Nigerian Journal of Pharmaceutical and Applied Science Research, 9(1): 63-68, January 2020 (ISSN 1485-8059 (Available at www.nijophasr.com)

Evaluation of Antibacterial Activity of Tetmosol® against some Clinical Isolates

Clement O. Anie [1*], Johnson D. Jemikalajah [2], Merit O. Meshack[1], Michael Oghenejobo[1]

[1]Department Of Pharmaceutical Microbiology, Faculty Of Pharmacy, Delta State University, Abraka, Delta State, Nigeria .
[2]Department of Microbiology, Faculty of Science Delta State University Abraka, Delta State, Nigeria.

ABSTRACT

The study was carried out to evaluate the antibacterial activities of Tetmosol® using some clinical bacterial isolates. The broth dilution and agar well diffusion methods were used to determine the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The result indicated that the sample of tetmosol was effective as a disinfectant with broad spectrum of activity. The MIC of Tetmosol using broth technique was 1.2125 %v/v, 0.60625 %v/v, O.60625 %v/v, 0.60625 %v/v for *Staphylococcus aureus, Streptococcus pyogene, Escherichia coli, Pseudomonas aeruginosa* respectively. The MBC using agar technique was 6% and that of broth technique 9%. Susceptibility testing of the microorganism to Tetmosol, showed the highest zones of inhibition were 11 mm,14 mm, 12 mm, 10 mm for *Staphylococcus aureus, Streptococcus pyogene, Escherichia coli, Pseudomonas aeruginosa* respectively. The phenol coefficient of Tetmosol was found to be 9.1 which indicated that Tetmosol was more potent than phenol. The use of Tetmosol® in hospital, water bath and clinics in Nigeria is recommended.

Keywords: Evaluation of antibacterial, Activity, Tetmosol®, Disinfectant, Clinical isolates,

INTRODUCTION

Microorganisms are known to cause several infectious diseases and as well death. They include: bacteria, algae, protozoa, fungi, and viruses. Microorganisms are ubiquitous, that is, they can be found everywhere. Over the years, it has been observed that microbial resistance to available antimicrobial agents have continued to be on the increase. This has led to the evaluation of potential antimicrobial action and inhibitory and lethal effects of therapeutic agents, and their importance in laboratory procedures Walsh et al. 2003. The population and prevalence of these organisms has been reduced by various methods. The devised methods include disinfection, immunization, sterilization and chemotherapy (Block, 1991). Disinfection is the process of removing microorganisms including potentially pathogenic ones, from the surface of inanimate objects (Block, 2001). The main objective of disinfection is to reduce the number of these pathogenic organisms in a potential source of infection to below what is required to cause infections using chemical agents such as disinfectants, preservatives or antiseptics. An antiseptic is a chemical agent that slows or stops the growth of micro-organisms (germs) on external surfaces of the body and helps to prevent infections. Most chemical agents can be used as both antiseptic and disinfectant. Since microorganisms are ubiquitous, they are present in water, on the skin, and on mucous membranes which makes them capable of causing infections. So, antiseptics and disinfectants are used mainly to reduce levels of microorganisms. They are added to bathing water, used for wound dressing of cuts, abrasions and burns, and other domestic activities such as toilet and general house cleaning Akimitsu *et al*., 1999. The degree of effectiveness of these antiseptics and disinfectants are due to their active ingredients. They are used externally at a dilution recommended by the manufacturer. Disinfectant is an agent that frees from infection, usually a chemical agent but sometimes may be a physical one, that destroys disease or other harmful microorganisms but may not kill bacterial spores. It refers to substances applied to inanimate objects. For convenience, inanimate objects are considered here as including carcasses of animals which have died from contagious diseases, long identified as the principal (or even the sole) source of virulent material (Block, 1991).

MATERIAL AND METHODS.

This study was carried out in the pharmaceutical microbiological laboratory of Faculty of Pharmacy, Delta State University Abraka, Delta State, Nigeria.

Materials and reagents used

Media: Nutrient agar, Nutrient broth, MacConkey, agar, Blood agar, Mueller Hinton agar, peptone water and mannitol agar.(Manufactured by Hi media laboratories Pvt,Ltd, Mumbai, India). Test disinfectant is Tetmosol.

Instruments: Autoclave, Incubator, weighing balance, water bath, microscope, centrifuge and refrigerator.

*Correspondence author: oliseloke@yahoo.com Tel: 08066390868

Identification of microorganisms by biochemical tests

Isolated from environment of Delta State University health Centre, laboratory, washing sinks. Biochemical tests were conducted to identify, distinguish and characterize various microorganisms, using standard technique (Cheesbrough, 2006).

Antibacterial Susceptibility Test by Disc Diffusion Technique.

Mueller Hinton agar was prepared by dissolving 3.8 g in 100 ml of water and sterilized. Allowed to cool and then 19.9 ml of MHA containing 0.1 ml of the respective overnight culture of the MacFarland standard test organisms was poured into four plates labeled respectively according to the test organisms and left to solidify. A solution of the test antiseptic disinfectant was prepared according to the manufacturer's recommendation by measuring 4.85 ml into 100 ml of sterile water into a beaker. Next sterile filter disc (6mm each) were collected using a sterile forceps and soaked in the test solution, they were then picked and drained of the excess liquid and then placed on each MHA plates and pressed lightly. The procedure carried out in an aseptic environment. The plates were then incubated at 37°C for 24 hrs (Cheesbrough, 2006).

Determination of the susceptibility of the microorganisms using Agar well method

Mueller Hinton agar was prepared and sterilized. After cooling for about 45°C, 20 ml was measured into sterile petri-dishes and allowed to solidify. The test organism was obtained using sterile swab stick and swabbed on the plate. A sterile cork borer was used to bore 6 mm diameter wells in the agar and the various concentration of test disinfectant were used to fill each hole carefully using a sterile syringe. They were left for 30 minutes to diffuse and then incubated for 37°C for 24 hours after which the zones of inhibitions were measured.(Cheesbrough, 2006).

Broth dilution technique:

Double strength nutrient broth was prepared by dissolving 2.6 g in 100 ml of water and sterilized, then 5ml of the double strength nutrient broth was measured into a test tube and labeled A1. Single strength nutrient broth was prepared by dissolving 1.3 g in 100 ml of water and sterilized, then four test tubes were labelledA2 to A5 and 5 ml of the single strength nutrient broth was added into each of them. Next, 5mls of 4.85 % of Tetmosol solution was added to A1 and mixed well. Then 5mls was collect from A1 and transferred to A2 using a sterile syringe and mixed well. This was repeated till A5. A loop full of the test organisms was then inoculated into the respective test tubes (A1-A5) using a Sterile inoculating wire loop and incubated at37°C for 48 hours. This procedure was carried out for each organism respectively in an aseptic environment. The tube containing the lowest concentration of Tetmosol disinfectant that inhibited growth for each test organism was taken as the MIC (Cheesbrough, 2006).

Agar Dilution Technique:

Different concentrations of the Tetmosol disinfectant were prepared and 1ml of each concentration was mixed with 19 ml of Mueller Hinton Agar(prepared by dissolving 3.8 g in 100 ml of water and sterilized),into labeled petri dish soap respectively. The petri dishes were left for a while to solidify and for proper diffusion of the disinfectant. Then overnight culture of the test organism were streaked into respective plates using a sterile wire loop and incubated at 37 °C for 24 hours. The procedure was carried out in an aseptic environment. The lowest concentration of disinfectant that inhibited growth for each test organism was taken as the MIC (Cheesbrough, 2006).

Determination of MIC and MBC

Double strength nutrient broth was prepared (by measuring 2.6 g in 100 ml of water) and sterilized. Additions were made in sterile test tubes to obtain final concentration. The test tubes were then incubated at 37°C for 48 hours and observed for growth. The procedure was carried out in an aseptic environment. The test-tube with the lowest concentration of Tetmosol that inhibited growth was taken as the MIC. While MIC was used to carry out the MBC. The MBC was the least concentration where there was no growth.

Determination of the MIC and MBC of Tetmosol using agar dilution method:

Double strength nutrient agar was prepared (by measuring 5.6 g in 100 ml of water and sterilized then it was allowed to cool to about 45°C,and additions were made in sterile petri-dishes to obtain the final concentrations. The petri-dishes were left to solidify then 0.1 ml of the test organism was added into the various plates using a sterile syringe. The petri-dish soap were incubated at 37°C for 24 hours and growth was observed. This procedure was carried out in an aseptic environment. The petri-dish that has the lowest concentration of Tetmosol that inhibited growth of the test organism is taken as the MIC.

Growth rate of the microorganisms

An overnight culture of the test organisms was diluted with sterile water to a concentration of 10-7 into a sterile test tube. 1 ml was then taken and added to sterilized molten nutrient agar 19mls and

rocked together in the petri-dish. The plate was then labeled 0 hr.The procedure was repeated at intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hours for each organism and the plates labeled respectively. The petri dish soap we're incubated at 37°C for 24 hours and distinct colonies indicating growth were counted.

In-Use Dilution (Killing Time)

A Ten (10) liter bucket was filled with water and 12 ml of Test disinfectant was added according to the manufacturer's recommendation. Then 1ml of the solution was measured immediately into sterile plate and 19 ml of molten nutrient agar was added to the plate labeled at zero (0) hour. The plate was rocked gently and left to solidify. This procedure was repeated at contact times of 3,5,10,20 and 30 minutes respectively and the procedure was carried out in duplicates in an aseptic environment. The plates were then incubated at 37°C for twenty-four hours.

Phenol coefficient test:

Twenty sterilized test tubes were placed in five rows and four columns, and they were labeled A1-A4,B1-B4,C1-C4,D1-D4,E1-E4. Five (5 ml) of sterilized nutrient broth was measured into another 5 test tubes. Stock solutions of 1/70,1/80,1/90,1/100,1/110 of the Phenol were prepared and 1ml each measured into sterile test tubes and labeled A,B,C,D,E respectively. Next 1ml of the *Salmonella typhi* was measured using a sterile syringe and 0.2 ml was put into tubes A-E respectively at time intervals of 30 seconds from the first inoculation. A wire loop was then used to inoculate the mixture into corresponding test tubes at time intervals of 30 seconds.(i.e a loop full from tube A into test tube A1,tube B into test tube B1,tube C into test tube C1,tube D into test tube D1,tube E into test tube E1,tube A into test tube A2 and continued until E4 was inoculated, all at time intervals of 30 seconds from the first inoculation. The test tubes were covered and incubated at 37°C for 24 hours and growth was observed.

Phenol coefficient test of the Tetmosol disinfectant.

The same procedure was repeated for test disinfectant using 20 sterile test tubes labeled K1-K4,L1-L4,M1-M4,N1-N4,O1-O4. Stock solution of test disinfectant was also prepared 1/600,1/800,1/1000,1/1200 and1/1400 in sterile test tubes labeled K,L,M,N and O respectively.

RESULTS

Microbial resistance to commonly used disinfectants within the community, hospital and Industry settings has become emerging public health worry. The Tetmosol had different activities on various microorganisms in broth and agar media, where *S.aureus* had the same growth result at the concentrations of 0.60625 %v/v and 1.2125 %v/v but not in 4.85 %v/v and 2.425 %v/v concentrations in both broth and agar medium. While *Streptococcus pyogenes, Pseudomonas aeruginosa and Escherichia coli* only had growth at 0.60625 %v/v, but not at other concentrations in broth medium, but in the agar medium growth was observed at 0.60625 %v/v and 1.2125 %v/v concentrations, except *Streptococcus pyogenes,* where growth was observed at 2.425 %v/v as shown in Tables 1 and 2.

Table 1: Effect of Tetmosol on the microorganisms using broth medium.

Microorganism	Concentration			
	4.85 %v/v	2.425 %v/v	1.2125 %v/v	0.6062 5%v/v
Staphylococcus aureus	_	_	+	+
Streptococcus pyogenes	_	_	_	+
Pseudomonas aeruginosa	_	_	_	+
Escherichia coli	_	_	_	+

+ = growth,. = no growth

Table 2: Effect of Tetmosol on the microorganisms using agar medium.

Microorganism	Concentration			
	4.85 %v/v	2.425 %v/v	1.2125 %v/v	0.60625 %v/v
Staphylococcus aureus	_	_	+	+
Streptococcus pyogenes	_	+	+	+
olPseudomonas aeruginosa	_	_	+	+
Escherichia coli	_	_	+	+

Key :+=growth,—=no growth

The MIC of Tetmosol (Table 3) using broth technique was 1.2125 %v/v, 0.60625%v/v, O.60625 %v/v, 0.60625 %v/v for *Staphylococcus aureus, Streptococcus pyogene, Escherichia coli, Pseudomonas aeruginosa* respectively with MIC using the agar technique was 2.425 v/v% of *Staphylococcus aureus, Streptococcus pyogene, Escherichia coli, Pseudomonas aeruginosa* respectively while the MBCs are higher as depicted in Table 3 .

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of Tetmosol disinfectant against the various organisms

Microorganism	MIC		MBC	
	Broth medium	Agar medium	Broth medium	Agar medium
Staphylococcus aureus	1.2125	2.425	2.425	2.425
Streptococcus pyogenes	0.60625	2.425	1.2125	4.85
Pseudomonas aeruginosa	0.60625	4.85	1.2125	4.85
Escherichia coli	0.60625	2.425	1.21625	2.425

The IZD of the various organisms are different with the *Pseudomonas aeruginosa* the lowest 10 mm,

Staphylococcus aureus 11 mm, *Escherichia coli* 12 mm and highest being *Streptococcus pyogenes* 14 mm as shown in Table 4.

Table 4: Zone of inhibition of tetmosol against the organisms using disc diffusion method

Microorganism	Zone of inhibition
Staphylococcus aureus	11
Streptococcus pyogenes	14
Pseudomonas aeruginosa	10
Escherichia coli	12

Concentration of Test used =4.85 %v/v

Results for the various test microorganisms indicates growth from time 0 hr–1 hr which indicates the lag phase, then there was increase in growth from 1 hr–2 hrs was observed indicating the exponential phase, thereafter was static growth from 2 hrs–6 hrs which indicates the stationary phase, and successive decrease in growth was observed after 6 hrs.

Table 5: Growth rate of the microorganisms.

Time	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*E.coli*
	Average number of colonies	Average number of colonies	Average number of colonies	Average number of colonies
0 hr	41	127	44	50
1 hr	45	127	45	51
2 hr	100	297	118	98
3 hr	99	298	110	100
4 hr	98	297	112	103
5 hr	98	296	111	97
6 hr	56	152	67	52
7 hr	34	198	37	34
8 hr	21	60	23	35

Dilution factor=10^{-7}

From the results (Table 8), bathing water containing test disinfectant Tetmosol showed that at different contact time different levels of efficacy (at 0 min ,there was growth and at 3 mins there was growth also but at 5 mins no growth was observed. Therefore, 5 mins is the killing time.

Table 6: In-use dilution; Killing time.

Time	Plate 1	Plate 2
0 min	+	+
3 mins	+	+
5 mins	_	_
10 mins	_	_
20 mins	_	_
30 mins	_	_

Key :+ = growth, - = no growth

The results (Table 7) indicate that the highest dilution factor of Phenol killing *salmonella typhi* in 7.5 minutes but not 5 minutes is 110 at tube E

Table 7: Phenol Coefficient of test disinfectant

Tube	A (1/70)	B (1/80)	C (1/90)	D (1/100)	E (1/110)	Time (min)
1	_	+	_	+	_	2.5
2	_	_	_	+	_	5.0
3	_	_	_	_	_	7.5
4	_	_	_	+	_	10

The results indicate that the highest dilution factor of Tetmosol killing *Salmonella typhi* in 7.5 minutes but not 5 minutes is 1000 at tube M. The phenol coefficient of test antiseptic disinfectant, given as the highest dilution factor of test antiseptic killing *salmonella typhi*in 7.5 minutes but not 5 minutes divided by the dilution factor of phenol having the same effect, was at M_3/E_3 (1000/110) that is 9.1. This shows that Tetmosol is 9.1 times more potent than phenol.

Table 8: Phenol Coefficient of Tetmosol for disinfectant

Tube	K (1/600)	L (1/800)	M (1/1000)	N (1/1200)	O (1/1400)	Time (min)
1	_	+	+	_	+	2.5
2	_	_	_	+	+	5.0
3	+	+	_	+	+	7.5
4	_	_	_	_	+	101

Key: +=growth = no growth

DISCUSSION

The results from the research carried out on the antibacterial activity of Tetmosol disinfectant an antiseptic commercial brand used commonly in the disinfection of bathing water against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa*and *Escherichia coli* showed that test disinfectant is active as a disinfectant with broad spectrum of activity when used as directed by the manufacturers dilution of 2 caps full(equivalent to 12 ml) in 10 litres of water at contact time of less than 5minutes as depicted in Table 6. This research ascertains that the concentration of the Tetmosol affects the efficacy of the Tetmosol. This is further corroborated by the MIC and susceptibility test results. These results indicated that the effect of the disinfectant dependent on the concentration used, that is, an increase in the concentration of disinfectant produced an increasing effect on the microorganisms from the MIC gotten from the broth dilution and agar technique, minor variations were observed with the broth dilution being a little lower. The reason for these variations may be due to factors such as the medium and the nutrient present in the medium which differ. Results of the MIC vary because the organisms are different and their susceptibility to biocide varies between different groups and species of microorganism (Russell, 2004).The minimum Bactericidal Concentration (MBC) which was gotten from the MIC of each Tetmosol against *Staphylococcus aureus, Streptococcu spyogenes, Pseudomonas aeruginos a*nd *Escherichia coli.* The MIC concentration does not actually kills the bacteria present already in d medium but the concentration that will inhibit growth. However, the minimum Bactericidal concentration is the concentration of the disinfectant that kills the bacteria. The MBC

values of the disinfectant is higher compared to that of the MIC and this is agreeable with similar studies carried out (Ashley, 1983). From the study carried out on the growth rate of the various test microorganisms as shown in Table 5 (*Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli*) it shows that the condition of the organisms affects the lag phase, the lower the minimum inhibitory concentration (Bacanova, 2008).The log phases of the test organisms shows that the cells are dividing at constant time, whereas depletion in the static stationary phase indicates loss of essential nutrient there by resulting in cell death. In the course of this investigation (Table 4), *Pseudomonas aeruginosa* was shown to be the most resistant organism to test disinfectant Tetmosol with no zones of inhibition below the manufacturer's recommendation during susceptibility testing to the disinfectant. This study agrees with previous researches you which indicates *Pseudomonas aeruginosa* as one of the organisms implicated in nosocomial infection outbreaks in hospitals especially in the intensive Care unit. *P. aeruginosa* isolates from ICU patients also trend toward higher rates of β-lactam resistance than general trends for hospitalized patients and it's susceptibility is limited to few antimicrobial agents, it has constantly be reported to show resistance not only to antibiotic but disinfectant also (Olayemi and Obayen, 1994, Singleton, 1999, McCracken and Cawson, 1983). From the result gotten from the phenol coefficient of Tetmosol disinfectant which was given as the highest dilution factor of Tetmosol killing *Salmonella typhi* in 7.5 minutes and not 5 minutes divided by the dilution factor of phenol having the same effect was 9.1. This shows that tetmosol is 9.1 times more potent than phenol.

CONCLUSION

From the investigation carried out, it can be concluded that the claims of the company or label of Tetmosol disinfectant having a broad spectrum of microbial activities at the manufacturer's recommended dilution has been tested using microorganisms that are commonly found in our environment; two gram positive organisms (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two gram negative organisms (*Pseudomonas aeruginosa*and *Escherichia coli),* the disinfectant was shown to be active against both gram positive and gram negative organisms. The manufacturer's claim of test disinfectant Tetmosol to be effective within 5 minutes has been verified as the disinfectant was effective at 5 minutes contact time against the microorganisms.

REFERENCES

Walsh SE, Maillard j, Russel AD, Catrenich CE, Chabanneau DL,Bratolo RG(2003).Development of bacterial resistance to several biocides and effects on antibiotic susceptibility *J Hosp Infect***.** 55(2):98-107.

Block SS (1991).Disinfection, sterilization and preservation (4th Edn.)Lea and Febiger.

Block SS (2001). Disinfection, sterilization and preservation"(5th Edn.) Lippincott Williams and Wilkins Philadelphia and London.

Akimitsu N,Hamamoto H, Inoue RI, shoji M,Akamine A,Takemorikl, Hamasaki N, sekimizu K (1999). Increase in Resistance of methicillin-resistant Staphylococcus aureus to B-lactamams caused by mutation conferring resistance to benzaalkonium chloride, a disinfectant widely used in hospitals " Antimicrobial Agents chemotherapy 43:3042-3043

Rutala WA (1997).Disinfection, sterilization and waste disposal prevention and control of Nosocomial infections. wenzel R.P.,ed. Baltimore: Williams and wilkins. 539-93.

Joswick HL, Corner TR, Silveenale J N, Gerhardt P (1971) - Antimicrobial actions of hexachlorophene: release of cytoplasmic materials. Journal of Bacteriology. 108: 492-500

Russell AD (2004). Factors influencing the efficacy of germicides. In: Rutala WA, ed. Disinfection, sterilization and antisepsis: Principles, practices, challenges, and new research. Washington DC: Association for Professionals in Infection Control and Epidemiology.162-70.

Gillis RJ, Schmidt WC(1983). Scanning electron microscopy *of* spores *on* inoculated product surfaces.MD 1983*:*46-9.MD:46-9

Russell AD(1998). Bacterial resistance to disinfectants: Present knowledge and future problems. J. Hosp. Infect. 43:S57-S68

Russell AD, Russell NJ (1995). Biocides: activity, action and resistance. In: Hunter PA, Darby GK, Russell NJ, eds. Fifty years of antimicrobials: past perspectives and future trends. England: Cambridge University Press, 327-65.

Rutala WA, Cole EC (1987). Ineffectiveness of hospital disinfectants against bacteria: a collaborative study. *Infect. Control* 8:501-6.

Russell AD, McDonnell G. (2000). Concentration: a major factor in studying biocidal action. J. Hosp. Infect. 44:1-3.

Rutala W.A (1999) Selection and use of disinfectants in healthcare. In: Mayhall CG, ed. Infect. Control and Hosp. Epidemiol. Philadelphia: Lippincott Williams & Wilkins,1161-87.

Lewis D.L, Arens M(1995). Resistance of microorganisms to disinfection in dental and medical devices. *Nat. Med*. 1:956-8.

Rideal S, Walker J T A (1903).The standardisation of disinfectants" J. R. Sanit. Inst .24 : 424–441.

Andrews J M (2001). Determination of minimum inhibitory concentrations".Journal of Antimicrobial Chemotherapy. 48: 5–16.

Singleton P (1999). Bacteria in Biology,Biotechnology and Medicine (5th ed.). Wiley. 444–454.

Ashley KL (1983). The antibacterial properties of two commonly used Mouth washes Corsodyl and oraldine Journal of *Applied Bacteriology*. 56:221–225

Bacanova (2008). Growth rate of test microorganisms. Available at: http://www.ifr.ac.uk/bacanova/project_backg.htm