Resistance of Large-Volume Parenteral Containers to Forced Microbial Contamination

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Abstract □ The hypothesis that contamination of large-volume parenteral containers may be due to improper handling technique was examined. Extensive studies were implemented involving carefully controlled conditions for both test (900) and control (450) units. Test containers were immersed in bacterial broth preparations in intact and "partially uncapped" conditions and were not swabbed; control containers were handled under "ideal" conditions except that they were not swabbed. A new administration set was used for each container examined and was opened only at the time of examination. Statistical evaluation of the results indicated that certain procedures may indeed result in contamination in many cases, whereas there was no significant contamination of any of the three systems tested when recommended procedures, even without swabbing, were followed.

Keyphrases □ Large-volume parenteral containers—resistance to forced microbial contamination □ Contamination, microbial—resistance of large-volume parenteral containers □ Sterility, large-volume parenteral containers—resistance to forced microbial contamination □ Parenteral containers, large volume—resistance to forced microbial contamination

The ability of large-volume parenterals and their companion administration sets to serve as sustaining and transmitting media of serious infection has been the subject of recent controversy. Various manufacturers have claimed that the proper use of their intravenous fluids and administration sets would forestall all contamination. However, several studies dealing with contamination of these fluids under conditions of normal use have raised questions both for and against such claims (1–5).

The purpose of this study was to determine whether large-volume parenteral containers (marketed by three different manufacturers[1]), used with their recommended administration sets, could resist bacterial contamination under adverse conditions.

EXPERIMENTAL

Systems Used—In this investigation, three types of containers were examined: A, nonfiltered air system, vacuum packed; B, filtered air system, vacuum packed; and C, open air system.

The administration set used for a particular container was designed by the manufacturer for that container. This condition was important since the study was designed to investigate microbial contamination of the intravenous liquid before it enters the venous system and since each set is designed by the manufacturer to maintain sterility for its specific system.

All liquids used in this study were lactated Ringer's solution, and all containers were of the 250-ml. size. One lot number for each type of container was used for both the test and controls. Analysis of preliminary results was used to determine a statistically valid sampling for the following studies.

Staphylococcus aureus was used as the test organism, since preliminary studies showed that this organism would propagate in lactated Ringer's solution. Propagation in lactated Ringer's solution was ensured by inoculation with microliter quantities of a known culture followed by both plating and filtration of the samples. In both cases, growth curves were found with extended lag times. Broths were prepared by inoculation from pure *S. aureus* (S-209) colonies and consisted of: trypticase soy broth (B.B.L.), 3.0 ml.; and distilled water, 100.0 ml.

A 24-hr. *S. aureus* broth was used for immersion of all test bottles. To determine the amount of contamination in the broth, a viable count method (6) was used to count the number of organisms. The counts taken ensured that the broth contained at least 1.7 billion organisms/ml.

Procedure—These forced contamination studies were divided into two categories: I, fully capped bottles (intact closures); and II, partially uncapped bottles (first closure removed). For example, in Category II, Type A bottles were uncapped to the rubber diaphragm, Type B bottles were uncapped to the rubber bung, and Type C containers were stripped to the screw cap.

Since no method was available to measure forced contamination of large-volume containers, the following techniques were developed and found to yield reproducible results.

Each bottle was immersed, mouth down, to about one-fourth of its length into the bacterial broth solution. The bottles were dipped in alternating order (Types A, B, and C), and the broth was continuously stirred to eliminate the possibility of bacterial sedimentation. After dipping in the broth, the bottles were placed in cases which were closed and spot taped. Each group of 24 test bottles and 12 control bottles was designated as a "run." The test bottles for several runs were dipped at the same time. Dipping was followed by a drying period of up to 10 days, after which the infusion sets were attached to the bottles one run at a time. Under a laminar flow hood, each set was attached as recommended by the manufacturer except that no wiping or swabbing procedure was used.

Bottles with attached infusion sets were shaken and inverted several times before being incubated[2] at 36 ± 1° for 15–24 hr. The control groups, in unopened cases, were placed in the incubator along with the tests.

Detection of bacteria in intravenous fluids was carried out by modifying several known techniques (7–12). After incubation, the contents of each bottle were passed through a Millipore filter apparatus which consisted of a Swinnex-25 filter assembly equipped with MF type HA black Millipore filter disks with grid, 0.45-μ openings, 25-mm. diameter, and a gasket. The entire apparatus was prepared and sterilized preceding filtration. Twenty-six filter assemblies were sterilized as a batch. One filter from each batch was removed under aseptic conditions, cultured, and examined for bacterial contamination. In a laminar flow hood, the effluent end of the Millipore filter assembly was connected to a vacuum line with a liquid trap. A vacuum was pulled, and the fluid was drawn from the intravenous bottle through the filter assembly into a conical flask. The organisms were collected on the filter disk located within the Swinnex-25 assembly. During each run and after filtration, each intact filter assembly was placed in a predetermined area prepared for it under the hood. The filter disk representing each bottle was then removed from the assembly and cultured using sterile technique (7–9).

Bacterial colony counts were taken from each filter on each of the first 3 days following filtration. The bacterial counting method (6) ensured accurate counts up to 300 organisms or colonies, beyond which estimates should be reasonably accurate to about 600 organisms. Although counts were made, they are not presented in this report since results were considered only as contaminated or not contaminated—an all or none approach to contamination.

Each test run consisted of eight of each type container, and each control run consisted of four of each type container. The control

[1] Abbott Laboratories, Baxter Laboratories, and Cutter Laboratories.

[2] In a Precision Thelco model 60 oven.

Table I—Fraction Contaminated[a] of Fully Capped Containers

	Type of Test Container			Type of Control Container		
	A	B	C	A	B	C
Series I	21/104	0/104	1/103	1/52	0/52	0/52
Series II	20/96	0/96	1/96	3/48	0/48	0/48
Total	41/200	0/200	2/199	4/100	0/100	0/100

[a] Containers yielding one or more colonies were considered contaminated.

Table II—Fraction Contaminated[a] of Partially Uncapped Containers

Type of Container	Test Units	Control Units
A	64/104	0/52
B	67/104	1/52
C	1/104	0/52

[a] Containers yielding one or more colonies were considered contaminated.

containers remained in their cartons as received from the manufacturer and were separated from the test groups. These bottles were exposed to normal room conditions and to a minimal amount of handling.

To ensure the possibility of potential contamination of intravenous bottles, the cap area of 36 bottles, after being immersed in the broth and dried for 7- and 8-day intervals, was washed with a known quantity of sterile water. These bottles yielded a calculated minimum count of 50,000 viable organisms when an aliquot was plated.

RESULTS AND DISCUSSION

Fully Capped Study—This portion of the experimentation was carried out in two segments (Series I and Series II) separated in time by 1 month. Each series and each test group were of approximately equal size, and control and test units were examined in an identical manner.

Two hundred each of the three types of containers were initially used as test units, and 100 of each type container were used as controls. In Table I, it can be seen that Types B and C test containers resisted contamination to a greater degree than A. Table I also shows that there was a similarity in the contamination rate of the 52 controls used in each case.

Table II was constructed from data collected from partially uncapped, stressed containers. From this table, it can be seen that containers of Types A and B were equally susceptible to contamination. The controls yielded almost identical results to those found in Table I. The results of colony counts made daily for 3 days after filtration are not reported here; only contamination and noncontamination are compared.

From these results, a statistical analysis of the data was carried out. The data from Table I consist of a set of proportions from bottles (both test and control) that were contaminated. An appropriate significance test for the comparison of several proportions when all or a part of the fractions are quite small (*e.g.*, 1/103 and 0/52 for Type C in the fully capped Series I) is the arc sin or $\sin^{-1}$ transformation (13, 14). If the actual rates of contamination are identical, the Brownlee (13) equation may be augmented so that:

$$Z = \frac{\sin^{-1}[(2n_1/N_1) - 1] - \sin^{-1}[(2n_2/N_2) - 1]}{\sqrt{(1/N_1) + (1/N_2)}} \quad \text{(Eq. 1)}$$

Table III—*Z* Values from Results in Table I[a]

Type of Test Container			Type of Control Container		
A	B	C	A	B	C
−0.10[b]	0.00[b]	0.05[b]	1.14[c]	0.00[c]	0.00[c]

[a] Comparison of Sections I and II. [b] No significant difference. [c] No difference.

Table IV—Pairwise Comparison of Fractions Contaminated[a]

Type Compared	Probability of Differences	
	In Test Units	In Control Units
C *versus* A	1×10^{-12}[b]	4.4×10^{-3}[b]
C *versus* B	4.55×10^{-2}[c]	No difference
A *versus* B	1×10^{-13}[b]	4.4×10^{-3}[b]

[a] Treatment of data from Table I. [b] Significant difference. [c] Possible significant difference.

Table V—Comparison of Test *versus* Control Containers[a]

Type of Container	Probability of Differences between Test and Controls	
	Probability of Difference	Significance of Difference
A	5.9×10^{-6}	Definite significance
B	5.0×10^{-1}	No significance
C	5.15×10^{-2}	Possible significance

[a] Treatment of data from Table I.

The quantity will have, to a good approximation, *normal probability distribution* with a mean value of zero and a standard deviation of one. Since no differences (Table III) were found between Sections I and II for both tests and controls of all types, the remainder of the analysis on this data can be done on the combined sections. Thus, fractions of tests contaminated on a pairwise basis (Table IV) indicate a tendency, under controlled conditions, for Type A containers to be more susceptible to contamination than those of either Type B or C.

Table V shows that there is a significant difference in the contamination of test and control units in the case of Type A containers, whereas there is no significance in Type C and only a possibility of significance for Type B. The finding of "possibly significant" for Type B containers is appropriate since federal regulations permit no contamination and commonly used statistical calculations would have, indeed, shown no significance.

A similar analysis of the data in Table II, for partially uncapped containers, shows that there is a definite increase in contaminated test containers compared to controls for Types A and B, whereas there is no significant difference between tests and controls for Type C.

A pairwise comparison of the three types of containers indicates that there is no difference in relative contamination between Types A and B and that Type C is less apt to be contaminated than either Type A or B under the test conditions.

From a practical point of view, one may extrapolate the findings of this experimentation to support the contention that contamination can occur if containers are improperly managed during preadministration. For example, to allow containers to remain in a partially opened state in a nonsterile area should significantly contribute to the loss of sterility. The study also shows that in an aseptic environment such as a laminar flow hood, there is little chance for contamination of the types of containers tested (Tables I and IV).

CONCLUSIONS

It was found that all three types of containers were well protected from bacterial contamination when procedures recommended by the manufacturers were used. The Type C open air system was least susceptible to contamination under stress conditions.

Table VI—Summary of 99% Confidence Intervals for True Contamination Rates under Experimental Conditions

	Type A		Type B		Type C	
	Test	Control	Test	Control	Test	Control
Capped	14–28%	1–12%	0–3%	0–5%	0–5%	0–5%
Partially uncapped	48–73%	0–10%	51–76%	0–14%	0–7%	0–10%

On the basis of the results reported here, one may speculate that contamination, emanating from outside the containers, can be the result of poor handling techniques. It may further be concluded that under the stress conditions studied, fully capped Type A containers were contaminated to a greater extent than those of Types B and C. It was also observed that with the containers partially uncapped, Types A and B were contaminated more frequently than was Type C. Furthermore, there was no difference between contamination rates for the control units.

It is the recommendation of the authors that regardless of the type of container used, or the precautions and engineering by the manufacturer, it is imperative that aseptic techniques be employed from the time the container is removed from the carton to the time infusion is completed.

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2-(Aminoethanesulfonylamino)thiazole and Related Compounds I: Stability, Absorption, Excretion, and Some Pharmacological Activities

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Abstract □ Decomposition rate constants of nicotinoyltauraminothiazole in aqueous solution at high temperatures were determined. Unchanged nicotinoyltauraminothiazole and three decomposition products (tauraminothiazole, aminothiazole, and an unknown substance) were detected by TLC. Nicotinoyltauraminothiazole has both analgesic and anti-inflammatory activities. Blood levels, tissue distribution, and excretion of nicotinoyltauraminothiazole in animals were also investigated. In urine of rabbits after the administration of the chemical, unchanged compound, tauraminothiazole, and a metabolite of undetermined structure were isolated.

Keyphrases □ Nicotinoyltauraminothiazole—stability and biopharmaceutical studies □ 2-(Aminoethanesulfonylamino)thiazoles—stability and biopharmaceutical studies, nicotinoyltauraminothiazole □ Anti-inflammatory activity—nicotinoyltauraminothiazole

Only a few thiazole derivatives are marketed drugs. These include dithiazanine iodide (anthelmintic) (1), succinylsulfathiazole (sulfonamide) (2), sulfathiazole (sulfonamide) (3), thiabendazole (anthelmintic) (4), and thiamine derivatives (vitamin).

Although tauraminothiazole [2-(aminoethanesulfonylamino)thiazole] was synthesized by Winterbottom *et al.* (5), nicotinoyltauraminothiazole is a new compound and was synthesized in a series of 2-aminoethanesulfonic acid derivatives (6–8). If taurine is liberated partially from nicotinoyltauraminothiazole *in vivo*, the surface activity of taurine must have some effect on the activity of the remaining components (2-aminothiazole and/or unchanged nicotinoyltauraminothiazole). It was established that the bond between the amino group and the 2-aminoethanesulfonyl group cannot be ruptured easily in rabbits; liberation of taurine from the nicotinoyltauraminothiazole will be examined later in man.

In the present work, the stability, acute toxicity, and some pharmacological activities of nicotinoyltauraminothiazole were investigated. Its absorption, excretion, distribution, and metabolic pathway were also studied.

EXPERIMENTAL

Sample for Kinetic Study of Stability—About 1 ml. of a 0.8% nicotinoyltauraminothiazole hydrochloride solution was placed in a colorless ampul (1-ml. capacity). The samples were stored at 50, 60, and 70 ± 1°, and the solutions were assayed at various times.