Microbial Monitoring* **

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The "tubular" schematic of mammalian structure is a well-known concept that has been in use for years, but nowhere is this concept more useful than in understanding the man microbial interphase (fig. 1). The diagram emphasizes the fact that humans are subjected to microbial invasion from two areas where bacterial populations exist in large numbers. The first of these is the internal flora of the respiratory, intestinal and lower urinary tract which, along with the skin organisms, make up the endogenous microorganisms of the body. The second area, the periphery of the diagram, contributes the external or exogenous flora. The numbers and types of organisms found here are unpredictable since they depend upon environmental factors. Infections which are caused by the endogenous flora are considered opportunistic infections, while those derived from the exogenous flora are termed nosocomial infections.

The need for monitoring both areas for alteration in microbial flora, which may result in invasion or adverse effect on internal organs of the host, has been an accepted scientific fact of modern medicine for years. Questions remain, however, on how much monitoring needs to be done. What areas of the patient and the environment should be tested, and what are the practical uses of the results of such monitoring? Before attempting to answer these questions, let me give you an overview of how microbial monitoring has been used at the Medical College of Virginia hospitals and other institutions to identify and solve problems related to nosocomial and opportunistic infection.

Changes in Etiology of Bacteremia. One area that has been under constant surveillance in our institution is that of blood cultures or bacteremia. The numbers and types of microorganisms present in blood cultures have been recorded over the last 20 years. The data document that there have been profound changes in types of bacteria primarily associated with this infection (fig. 2). This graph shows the percentage of patients from whom gram-positive and gram-negative organisms were isolated during a 20-year period at the Medical College of Virginia hospitals. Since 1960, the percentage of patients with gram-negative bacteremia has continuously increased, while during the same period the percentage of patients subjected to gram-positive bacteremia declined (fig. 3). This graph demonstrates the number of patients who had bacteremias with some of the more common organisms associated with this type of infection. Perhaps the most striking results are those for 1970. In this year, the number of bacteremias by *Escherichia, Klebsiella, Enterobacter* and *Serratia* was in the neighborhood of 260 patients. In the same year, there were about 60 *Staphylococcus aureus* bacteremias, a ratio of over four gram-negative bacteremias for every one staphylococcus bacteremia. It is also distressing to note that there has been no decrease in the upward slope of the gram-negative organisms over the last 20-year period but rather an acceleration in this upward slope for the last five-year period.

Alterations of Endogenous Flora. The reason for this shift in the etiology of bacteremia is not known. A number of possibilities immediately present themselves, such as age of the population tested, types of surgical procedures performed and types of medication given. All may play a role in the alteration of results. In regard to medication, particular attention has been paid to such agents as corticosteroids, as they have been related to changes in the immunological response of the host which may allow alteration in microbial flora.

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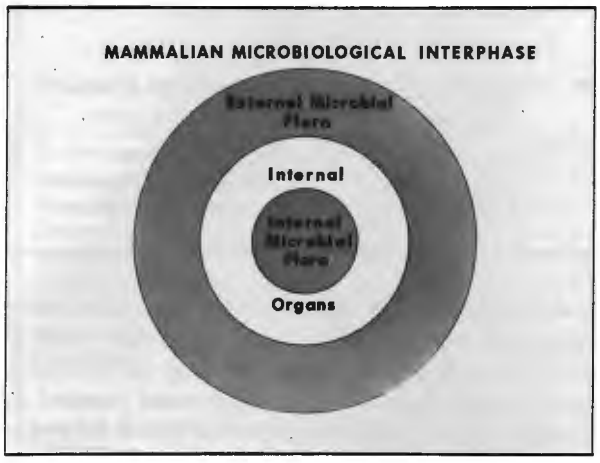

Fig. 1

More recently it has become evident that antibiotics may be responsible for allowing endogenous organisms to manifest pathogenicity. There is evidence that many of the gram-negative infections are endogenous in source, and the organisms of these infections in the immunologically depressed or debilitated patients are selected for by the use of antibiotics. An example of this is shown in figure 4.

The study was done in cooperation with Dr. B. W. Haynes of the Department of Surgery at the Medical College of Virginia. A patient, who had suffered a 23% total body burn, was given penicillin to protect him from the common exogenous pyogenic organisms such as *Staphylococcus aureus* and *beta hemolytic streptococcus*. Bacterial monitoring of the

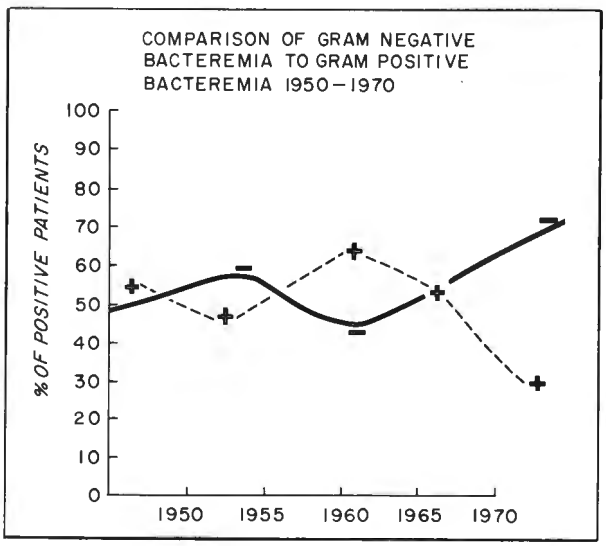

Fig. 2

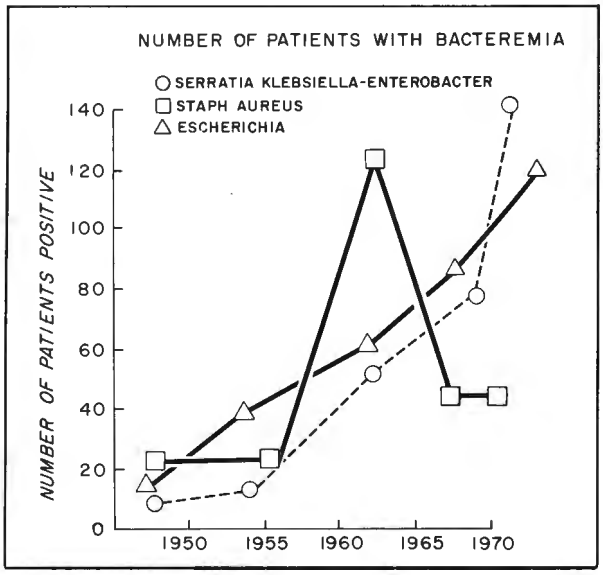

Fig. 3

burn lesion, using a standard technique, shows the success of this therapy, for early in the course of this patient's treatment both of these organisms were present but did not colonize the burn lesion (2). Penicillin, however, did not stop colonization of the lesion by gram-negative organisms consistent with the endogenous flora of the patient. Such organisms as *Proteus, Enterobacter* and *Pseudomonas* rapidly increased in number and only decreased after eschar removal with healing of the lesion. Only then was there a return of gram-positive organisms associated with normal flora (fig. 5). When broad-spectrum antibiotics are included in the antibiotic regimen, there may occur an emergence of a large number of organisms resistant to all the antibiotics used. In this

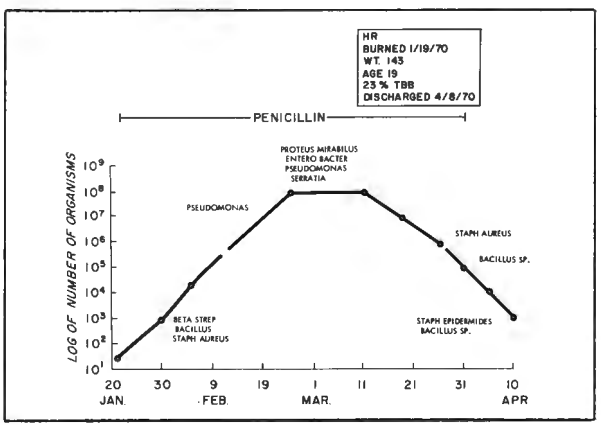

Fig. 4

patient, penicillin, streptomycin and gentamicin were given. The burn area was colonized by neither gram-positive nor gram-negative bacteria but rather by the yeast, *Candida albicans,* an organism known for its resistance to antibiotics. Again, a normal gram-positive flora was not established until eschar removal and healing of the lesion.

In our laboratory, we have been able to alter the normal flora of the hamster by giving large doses of oxicillin. In the upper respiratory tract of ten treated animals, there was a decline in the number of *alpha hemolytic Streptococcus* and *Neisseria,* with an increase in the number of *Escherichia, Proteus,* and *Enterobacter.* In three of the ten test animals, gram-negative bacteremias were demonstrated by heart blood cultures and in three of the ten test animals, death occurred from gram-negative pneumonia within 72 hours of treatment. The normal untreated controls during this period remained healthy and had no evidence of infection (6).

The medical literature also describes numerous examples of alteration in the etiological agents of infections which may be associated with treatment. Rogers (8) contrasts the etiologic agents of pneumonia found during a preantibiotic era (1938–1940) with that of a postantibiotic era (1955–1958). In the first period, there was an 8% incidence of gram-negative pneumonia, while in the latter period during the antibiotic era there was a 38% incidence of this type of infection. Lerner (5)

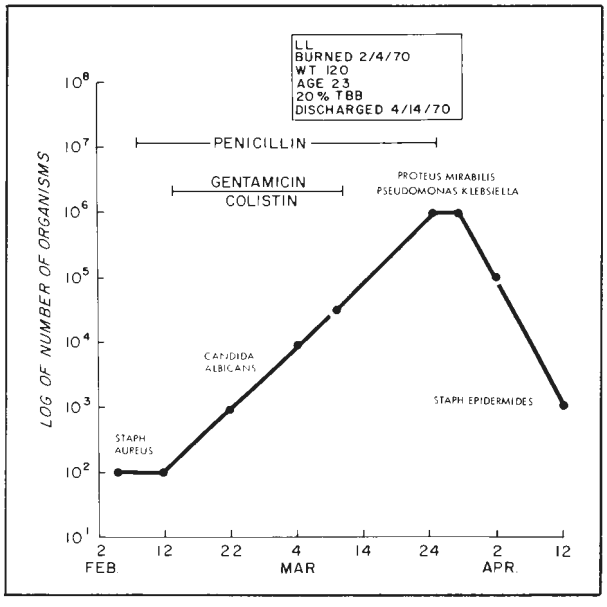

Fig. 5

TABLE 1

TYPES OF THERAPEUTIC REGIMEN ASSOCIATED WITH ENDOGENOUS MICROBIAL FLORA ALTERATIONS

Irradiation	Corticosteroids
Antimetabolites	Immunosuppressants
Antibiotics	Dermal preparations

also has reported an increase in gram-negative pneumonia in nonhospitalized patients. Price (7), in 1970, presented strong evidence that the extensive use of antibiotics in a neurology ward resulted in an epidemic of *Klebsiella* infection which only subsided with the withdrawal of antibiotics from ward use and a more judicious use of antibiotics after the withdrawal period. Can one predict with certainty whether a particular therapy will cause serious overgrowth? Of course, the answer is "No." One can, however, be alert to the therapeutic regimen that has a potential to cause such trouble (Table 1). This table shows those therapeutic regimens which have been associated with gram-negative opportunistic infection (1, 4). The list is by no means exhaustive, and many other examples can be found. Those listed in this table are known to have been associated with gram-negative infections at this institution or they have been cited repeatedly in literature. The common causes for alteration in flora according to these sources are: irradiation, antimetabolites, antibiotics, corticosteroids, immunosuppressants and dermal preparations.

The combined findings leave little doubt that the physician must appreciate the microbial ecology of the human and treat it as much as possible with an agent that will not drastically alter the normal microbial flora. In other words, the attending physician should be an ecologist concerned with the very personalized microflora of his patient. This is not a very radical statement since the medical profession

TABLE 2

TYPES OF PROCEDURES ASSOCIATED WITH EXOGENOUS MICROBIAL INFECTIONS

Intravenous infusion	Respirators
Dialysis	Incubators
Parental feeding	Suturing
Catheterization of blood vessel	Punch biopsies
Catheterization of urinary tract	Bone marrow aspiration

TABLE 3

FREQUENCY OF ISOLATION FROM 100 SKIN CULTURES OF VARIOUS SPECIES OF BACTERIA

Staphylococcus epidermidis	86 (85%)
Bacillus species	42 (42%)
Streptococci	29 (29%)
Enterococci	16 (15%)
Diphtheroids	13 (13%)
Herellea	4 (4%)
Escherichia	2 (2%)
Staphylococcus aureus	2 (2%)
No growth	4 (4%)

Herellea vaginicola isolated from skin of two patients on hospital day 1 for one patient and hospital days 26 and 29 for another.

Escherichia isolated from skin of two patients on hospital days 4 and 11, respectively.

(All gram-bacilli were isolated from upper extremities.)

has been concerned with the total environment of the patient for centuries.

Introduction of Exogenous Microbes. I have spent the time so far discussing only endogenous flora. What about exogenous flora or nosocomial infection? What role do these agents play in present day disease? One aspect of this problem is the number of new medical techniques and devices that require direct communication into the internal organs. Not only have a large number of new devices been added in the last 20 years, but also there has been a vast increase in the use of older procedures such as venipunctures. These procedures, unless careful aseptic technique is employed, serve as vehicles for the entry of exogenous organisms into the internal organs. In Table 2, you will find the techniques that have been repeatedly associated with nosocomial infections (1, 4). As in the last table, the list is not exhaustive and there are other procedures which have been associated with this condition. The role that these manipulations may play in contributing to nosocomial infections cannot be overemphasized. Let me give you two examples of how these procedures may allow the entry of organisms into the vascular system. The first example has to do with the effect of venipuncture on 100 hospitalized patients. This work was done in conjunction with Dr. Richard Duma of the Division of Infectious Disease. We have listed in Table 3, the types of organisms recovered from the antecubital area of the right arm, prior to cleaning the site for venipuncture. *Staph epidermidis* and *Bacillus species* were most commonly recovered, followed surprisingly by *Enteric streptococci, Herellea* and *Escherichia*. The purpose of the venipuncture was for obtaining blood cultures. This allowed, therefore, the correlation of the organisms recovered from blood cultures with those found on the skin surface. Table 4 shows the results of this correlation. Ten individuals had positive blood cultures; in eight of these, there was no

TABLE 4

RELATIONSHIP OF POSITIVE BLOOD CULTURES TO CULTURES OF OVERLYING SKIN IN 10 PATIENTS

Culture number	Blood culture (organisms)	Skin culture (organisms)	Site of cultures	Day of hospitalization
1	*Pneumococcus*	*S. epid.*, Diphth.	L. & R. A.	1
2	*Pneumococcus*	*S. epid.*, Bacillus sp., α strep. (Ent.)	L. & R. A.	1
3	*Streptococcus*	*S. epid.*, Bacillus sp. (α strep. cultured from skin of opposite arm)	L. A.	1
4	*Anerobic diphtheroids*	*S. epid.* & Bacillus sp.	L. A.	3
5	*Bacillus* species	Bacillus sp.	L. & R. A.	10
6	*Micrococcus* species	*S. aur.*	R. A.	3
7	*Herellea vaginicola*	*H. vaginicola*, *S.* epid., α strep. (Ent.), & Bacillus sp.	L. & R. A.	26
8	*Cryptococcus*	*S. epid.*	L. A.	1
9	*Escherichia* species & *Cryptococcus*	*S. epid.*, & Bacillus sp.	L. & R. H.	16
10	*Aspergillus*	*S. epid.*, & Bacillus sp.	R. A.	1

L—Left, R—Right, A—Arm, H—Hand

TABLE 5

COMPARISON OF COMMON ORGANISMS ISOLATED FROM BLOOD CULTURES 1969–1968

Genus	Number of Patients 1969	1968	% Change
Escherichia	84	70	+20
Aerobacter, *Klebsiella*, *Serratia*	102	109	−7
Proteus	30	27	+10
Pseudomonas	38	40	−5
Staph aureus	34	37	−8
Pneumococcus	58	47	+23
Number of cultures taken	11,380	11,117	+1

relationship between the organisms recovered from blood cultures and those isolated from the skin. But in two patients, numbers three and seven in Table 4, the same organisms found on the skin were also present in the blood cultures, indicating that the needle at venipuncture may have been contaminated at the site of entry and may have pushed organisms into the vessel. The data certainly indicate that this occurs infrequently, but the mere fact that it can occur at all should sensitize medical personnel to the need for good aseptic technique.

The second example that we will use to demonstrate how nosocomial infection can be spread will involve the use of i.v. infusion (Table 5). This table shows the percent increase of common bacteremia organisms for the years 1969–1968. You will note that when the two periods are compared, there has been a small increase in many of the organisms, but well within the percent increase that was discussed previously. Table 6 compares the first six-month period of 1969 and the first six-month period of 1970 for the same organisms shown in the previous table. You will note that there is a tremendous increase in the percentage of recovery, particularly with the gram-negative organisms. During this 1970 period, Dr. Richard Duma and Dr. Jack Warner (3) did an extensive in-use study on the i.v. systems throughout the hospital. They sampled

TABLE 6

COMPARISON OF MICROORGANISMS CULTURED FROM BLOOD ACCORDING TO NUMBER OF PATIENTS AND CULTURES JANUARY TO JUNE 1969–1970

Genus	Number of Patients 1969	1970	% Change
Staphylococcus aureus	14	33	+135
Herellea	5	11	+120
Aerobacter	10	22	+120
Candida	12	23	+91
Klebsiella	31	58	+87
Proteus	14	21	+50
Escherichia	42	55	+30
Alpha *Streptococcus*	27	32	+19
Serratia	10	10	0
Pneumococcus	33	28	−16
Number of cultures	5,584	6,688	+19

TABLE 7

BACTERIOLOGIC STUDIES ON 24 VOLUME CONTROL INTRAVENOUS INFUSION SETS IN 24 PATIENTS

Set Number	Type of Set	Culture Results: Inside of Cylinder	Inside of Bottle Cap
3A	25		*Corynebacterium*
6A	25		*Mima*
7	25	*Mima*	*Mima*
9	50		*Klebsiella*
10	50		*S. epidermidis*
12	50		*Pseudomonas* *E. coli*
14	50	*Serratia*	*Serratia*
16	75	*Enterobacter*	*Enterobacter* *Serratia* *E. coli*
17	25		*Flavobacterium* *E. coli*
20	50		*Corynebacterium*
23	50		*Flavobacterium*
24	25	*E. epidermidis*	
25	50	*Flavobacterium*	*Bacillus species*
26	50	Hemolytic *Streptococcus*	
31	50	*Candida species*	*Candida species*
37	50		*Mima*
38	25	*Corynebacterium*	
41	50		*Flavobacterium* *Candida species*
48	25		*Enterobacter*
55	25		*S. epidermidis*
58	25	*Corynebacterium*	
60	50		*Bacillus species*
61	50		*S. epidermidis*
62	50		*Flavobacterium*

Blank space represents sterile culture.

the internal reservoir of the i.v. sets while they were being used on the wards (Table 7). The results of this survey demonstrated that many of the same organisms which increased in 1970 were to be found as contaminant in the i.v. fluid itself. A correction of this situation brought a decline the next year in the *Klebsiella, Enterobacter,* and *Serratia* organisms isolated from bacteremia.

Studies such as these are found throughout the literature and are associated with many procedures. We all tend to neglect these results, however, thinking our ward and office procedures are above these dreaded possibilities; unfortunately, unless constant in-use monitoring of these procedures is done, we just do not know that they are.

Value of Microbial Monitoring. With this overview as background, let me restate my original questions and answer them as best I can. What patients need to be monitored bacteriologically? Any debilitated individual under therapy or subjected to the procedures described here should be carefully followed by clinical and laboratory methods for the possibility of an emerging opportunistic or nosocomial infection. What areas need to be monitored? All of the patient's environment conducive to microbial growth should be tested to insure that proper housekeeping procedures are being carried out but more importantly, equipment and infusions which go directly into the internal organs should be tested for sterility *while in use.* How often should monitoring be done? As often as necessary to convince the skeptical physician that good aseptic technique in microbial clean environment is available for his patient. The patient himself should be monitored also to insure that endogenous organisms are not adversely affecting him. What is the practical value of the results of such monitoring? One direct benefit to the patient is that such testing can alert the physician that a particular therapeutic regimen may be altering the microbial flora. There are also indirect benefits as problems are identified and solved by good in-use monitoring at the patient levels. There will be a decrease in the number of organisms with which the patient comes into contact. This should allow for a decline in the number of infections. Lastly, but perhaps most importantly, microbial monitoring of numerous patients allows the acquisition of valuable epidemiological data which contribute to our understanding of the dynamic ever-changing world of biology and medicine.

REFERENCES

1. Bartlett, R. C., Hammond, J. B. and Wiehersham, V. *Hospital Associated Infections.* American Society Clinical Pathology, 1971.

2. Brentano, L. and Gravens, D. L. A method for the quantitation of bacteria in burn wounds. *Appl. Microbiol.* 15:670, 1967.

3. Duma, R. J., Warner, J. and Dalton, H. P. Septicemia from intravenous infusions. *N. Engl. J. Med.* 284:257, 1971.

4. Klainer, A. S. and Beisel, W. R. Opportunistic infections: A review. *Am. J. Med. Sci.* 258:431, 1969.

5. Lerner, M. A. and Tillotson, J. A. Pneumonia caused by gram-negative bacilli. *Mich. Med. J.* 67:35, 1968.

6. Muhovick, M., Sr. A study on the endogenous flora of the hamster. *Master Thesis.* Dept. of Pathol., Med. Coll. Va., 1973.

7. Price, O. J. E. and Sleigh, J. P. Control of infections due to K-aerogenes in a neurosurgical unit by withdrawal of all antibiotics. *Lancet* 2:1213, 1970.

8. Rogers, D. E. The changing pattern of life-threatening microbial disease. *N. Engl. J. Med.* 261:540, 1965.