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Statistical analysis of staphylococcal infection in hospital patients in relation to use of antibiotics and other factors

By A. W. VOORS and G. T. STEWART
Department of Epidemiology, School of Public Health, University of North Carolina, and the North Carolina Memorial Hospital

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INTRODUCTION

The increasing importance of hospital cross-infection as a by-product of improved drug therapy entails the need for operational indices of the epidemicity of such infection. It is only by using such indices that institutional measures intended to decrease hospital infection can be evaluated objectively. Unfortunately, it is often impossible to conclude for a given strain of micro-organisms isolated from a hospital patient whether it is the result of: (*a*) infection of the patient before his admission, (*b*) activation of an organism which had colonized passively in the patient before his admission, or (*c*) actual infection in the hospital. However, it is generally suspected that many of the penicillin-resistant staphylococci isolated from hospital patients belong to the last category.

In this respect, the following finding is of interest. The coagulase or mannite positive staphylococci isolated from hospital patients can be plotted in a frequency curve according to the size of the growth-free zone in a disk test for sensitivity to penicillin-G. This curve is trimodal (Fig. 1),* implying that there are three populations of such staphylococci with distinctly differing degrees of resistance to penicillin-G: (1) the sensitive; (2) the moderately resistant; (3) the highly resistant (Lindberg & Stewart, 1965). Strains capable of producing penicillinase, spontaneously or inducibly, which are therefore advantageously placed when penicillin is being used, are found predominantly in the third category.

The objective of this study is to investigate whether this distinction offers an operational index of epidemicity of staphylococcal hospital infection which could be used in the evaluation of control programmes. This is done by seeing whether there is an ecological difference between staphylococci highly resistant to penicillin and those of moderate resistance. The ecological determinants under study are: (1) duration of hospital stay before the isolation of the organism; (2) use of penicillin-G during this stay; and (3) use of, combined with resistance to, other anti-

* It can be argued that the mode in the left of the figure is not obvious since it corresponds to a large interval on the measurement scale. Technical restrictions of the disk method used did not allow further specification of zone sizes smaller than a millimeter. However, even if this were possible and a frequency curve were plotted on a logarithmic scale allowing for a large number of intervals for this 'mode', the presence of at least one mode below size 10 could hardly be denied in view of the large total of such observations, practically irrespectively of the shape of the curve.

biotics. If it transpires that one of the two penicillin-resistant groups is not introduced from outside the hospital and that antibiotic use has no effect upon the incidence of this group, then this will suggest hospital infection as the sole explanation of the existence of this resistance group, in which case the incidence of this group can be used as a specific index of hospital infection.

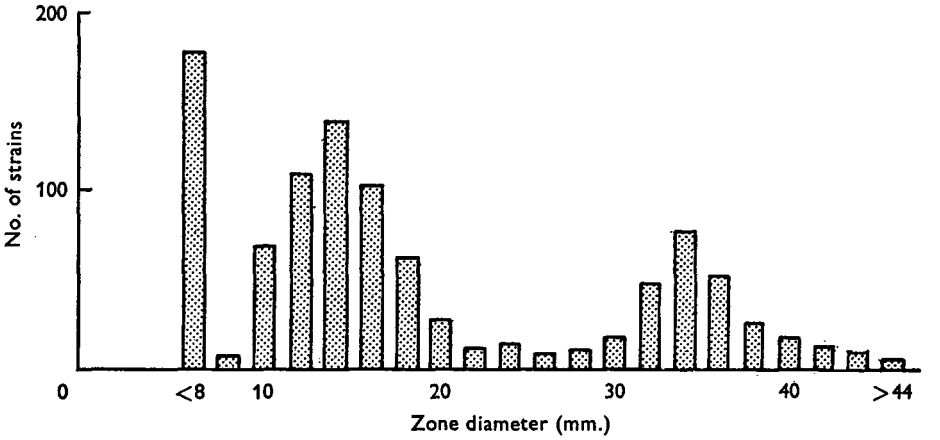

Fig. 1. Zones of inhibition produced in 1000 strains of *Staphylococcus aureus* by penicillin-G (10 units) disks. (Consecutive isolates in North Carolina Memorial Hospital during the period of surveillance.)

MATERIALS AND METHODS

From October 1964 until October 1965 all strains of coagulase or mannite positive staphylococci isolated in the North Carolina Memorial Hospital were referred from the diagnostic laboratory to the epidemiologic laboratory in the School of Public Health. Clinical data about the patient from whom each strain was isolated were obtained from the ward or case-chart. Each strain was examined for sensitivity to several antibiotics by the 10-unit disk method, and tested for penicillinase production by (*a*) a membrane-plate technique (Holt & Stewart, 1963), and (*b*) an indicator-dye method (Novick & Richmond, 1965). The characteristics for a given strain, including those of the patient from whom it was isolated, were punched in a card (Table 1). Further strains from this patient were similarly entered in a fresh card. A total of 2007 strains were thus documented in more or less detail. For each patient the clinical information was completed after the patient's discharge.

The data were examined with two questions in mind: (1) How often were patients admitted with staphylococci either moderately or highly resistant? (2) Could antibiotic use be excluded as a determinant of one of the two resistance groups of strains?

Table 1. *Partial code sheet, staphylococcal hospital infection study North Carolina Memorial Hospital*, 1964–5

Rank order of staphylococcus isolated
Patient identification number
Date of admission
Days after admission this organism isolated (= selected period)
Use of penicillin-G during selected period
Use of chloramphenicol during selected period
Use of tetracyclin during selected period
Use of kanamycin during selected period
Use of erythromycin during selected period
Zone size of disk test (penicillin-G)
Result of test for β-lactamase (membrane)
Result of test for β-lactamase (dye)
Sensitive or resistant to chloramphenicol
Sensitive or resistant to tetracycline
Sensitive or resistant to kanamycin
Sensitive or resistant to erythromycin

How often were patients admitted with staphylococci either moderately or highly resistant?

No systematic data were available concerning the strains from patients before admission. Data on staphylococcal infection in the Intensive and Special Care Service of North Carolina Memorial Hospital collected from August 1964 through April 1965 give the impression that the median time interval between infection and detection as practised here is not longer than 6 days (Voors, 1967*a*). Hence the strains isolated from patients 6 days or less after their admission were analysed separately from those from patients having stayed 7 days or more. Thus, the proportion of all strains that is highly resistant was calculated for each duration-of-stay group, and the same was done for the moderately resistant strains. Here the strains at risk of becoming or being moderately resistant are limited to the sensitive and moderately resistant groups, and do not include the highly resistant according to current bacteriological theory. Hence, here the moderately resistant proportion of the sum of moderately resistant and sensitive strains was calculated for each duration of stay before isolation of these strains. The resulting proportions are called 'proportion of strains highly resistant' and 'proportion of strains moderately resistant' respectively. These four proportions were compared by chi-square technique. The legitimacy of combining the various durations of stay within the four groups was assessed by analysis of variance.

Could antibiotic use be excluded as a determinant of either moderately or highly resistant strains?

The number of highly resistant strains was compared with the sum of the moderately resistant and sensitive strains for various combinations of characteristics measured. Likewise, the number of moderately resistant strains was compared with the number of penicillin sensitive strains. These data were tested for independence by chi-square technique.

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RESULTS

How often were patients admitted with staphylococci either moderately or highly resistant?

The number of strains is tabulated by degree of penicillin resistance and by duration of prior stay in Table 2.

Table 2. *Staphylococcal infections in hospital. Strains by degree of resistance to penicillin-G and by duration of stay prior to isolation*

Duration of prior stay (days)	Degree of resistance of strain			
	High	Moderate	None	Total
0	65	298	221	584
1	15	34	37	86
2	3	18	17	38
3	0	23	9	32
4	5	3	9	27
5	8	14	10	32
6	0	9	1	10
7	3	12	2	17
8	5	11	0	16
9	5	15	3	23
10–19	57	73	23	153
20–29	18	39	7	64
30–39	17	25	2	44
40–49	14	1	3	18
50–59	7	1	3	11
60–69	1	9	3	13
70–79	0	3	1	4
80–89	0	0	0	0
90+	2	5	0	7
Total	225	603	351	1179

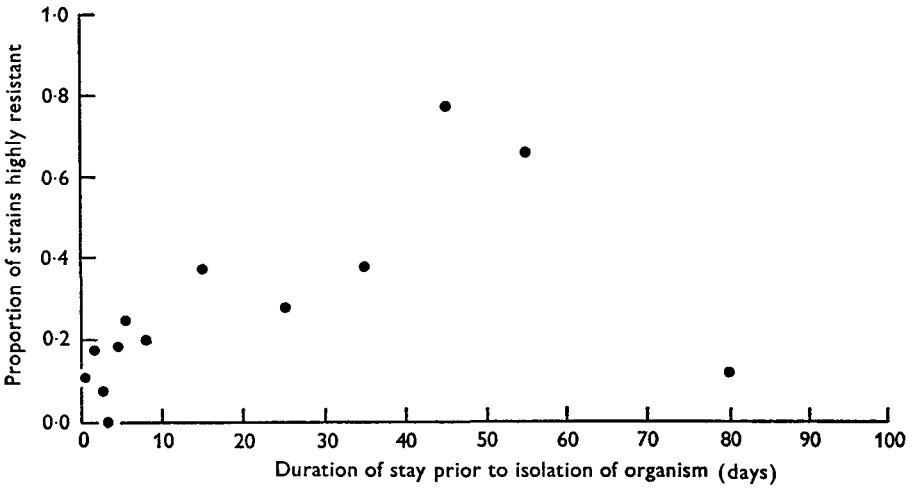

Fig. 2. Proportion of staphylococcal strains highly resistant to penicillin-G, by duration of prior stay in hospital.

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The proportion of strains highly resistant is plotted by duration of prior stay in Fig. 2 and of strains moderately resistant in Fig. 3. Each of these two groups of proportions was divided into a category with stay durations of 6 days or less and one of 7 days or more.

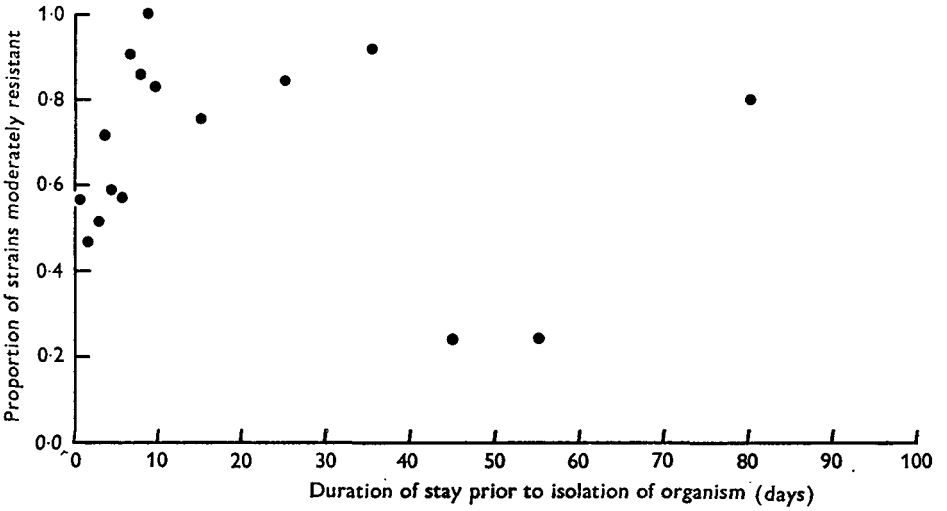

Fig. 3. Proportion of staphylococcal strains moderately resistant to penicillin-G, by duration of prior stay in hospital.

Table 3. *Staphylococcal infections in hospital. Strains by degree of resistance to penicillin-G, by use of penicillin-G, and by duration of prior hospital stay*

Days of prior hospital stay	Use of Penicillin-G	Resistance to penicillin-G			
		High	Moderate	None	Total
0–5	+	30	167	118	315
	−	37	126	111	274
6–19	+	43	69	10	122
	−	27	50	19	96
20+	+	32	48	7	87
	−	26	34	12	72
Total	+	105	284	135	524
	−	90	210	142	442

When a weighted regression of the proportions against the duration of stay was performed, the weight being inversely proportional to the estimated variance [i.e. if the proportion is $p_i = r_i/n_i$ for any duration-of-stay i, then the weight is

$$w_i = \frac{n_i^3}{r_i(n_i - r_i)} = \frac{n_i}{p_i(1-p_i)}\Big],$$

it was found in all four groups analysed that neither the linear nor the quadratic term was significant at the 5% level when tested against the residual from a quadratic trend. Thus, the data are not inconsistent with the contention that after the sixth day of admission the proportions remain fairly constant.

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By chi-square technique it was found that the short-stay long-stay ratio of proportions highly resistant was very significantly smaller than that of proportions moderately resistant. The data (Fig. 2) suggest that few if any of the highly resistant strains were acquired before admission.

Could antibiotic use be excluded as a determinant of either moderately or highly resistant strains?

The number of strains by degree of resistance, duration of prior stay and use of penicillin-G during this stay are given in Table 3.

Table 4. *Strains by degree of resistance to penicillin-G, by duration of prior hospital stay, and by use* of chloramphenicol*

Days of prior hospital stay	Use of* chloram-phenicol	Resistance to penicillin-G			
		High	Moderate	None	Total
0–19	+	23	21	0	44
	−	114	391	258	763
20+	+	19	13	2	34
	−	39	69	17	125
Total	+	42	34	2	78
	−	153	460	275	888

* Combined with resistance to chloramphenicol.

Table 5. *Strains by degree of resistance to penicillin-G, by duration of prior hospital stay, and by use* of tetracycline*

Days of prior hospital stay	Use of tetra-cycline	Resistance to penicillin-G			
		High	Moderate	None	Total
0–19	+	24	40	0	64
	−	113	372	258	743
20+	+	19	32	1	52
	−	39	50	18	107
Total	+	43	72	1	116
	−	152	422	276	850

* Combined with resistance to tetracycline.

Application of the chi-square test for independence to this table as described above indicates that, with regard to the number of strains, the relative frequency of high resistance is not significantly dependent upon use of penicillin-G in these patients at the 5% level of significance. However, the effect of moderate resistance is dependent on the effect of use of penicillin-G at the 5% level of significance.

The numbers of strains by degree of resistance, duration of prior stay and use of four other common antibiotics (chloramphenicol, tetracycline, kanamycin and erythromycin) are given in Tables 4–7. Application of the chi-square test in the same manner as described above results in rejection of the null-hypothesis at the 5% level of significance for all four antibiotics and both resistance groups.

Table 6. *Strains by degree of resistance to penicillin-G, by duration of prior hospital stay, and by use* of kanamycin*

Days of prior hospital stay	Use of* kana-mycin	Resistance to penicillin-G			
		High	Moderate	None	Total
0–19	+	8	5	0	13
	−	129	407	258	794
20+	+	5	12	0	17
	−	53	70	19	142
Total	+	13	17	0	30
	−	182	477	277	936

* Combined with resistance to kanamycin.

Table 7. *Strains by degree of resistance to penicillin-G, by duration of prior hospital stay, and by use* of erythromycin*

Days of prior hospital stay	Use of* erythro-mycin	Resistance to penicillin-G			
		High	Moderate	None	Total
0–19	+	5	7	0	12
	−	132	405	258	795
20+	+	12	11	0	23
	−	46	71	19	136
Total	+	17	18	0	35
	−	178	476	277	931

* Combined with resistance to erythromycin.

INTERPRETATION OF RESULTS

There is a prevailing impression that staphylococci with high resistance to penicillin-G belong to the hospital environment and are seldom introduced from outside. The data here support this impression.

The next question is whether the acquisition of such staphylococcal strains is influenced by their production of penicillinase when penicillin is given to a patient who might have been infected with a predominantly sensitive strain before his admission, or is due to infection within the hospital. An attempt to answer this question is made by assessing the association between use of antibiotics and degree of resistance to penicillin-G.

If attention is focused on strains of staphylococci with high resistance to penicillin-G, the present study suggests that use of this drug is not associated with an excessive isolation rate of these strains over other strains of lesser resistance. This is not true, however, for the use of four other antibiotics (chloramphenicol, tetracyclines, kanamycin and erythromycin); isolation of strains highly resistant to penicillin-G is associated with the use of any one of the four other antibiotics just mentioned. Hence we may speculate that use of penicillin-G does not favour the acquisition (by its user individually) of staphylococci highly resistant to this drug, although since the organisms concerned were, for the most part, shown in

the study to be producers of penicillinase, the biologic effect of use over a period must have constituted a positive selective pressure favouring persistence of these strains in the hospital environment: use of other antibiotics, however, was found to be associated with penicillin resistance as indicated by an excessive rate of highly penicillin-resistant organisms isolated from the users of these antibiotics. It is therefore possible that this use of non-penicillin drugs is actually favouring penicillin resistance.

If attention is focused on strains of staphylococci with moderate resistance to penicillin-G, both use of this drug and of the four other antibiotics is significantly associated with this resistance. In the case of penicillin-G, this association is stronger here than in the highly resistant strains.

These findings suggest that the highly penicillin-resistant staphylococci in hospital patients, who did not receive other antibiotics to which these strains are also resistant, are rarely introduced into the hospital by a newly admitted patient and are rarely induced in a patient by his use of penicillin-G in the circumstances of the present study. Hence they must be largely due to infection after admission, or related to use of antibiotics other than penicillin-G.

IMPLICATION

Under the conditions as inferred above, the threshold theorem (Bailey, 1957) predicts that, as soon as, for a ward or service, the product of rate of contact between individuals and expected duration of infectivity after infection decreases below the value one (the 'epidemic threshold'), the incidence of the relevant strains will virtually drop to zero. The model on which the threshold theorem is based is not invalidated by abandoning the assumption of closed communities (Voors, 1967*b*). Therefore, the proportion of highly penicillin-resistant staphylococci from patients who did not receive broad-spectrum antibiotics could be used as an index of success in a control programme of hospital infection.

Thus, there may be practical merit in routinely distinguishing between the three degrees of resistance to penicillin-G in staphylococci. The two resistant groups can be easily separated by their position on the zone-frequency curve (Fig. 1). In view of the observed association it would be desirable in this respect to disregard the strains also resistant to other antibiotics and isolated from patients who used these antibiotics. However, it is proposed that, if the laboratory facilities are limited, the index of hospital infection be restricted to those highly penicillin-resistant strains isolated from patients who did not receive other antibiotics during their hospital stay. If fuller laboratory facilities are available, the epidemiologic consequences of using antibiotics can be additionally assessed from the shape of the trimodal curve and, especially, from changes in population with moderate or intermediate resistance to penicillin G.

SUMMARY

When staphylococcal strains from hospital patients are plotted in a frequency curve according to the size of the growth-free zone in a disk test for sensitivity to penicillin-G, this curve is trimodal. This implies that, besides the sensitive, there are two populations of resistant staphylococci: the moderately and the highly resistant, In a hospital-wide survey of coagulase or mannite positive staphylococci isolated in North Carolina Memorial Hospital between October 1964 and October 1965 it was inferred that: (1) there was a statistically highly significant difference between the distributions of the moderately and the highly resistant strains over duration of hospital stay prior to the time of isolation; (2) the highly resistant strains were seldom introduced from outside; and (3) there was no significant association between use of penicillin-G by a patient harbouring a strain and the frequency with which such a strain was found to be highly resistant to this drug. The latter finding is in contrast to significant associations found when the penicillin resistance is moderate, or when the drug used is other than penicillin-G. It is concluded that a fall in the incidence of staphylococcal strains highly resistant to penicillin-G in patients who did not receive other antibiotics may well be a valid index of the efficacy of programmes to control hospital infection. If so, there is a practical merit in adopting the routine of distinguishing between two groups of penicillin-resistant staphylococci, which behave, epidemiologically, as two distinct bacterial populations.

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