African Journal of Biomedical Research, Vol. 6; 91 – 94 (2003)

Original article

THE EFFECT OF A FIXED NON-MINERAL OIL ON THE ANTIBACTERIAL ACTIVITY OF AMPICILLIN TRIHYDRATE AGAINST RESISTANT CLINICAL STRAINS OF *STAPHYLOCOCCUS AUREUS*.

O.E ADELEKE* and J.O. OLAITAN

Microbiology Department, University of Agriculture, Abeokuta, Nigeria

The effect of King's Vegetable oil, a fixed non-mineral oil, on the antibacterial activity of ampicillin trihydrate, a water- insoluble form of ampicillin, was investigated against resistant clinical strains of Staphylococcus aureus. In the agar-diffusion method employed, 40% of the resistant clinical strains tested showed sensitivity to different oil-dispersed concentrations of ampicillin trihydrate, which ranged from 0.06µg/ml to 1.25µg/ml. The resistant strains were among the clinical strains detected with Beta-lactamase. This finding is presented as a preliminary report on the potentiality of employing an oil medium to effect a "cure" of antibiotic resistance in staphylococci besides the use of acridine dyes, ethidium bromide, ultraviolet radiation and other measures.

Keywords: King's vegetable oil, ampicillin trihydrate, staphylococcus aureus,

**Author for correspondence*

INTRODUCTION

Ampicillin trihydrate is a water-insoluble form of ampicillin for its activity against sensitive strains of *S. aureus* (Hugbo and Ruczaj, 1983). The poor water solubility profile of ampicillin trihydrate (Clarke,1986) implicates a poor antibacterial activity due to lack of effective concentration of drug molecules across the bacterial cell membrane. In a previous study (Adeleke and Agunbiade, 1991), ampicillin trihydrate dispersed in an oil medium was associated with a greater antibacterial activity against sensitive strains of *S.aureus*, than water-dispersed ampicillin trihydrate and the water-soluble form of ampicillin (ampicillin sodium) both in tube broth-dilution and agar-diffusion methods. These results were predicated upon reports of preferential distribution of some microorganisms and water-insoluble antimicrobial compounds into oily organic phase relative to aqueous phase, in partioning coeeficient based distribution experiments (Adeleke, 1991). The minimal water content of fixed non-mineral oils (Diem and Letner, 1974) was among the reasons suggested for the better antibacterial activity.

Resistance of *S. aureus* is atttributed largely to transducible R- plasmids which mediate production of Beta-lactamase in the resistant strains (Davis *et al*., 1973). The inactivation process of Beta-lactamase on susceptible Beta-lactam antibiotics such as ampicillin, is hydrolysis (Franklin and Snow, 1975). Application of oil in the dispersion of a water-insoluble antibiotic would be expected to avoid the hydrolytic reaction, thereby, facilitating the anti-bacterial activity of the compound. This property could therefore, find application in the elimination or "curring" of bacterial resistance to antibiotics that are susceptible to hydrolysis by Beta-lactamase, as an alternative to elimination with acridine dyes and ethidium bromide, some of which have mutagenic effects on bacterial cells (Bouanchaud *et al*. 1969).

In the present study, the effect of King's Vegetable oil, a fixed non-mineral oil, on the antibacterial activity of ampicillin trihydrate, a water- insoluble form of ampicillin, was investigated against resistant clinical strains of *Staphylococcus aureus*. This is with a view to finding out the potentiality of

employing an oil medium to effect a "cure" of antibiotic resistance in staphylococci besides the use of acridine dyes, ethidium bromide, ultraviolet radiation and other measures

MATERIALS AND METHODS

Microorganisms: Fifty (50) clinical strains of *S. aureus* and a standard strain,NCTC 6571,were used in this study. The clinical strains were isolates from specimens of different clinical manifestations obtained from the university College Hospital, Ibadan. All the strains were confirmed coagulase and deoxyribonuclease positive as well as fermentative in mannitol, both under aerobic and anaerobic incubations. They were maintained as purified cultures on tryptone soya agar (OXOID) slants and subcultured periodically.

Fixed non-mineral oil: King's vegetable oil (bleached palm olein), Devon Industries SDN RHD RS, was obtained locally. It was sterilised by dry heat in an oven sterilizer at 160^{o}C for 1hr.

Multodisk^{R} Agar-diffussion test: As specified for staphylococci (Brown and Blowers, 1978), a 4mm loop-full of an overnight nutrient broth (OXOID) culture of each strain of *S. aureus* was mixed with 3ml. of sterile nutrient broth, for a 1:1000 dilution of the culture. The mixture was used to flood a plate of dried Seed Agar antibiotic medium I (BBL) to form an overlay and excess of the mixture was siphoned out. A strip of Multodisk^{R} (OXOID) was placed on the medium followed by incubation at 37^{o}C for 24hrs. The multodisk^{R} heads carried penicillin G (1.6 i.u or 1µg), ampicillin (2µg), cloxacillin (5µg) and erythromycin (10µg).

Screening for Beta-lactamase production: All the strains of *S. aureus* were screened for the production of Beta-lactamase enzyme by the iodometric cell-suspension method (Sykes, 1978). The cell population in every cell-suspension prepared in phosphate bufferd penicillinG was estimated at10^{9} cells per ml.on Mcfarland turbidity standards (Bauer *et al*., 1966).

MIC of ampicillin trihydrate in an oil medium: Sterile distilled water served as a medium of transference of ampicillin trihydrate into sterile oil by removing 5ml. of aqueous 1,000µg/ml.concentration into 45g of sterile oil to give 100µg/ml.from which graded decreasing concentrations were prepared in nutrient broth. Each mixture was inoculated with 0.1ml.of 1:100 dilution of overnight broth culture of *S. aureus* NCTC 6571 and shaken on a vortex Mixer (Griffin) followed by incubation with appropriate controls at 37^{0}C for 24hrs.

Similar determination was carried out using sterile distilled water entirely as the dispersion medium for ampicillin trihydrate.

Agar-diffusion (well-in-seeded plate) test: Selected resistant clinical strains were exposed to three successively high concentrations of ampicillin trihydarte in oil medium including the MIC obtained for the standard strain, NCTC 6571. Wells were cut in duplicates in the seeded plate of each strain. Each well was filled with 0.1ml. of x10 of a particular concentration so as to obtain the required concentration in well. For instance, 0.1ml. of 0.6µg/ml. of oil-dispersed ampicillin trihydrate was filled into each of two wells to give a final concentration of 0.06µg/ml. The other two concentrations used were 0.125µg/ml and 1.25µg/ml. The plates were incubated after allowing a pre-incubation diffusion period of 2hrs at 37^{o}C for 48hrs.

RESULTS

Forty-seven (94%) of the 50 clinical strains of *S..aureus* were resistant to penicillin G and ampicillin while 16 (32%) of the strains were sensitive to cloxacillin. All the strains but one was sensitive to erythromycin. Most of the resistant strains were from pyoderma which also represented 42% of the specimen collection sources. Beta-lactamase was detected in 80% of the 47

resistant strains, leaving only a strain without the enzyme.

The minimum inhibitory concentration (MIC) obtained for ampicillin trihydrate dispersed in oil medium against *S. aureus* NCTC6571, was 0.06µg/ml. against 0.25µg/ml. for aqueous ampicillin trihydrate (Table 1). In the subsequent Agar-diffusion (well-in-seeded plate) sensitivity test using 0.06, 0.125 and 1.25µg/ml.in oil medium against selected resistant clinical strains, sensitivity of 40% of the strains test was recorded to one or more of the three concentrations (Table 2).

DISCUSSION

The preponderance of Beta-lactamase producers among the resistant clinical strains of *S. aureus* underscores the menace associated with the implication of B-lactamase in bacterial resistance to susceptible antibiotics (Oyelese and Oyewo, 1995). This observation further paints an apprehensive picture with only 16 of the 47 resistant strains showing sensitivity to cloxacillin, a Beta-lactamase stable penicillin antibiotic. However, while virtually all the strains were sensitive to erythromycin, a macrolide antibiotic, it is interesting to note that 40% of the resistant clinical strains tested for elimination of antibiotic resistance, to different concentrations of ampicillin trihydarte in oil medium, reverted to sensitive strains. This result suggests the potentials of using fixed non-mineral oils, especially King's Vegetable oil, in the elimination of staphylococcal resistance to Beta-lactamase sensitive antibiotics such as the parent penicillins and some semi-synthetic Beta-lactam antibiotics. This would be a radical departure from the use of "traditional" curring agents such as acriflavine, acridine orange, mepacrine and ethidium bromide (Bounchaud *et al*., 1969; Wantanabe and

Table1
MIC of ampicilin trihydrate B. P. in aqueous medium and oil medium against *S. aureus* NCTC 6571

Type of medium	Antibiotic concentration (µg/ml.)						MIC (µg/ml.)
	1	0.5	0.25	0.125	0.0625	0.031	
Aqueous medium	-	-	-	+	+	+	0.25
Oil medium	-	-	-	-	-	+	0.065

KEY: MIC = minimum inhibitory concentration; NCTC = National collection of typed cultures
+ = growth; - = no growth

Table 2
Agar-diffusion test on resistant clinical strains of *S. aureus* with oil-dispersed ampicillin trihydrate

*Strain of *S. aureus*	Antibiotic concentration (µg/ml.)			Oil (5g/ml.)
	0.06	0.125	1.0	
4611	-[a]	12mm**	15mm	-[b]
6370	15mm	15.5mm	16.6mm	-
1096	-	-	-	-
4747	-	-	-	-
5843	13mm	14mm	-	-
UR-0F	-	-	-	-
2018	-	-	-	-
700	-	-	-	-
1566	-	-	-	-
14.5mm	14.5mm	16.5mm	18mm	-

KEY: ** = Zone of inhibition in millimetres; a = No zone of inhibition; b = No growth in oil
*= Strain of *S. aureus* with resistance to penicillinG, ampicillin, cloxacillin and erythromycin.

Fukasawa, 1960d; Watanabe and Fukasawa, 1961b; Levy and Watanabe, 1966). Some of these agents such as ethidium bromide are mutagenic (Bouachaud *et al.,* 1969; Naomi, 1978), as such, their curring effect only constitutes an evidence but not a proof that a resistance is plasmid mediated (Naomi, 1978). Moreover, the curring effect of these agents on resistant bacteria is yet to be associated with application in clinical situations. Interestingly, metabolisable fixed non-mineral oils are commonly used in the formulation of small-volume oily injections (Allwood, 1983).

REFERENCES

Adeleke, O. E. (1991). Fixed oils as microbial dispersion and susceptibilityn testing media. An M. Sc. Thesis in the Department of Pharmaceutical Microbiology, University of Benin, Nigeria.

Adeleke, O. E. and Agunbiade, R. A.(1999). A fixed non-mineral oil in broth dilution and Agar-diffusion for evaluating antibacterial activity of ampicillin trihydrate against sensitive strains of *Staphylococcus aureus*. Afr. J. Biomed. Res. 2:69-73.

Allwood, M.C. (1983). Sterile Pharmaceutical products. In: Hug, W.B. and Russel, A.D., (eds) Pharmaceutical Microbiology 3rd edn. Blackwell Scientific Publications, Oxford, pp. 386-401.

Bauer, A W., Kirby, W. M., Sheris, J. C. and Turck, M (1966) Antibiotic susceptibility testing by a standardised single disc method Am. J. of Clin. Path. 45: 149-158.

Bouaanchaud, H. A., Scavizzi, M. R. and Chabbert, Y. A. (1969). Elimination by ethidium bromide of antibiotic resistance in Enterobacteria and Staphylococci. J. Gen. Microbiol. 54: 417-425.

Brown, D. and Blowers, R. (1978). Disc method of sensitivity testing and other semiguantitative methods. In: Reeves, D. S. Philliphs, I. Williams, J. D. and Wise, R. (eds.). Laboratory methods in antimicrobial chemotherapy. Churchill Livingstone Edinburgh, London and New York.

Clarke, (1986) Clarke's Isolation and Identification of Drugs in pharmaceuticals, body fluids, and postmortem materials. 2nd edn. The pharmaceutical Press, London.

Davis, B. D., Dulbecco, R. Eisen, H. N. Ginberg, H. S. and Wood, W. B. (1973) Microbiology. 2nd edn. LondonandNewYork-HarperandRow.

Diem, K and Letner, C. (1974). Geigy Scientific Tables GEIGY PHARMACEUTICALS, CIBA-GEIGY CORPORATION Ardsley New York. Pp.506-510.

Franklin, T. J. and Snow, G. A.(1975). BiochemistryofAntimicrobial action, 2nd edn. Chapmanand Hall, London.

Hugbo, P. G. and Ruczaj, Z. (1983). Five decades of Beta-lactam antibiotics, West African J. Pharmacol. and Drug Res. 6(1): 69-93.

Levy, S. B. and Watanabe, T. (1966). Mepacrine and transfer of R-factor (correspondence). Lancet 11:1138.

Naomi, D. (1978). Plasmid determined drug resistance In: Reevees, D. S.; Phillips, I. Williams, J. D. And Wise, R.(eds.), Laboratory methods in antimicrobial chemotheraphy. Churchill Livingstone Edinburg London and NewYork, pp.56-59.

Oyelese, A. O. and Oyewo, E. A. (1995). The menace of Beta-lactamase production on antibiotic prescription in community acquired infections in Nigeria. Afr. J. Med. Sci. 24: 125-130

Sykes, R. B. (1978). Methods for detecting Beta- lactamase. In: Reevees, D. S.; Phillips, I., Williams, J. D. And Wise, R.(eds.), Laboratory methods in antimicrobial chemotheraphy. Churchill Livingstone Edinburg London and New York, pp.44-75.

Watanabe, T. and Fukasawa, T. (1960a). Episome resistance factors in Enterobacteriaceae. III Elimination of resistance factors bytreatment with acridine dyes. (In Japanese). Med. Biol. (tokyo) 56:71-74.

Watanabe, T. and Fukasawa, T. (1961b).Episome mediated transfer of drug resistance in Enterobacteriaceae. III Elimination of resistance factors by treatment with acridine dyes. (In Japanese). Med. Biol. (Tokyo) 56:71-74.

Received: May 2001
Accepted in final form: February 2003