Microbial Contamination of Nonsterile Antibiotic Market Samples: A Survey

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Abstract □ Nonsterile antibiotic samples on the U. S. market were examined for microbial contamination in a survey that included 261 batches from 38 different manufacturers. All the drugs tested were of acceptable hygienic quality in that their microbial content was minimal and innocuous.

Keyphrases □ Antibiotics, nonsterile market samples—microbial contamination, survey □ Microbial contamination—survey of nonsterile antibiotic market samples □ Contaminants, in nonsterile antibiotics—survey of market samples

Since the 1964 inquiry by the Royal Swedish Medical Board into microbiological contamination of medical preparations (1), there has been growing concern about the possibility of excessive and undesirable microbial contamination of nonsterile drugs. In Sweden, severe eye disorders caused by cortisone ointments contaminated with *Pseudomonas aeruginosa* were among the human infections found due to contaminated nonsterile drugs. Cases of salmonellosis due to thyroid tablets contaminated with *Salmonella* were also reported. These findings led to official Swedish recommendations in 1966 on "Production, Hygiene, and Bacteriological Control in the Manufacture of Pharmaceuticals" (2).

In other countries, infections in man caused by contaminated oral or topical pharmaceutical preparations have been reported. Phillips (3) described four cases of lung infection caused by contaminated lignocaine ointment used for the lubrication of endotracheal catheters. Hospital outbreaks of *Salmonella cubana* infections in both the United States and the United Kingdom in 1966 were traced to the administration of capsule products containing contaminated carmine (4).

Since contamination of nonsterile drugs has been found to be widespread, other countries have issued regulations designed to improve the sanitary quality of these drugs. The Czechoslovakian Pharmacopeia (5) sets a total count limitation on the number of permissible microorganisms per gram or milliliter of various preparations. The *General Notices on Added Substances* in the 1968 BP (6) contains a warning that such substances should be free from harmful organisms. USP XVIII provides tests for estimating the numbers of viable aerobic microorganisms present and for freedom from designated microbial species in pharmaceutical articles of all kinds, from raw materials to the finished dosage forms (7). The International Pharmacopeia (8) specifies that microbiological contamination of nonsterile drugs should be no greater than that permitted for foods under the law of the country concerned.

The USP and NF defer to the Antibiotic Regulations for standards of potency and purity for antibiotic drugs. In these Regulations, there are no specified tolerances for microbial contamination of nonsterile antibiotics, except for one topical preparation (9). The microorganism count for that preparation must not exceed 10 per gram of powder, or per container if it is packaged with inert gases. Even though other certifiable antibiotic products are not regularly examined, they are required under Section 501(a) of the Federal Food, Drug, and Cosmetic Act to be free from harmful microorganisms. The Good Manufacturing Practice Regulations (10) stipulate that a drug must possess the quality and purity characteristics it purports or is represented to possess. An antibiotic and all its dosage forms must, therefore, be manufactured in a facility that provides adequate microbiological controls to preclude contamination by extraneous adulterants and prevent the dissemination of microorganisms from one area to another.

In 1968, several batches of an antifungal antibiotic powder were found to be grossly contaminated with microorganisms (11). Investigational studies revealed that the use of this grossly contaminated powder in the formulation of tablets or ointments resulted in highly contaminated products. In addition, an antifungal topical lotion was found to be contaminated with *P. aeruginosa* (12). The raw materials used in the formulation of this preparation were tested, and these organisms were not detected; therefore, the manufacturing facilities or the personnel were suspected as the source of the contamination. As a result of these findings, six other antibiotic powders were screened for microbial contamination. No gross contamination was detected in this limited number of samples of nafcillin, bacitracin, zinc bacitracin, neomycin, penicillin G, and tyrothricin. However, with the increased concern about the microbial purity of nonsterile drugs, a more extensive survey was made to determine the level of contamination in nonsterile antibiotic drugs on the market.

EXPERIMENTAL

Since antibacterial and antifungal antibiotics prevent the growth of some microorganisms, these products cannot be effectively tested for microbial contamination by the usual procedures used for other drugs, cosmetics, or foods. Special methods (11, 13, 14) have been developed for testing antibiotic preparations for sterility and microorganism count. Therefore, for the initial screening of the antibiotic samples tested in this survey, a sterility test was performed by the method described in the Antibiotic Regulations (15). The antibiotic products were divided into two categories: those that could be tested by the membrane filtration method after dissolution in water or another solvent, and those insoluble products for which the direct method was used. If no growth was observed after the 7-day incubation period at the appropriate temperatures, the product was reported as sterile. If contamination was found by the sterility test, additional tests were performed to identify and quantitate the number of viable microorganisms.

Precautionary measures, including performing the tests under a laminar flow hood, were taken to avoid adventitious contamination. A total aerobic microbial count was performed on all preparations found contaminated in the original screening test. All the products were sufficiently soluble or translucent to permit the use of the plate method for total aerobic microbial count.

Table I—Results of Survey of Marketed Nonsterile Antibiotic Samples for Microbial Contamination

Sample	Number of Batches Tested	Number of Batches Contaminated[a]	Types of Organisms Recovered from Contaminated Batches[b]	Number of Batches per Contaminant Type
Oral preparations:				
Potassium penicillin G tablets	5	3	*Staphylococcus epidermidis*	2
			Bacillus subtilis	1
Ampicillin capsules	4	2	*Bacillus circulans*	2
Tetracycline hydrochloride capsules	26	10	*S. epidermidis*	7
			Penicillium sp.	1
			Saccharomyces cerevisiae	2
Sodium novobiocin capsules	4	0		
Oxytetracycline hydrochloride capsules	4	0		
Trioleandomycin capsules	2	0		
Methacycline hydrochloride capsules	2	1	*S. epidermidis*	1
Lincomycin hydrochloride capsules	2	0		
Tetracycline syrup	6	0		
Erythromycin syrup	4	0		
Chloramphenicol palmitate syrup	2	0		
Lotions and topical ointments:				
Nystatin lotion	15	2	*S. epidermidis*	2
Nystatin ointment	33	17	*S. epidermidis*	10
			Bacillus sp.	7
Bacitracin–neomycin–polymyxin ointment	7	1	*Aspergillus niger*	1
Ophthalmic ointments:				
Neomycin–polymyxin	1	0		
Potassium penicillin G	1	1	*S. epidermidis*	1
Oxytetracycline hydrochloride–polymyxin B	1	0		
Bulk antibiotic powders:				
Sodium ampicillin	4	0		
Chloramphenicol	2	1	*S. cerevisiae*	1
Dihydrostreptomycin sulfate and streptomycin sulfate	10	0		
Potassium penicillin G	18	5	*Bacillus* sp.	3
			Mold (unidentified)	1
			S. cerevisiae	1
Oxytetracycline base or hydrochloride	18	2	*Rhizopus* sp.	2
Tetracycline base	35	9	*S. epidermidis*	8
			Mold (unidentified)	1
Neomycin sulfate	7	0		
Nystatin	2	2	Gram-negative coccus bacillus	1
			Bacillus sp.	1
Griseofulvin	2	1	*Bacillus* sp.	1
Amphotericin	2	1	*Bacillus* sp.	1
Lincomycin hydrochloride	4	1	*Bacillus* sp.	1
Gramicidin	3	0		
Bacitracin	2	0		
Sodium cloxacillin	1	0		
Kanamycin sulfate	3	0		
Tyrothricin	1	1	*Penicillium* sp.	1
Sodium novobiocin	1	1	Mold (unidentified)	1
Chlortetracycline hydrochloride	2	0		
Streptomycin sulfate	3	1	*Bacillus subtilis*	1
Erythromycin	8	1	*Mucor* sp.	1
Polymyxin B sulfate	2	0		
Penicillin G diethylaminoethyl ester hydrochloride	1	0		
Dihydrostreptomycin sulfate	5	0		
Triacetyloleandomycin	3	0		
Sodium penicillin O	1	0		
Zinc bacitracin	1	0		
Ampicillin trihydrate	1	1	*Penicillium chrysogenum*	1
Total	261[c]	64		

[a] All contaminated batches had less than 50 microorganisms per gram or milliliter. [b] All contaminated samples were further examined and found negative for the presence of *Pseudomonas, E. coli, S. aureus* (coagulase-positive), and *Salmonella*. The 261 batches were made by 38 different manufacturers.

Materials—The soybean–casein digest agar medium was prepared according to the USP XVIII. Peptone water was prepared by dissolving 1.0 g. of peptic digest of animal tissue USP in sufficient distilled water to make 1000 ml., and 99-ml. portions were dispensed into flasks and sterilized at 121° for 20 min. The final pH was 7.1 ±0.1.

Method I: Insoluble Antibiotics—A 1.0-g. portion of powder or 1 ml. of a liquid sample was added to 9 ml. of sterile peptone water. A number of 10-fold serial dilutions were prepared by aseptically transferring 1 ml. of the sample suspension to 9 ml. of sterile peptone water. From each dilution, 1 ml. was removed and added to separate sterile 15 × 150-mm. petri dishes; 40 ml. of sterile melted soybean–casein digest agar, which had been cooled to approximately 45°, was then added to each dish. The agar was swirled in the plate, covered, and allowed to harden. The plates were inverted and incubated at 35–37° for 48–72 hr., and the colonies on each plate

were then counted. If a count of less than 10/ml. was expected, the measured quantity of sample was added directly to the agar without making the dilutions in peptone water.

Method II: Soluble Antibiotics—A membrane filter of 0.45-μ porosity was placed in a filter funnel attached to a vacuum flask. Then 1.0 g. of powder or 1 ml. of a solution was added aseptically to 200 ml. of sterile peptone water; powders were allowed to dissolve. The solution was immediately vacuum filtered through the membrane. The membrane was washed three times with 100 ml. of sterile peptone water and allowed to dry by continuing the vacuum for 5 min. after filtration. The membrane was then placed on the surface of 20 ml. of solidified soybean–casein digest agar in a petri dish. The plate was inverted and incubated for 48–72 hr. at 35–37°, and any colonies that appeared on the membrane were counted.

Method III: Petrolatum-Based Antibiotic Ointments—The test procedure described for ointments in the USP XVIII (7) was used with the following exceptions. If the ointment contained penicillin, approximately 10,000 Levy units of penicillinase was added to both the thioglycollate and soybean–casein digest test media; if the ointment contained neomycin, 27.5 g. of sodium chloride and 1 g. of ascorbic acid were added to the thioglycollate test medium, and 30 g. of sodium chloride and 1.0 g. of ascorbic acid were added to the soybean–casein digest broth.

As stated previously, the concept of monitoring nonsterile pharmaceutical products was introduced in the USP XVIII as a means of ensuring that the manufacturing process is under control and the product will not contain excessive amounts of either bacteria or molds. To implement this concept, specific microbiological monitoring procedures were prescribed in the USP for the first time. In addition to providing tests for estimating the number of viable aerobic microorganisms, the USP XVIII also describes procedures for detecting four pathogenic organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species, and *Escherichia coli*. Since the USP XVIII designated these four indicator organisms as undesirable contaminants, the contaminating microorganisms recovered in this survey were submitted to microbiological tests for identity. If the contaminant was found to be a Gram-negative bacillus or Gram-negative coccus-bacillus, it was transferred to the selective agars described in the USP XVIII for the tests for *Salmonella* species, *P. aeruginosa*, and *E. coli*. Upon examination of the colonies on the selective agars, if none showed colonial morphology characteristic of the respective organisms, the product was reported to be free from these bacteria. If a Gram-positive coccus was recovered in the sterility test, it was transferred to the selective Vogel–Johnson agar medium. Colonies on this medium suspected of being *S. aureus* were tested for coagulase production. If no coagulation was observed, the sample was reported to be free from coagulase-positive *S. aureus*.

RESULTS

A total of 261 batches of nonsterile antibiotics from 38 different manufacturers were examined for microbial contamination; all were found to be of satisfactory quality. As shown in Table I, no microbial growth was observed in 197 (75.5%) of the batches tested. For the 64 samples in which some growth was detected, the total aerobic counts were made and, in all cases, were less than 50 organisms per gram or milliliter. Of the 64 batches in which contamination was detected, only seven of the contaminants required plating on differential media. Six contaminants were tested to assure freedom from coagulase-positive *S. aureus* and one contaminant was tested on the various differential media to assure freedom from *P. aeruginosa*, *E. coli*, and *Salmonella*. All these batches were found to be free from coagulase-positive *S. aureus*, *E. coli*, *Salmonella* sp., and *P. aeruginosa* when tested by the USP XVIII microbial limit tests.

DISCUSSION

Since it has been shown that contaminated drugs can mediate infection in man, harmful organisms should be absent from nonsterile antibiotic preparations. Therefore, this survey was conducted to obtain general information on the microbial content of nonsterile antibiotic market samples. Experience has shown that antibiotic preparations can be produced with a low microbial content if the raw materials are not grossly contaminated and the manufacturer adheres to strict industrial hygienic procedures. Good Manufacturing Practice Regulations (9) consider that microbiological quality controls of water, air, equipment, and personnel are essential for all drugs.

Although these drugs have no sterility requirement, since they are not purported to be sterile, approximately 75% of the 261 batches tested were found to be sterile. In the remainder that were found contaminated, the number of microorganisms per gram or milliliter was extremely small. This survey showed that nonsterile antibiotic drugs sold in the United States are generally of acceptable hygienic quality in that their microbial content is minimal and innocuous. However, manufacturers should continue to monitor these preparations since past experience has shown that samples may occasionally become heavily contaminated.

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