)	24(S)	ND	ND
<i>Streptococcus pyogene3</i>	14(R)	32(S)	24(S)	ND	ND
<i>Streptococcus pyogene4</i>	22(S)	12(R)	12(R)	ND	ND
<i>Streptococcus pyogene5</i>	14(R)	20(S)	22(S)	ND	ND
<i>Staphylococcus aureus 1</i>	25(S)	12(R)	14(R)	ND	ND
<i>Staphylococcus aureus 2</i>	18(S)	14(R)	12(R)	ND	ND
<i>Staphylococcus aureus 3</i>	14(R)	27(S)	24(S)	ND	ND
<i>Staphylococcus aureus 4</i>	14(R)	20(S)	25(S)	ND	ND
<i>Staphylococcus aureus 5</i>	25(S)	24(S)	26(S)	ND	ND
<i>Staph. aureus ATCC29213</i>	28(S)	24(S)	12(R)	ND	ND
<i>Candida albicans 1</i>	ND	ND	ND	28(S)	14(R)
<i>Candida albicans 2</i>	ND	ND	ND	28(S)	25(S)
<i>Candida albicans 3</i>	ND	ND	ND	32(S)	25(S)
<i>Candida albicans 4</i>	ND	ND	ND	28(S)	30(S)
<i>Candida albicans 5</i>	ND	ND	ND	25(S)	32(S)

Key: Sensitive: +, Resistant: -, ND = Not Determined due to the nature of organisms concerned.

Bact: Bactroban, Gent: Gentamicin, Chlo: Chloramphenicol, Nyst: Nystatin, WF: Whitefield

of the five isolates of *Streptococcus pyogenes* 5 were resistant with the exception of *Streptococcus pyogenes* 5. Three of the five isolates of *Staphylococcus aureus* and a typed strain of *Staphylococcus aureus* were susceptible to bactroban. Gentamicin, an aminoglycoside had no activity on *Pseudomonas aeruginosa* but four of the five isolates of *Streptococcus pyogenes* were susceptible with the exception of *Streptococcus pyogene* 4 that is resistant to gentamicin. Also, only three of the five *Staphylococcus aureus* strains were susceptible to gentamicin ointments

ointment. All the isolates of *Candida albicans* were susceptible to nystatin and Whitefield's as shown in Table 1.

The MIC's values of bactroban against *Pseudomonas aeruginosa* ranged from 100µg/ml -160µg/ml were more significant than the MIC's values of the same organism on gentamicin and chloramphenicol that ranged between 200µg/ml - 400µg/ml for both ointments.

Table 2: Minimum inhibitory concentration of test antimicrobial ointments on selected organisms.

ORGANISMS	BACT	GENT	CHLO	NYST	WF
	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
<i>Pseudomonas aeruginosa</i> 1	160	400	200	ND	ND
<i>Pseudomonas aeruginosa</i> 2	160	280	200	ND	ND
<i>Pseudomonas aeruginosa</i> 3	100	320	180	ND	ND
<i>Pseudomonas aeruginosa</i> 4	100	260	400	ND	ND
<i>Pseudomonas aeruginosa</i> 5	140	200	200	ND	ND
<i>Streptococcus pyogene</i> 1	200	200	160	ND	ND
<i>Streptococcus pyogene</i> 2	180	200	200	ND	ND
<i>Streptococcus pyogene</i> 3	180	200	400	ND	ND
<i>Streptococcus pyogene</i> 4	180	200	200	ND	ND
<i>Streptococcus pyogene</i> 5	200	200	200	ND	ND
<i>Staphylococcus aureus</i> 1	100	50	250	ND	ND
<i>Staphylococcus aureus</i> 2	200	100	400	ND	ND
<i>Staphylococcus aureus</i> 3	200	100	400	ND	ND
<i>Staphylococcus aureus</i> 4	200	100	200	ND	ND
<i>Staphylococcus aureus</i> 5	200	100	200	ND	ND
<i>Staph. aureus</i> ATCC29213	100	50	400	ND	ND
<i>Candida albicans</i> 1	ND	ND	ND	160	200
<i>Candida albicans</i> 2	ND	ND	ND	100	180
<i>Candida albicans</i> 3	ND	ND	ND	100	240
<i>Candida albicans</i> 4	ND	ND	ND	140	200
<i>Candida albicans</i> 5	ND	ND	ND	120	200

Key: ND= Not Determined due to the nature of organisms concerned.

Bact: Bactroban, Gent: Gentamicin, Chlo: Chloramphenicol, Nyst: Nystatin, WF:Whitefield

tested. *Staphylococcus aureus* ATCC 29213 was susceptible to the ointment. All isolates of *Pseudomonas aeruginosa* were resistant to chloramphenicol ointment while four of the five isolates of *Streptococcus pyogenes* were susceptible to chloramphenicol. Three *Staphylococcus aureus* strains were susceptible while the remaining two and a typed strain were resistant to chloramphenicol

The minimum inhibitory concentrations of bactroban against *Streptococcus pyogene* ranged between 180µg/ml - 200µg/ml while the MIC values of the same gentamicin and chloramphenicol were between 160µg/ml - 400µg/ml against the isolates of *Streptococcus pyogene*.

Table 3: Minimum bactericidal concentration of test antimicrobial agents on selected organisms.

ORGANISMS	BACT	GEN	CHLO	NYST.	WF
	µg/ml	µg/ml	µg/ml	µg/ml	µg/r
<i>Pseudomonas aeruginosa</i>	180	400	400	ND	ND
<i>Streptococcus pyogene</i>	400	400	400	ND	ND
<i>Staph. aureus</i>	200	200	400	ND	ND
<i>Staph. aureus</i> ATCC29213	200	100	200	ND	ND
<i>Candida albicans</i>	ND	ND	ND	200	240

Key: ND= Not Determined due to the nature of organisms concerned.

Bact: Bactroban Gent: Gentamicin Chlo: Chloramphenicol Nyst: Nystatin WF:Whitefield

The MIC of bactroban against *Staphylococcus aureus* and a typed strain ranged between 100µg/ml - 200µg/ml while it varied from 50µg/ml - 100µg/ml against gentamicin and it ranged between 200µg/ml - 400µg/ml against chloramphenicol.

The MIC of nystatin against *Candida albicans* ranged between 100µg/ml -160µg/ml and for whitefield the MIC ranged between 180µg/ml -240µg/ml respectively as shown in Table 2.

The minimum bactericidal concentrations of the test antimicrobial agents were observed to be double the MIC. The MBC values of bactroban and gentamicin against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* was between 180µg/ml and 400µg/ml while the MBC values of the same agent was 200µg/ml each against both *Staphylococcus aureus* investigated. The MBC values of 400µg/ml were recorded for chloramphenicol against the clinical isolates of *Staphylococcus aureus* and while a typed strain of *Staphylococcus aureus* had MBC value of 200µg/ml. The MBC of 200µg/ml and 240µg/ml of nystatin and whitefield were obtained for *Candida albicans* as shown in Table 3.

The bactericidal activity of bactroban showed varied population reduction at a different concentration. It took 3 hours for bactroban to effect total population reduction to zero at 2MBC against *Pseudomonas aeruginosa* as shown in Figure 1 below.

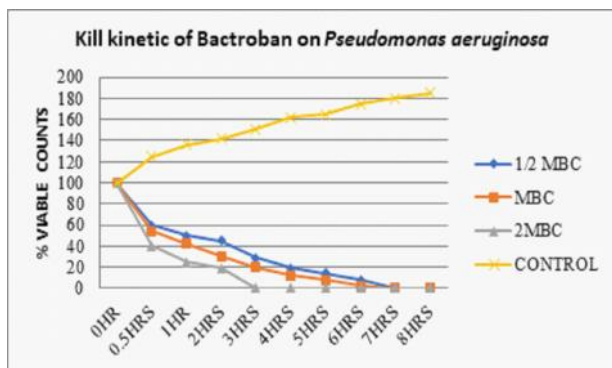


Figure 1: Kill kinetic of bactroban on *Pseudomonas aeruginosa*.

At 2MBC, it took 6 hours for bactroban to effect total population reduction to zero against *Streptococcus pyogenes* as showed in Figure 2.

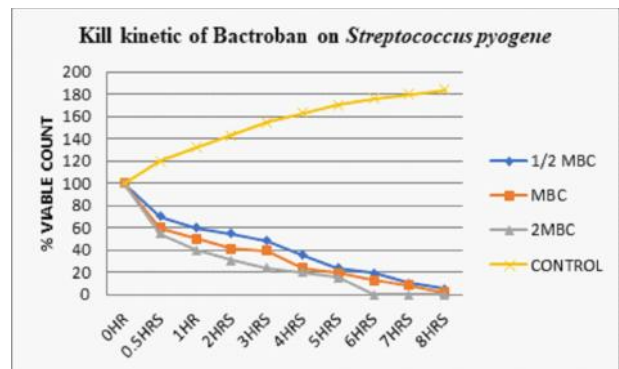


Fig. 2: Kill kinetic of bactroban on *Streptococcus pyogenes*.

At 2MBC, it took 7 hours for bactroban to effect total population reduction to zero against *Staphylococcus aureus* as shown in Figure 3 below.

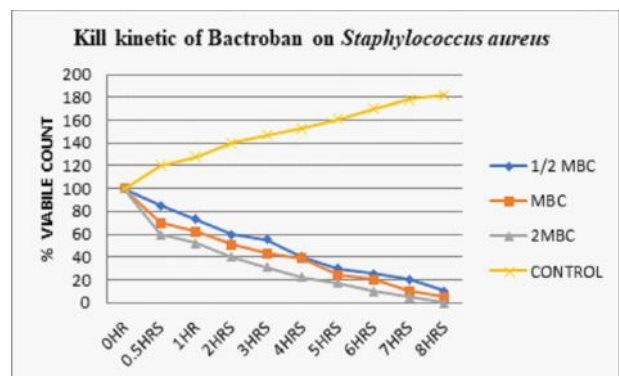


Fig. 3: Kill kinetic of bactroban on *Staphylococcus aureus*.

At 2MBC, it took 4 hours for bactroban to effect total population reduction to zero against *Staphylococcus aureus* 29213 as shown in Figure 4 below.

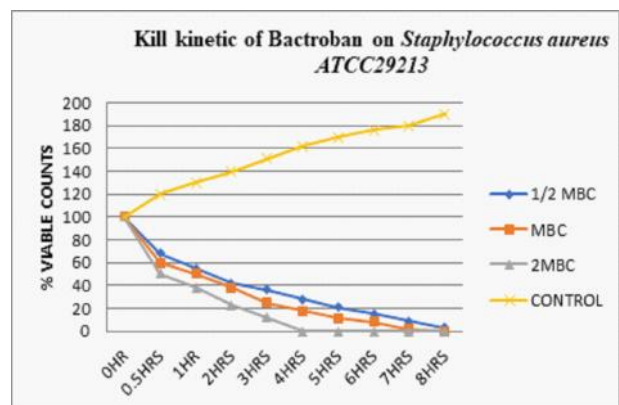


Fig. 4.: Kill kinetic of bactroban on *Staphylococcus aureus* 29213.

At 2MBC, it took 2 hours for nystatin to effect total population reduction to zero against *Candida albicans* as shown in Figure 5.

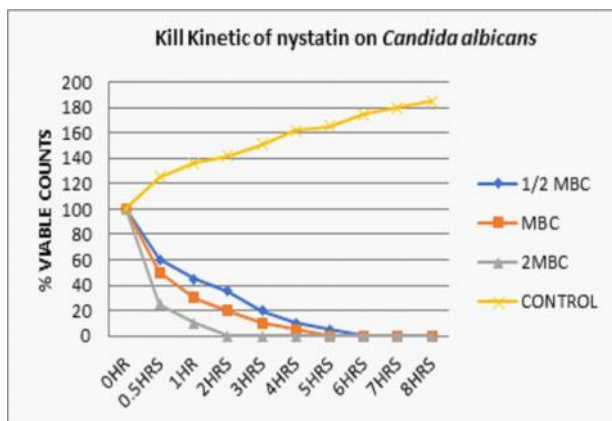


Fig. 5: Kill kinetic of nystatin on *Candida albicans*.

At 2MBC, it took 2 hours for whitefield to effect total population reduction to zero against *Candida albicans* as showed in figure 6 below

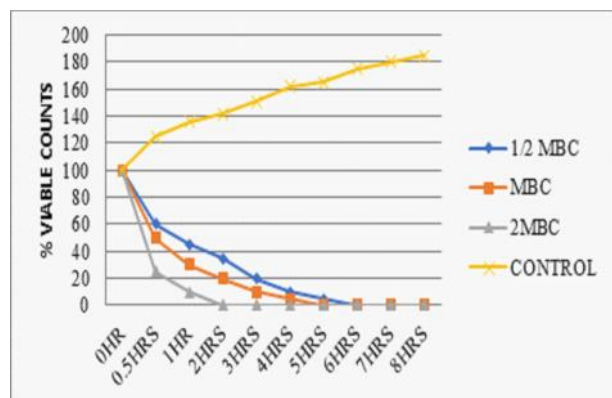


Fig. 6: Kill kinetic of whitefield on *Candida albicans*.

DISCUSSION.

In comparing antimicrobial ointments in an *in-vitro* experiment, minimum inhibitory concentration, minimum bactericidal concentration and kinetics of bactericidal activity of the agents are three important parameters to indicate antimicrobial activity of an agent. The three antibacterial ointments (bactroban, gentamicin and chloramphenicol) and two antifungal ointments (nystatin and Whitefield) were assessed for antimicrobial activity and they showed varied activity on selected clinical isolates¹⁵.

Bactroban (mupirocin), a naturally occurring antibiotic produced by fermentation of *Pseudomonas fluorescens*, is active against a wide range of both Gram negative and Gram positive organisms. It exerts its bacteriostatic activity at low concentrations and its bactericidal activity at high concentrations when applied locally. Bactroban showed antipseudomonas properties in this study, all the test isolates of *Pseudomonas aeruginosa* were susceptible to bactroban while four out of the five isolates of *Streptococcus pyogenes* 5 were resistant with

the exception of *Streptococcus pyogenes* 5. Three of the five isolates of *Staphylococcus aureus* and the typed strain of *Staphylococcus aureus* were susceptible to bactroban. This could be due its ability to inhibit protein synthesis by reversibly and specifically binding to isoleucyl-transfer-RNA synthetase¹⁶.

Gentamicin, an aminoglycosides, has been used in serious infections caused by bacteria that are resistant to other drugs. Gentamicin sulphate at a concentration of 0.1% has been used topically in creams for infected burns and skin lesions. It had no activity on *Pseudomonas aeruginosa* in this study, but four of the five isolates of *Streptococcus pyogenes* were susceptible to gentamicin with the exception of *Streptococcus pyogenes* 4 that is resistant to gentamicin. Also, three of the five *Staphylococcus aureus* strains were susceptible to gentamicin while *Staphylococcus aureus* 1 and 2 were resistant to the gentamicin ointment tested. *Staphylococcus aureus* ATCC 29213 was susceptible to the ointment, which could be attributed to overexposure of this antimicrobial agent to the organism that had facilitated its resistance¹⁷. Chloramphenicol is a broad-spectrum antibiotic originally isolated from *Streptomyces venezuelae* but now manufactured synthetically. It is primarily bacteriostatic and is indicated in the treatment of superficial ocular infection involving the conjunctivas caused by chloramphenicol susceptible organisms. All isolates of *Pseudomonas aeruginosa* in this study were resistant to chloramphenicol ointment while four of the five isolates of *Streptococcus pyogenes* were susceptible to chloramphenicol. Three *Staphylococcus aureus* were susceptible while the remaining two *Staphylococcus aureus* and a typed strain of *Staphylococcus aureus* were resistant to chloramphenicol ointment. This could be attributed to the inherent nature of the tests organisms, strain variation and abuse of this drug as a result of unguided use¹⁸.

All the isolates of *Candida albicans* were susceptible to nystatin and Whitefield's but at relatively varied concentrations which may be due to genetic composition of these isolates that made them susceptible and also could be attributed to the efficacy of these antifungal agents at the concentrations applied¹⁹.

In this study, bactroban and chloramphenicol showed antibacterial activity that compared favourably with each other. However, there was a little disparity in the minimum inhibitory concentration and minimum bactericidal values obtained for chloramphenicol. Bactroban and gentamicin ointments were found to be effective against typed strain of *Staphylococcus aureus* while chloramphenicol was less effective on *Staphylococcus aureus*²⁰

Nystatin and Whitefield showed antifungal activity against *Candida albicans* but with relatively higher than minimum bactericidal concentration. In this study, Whitefield's appears to be more effective than nystatin. The kill kinetic timing for bactroban (the only representative ointment selected in this study) varied from one test organisms to the other, it took 3 hours to achieved population reduction to zero at 2 MBC on *Pseudomonas aeruginosa*, a Gram negative bacterium while it took 6hr against *Streptococcus pyogenes*. This susceptibility could be attributed to cellular composition of the tests isolates. Bactroban took 7 hours that twice the MBC value to reduce the population of *Staphylococcus aureus* to zero in this study while the typed strain of *Staphylococcus aureus* subjected to the same test was reduced to zero within the time limit of 4 hours as showed in figure 3 and 4 above. Nystatin and Whitefield expressed their killing kinetic on *Candida albicans* at 2 hours in this study which could be traced their relatively closer MBC in this study²¹.

The results obtained in this study showed varied antimicrobial activity of the agents used on selected microbes. Over-exposure and preventive application of the antimicrobial agents coupled with the possibility of their addition to other creams or ointments might be responsible for the low activity of some of the ointments. Further research may be carried out on the antimicrobial ointments tested.

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