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Etiology of Bacteremia

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Blood-culture results for a 15-year period from a large southern hospital were tabulated and analyzed for alterations in the bacterial species associated with bacteremia. From 15,543 cultures, 2,410 positive cultures (15.6%) were obtained. These results were grouped into 5-year periods, and an alteration in the incidence of the agents was demonstrated. In the past 5 years, gram-negative bacilli have replaced gram-positive cocci as the most common agents of bacteremia, and several species not formerly associated with septicemia were found to be involved in a large number of cases. The importance of the clinical laboratory monitoring blood culture results was demonstrated as was the need for constant collaboration between the clinician and microbiologist.

The purpose of this report is to demonstrate the value of blood culture data as a clinical epidemiological tool. Reports by Finland and Jones and others have demonstrated the value of blood cultures in detecting the changing pattern and incidence of bacteremia (13, 19, 21). These reports, however, have been concerned mainly with the clinical and therapeutic aspects of bacteremia, without emphasizing the role that the clinical laboratory can play in establishing the epidemiology and the clinical significance of bacterial species associated with this condition.

MATERIALS AND METHODS

Blood was obtained by vena puncture with a sterile needle and syringe. Before drawing the sample, the area of puncture was wiped with green soap solution, alcohol, and tincture of iodine. Approximately 10 ml of blood was inoculated into a closed culture bottle by inserting the syringe needle through the rubber diaphragm cap which had been wiped with alcohol. It was recommended that three to five cultures be drawn on all patients suspected of having bacteremia (4).

The culture bottle contained Brain Heart Infusion (Difco) with 0.1% agar, penicillinase, and *p*-aminobenzoic acid. One side of the bottle was coated with a layer of Trypticase Soy Agar (BBL). This layer permitted the growth of bacterial colonies to be easily seen and aided in the macroscopic reading of the cultures. All specimens were incubated for 15 days at 37 C, unless growth was observed by gross examination before this time. At the end of the 15th day, the cultures were inoculated onto blood-agar plates before being discarded as negative. This blood culture system has been in use in this hospital for the past 16 years.

The survey was initiated by examining all culture results for the years 1950, 1955, 1960, and 1965. The number of positive cultures was recorded, as were the bacterial species. In addition, the number of patients supplying the positive cultures was noted.

The effect of seasonal factors on species variation was studied by dividing the results of the above-mentioned 4 years into quarters and examining repeated increased incidence during the warm or cold months.

The study was continued by examining 1,000 cultures per year for the 15-year period, 1951 to 1965. These results were tabulated and used to demonstrate whether any alteration in the incidence of certain bacterial species in bacteremia had occurred during the period studied.

RESULTS

The number of blood cultures taken has increased in every year of the survey (Table 1). As the total number of cultures increased, the number of patients having positive culture also increased. This increase does not appear to be closely related to the number of hospital admissions, for it has continued during the years when admissions had not significantly increased.

When the above 4 years were studied for seasonal variation in the causative agent of bacteremia, it was surprising that *Diplococcus pneumoniae* was the only organism, of the common species associated with bacteremia, to show a consistent seasonal variation. This organism increased from an average of 3.5% of the total positive cultures in the warmer months, April to September, to an average of 7.6% in the colder months, October to March. Among the less commonly isolated organisms, only *Neisseria meningitidis* had an increased incidence in the colder season, 13 of the 18 isolates being made during this period.

Table 2 demonstrates the relationship between

TABLE 1. *Incidence of bacteremia*

Assay	1950	1955	1960	1965
Total no. of cultures	1,419	2,681	4,566	6,814
Total positive cultures	188	377	755	1,091
Percentage of cultures positive	13.2	14.0	16.5	16.0
Total no. of patients sampled	1,135	2,010	3,015	3,951
Total no. of patients positive	171	309	506	631
Percentage of patients positive	15.0	15.4	16.8	15.9
Total no. of patients admitted	24,436	31,559	31,729	36,430
Incidence per 1,000 admissions	6.9	9.8	15.9	17.3
Percentage of admissions cultured	4.6	6.4	9.5	10.8

TABLE 2. *Relationship between percentage of patients positive and percentage of positive cultures (1965)*

Organism	Percentage of patients positive	Percentage of cultures positive
Staphylococcus epidermidis	28.0	20.4
Corynebacterium species	17.8	11.0
Escherichia coli	8.7	7.7
Diplococcus pneumoniae	7.7	8.6
Paracolon species	6.8	5.7
S. aureus	6.1	7.1
Klebsiella aerobacter	5.5	5.5
α-Hemolytic *Streptococcus*	5.4	5.4
Pseudomonas aeruginosa	4.8	5.9
Proteus species	4.1	4.8
β-Hemolytic *Streptococcus*	3.1	4.2
Herellea species	2.0	2.3

the percentage of positive cultures for 12 bacterial species commonly associated with bacteremia and the percentage of the patients from which the particular species were isolated. The percentage of positive cultures for any species was closely related to the percentage of patients harboring that organism. This relationship existed even with multiple positive cultures from a single patient because of the large number of cultures used in this survey.

The results of surveying the 15-year period, 1951 to 1965, are shown in Table 3. These figures are based on approximately 1,000 cultures for each of these years to make a total of 15,543 specimens. The number of positive cultures in this number was 2,410, which gave an overall positive percentage of 15.6.

Table 4 demonstrates the yearly percentage of positive cultures which fluctuated from a low of 12.8 in 1956 to a high of 19.4 in 1964. However, when these yearly percentages are grouped into 5-year periods, there is only a small variation of ±1.2% from the total recovery percentage of 15.6. These data suggest that there has been very little increase in the incidence of bacteremia over the 15-year period.

The four figures show the changing pattern of bacteremia in this hospital. The data of figures

TABLE 3. *Etiology of bacteremia over a 15-year period*

Etiological agents	Per cent
Staphylococcus epidermidis	23.0
S. aureus	12.0
Corynebacterium species	11.6
Escherichia coli	7.2
Diplococcus pneumoniae	6.4
Bacillus species	6.1
Aerobacter species	4.7
α-Hemolytic streptococci	4.6
Pseudomonas species	3.7
Proteus species	2.3
Salmonella species	2.3
β-Hemolytic streptococci	2.2
Paracolon species	2.1
Unidentified gram-negative *Bacillus*	1.4
2-Hemolytic streptococci	1.2
Neisseria meningitidis	1.0
Alcaligenes-Flavobacterium species	0.9
Clostridium species	0.8
Candida species	0.8
Herellea-Mima species	0.6
Haemophilus influenzae	0.5
Bacteroides species	0.5
Anaerobic streptococci, *Klebsiella pneumoniae*, *Cryptococcus neoformans*, *Nocardia asteroides*, *Neisseria gonorrhoeae*, *Neisseria flava*, *Neisseria sicca*	4.1
Total	100.0

[a] The period was from 1951 to 1965. There were 15,543 cultures, of which 2,410 (15.6%) were positive.

TABLE 4. *Percentage of positive blood cultures from 1951 to 1965*

Year	Per cent positive[a]	Per cent positive for 5-year periods
1951	15.0	15.6
1952	13.6	
1953	16.5	
1954	18.7	
1955	14.2	
1956	12.8	14.2
1957	15.8	
1958	14.0	
1959	13.5	
1960	14.8	
1961	16.8	16.8
1962	16.6	
1963	15.3	
1964	19.4	
1965	16.0	

[a] Percentage is based on 1,000 blood cultures per year.

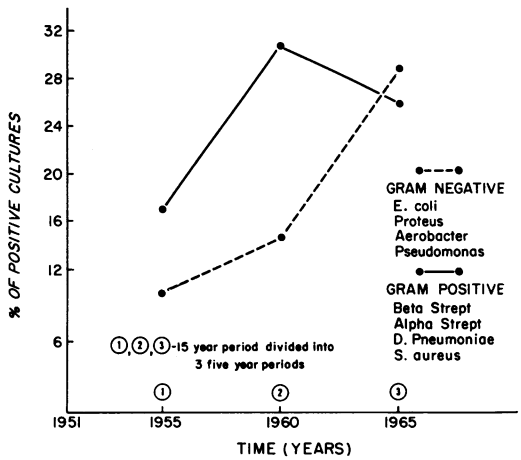

FIG. 1. *Combined percentage of positive blood cultures for four gram-positive and four gram-negative bacteria.*

were derived, as were the data in the last table, by dividing the 15 years covered by the study into 5-year periods, and they are expressed as percentages of the different organisms making up the total positive cultures for each of the three periods.

In Fig. 1, the data of four common gram-positive organisms, *Staphylococcus aureus*, *D. pneumoniae*, β-hemolytic *Streptococcus*, and α-hemolytic *Streptococcus*, are combined and compared to the combined total of four common gram-negative bacilli, *Escherichia coli*, *Aerobacter aerogenes*, *Proteus* species, and *Pseudomonas aeruginosa*. There was a rapid increase in gram-positive bacteremia in the 5-year period of 1956 to 1960 and then a slight decline in the period 1961 to 1965, but still well above the base period, 1951 to 1955. The gram-negative organisms in the base period were well below the gram-positive organisms in the frequency of occurrence, but increased in frequency in the next two periods such that by 1965 their incidence was greater than that of the gram-positive group.

That this alteration in frequency between the two groups is not due to a radical increase or decrease in any one species making up the combined group is demonstrated in Fig. 2. Although this graph is less dramatic than the first, it shows that the four gram-positive organisms individually increased or held steady during the third period. In contrast, the gram-negative bacteria individu-

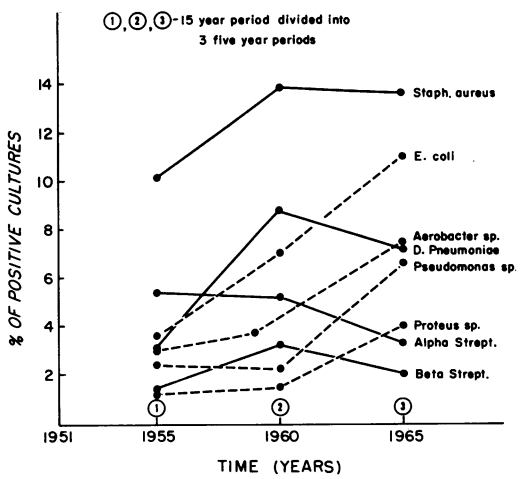

FIG. 2. *Alteration in the frequency of isolation of eight bacterial species from blood cultures.*

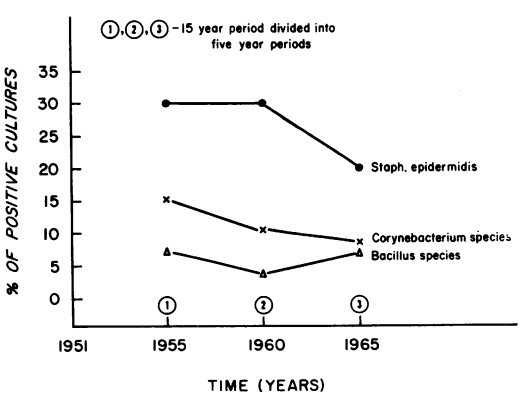

FIG. 3. *Variations in bacteremia.*

ally all increased in frequency in the second and third period.

Figure 3 demonstrates the incidence of three bacterial species which have been considered as contaminants by many investigators. Two of the species, *S. epidermidis* and *Corynebacterium* species, follow the same curve as the other gram-positive organisms, increasing in the second 5-year period and decreasing in the last period. Only one of the groups, *Bacillus* species, increased during the last period of 5 years.

Figure 4 shows an increase in the frequency of four less common gram-negative organisms, species of *Paracolobactrum*, *Herellea*, *Flavobacterium* and *Alcaligenes*. These organisms increased slightly during the 1956 to 1960 period, and then increased to a new high in the 1961 to 1965 period. The frequency of mixed cultures dramatically increased in the last period. γ-Hemolytic *Streptococcus*, the only gram-positive organism, classically associated with bacteremia, increased in the last 5-year period.

There were several genera in which there was no significant change in the frequency of recovery over the 15-year period. These genera were *Candida*, *Neisseria*, *Haemophilus*, *Salmonella*, and an organism described as an unidentified gram-negative *Bacillus*. This last group of organisms appeared every year, but to date it has not been placed in any suitable genus. A similar organism was reported by Tucker in 1962 (32).

Many species were isolated only rarely from blood cultures during the 15-year period. The organisms in this group were anaerobic *Streptococcus*, *Cryptococcus neoformans*, and species of *Bacteroides*, *Nocardia*, and *Clostridium*. Although it is true that these organisms taken as individual species were rarely isolated from blood, these so-called rare species made up over 1% of the total positive blood cultures when they were combined.

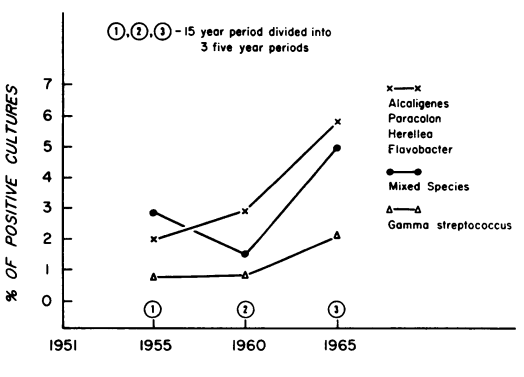

FIG. 4. *Variations in bacteremia.*

To test further the reproducibility of using blood culture results as an indicator of species frequency in bacteremia, the blood cultures of 80 diagnosed cases of bacterial endocarditis were examined. These cases all occurred during the 15 years covered by the survey, and all cultures were processed as in the general survey. The percentage of positive cultures for a particular species (Table 5) correlated well with the percentage of endocarditis patients infected with that species. All but two of the *S. epidermidis* cultures came from

TABLE 5. *Bacterial endocarditis cultures*[a] *from 1951 to 1965*

Organism	No. of cases	Per cent of positive cases	No. of positive cultures	Per cent of positive cultures
α-Hemolytic Streptococcus	31	38.9	211	46.5
Staphylococcus aureus	17	21.4	111	24.4
S. epidermidis	10	12.5	43	9.5
Enteric *Streptococcus*	6	7.5	28	6.2
β-Hemolytic Streptococcus	3	3.7	19	4.2
Candida species	2	2.5	6	1.3
γ-Hemolytic *Streptococcus*	2	2.5	8	1.8
Escherichia coli	2	2.5	6	1.3
Diplococcus pneumoniae	2	2.5	8	1.8
Neisseria flavescens	1	1.1	2	0.4
Haemophilus aprophilus	1	1.2	7	1.5
Corynebacterium species	1	1.2	3	0.7
Aerobacter species	1	1.2	1	0.2
Gram-negative *Bacillus*	1	1.2	1	0.2
Bacillus species	0	0	0	1
Total	80	100.0	454	100.0

[a] Total number of cultures 1,576.

TABLE 6. *Changing pattern of bacteremia from 1950 to 1965*[a]

Organism	Percentage of positive patients			
	1950	1955	1960	1965
Staphylococcus aureus	6.5	3.9	11.2	6.1
α-Hemolytic *Streptococcus*	6.5	2.6	2.6	5.4
α-Hemolytic *Streptococcus*	0.5	1.5	1.1	0.9
β-Hemolytic *Streptococcus*	1.6	2.1	1.3	3.1
Diplococcus pneumoniae	2.8	4.1	8.1	7.7
Total gram-positive	17.9	14.2	24.3	23.2
Escherichia coli	5.4	5.0	8.1	8.7
Proteus species	4.9	2.6	1.7	4.1
Klebsiella-Aerobacter	2.2	4.5	6.9	5.5
Paracolon group	2.8	2.1	1.5	6.8
Pseudomonas species	1.6	3.5	3.5	4.8
Total gram-negative	16.9	17.7	21.7	29.9

[a] Percentage based on 749 patients.

the 10 cases diagnosed as *S. epidermidis* endocarditis, and all three *Corynebacterium* cultures were from the same individual who was diagnosed as having *Corynebacterium endocarditis*.

Table 6 demonstrates the alteration in the causative agent of bacteremia on a patient basis. The table gives the percentage of positive patients for each species for the years 1950, 1955, 1960, and 1965. The results of this table parallel those of Fig. 1.

DISCUSSION

The chief value of using as a unit of measurement a definite number of cultures, such as 1,000 cultures per year, is to remove the variation in the sampling population caused by the fluctuations in the hospital admission rate. It is evident from this survey that more blood cultures are drawn every year on a larger percentage of the admissions. Under these conditions, it is difficult to establish whether there has been an actual increase in bacteremia or simply an increased awareness of it with a corresponding increase in the number of patients tested. The data presented here suggest that an increased awareness on the part of the clinician has resulted in a dramatic rise in the number of patients having positive cultures when expressed on the basis of a positive patient to patient admission ratio. However, when these data are examined on the basis of a positive patient to total patients sampled ratio or on the basis of positive cultures to total culture ratio, there is little significant difference.

The recovery of bacteria from blood cultures always raises the question of the significance of the particular organism. In order for the laboratory to help in the interpretation of this question, certain basic procedures have been established, such as the taking of multiple cultures and the need for multiple positive cultures to make a diagnosis of septicemia (4). In addition, the genus or species of the organisms have in the past been considered to be of fundamental importance for certain organisms and were automatically considered to be associated with bacteremia and pathogenic, whereas other species were considered saphrophytic and therefore contaminant (17, 26). It is apparent both from these data and the recent literature that separation of pathogenic and saprophytic bacteria on taxonomical grounds alone is misleading because of the dramatic alteration in the etiology of bacteremia (5, 6, 9, 11, 18). That alteration in the patterns of bacteremia is of importance was pointed out by Finland and Jones in 1959 (13) and again by McCabe and Jackson in 1962 (19, 20). In addition, other reports cite every organism listed in this study as a cause of bacteremia.

S. epidermidis was the most frequently isolated organism in this series and perhaps the hardest one to evaluate in regard to its clinical significance. The common explanation that this organism is always a skin contaminant is most unsuitable. Smith et al. in 1958 reported on 5 cases of bacterial endocarditis caused by this species and cited 90 other cases, which have appeared in the literature (30). Allison, Gerszten, and Dalton reported 10 cases of endocarditis attributed to *S. epidermidis* (2). In addition, Wilson and Hambutger reported on 9 deaths from *S. epidermidis* septicemia and considered this organism especially virulent in certain individuals (7, 35).

S. aureus was the second most frequently isolated organism over the 15-year period and was one of the most virulent bacteria associated with bacteremia. The data on the incidence of these organisms are similar to those in other reports (12, 34). In this survey, *S. aureus* bacteremia reached a peak during 1960 and 1961 and since that time has been declining. This is reflected by an increase in the second 5-year period and a slight decline in the third period (Fig. 2).

The significance of diphtheroids and *Bacillus* species in blood is usually considered to be similar to that of *S. epidermidis* in that they are commonly considered as skin contaminants. This view may be an oversimplification since these organisms have been repeatedly cited in the literature as the etiological agent of septicemia (9, 36) and since their incidence, like that of *S. epidermidis*, was significantly lower in the endocarditis study than in the general survey.

The significance of gram-negative bacteremia has been pointed out recently by many investigators, and there is little to be added in this regard (1, 10, 15, 19–28, 31). However, it is important to emphasize that the incidence of this type of bacteremia is still increasing, as demonstrated by this survey. The exact reason for this increase is not known, and there is no adequate explanation for this trend.

The increase of bacteremia in which two or more different bacterial species were isolated has increased markedly within the past 5 years. This observation has also been reported by Hochstein, Kirkham, and Young (16). It is paramount that the clinical laboratory recognize this trend, as one species present in greater number may mask the second species unless careful observations are made.

Although this paper reflects the actual pattern of bacteremia in this hospital, it is important to emphasize that each institution should examine

its own data because of the epidemiological importance of monitoring blood culture results.

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