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An estimate for the source of coagulase-negative staphylococci in blood cultures

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SUMMARY

An estimate for the source of coagulase-negative staphylococci in blood cultures is presented and its use illustrated in two populations. The method estimates the probability that a positive blood culture is positive due to contamination and the probability of it being positive due to bacteraemia. The effect of changes in efficiency of the blood culture technique on these estimates is demonstrated. One of the illustrative populations consists of 537 sample pairs from 273 patients, taken after open-heart surgery in 1978–80. The other consists of 500 consecutive sample pairs taken in one of two hospitals in 1983–4.

INTRODUCTION

In evaluating the clinical implications of positive blood cultures, a replicated finding is often assumed to be confirmatory for bacteraemia, unless a constant error in processing is present. Contamination is often excluded if a similar strain is not isolated from subsequent cultures. As bacteraemia will probably be detected in most cases if two samples are taken, this procedure of paired sampling is often used. This is especially relevant if it concerns a *Staphylococcus epidermidis* (SE), routinely found on the skin, in patients with an intravascular prosthetic device with its affinity for coagulase-negative staphylococci (CNS) and the difficulty in eradicating infections without removing the foreign body. A method to estimate the probability that CNS in the blood culture were actually present in the blood at the time the sample was taken, is presented. Although it is almost impossible to estimate the probability of missing CNS in the blood culture if bacteraemia is present in the patient, the effect of changes in this culture-efficiency on the estimate is also given in the formula. A method to estimate the probability that CNS in the blood culture is a contaminant of the sample and the effect of culture-efficiency on this estimate is also presented.

The use of these methods is illustrated in two different sample-populations. One is from patients recovering from open-heart surgery, a population in which the interpretation of CNS-positive blood cultures has important implications. The other is from samples as they arrive in the laboratory on a routine base, including different types of patients, different departments and different hospitals. These

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populations are only included for illustrative purposes, to show the usefulness of the methods presented in this paper; not to compare or discuss incidences of bacteraemia in these patients.

MATERIAL AND METHODS

Estimates

The equations leading to the estimates are based on the theory of probability and two assumptions. The first assumption is that if cultures are considered positive due to contamination, the probability of positivity in one sample is independent of the probability of positivity for the other sample in the sample pair. This assumption excludes the possibility that both samples are contaminated by a similar strain of CNS. This seems a fair assumption, as each blood sample is drawn from a different arm of the patient. The second assumption is that if cultures are considered positive due to bacteraemia, the probability of positivity of one sample is similar to the probability of positivity in the other sample in the sample pair. This assumption states that by taking two blood samples from a patient with bacteraemia one has CNS in both samples, or the probability of CNS in both samples will be equal. This assumption is a fair one if bacteraemia persists during the period the samples are taken. This period is limited to minutes in the sample from open-heart surgery patients and to 1 h at most in the sample from the two hospitals.

The probability of a sample pair consisting of two samples positive for similar CNS can be equated as

$$p(+/+) = p_b + (1-p_b)\,p_c^2,$$

p_b is the probability of a sample being positive due to bacteraemia, and p_c the probability that a contaminant strain is cultured and a bacteraemia strain is not found; the possibility of both being cultured is not considered. The first part of the formula presents the probability of a pair being double-positive due to bacteraemia, p_b. The second part presents the probability of a pair being double-positive due to contamination among sample pairs not positive due to bacteraemia, $(1-p_b)\,p_c^2$.

The probability of a sample pair consisting of a positive and negative sample can be equated as

$$p(+/-) = 2\,(1-p_b)\,p_c\,(1-p_c).$$

The probability of one sample positive due to contamination, p_c, and the other sample not positive due to contamination, $(1-p_c)$, among sample pairs not positive due to bacteraemia, $(1-p_b)$, is multiplied by two, to include $+/-$ as well as $-/+$ sample pairs.

The probability of a sample pair consisting of two negative samples can be equated as

$$p(-/-) = (1-p_b)\,(-p_c)^2.$$

This probability consists of the probability of both samples of a pair being not positive due to contamination, $(1-p_c)^2$, in the sample pairs not positive to bacteraemia, $(1-p_b)$. These equations are based on the assumption that any CNS

present will lead to a positive blood culture. It is not realistic to assume such a perfect efficiency of culture technique. Although it may be hard to estimate the actual efficiency in the routine of a laboratory of medical microbiology, one can set estimates at arbitrary levels and calculate the effect of varying the efficiency on p_b and p_c. Efficiency of culture technique (e) is defined as the probability that bacteria present in the sample lead to a positive culture.

The probability of a sample pair consisting of two positive samples, taking efficiency into account, can be equated as

$$p(+/+) = p_b e^2 + (1-p_b) p_c^2 e^2.$$

This probability consists of the probability of a double-positive sample pair due to bacteraemia giving the efficiency, $p_b e^2$, and the probability of both samples being positive due to contamination giving the efficiency $p_c^2 e^2$, among the sample pairs not positive due to bacteraemia, $(1-p_b)$.

The probability of a sample pair consisting of a positive and a negative sample, taking efficiency into account, can be equated as

$$p(+/-) = p_b\{2e(1-e)\} + (1-p_b)\{2p_c^2 e(1-e) + 2p_c(e_1-p_c)\}.$$

This situation exists if the first sample is positive due to bacteraemia and its partner false-negative or the other way around, $p_b\{2e(1-e)\}$. Among the other sample pairs, $(1-p_b)$, a similar situation exists if either both samples of a pair are contaminated, $2p_c e(1-e)$, or only one is positive due to contamination and the other true-negative, $2p_c e(1-p_c)$.

The probability of a sample pair consisting of two negative samples, taking efficiency into account, can be equated as

$$p(-/-) = p_b(1-e)^2 + (1-p_b)\{p_c^2(1-e)^2 + 2p_c(1-p_c)(1-e) + (1-p_c)^2\}.$$

This situation exists if with bacteraemia both samples are false-negative, $p_b (1-e)^2$. If no bacteraemia is present, $(1-p_b)$, the situation can occur if both samples are contaminated and false-negative, $p_c^2(1-e)^2$; if one sample is true-negative and the other false-negative, $2p_c(1-p_c)(1-e)$; or if both samples are not contaminated $(1-p_c)^2$.

In a population of paired samples, $p\,(+/+)$, $p\,(+/-)$ and $p\,(-/-)$ are the *a posteriori* probabilities or the observed rates. If these probabilities are known, one has three equations with two unknowns (p_b and p_c) which can be calculated by conventional mathematical methods. In this study these probabilities have been calculated for the two illustrating sample populations and several degrees of efficiency by computer.

Sampling of patients

The first illustrating sample consists of 357 pairs of blood samples taken from 273 patients after open-heart surgery in the Catharina Hospital at Eindhoven, the Netherlands, in the period 1978–80. Blood cultures were taken postoperatively if the body temperature rose above 38·5 °C or if signs or symptoms suggestive for septicaemia or intravascular infection were present. Blood samples were obtained by concurrent venepuncture from the antecubital veins of the left and the right arm of the patient. Ten millilitres of blood were taken for each culture, 5 ml for

each bottle (Brain Heart Infusion (BHI) – sample and Liquoid Roche (LR) – sample). Before each venepuncture the skin was cleansed with iodine (1% w/v) in ethanol (70% v/v). The two venepunctures were always done one immediately after the other.

The second illustrating sample consists of 500 consecutive sample pairs taken in a hospital routine in the Catharina Hospital or the Diaconessenhuis at Eindhoven in 1983 and 1984. They were taken by many different physicians and on a great variety of indications, including suspected endocarditis as well as suspected septicaemia. Only pairs of samples collected within an hour at most were admitted. The routines for cleansing the skin were similar to the one described above.

Bacteriological methods

Blood cultures were processed as follows. From the first bottle, (the BHI sample), containing 15 ml Brain Heart Infusion (BHI) broth, with sufficient penicillinase to counteract 10000 units of penicillin, three pour plates were prepared using Blood Agar Base No. 2 (Oxoid Ltd, Basingstoke, England), containing glucose (1% w/v). The second bottle (the LR sample), containing liquoid and para-aminobenzoic acid, was subcultured into Brewer Thioglycollate broth (Difco, Detroit, USA) and into BHI broth. In addition, a pour plate using Blood Agar Base No. 2 containing glucose (1% w/v) was prepared. The agar plates were incubated at 37 °C for 3 days and the broth media for 7 days. Three pour plates (one from the LR sample and two from the BHI sample) were incubated in an atmosphere of 5% CO_2. These were inspected daily. Subcultures were made when growth was seen. After 5 days, blind subculturing of the media was done on Blood Agar Base No. 2 containing heated sheep blood (5%) and penicillin (0·1 U), on a medium consisting of four parts Oxoid G.C. Agar Base and one part Veal infusion medium (Difco) containing 5% heated sheep blood agar and in a Brewer Thioglycollate broth.

The latter medium was incubated for 5 days, the other media were incubated for 2 days. If growth was seen or if Gram-stained smears indicated the presence of micro-organisms, subcultures were performed on appropriate microbiological culture media. Antimicrobial susceptibility testing was performed using the Disk Diffusion Method (Bauer *et al.* (1966)), if necessary supplemented by dilution methods (Ericsson & Sherris, (1971)). For staphylococci these tests included penicillin, cloxacillin, tetracyclin, gentamicin, cephalothin, erythromycin, clindamycin and trimethoprim.

RESULTS

The total number of patients in the first illustrative sample in this study, the one in the department of cardio-pulmonary surgery conducted in 1978–80, was 273. In 44 patients a Björg–Shiley prosthetic valve was implanted. Paired blood samples were taken from these 44 patients almost three times as often as from the other 229 patients (Table 1).

Coagulase-negative staphylococci (CNS) were not cultured from 442 (82·3%) of the 537 sampled pairs. From 88 sampled pairs (16·4%) CNS were isolated from only one of the two samples, and in seven sampled pairs both samples were positive

Table 1. *Patients having paired-sampling for blood cultures after open-heart surgery (1978–80)*

Open-heart surgery	Patients	Pairs	Pairs/patient
Without prosthetic valve implantation	229	354	1·5
With prosthetic valve implantation	44	183	4·2
Totals	273	537	2·0

Table 2. *CNS from 500 sample pairs from patients obtained in 1983–4.*

	Positive samples in each pair		
	None	One	Both
Staphylococcus epidermidis (SE)			
Number	465	30	5
Percentage	93·0	6·0	1·0
Staphylococcus saprophyticus (SS)			
Number	498	2	0
Percentage	99·6	0·4	0·0
Other coagulase-negative staphylococci (CNS, s.s.)			
Number	473	25	2
Percentage	94·6	5·0	0·4

for CNS (1·3%). In the estimating formula: $p\,(+/+) = 0{\cdot}013$; $p\,(+/-) = 0{\cdot}164$ and $p\,(-/-) = 0{\cdot}823$. The sample pairs consisting of two positive samples were collected from seven patients. None of the patients had a double-positive sample pair twice. Six out of these seven patients had no symptoms or signs of intravascular infection, and had no antimicrobial treatment as a result of these blood cultures. In each pair of samples the CNS isolated differed in susceptibility pattern from its partner.

One patient had a double-positive sample pair with identical susceptibility pattern. This patient was a man, 52 years old. Blood cultures were taken for persisting low grade fever 15 days after replacement of his aortic valve by a Björg-Shiley prosthesis for insufficiency of unknown origin. Together with sustained leucocytosis and development of paravalvular leakage, the diagnosis prosthetic valve endocarditis was considered to be definite enough to warrant antimicrobial treatment. Three months later leakage around the prosthesis led to serious dyspnoea, and his prosthesis was replaced by a Hancock prosthesis. During surgery annular vegetations were noted, where they were not seen during the first operation, so endocarditis is almost certain.

The second illustrating sample in this study was taken 5 years after the first one. At this time, when the 500 sample pairs were collected from the two hospitals, the coagulase-negative staphylococci (CNS, s.l.) were differentiated into *Staphylococcus epidermidis* (SE), *Staphylococcus saprophyticus* (SS) and other coagulase-negative staphylococci (CNS, s.s.). The number of pairs with one sample positive for one of them is 57 (11·4%), and in seven pairs the samples were both positive (3·5%). There are marked differences in incidents between the three subgroups, however (Table 2). For SE the parameters in the estimating formula are:

Table 3. *Probabilities (%) of blood cultures being positive for CNS due to contamination (p_c)*

	Efficiency of blood culture technique (e)				
	0·5	0·8	0·9	0·95	1·0
Open-heart surgery (1978–80)					
CNS, s.l.	10·7	9·1	9·0	9·0	9·1
Two hospitals (1983–4)					
SE	2·9	3·0	3·0	3·1	3·1
CNS, s.s.	2·9	2·6	2·6	2·6	2·6

Table 4. *Probabilities (%) of blood cultures being positive for CNS due to bacteraemia (p_b)*

	Efficiency of blood culture technique (e)				
	0·5	0·8	0·9	0·95	1·0
Open-heart surgery (1978–80)					
CNS, s.l.	5·1	1·2	0·8	0·6	0·5
Two hospitals (1983–4)					
SE	3·0	1·5	1·1	1·0	0·9
CNS, s.s.	1·5	0·6	0·4	0·4	0·3

$p\,(+/+) = 0{\cdot}010$; $p\,(+/-) = 0{\cdot}060$ and $p\,(-/-) = 0{\cdot}030$; and for CNS, s.s.: $p\,(+/+) = 0{\cdot}004$; $p\,(+/-) = 0{\cdot}050$ and $p\,(-/-) = 0{\cdot}046$.

The susceptibility patterns of each of the five sample pairs positive for SE were similar to each other. Two sample pairs were from two patients on tumour chemotherapy, one of these patients also had radiotherapy. With the consequent diminished host resistance in mind, many blood samples were taken during periods of long standing low grade fever. No antimicrobials were used in connection with the positive blood samples, and no signs of intravascular infection were noted during a follow-up period of almost a year in either patient.

The other three for SE double-positive pairs were from three patients. One was a woman, 68 years old. Blood samples were taken during a period with several serious postoperative complications after the mitral, as well as the aortic valve had been replaced by Ionescu-Shiley prostheses. Vancomycin was given after three samples showed to be positive for SE. Her general condition gradually improved, and no signs of intravascular infection were noted. Bacteraemia as the source of SE in these samples is considered dubious.

In the other two patients the intravascular origin of the SE cultured is also considered dubious, as both lived only a few days after the samples had been taken. One was a 78-year-old adipose diabetic in intractible coma. The other was a man, 54 years old, with pancytopenia as a result of cytostatic treatment for acute myeloblastic leucaemia. The two pairs double-positive for CNS were from the same person, a man, 55 years of age, known to have non-Hodgkin lymphoma for many years. Admitted for general malaise and low grade fever 1 year after implantation of an aortic bifurcation prosthesis and 2 years after desobstruction

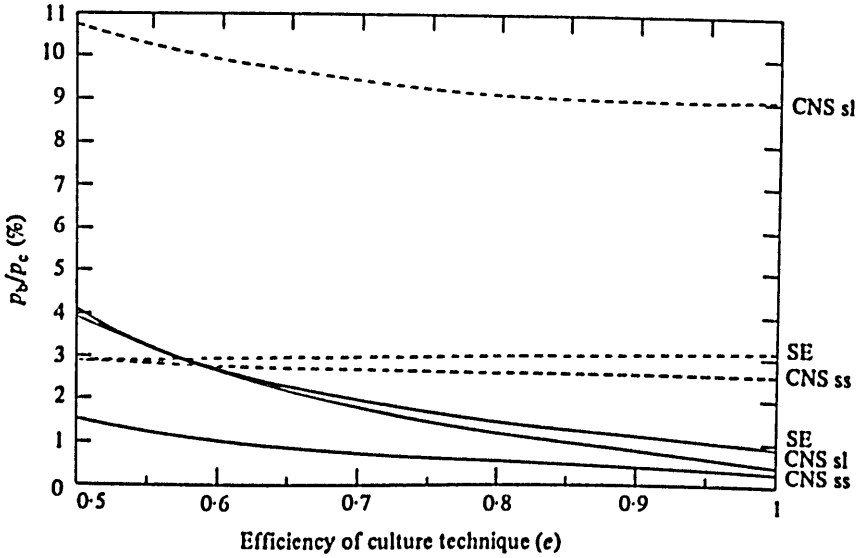

Fig. 1 The probabilities of contamination (p_c) and bacteraemia (p_b) as source of coagulase-negative staphylococci in blood cultures (see text). --- p_c; ——, p_b.

of an internal carotid artery, and anonyma artery. A systolic murmur, not noted previously, was heard. This, and periods with erythrocyturia indicated intravascular infection. After both pairs of blood samples showed growth of staphylococci with similar susceptibility pattern, he was treated with vancomycin and rifampicin for 45 days. No signs of intravascular infection persisted or reappeared, although treatment with prednisone and chlorambucil was initiated soon afterwards. Here the intravascular origin of the positive blood cultures was confirmed by the clinical data. The probabilities of positivity of a sample being positive for CNS due to contamination (p_c) in each part of the study is shown in Table 3. Probabilities of positivity due to bacteraemia (p_b) calculated in a similar way is shown in Table 4. The effect of variation of the efficiency of the culture technique (e) on both probabilities (p_c and p_b) is also demonstrated in Fig. 1.

DISCUSSION

The method described in this paper allows an estimate of p_b and p_c of a single blood culture, if results of sample-pairs in the same sample-population are known. If two or more blood cultures taken one after the other from the same patient show growth of coagulase-negative staphylococci, one might conclude persistent presence of these bacteria in the patients blood for the period the blood samples were taken. If its presence is transient during this period, positivity due to bacteraemia will behave like positivity due to contamination in the model used in this study. In the part of the sample taken in 1978–80 the venepunctures of a sample-pair were always done one immediately after the other. In the part of the sample taken in 1983–4, the interval was more varied, up to 1 h. The population from which the latter samples came is also more diverse than the first one. This, and many unspecified changes in the course of years, invalidates comparison of the results in

both samples in this study. They are not included for comparison, but for illustrating the use of the method to estimate p_b and p_c.

A critical assumption is that one that similarity of antimicrobial susceptibility pattern means that the same strain is isolated twice. The application of antibiograms to the problem of identifying coagulase-negative staphylococci is widespread. Its validity depends on the number of antibiotics involved, cross-resistance between these antibiotics, and the variability in the population. The latter will be limited during epidemic presence of the staphylococcus, especially if it is a common-source epidemic.

In the first sample in this study the staphylococci were also phage typed and biotyped (Staal *et al.* (1981)). One of the conclusions of that and similar studies is that neither phage typing, nor biotyping is a powerful discriminant of coagulase-negative staphylococci-phenotypes (Christensen *et al.* (1983)). In spite of the practical and theoretical objections against this application of antibiograms, this approach is found to be the most rewarding compared to phage and biotyping. It is often the only one available in a routine hospital microbiology laboratory at the time decisions about treating the patients have to be made.

The patient with a sample pair double-positive for similar coagulase-negative staphylococci in the first sample in this study was the only one showing evident intravascular infection amongst all patients with positive sample pairs. In the second sample in this study the two double-positive sample pairs were from the same person; the only person with evident intravascular infection. The prediction of bacteraemia on the basis of similarity of antibiograms was thus confirmed by the clinical histories of these two patients after the blood samples had been collected.

If staphylococci are missed due to a low efficiency of the culture technique, some of the sample pairs double-positive due to bacteraemia will be single-positive. This contributes to the number of single-positive sample pairs. This increase will be counteracted by the loss of single-positive sample pairs due to contamination. If these two level out, the effect on the estimate of p_c will be minimal, as is the case for SE and CNS, s.s. (Fig. 1). Nevertheless improving culture technique will lower p_b in all instances, improving its value in diagnosing bacteraemia.

In conclusion, taking sample pairs instead of single samples for blood cultures provides the clinical advantage of differentiating contamination from bacteraemia if coagulase-negative staphylococci are cultured. It also provides means to estimate the incidence of contamination and the incidence of bacteraemia and to illustrate how these estimates are effected by changes in the efficiency of blood culture technique. If a single blood culture is positive for coagulase-negative staphylococci, one can estimate the probability of bacteraemia in this patient, provided the results of paired-samples from the same patient-population are known.

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