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Original Research Article

Effect of storage on the Time- Kill Qualities of Ampicillin

Udobi, Chinweizu Ejikeme* and Otudoh Chidiebere

Pharmaceutical Microbiology Unit, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria
**Corresponding author*

A B S T R A C T

The attention of researchers has been drawn to the fact that poor storage of ampicillin instead of development of resistance may be responsible for the high level non susceptibility of most organisms to ampicillin observed. This work was therefore, done to study the effect of storing ampicillin under conditions outside that recommended by the manufacturers on its time-kill qualities. Ampicillin capsules stored at different conditions for a period of two months viz-Heat (40-45^0c), Heat dust (Above 45^0c in a dusty environment) and cool (below 25^0c) were used to challenge the growth of *Staphylococcus aureus* NCTC 6571 chosen from amongst a wide range of organisms confirmed to be sensitive to ampicillin because of its observed high susceptibility. Results obtained showed a significant reduction in the killing ability of ampicillin against the test organism over a specified period in conditions exceeding standard storage conditions for ampicillin. This was shown by an increase in bacterial number when the ampicillin stored at above 45^0c was used to challenge the test organism from $3.0x10^3$ to $100x10^5$ and from $4.0x10^3$ to $97.0x10^5$ when that stored between 40-45^0c was used within 24 hours of incubation while those stored at the recommended conditions showed virtually a bacteriostatic effect. Yes, storage of ampicillin at conditions outside the recommended, affects its killing ability negatively.

K e y w o r d s

Storage, Ampicillin, Heat, Killing, *Staphylococcus aureus*, Recommended

Introduction

Infections caused by microorganisms, sometimes, fail to respond to standard treatment resulting in prolonged illness (Lippincott, 2007). This is often attributed to resistance by microorganisms ignoring the possibility of loss of potency caused by poor storage especially in areas where storage facilities are poor.

Generally, pharmaceutical manufacturers recommend that most of their products be stored at a controlled temperature range. When storage conditions exceed or go below these ranges, medicines will either change physically, loose potency or even break down to unwanted products with potential harmful effects. If medicines are not properly stored, they may not work in the way they were intended and so pose a potential risk to the health and well being of the persons receiving the medicines (Rhodes and Kommanaboyina, 1999).

In most parts of Nigeria and a greater part of sub Saharan Africa, The temperature for most months of the year will be up to 30^0c. The corners of the room and cupboards or wherever these medicines are kept may be even hotter. Medicines meant to be stored at temperatures lower than this are most likely to be affected. When such medicines are used to challenge organisms, their inability to either kill or inhibit their multiplication will be thought to be due to resistance development by the organisms.

The storage temperature of medicines is one of the most important factors that can affect its stability. Some products can be irreversibly degraded within a brief period of temperature change (WHO, 2003). During heat waves for instance, storage locations can heat up above recommended ranges causing medicines to degrade and possibly lose potency or breakdown into unknown products with potential harmful effects.

Ampicillin is a beta Lactam antibiotic that belongs to the Penicillins which is a group of naturally occurring and semi-synthetic antibiotics (Udobi and Onaolapo, 2008). It is active against both Gram positive and Gram negative organisms. Its mode of action is by the prevention of the incorporation of N-acetyl muramic acid into the mucopeptide layer of the cell wall thereby preventing the completion of this important component of the wall which bacteria will require for its rigidity (Dale and Rang, 2007). Ampicillin has been used extensively for the treatment of bacterial infections since 1961 but resistance to this agent by most organisms has been extensively reported since then.

While not doubting that resistance to ampicillin has been developed by a wide range of organisms through different processes, this work attempts to establish that poor storage of ampicillin may have contributed to the high level of non susceptibility reported and subsequent ailment prolongation when ampicillin is used for therapy.

Materials and Methods

The study was carried out in the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy of the University of Uyo, Nigeria

The Ampicillin capsules used were obtained from a registered Pharmacy shop in Uyo, Akwa Ibom state, Nigeria where they were properly stored and maintained under proper conditions. They were kept for a period of two months under three different conditions namely:

A= cool-------Below 25^0c
B=heat ---------Between 40 and 45^0c
C=heat dust------------- above 45^0c and in a dusty environment

A wide range of organisms which include standard and clinical isolates of *Staphylococcus aureus*, *Escherichia* coli, *Pseudomonas aeruginosa* and *Bacillus subtilis* were used. The clinical isolates were obtained from the Microbiology Laboratory of the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria while the Standard isolates were obtained from the stock of organisms in the Pharmaceutical Microbiology laboratory, Faculty of Pharmacy, University of Uyo. The organism that showed the best activity with ampicillin was eventually selected as the test organism for the time-kill study.

Preparation/Preservation of Test Organism

The test organism *Staphylococcus aureus*

NCTC 6571 selected based on its high susceptibility to ampicillin was preserved by culturing on nutrient agar slants and stored in the refrigerator at 4^0c. They were however, constantly sub- cultured at regular intervals to maintain the organisms in a pure state.

Susceptibility Test

The Agar-plate diffusion method was used for this test. 20ml of molten nutrient agar was aseptically dispensed into sterile nutrient agar plates and allowed to solidify. A sterile spatula was used to make a longitudinal ditch across the solid agar after the test organism had been introduced and spread across the plate using a sterile spreader. 0.1ml of 250mg/ml of ampicillin trihydrate solution was introduced into the ditch and allowed one hour diffusion time at room temperature. The plate was then incubated at 37^0c for 24 hours after which the zone of inhibition was measured.

Time – Kill Assay

Four bottles each containing 9mls of sterile nutrient broth were labelled A B C and D. To each of bottles A B and C was correspondingly added 1ml of 250mg/ml of ampicillin solutions A B and C made by dissolving the stored ampicillin samples in water. A drop of an overnight culture of the test organism was added to the tubes A,B and C while D served as the control(With no organism added). The bottles were incubated in a water bath at 37^0c for 24hours. In between, viable counts were taken at intervals by withdrawing 0.1ml of the mixture in the bottle and diluting in sterile water. Dilutions were then plated out on nutrient agar plates and incubated at 37^0c for 24 hours. Colonies were counted and colony forming units calculated.

Results and Discussion

Selection of Test Organism

Staphylococcus aureus NCTC 6571 was selected as the test organism for the time-kill assay because it was found to have the best activity with Ampicillin when compared with all the organisms tested. (Table 1)

The conditions under which ampicillin is manufactured and stored can have a major impact on their quality (Kiyingi and Laumo, 1993). According to WHO directives, ampicillin capsules should be stored in a cool and dry place at a temperature not exceeding 25^0c unless otherwise specified. This will help the product to maintan its potency and ability to inhibit bacterial cell wall function. (Kays *et* al, 1999).

Nine different organisms (Both clinical and standard) confirmed to be sensitive to ampicillin were equally challenged with the test agent(ampicillin).*Staphylococcus aureus* NCTC 6571 was found to be most susceptible and was therefore chosen as the test organism (Table 1).

Results obtained from the time-kill studies showed an increase in the number of colonies with time instead of a decrease when ampicillin stored at temperature exceeding stipulated storage temperature for ampicillin was used to challenge the growth of test organism. More colonies were observed where the drug was exposed to heat and dust at the temperature above 45^0c. This must have been brought about by loss in the ability to kill or inhibit the organism over time resulting from exposure to conditions not favourable for maintaining its potency. This is because the same drug had shown good activity before storage (Table 1). Storage of the drug at temperature below 25^0c which falls within the BP standard range of 15°C - 30°C however showed a static (minimal) growth in colonies indicating a bacteriostatic effect when stored under such conditions (Table 2).

Storing medications surely can be very challenging because of the intricacies and the unique requirements that may be involved. The effect of improper storage can be even more devastating. When it brings about loss of activity and efficacy, it can lead to long periods of treatment and stay in hospitals and for people with very serious illness, it can be even more dangerous because medicine that does not work can be a treat to life.

Results of this work confirms that improper storage of ampicillin affects its ability to kill or even retard the growth of the organisms it is meant to control over time (Table 3).

Table.1 Result of susceptibility test showing activity of ampicillin against a range of organisms tested

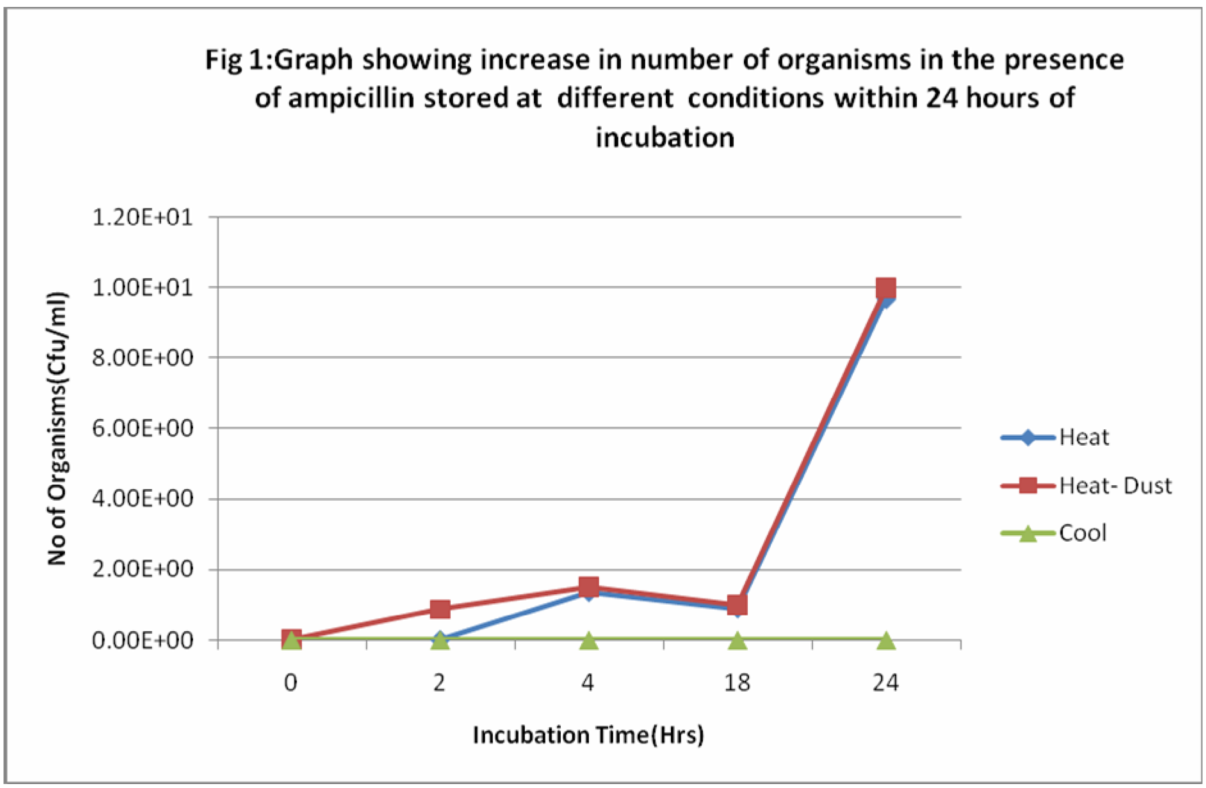
Organism	Zone of Inhibition(mm)
Staphylococcus aureus ATCC 25923	13
Staphylococcus aureus NCTC 6571	23
Staphylococcus aureus (Clinical isolate)	20
Shigella dysentarae Clinical isolate	19
Bacillus subtilis NCTC8553	10
Escherichia coli NCTC 10418	15
Escherichia coli NCTC 25922	5

Table.2 Showing the number of colonies after test organism was challenged with ampicillin stored at different conditions over a 24 hour incubation period.

Dilution	No of colonies at different storage conditions														
	Heat					Heat-Dust					Cool				
	Incubation period(Hrs)					Incubation period(Hrs)					Incubation period(Hrs)				
	0	2	4	18	24	0	2	4	18	24	0	2	4	18	24
10^{-4}	-	1	17	18	19	-	5	20	25	34	-	-	5	3	2
10^{-3}	4	5	74	89	97	3	10	80	98	100	3	-	5	5	4
10^{-1}	NC	6	136	145	148	NC	86	150	165	165	NC	5	5	5	4

Table.3 Showing number of organisms in the presence of ampicillin stored under different conditions within 24 hours of incubation

Incubation Time(Hrs)	No of organisms(cfu/ml) at different storage conditions		
	Heat	Heat-Dust	Cool
0	4.0 $x10^3$	3.0 $x10^3$	3.0 $x10^3$
2	5.0 $x10^3$	8.6 $x10^3$	5.0 $x10^2$
4	13.6 $x10^3$	15.0 $x10^3$	5.0 $x10^2$
18	89.0 $x10^4$	98.0 $x10^4$	5.0 10^2
24	97.0 $x10^5$	100 $x10^5$	4.0 $x10^2$



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