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Drug Resistance as Influenced by Inactivated Sensitivity Discs[1]

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Reports of staphylococci resistant to the semisynthetic penicillins stimulated a study of the factors influencing the stability of the drugs in discs. The behavior of penicillin G, methicillin, oxacillin, cloxacillin, and cephalothin discs under different humidity and temperature conditions is described. Humidity was found to be the most significant factor in drug inactivation. Storage of discs in a vacuum desiccator at −20 C provides maximal antibiotic stability.

The extreme instability of penicillin, especially in dilute solutions, is well known. Humidity and temperature have been considered equally important factors in the storage of penicillin. It has been shown that penicillin in aqueous solution undergoes an appreciable loss of activity in a few days at room temperature and is completely destroyed in a few minutes at 100 C (2).

With the introduction of the β-lactamase resistant, semisynthetic penicillins and the reports of appreciable resistance to these drugs, the question of the stability of the drugs in the sensitivity discs was raised (1, 3). In spite of careful manufacture and packaging, resulting in the general distribution of trustworthy discs, inactivated discs might be responsible for some of the resistance reported.

It was the object of this study to determine the amount of deterioration of the drugs in discs containing penicillin G, methicillin, oxacillin, sodium cloxacillin, and cephalothin under some of the diverse storage conditions possible in the clinical laboratory. This information might define more exactly guidelines for optimal storage and handling of sensitivity discs.

MATERIALS AND METHODS

All sensitivity discs used in this study were obtained from the Baltimore Biological Laboratories and the discs of each antibiotic were selected from the same assayed lot. Concentrations of the discs were: penicillin G, 10 IU; methicillin, 5 μg; oxacillin, 1 μg; cloxacillin, 2 μg; and cephalothin, 30 μg. Prior to use all discs were randomly assayed for uniformity and reactivity. A strain of *Sarcina lutea* ATCC 9341, which was sensitive to 0.002 IU of penicillin G per ml by the minimal inhibitory concentration method, was used for the drug assays. An 18-hr Trypticase Soy Broth culture was used to seed the assay plates. The swab method was used to apply the inoculum.

Storage of the discs to be studied was accomplished at four different temperatures: −20, 4 to 6, 37, and 56 C. In addition, the discs were stored at three conditions of humidity contact.

Condition A (without desiccant). The discs were placed in a 30-ml screw-cap bottle which was placed in a screw-cap pint jar.

Condition B (with desiccant). This was the same as Condition A, except that 25 g of drierite was placed in the pint jar. The 30-ml bottle containing the discs was placed in the desiccant.

Condition C (high humidity). The discs, in an open 30-ml bottle, were placed in a pint screw-cap jar containing 40 ml of water.

The humidity condition, influenced by the temperature, was determined by the direct-reading Electro-Hygrometer (Will Scientific, Inc., Rochester, N.Y.) with the electrode placed in the pint jar used for the disc storage. Sampling of all discs was done in duplicate and the average was determined. At the start of the study, because the deterioration rate was unknown, the discs were assayed twice daily. Later, this was decreased to daily, and during the 5th week only a single sampling was assayed.

RESULTS

Random sampling of the discs prior to testing under the various storage conditions gave the following zone sizes (in millimeters) when assayed with the test organism: penicillin G, 38; methicillin, 28.5; oxacillin, 27; cloxacillin, 27; and cephalothin, 34.4.

During the assay period of 35 days, the discs of the five antibiotics under the three conditions of humidity contact at −20 C showed remarkably little change in zone size, although water was present in the form of ice in storage condition C.

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No antibiotic loss was observed. The stability of the various drugs at −20 C without desiccant is illustrated by Fig. 1.

At 4 to 6 C, with and without desiccant, the antibiotics were stable to nearly the degree demonstrated at −20 C. Some deterioration of the antibiotics occurred when the discs were stored at 4 to 6 C at 80% relative humidity. These changes, which might be significant for long-term storage,

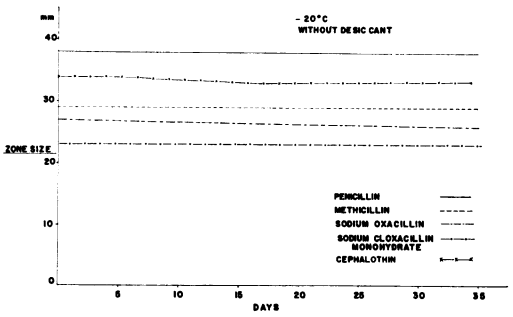

FIG. 1. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at −20 C without desiccant.*

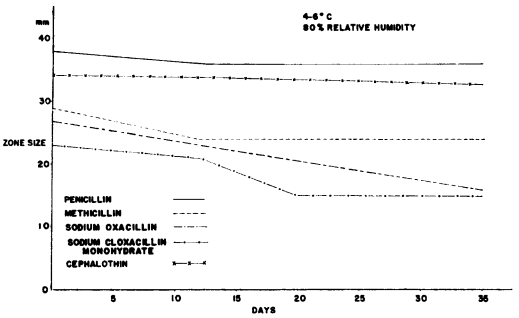

FIG. 2. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at 4 to 6 C and 80% relative humidity.*

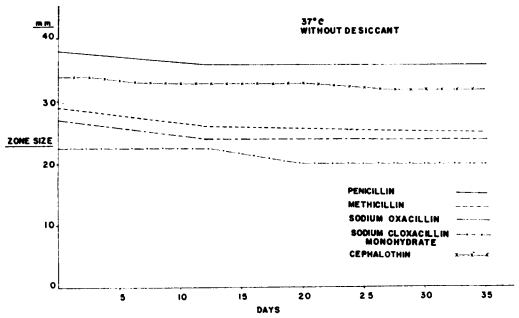

FIG. 3. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at 37 C without desiccant.*

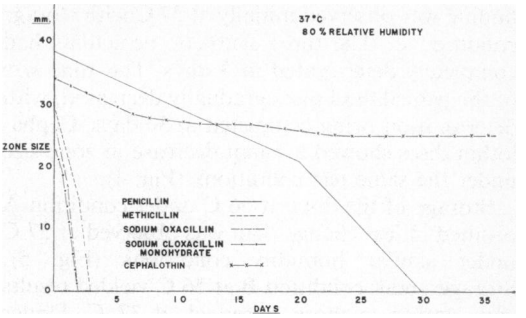

FIG. 4. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at 37 C and 80% relative humidity.*

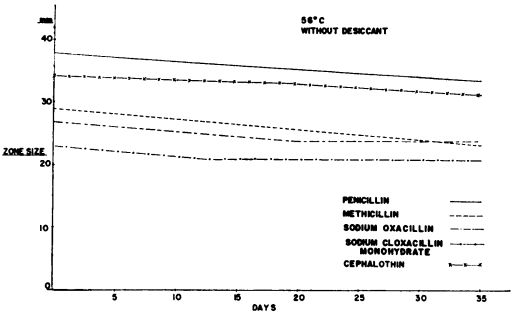

FIG. 5. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at 56 C without desiccant.*

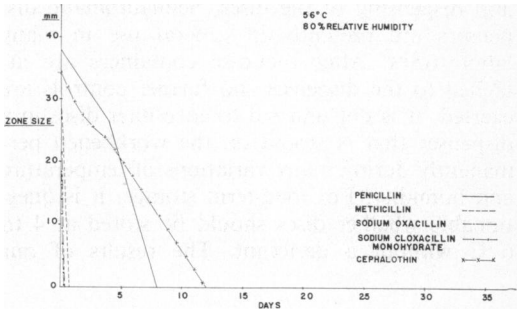

FIG. 6. *Stability of penicillin, methicillin, sodium oxacillin, sodium cloxacillin monohydrate, and cephalothin discs at 56 C and 80% relative humidity.*

are presented in Fig. 2. Cephalothin and penicillin G were most stable under these conditions.

At 37 C under storage condition A, little significant change was recorded in zone size during the assay period (Fig. 3). Under condition B, somewhat more deterioration occurred, possibly because of some moisture uptake by the desiccant, with subsequent reaction with the antibiotics. The major change and significant

finding was observed initially at 37 C with storage condition C. The three synthetic penicillins had completely deteriorated in 3 days. The zone size of the penicillin G discs gradually decreased, with deterioration being completed in 30 days. Cephalothin discs showed a 7-mm decrease in zone size under the same test conditions (Fig. 4).

Storage of the discs at 56 C under condition A resulted in less change than was observed at 37 C under similar humidity conditions (Fig. 5). Storage under condition B at 56 C yielded results very similar to those observed at 37 C. Under condition C, the three synthetic penicillins retained no activity at the end of 1 day, with penicillin G being completely inactivated in 8 days. Cephalothin was most stable, retaining some activity for 12 days under these extreme storage conditions (Fig. 6).

DISCUSSION

Controls exerted by the Food and Drug Administration and certification before discs may be released to the market have resulted in the general distribution, by the different manufacturers, of discs of excellent quality. Careful manufacture and optimal storage with desiccant in the containers have increased the trust placed in the results of sensitivity tests accomplished by the disc method. The remaining serious deficiency is the breakdown of precautions in handling labile antibiotics after receipt in the clinical laboratory. The difficulty lies in improper storage and dispensing of the discs. Semiautomatic dispensers are presently in general use in many laboratories. After the disc containers are attached to the dispenser, no further controls are exerted. It is not unusual to encounter discs in a dispenser that is stored on the workbench permanently during many variations of temperature and humidity. For long-term storage, it is questionable whether discs should be stored at 4 to 6 C without a desiccant. The results of our observations with discs stored at 37 C and 80% relative humidity show that proper storage is mandatory in geographical locations duplicating these conditions as a normal climatic characteristic. Reports of resistance to the synthetic penicillins, especially with the staphylococci, must be interpreted with the knowledge that the sensitivity discs have been stored correctly. The role of temperature, as shown by the relative stability of the different drugs at the higher temperatures, appears to be of secondary importance if humidity is kept low. High humidity seems the major consideration in sensitivity disc stability.

To eliminate the possibility of antibiotic deterioration, storage should be in a cool, dry place. Optimal storage conditions are met by using a calcium chloride or drierite desiccator with vacuum in a deep freeze. Even in the best storage conditions, the desiccator with the discs must be removed from the freezer at least 1 hr before the discs are to be used. This allows the discs to come to room temperature before removal from the desiccator, thus preventing condensation with subsequent antibiotic inactivation.

ACKNOWLEDGMENT

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