Etiologic Diagnosis of Chronic Osteomyelitis

A Prospective Study

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Background: Although bone specimens were established 25 years ago as the gold standard for etiologic diagnosis of chronic osteomyelitis, recent studies suggest that non-bone specimens are as accurate as bone to identify the causative agent. We examined concordance rates between cultures from nonbone and bone specimens in 100 patients.

Methods: Prospective study conducted at Hospital Universitario San Vicente de Paul, a 750-bed university-based hospital located in Medellín, Colombia. We included patients with chronic osteomyelitis who had been free of antibiotic therapy for at least 48 hours, excluding those with diabetic foot and decubitus ulcers. At least 1 nonbone and 1 bone specimen were taken from each individual and subjected to complete microbiologic analysis.

Results: Bone cultures allowed agent identification in 94% of cases, including anaerobic bacteria in 14%. Cultures of nonbone and bone specimens gave identical results in 30% of patients, with slightly better concordance in chronic osteomyelitis caused by *Staphylococcus aureus* (42%) than by all other bacterial species (22%). However, statistical concordance determined by the Cohen kappa statistic was less than 0 (-0.0092 ± 0.0324), indicating that the observed concordance was no better than that expected by chance alone ($P > .99$).

Conclusions: Appropriate diagnosis and therapy of chronic osteomyelitis require microbiologic cultures of the infected bone. Nonbone specimens are not valid for this purpose.

Arch Intern Med. 2006;166:95-100

THE INFECTION OF BONE THAT contains bone marrow, called osteomyelitis, is as old as humankind and continues to be an important problem for modern medicine owing to its high morbidity and sequelae.[1,2] Acute osteomyelitis evolves over the course of days to a few weeks and can be cured with antibiotic therapy alone. Chronic osteomyelitis (COM), on the other hand, is a relapsing and persistent infection that evolves over months to years and is characterized by low-grade inflammation, presence of dead bone (sequestrum), new bone apposition, and fistulous tracts.[3] The most important point in relation to chronic bone infections is the difficulty to correctly establish the etiologic agent and the proper treatment to cure the patient.[4] Nowadays, to arrest COM, most experts consider it essential to provide adequate drainage, thorough debridement, obliteration of dead space and soft tissue, wound protection, and intravenous antibiotic treatment for at least 4 to 6 weeks.[2-4] Proper selection of therapy should always be made on the basis of correct identification of the causative organism(s) and knowledge of the pattern of susceptibility.[2-5]

The choice of bone as the ideal specimen for microbiologic diagnosis of osteomyelitis is based on common sense and a classic retrospective study of 40 patients published by Mackowiak et al[6] 27 years ago. More recent studies came to a similar conclusion, but data collection was retrospective, included a small number of patients, or had different objectives.[7-10] The difficulties inherent in the process of obtaining bone specimens led to new approaches during the last decade, with 3 studies concluding that specimens from sinus tracts and other soft tissue were as accurate as bone to identify the etiologic agents of osteomyelitis.[11-13] Together, these 3 studies evaluated 155 patients, but some experts disagree with these findings and insist that bone specimens must be the gold standard for etiologic diagnosis of COM.[14-16] In fact, it is difficult to draw definitive conclusions from these articles because they have diverse methodologic flaws, thoroughly exposed elsewhere.[7]

The lack of agreement on the best sample to use for microbiologic diagno-

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sis of COM demands new research on the topic because mistakes in the identification of the pathogen will lead to wrong treatment, contributing further to the "no cure" stigma of this expensive illness.[5] The aim of the present prospective study is to determine the microbiologic concordance of nonbone with bone specimens, taking the latter as the gold standard.

STUDY DESIGN AND PATIENTS

From February 2001 to December 2002, patients of any age, sex, ethnicity, and economic condition hospitalized with a diagnosis of osteomyelitis at Hospital Universitario San Vicente de Paul in Medellín, Colombia, were prospectively screened for COM. *Chronic osteomyelitis* was defined as a bone infection that was worse or had not improved clinically or microbiologically after 1 month of evolution, independent of the presence or absence of surgical and/or antimicrobial therapy. This study was approved by the human research ethics committees of the University of Antioquia Medical School and Hospital Universitario San Vicente de Paul.

PROCEDURES

Patients fulfilling our definition of COM were assigned to the same process: (1) interruption of all antibiotic therapy at least 48 hours before specimen collection; (2) surgical biopsy of the infected bone for histopathologic and microbiologic diagnosis, accepting specimens only from bone marrow, sequestra, and cortical bone, and rejecting debridement material even if it contained bone tissue; and (3) microbiologic study of nonbone specimens directly related to the infected bone, collected during surgery or in the ward following standard aseptic procedures, including soft tissue biopsy, swabs from surgical wounds, pus draining through sinus tracts or orifices left by orthopedic pins, and pus aspirated from soft tissue surrounding the infected bone. All these criteria were directly verified by the research group.

Orthopedists were further required to specify whether surgical access to the infected bone was made through intact skin or through infected soft tissue. Since surgical incision through infected soft tissue can contaminate the bone, we implemented a bone biopsy protocol designed to reduce contamination to a minimum. Briefly, the surgeon performed a thorough wash and debridement of the infected soft tissue before accessing the bone, a specimen from which was taken only after discarding superficial cortical bone by curettage. To determine if access through infected soft tissue was more commonly associated with polymicrobial bone cultures (a sign of potential bone contamination), we compared such outcome with that of bone biopsy specimens taken by incisions through healthy skin. Patients with diabetic foot and decubitus ulcers were excluded because the value of bone cultures is clear in the first and uncertain in the second condition.[17,18]

Patients were registered in a database that included age, sex, employment, hospitalization time, bone involved, time with COM, mechanism of bone infection, clinical condition of the skin incised by the surgeon to take the bone specimen, type of specimens cultured, and microbiologic identification and susceptibility pattern of organisms grown in aerobic (Vitek Systems; bioMerieux, Hazelwood, Mo) and anaerobic atmosphere (Rapid ID32A, bioMerieux). Routine and standard laboratory techniques for transportation and culture of aerobic and anaerobic microorganisms were followed, including special stains for bacteria, fungi, and myco-

bacteria.[19] Intravenous (IV) antibiotic therapy was begun once the susceptibility pattern of the bone-infecting organisms was established and continued for 28 to 42 days. In cases where internal osteosynthesis material was left in place, suppressive antibiotic therapy followed IV treatment, stopping 4 weeks after such material was removed. To evaluate the success of therapy, available patients were observed by the researchers at the outpatient unit or by telephone interviews for a minimum of 2 and up to 3 years.

STATISTICAL ANALYSIS

Isolates from the infected bone were compared with isolates from nonbone specimens for each patient. Nonbone specimens were considered concordant with the bone when they grew exactly the same pathogens isolated from the bone and had identical susceptibility patterns. Concordance was calculated for all causes and for COM caused by *Staphylococcus aureus*, the agent most commonly isolated from infected bone.

We used the Cohen kappa statistic as the measure of concordance for the dichotomous data produced by bone and nonbone specimens and quantified the probability that the value found for kappa differed statistically from 0 by computing P values using the 1-sided test validated by Landis and Koch.[20] For $P \le .05$, the null hypothesis (kappa ≤ 0) was rejected. Kappa values of 0.0 to 0.40, 0.41 to 0.60, 0.61 to 0.80, and 0.81 to 1.0 represent "marginal," "moderate," "substantial," and "almost perfect" agreement, respectively. On the other hand, for $P > .05$, the null hypothesis was accepted, concluding that both diagnostic approaches are independent, ie, the observed agreement is no better than would be expected by chance alone.

To determine if conditions of the skin incised by the surgeon to take the bone biopsy specimen (healthy vs infected skin) had any influence in the number of microorganisms isolated from the bone (monomicrobial vs polymicrobial COM), we constructed 2×2 contingency tables and determined the significance of the differences by Yates-corrected χ^2 analysis. For data management and analysis, we used Microsoft Excel 2003 (Microsoft Corp, Redmond, Wash), Epi-Info, version 3.3 (CDC, Atlanta, Ga), InStat version 3.06 (GraphPad Software, San Diego, Calif), and StatXact-5 (Cytel Software Corp, Boston, Mass).

During the study period, 121 patients were diagnosed as having osteomyelitis not related to diabetic foot or decubitus ulcers. Twenty-one patients were excluded because antibiotic treatment was not stopped 48 hours before the bone biopsy (n=13), evolution of osteomyelitis was shorter than 1 month (n=5), and lack of nonbone (n=2) or bone specimens (n=1 patient). One hundred patients fulfilled the inclusion criteria, 72 male and 28 female, aged 9 to 83 years (mean±SD age, 38±18 years). The COM had evolved over a period of 1 to 384 months before diagnosis (median, 6 months), and both specimens were taken after 1 to 120 days of hospitalization (median, 5 days).

Tibia (34%) and femur (33%) were the most frequent foci of the COM, followed by fibula (8%, 5 associated with the tibia), iliac crest (7%), vertebra (3%), humerus (3%, 1 associated with the ulna), and other medullar bones (12%). In most cases (n=93), bacteria reached the bone by local spread from a contiguous source of infection; the other 7 patients had hematogenous osteomyelitis. Contiguous sources of infection appeared af-

ter violent trauma (n=64), surgical procedures (n=25), and infection of neighboring joints or skin structures (n=4).

Table 1 lists the type of specimens cultured. Bone specimens were taken from all patients during open surgery and subjected to complete microbiologic study. Selection of the bone structure best suited for culture was made by the surgeon based on the presence of macroscopic signs of infection. As soon as possible before or after the bone biopsy, a member of the research group also took 1 nonbone specimen per patient from soft tissue with signs of infection or colonization.

Table 2 lists the bacteria and fungi obtained from cultures of bone and nonbone specimens and details the results of concordance analysis categorized by bone organisms (n=150) and by patients (n=100). The **Figure** illustrates the causes of COM based on bone cultures and the agreement of nonbone analyses for each etiologic group. Bone cultures allowed isolation and identification of the cause of COM for 94 patients; the other 6 subjects had COM demonstrated by bone histopathologic analysis, but no organisms were visualized or isolated from their bone specimens, including aerobic and anaerobic bacteria, *Mycobacterium* species, and fungi. Bone cultures from these 94 patients produced 150 isolates: *S aureus* was the most frequent (43/150, 29%), followed by 10 different species of the Enterobacteriaceae family (n=24, 16%), *Enterococcus* species (n=22, 14.67%), anaerobic bacteria (n=16, 10.67%), and others, including *Pseudomonas aeruginosa*, coagulase-negative staphylococci, *Streptococcus* species, and *Acinetobacter calcoaceticus-baumannii* complex. Anaerobic bone cultures were done for 92 patients, and 13 (14%) of them produced 16 organisms primarily associated with violent trauma. The first bone specimen gave negative cultures in 10 patients. Follow-up showed that 2 had anaerobic COM (1 by *Peptostreptococcus prevotii* and 1 by unclassified anaerobe gram-negative coccobacilli); 2 had aerobic bacteria isolated from the bone in samples from a second biopsy; and 6 persisted with negative bone cultures.

Nonbone specimens were also negative for 11 patients but produced 116 isolates from the other 89 subjects. *Staphylococcus aureus* was again the most frequently found organism (44/116, 38%), followed by Enterobacteriaceae (n=22, 19%), coagulase-negative staphylococci and *P aeruginosa* (n=13 for each, 11%), and others like *Enterococcus* species, *Streptococcus* species, other aerobes, anaerobes, and fungi. Concordance analysis by microorganism was 19.33% for all causes and 41.86% for *S aureus*, the highest for any bacterial species (Table 2). Cohen kappa values computed for COM caused by *S aureus* or any other cause were all close to 0, indicating no microbiologic correlation of nonbone with bone specimens (Table 2).

Concordance analysis by patients (**Table 3**) showed that 26 had exactly the same organisms in nonbone and bone specimens (true agreement with positive bone findings, 26%), and 68 had different microorganisms in each kind of specimen (disagreement with positive bone findings, 68%). On the other hand, 2 patients had positive nonbone findings but sterile bone cultures (disagreement with negative bone findings, 2%), while 4 had nega-

tive cultures in both specimens (true agreement with negative bone findings, 4%). The diagnostic accuracy of nonbone specimens, as determined by the rate of microbiologic agreement with bone cultures, was 30%. The Cohen kappa value was lower than 0 (-0.0092 ± 0.0324; $P > .99$), confirming that the observed agreement of 30% was the same as that expected by chance.

Two specific subgroups of patients with COM for whom nonbone specimens are supposedly well suited to etiologic diagnosis also showed a lack of concordance with the bone based on the Cohen kappa test: COM caused by *S aureus* (vs other causes) and COM caused by local spread of a neighboring infection (vs hematogenous COM). Similarly, we found complete independence between some specimens considered concordant by nature, ie, sequestra (bone) and sinus tracts (nonbone specimen). Concordance was also absent in patients for whom bone specimens had to be taken by incising infected or healthy skin and soft tissues. **Table 4** lists the rate of concordance and respective kappa values for these subgroups, none statistically different from 0. In other words, the results from cultures of nonbone specimens are completely independent from bone specimens, without regard for the group analyzed.

There was no difference in the frequency of polymicrobial COM between patients with healthy vs colonized or infected soft tissue, which suggests that the conditions of the skin incised by the surgeon to take the bone biopsy specimen had no influence on the concordance rates found by this study (**Table 5**).

In addition to surgical treatment, all patients received at least 28 days of IV antibiotic therapy based on the antibiograms of bone isolates. Microorganisms isolated from soft tissue were not considered for antibiotic treatment except in cases with clinical signs of skin and soft tissue infection that were treated for 5 to 10 days. Only 26 subjects were available for follow-up for a period of 2 to 3 years: 18 patients (69%) were cured (15 without sequelae, 2 with chronic pain, and 1 with limb amputation); 6 patients (23%) still had COM at the third year; and the other 2 (8%) died for reasons not related to COM but without certainty about the cure of their bone infections.

Histopathologic studies were performed for 39 patients, including all 6 with bone-negative cultures. Chronic

Table 1. Specimens Taken From Study Patients With Chronic Osteomyelitis

Specimen	Number
Bone	100
Cortical bone biopsy	83
Sequestra	13
Bone marrow	7
Nonbone	100
Soft tissue: pus swabs	49
Infected surgical wound swabs	29
Sinus tract: pus swabs	17
Orthopedic pin orifices: pus swabs	3
Soft tissue: pus aspirates	2

Table 2. Etiology by Bone Cultures of 100 Patients With Chronic Osteomyelitis

Microorganisms	No. of Isolates		Concordance*			
	Bone Cultures	Nonbone Cultures	By Organism, No. Found/ Total No. (%)	Cohen Kappa	By Patient, No. (%) (n = 100)	Cohen Kappa
Gram-positive aerobes	89	75	24/89 (27)	−0.5460	22 (22)	−0.3600
Staphylococcus aureus	43	44	18/43 (42)	0.1744	18 (18)	−0.0370
Enterococcus faecalis	19	9	3/19 (16)	0.1446	2 (2)†	0.1611
Staphylococcus epidermidis	5	7	1/5 (20)	0.1329	1 (1)	0.1150
Other coagulase-negative staphylococci	8	6	0/8 (0)	−0.0470	0 (0)	−0.0073
Streptococcus agalactie	3	2	2/3 (67)		1 (1)†	
Streptococcus constellatus	2	2	0		0	
Enterococcus faecium	2	0	0		0	
Enterococcus avium	1	1	0		0	
Streptococcus dysgalactiae	1	1	0		0	
Streptococcus oralis	1	0	0		0	
Streptococcus mitis	1	0	0		0	
Streptococcus salivarious	1	0	0		0	
Streptococcus bovis	1	1	0		0	
Streptococcus intermedius	0	1	0		0	
Streptococcus sanguis	0	1	0		0	
Gram-positive cocci‡	1	0	0		0	
Gram-negative aerobes	45	39	5/45 (11)	−0.2210	4 (4)	−0.0670
Pseudomonas aeruginosa	14	13	2/14 (14)	0.0640	2 (2)	0.0154
Escherichia coli	9	11	1/9 (11)	0.0364	1 (1)	0.0011
Acinetobacter baumannii	5	3	1/5 (20)	0.2307	0†	0.2207
Enterobacter cloacae	4	3	0		0	
Proteus penneri	3	0	0		0	
Morganela morganii	2	1	0		0	
Proteu mirabilis	2	3	1/2 (50)		1 (1)	
Proteus vulgaris	1	1	0		0	
Pseudomonas fluorescens	1	0	0		0	
Acinetobacter lwoffi	1	0	0		0	
Enterobacter aerogenes	1	0	0		0	
Serratia fonticola	1	0	0		0	
Klebsiella oxytoca	1	0	0		0	
Klebsiella pneumoniae	0	2	0		0	
Providencia rettgeri	0	1	0		0	
Comamonas acidovorans	0	1	0		0	
Anaerobes	16	1	0	−0.0120	0	−0.0200
Clostridium difficile	4	0	0		0	
Propionibacterium acnes	2	0	0		0	
Bacteroides urealyticus	2	0	0		0	
Porphyromonas species	2	0	0		0	
Peptostreptococcus prevotii	1	0	0		0	
Clostridium perfringens	1	0	0		0	
Gram-positive coccobacilli‡	1	0	0		0	
Gram-negative coccobacilli‡	3	1	0		0	
Fungi	0	1	0		0	
Candida albicans	0	1	0		0	
Sterile cultures	6	11	4/6 (67)	0.4428	4 (4)	−0.1460
Total	150	116	29/150 (19)	−0.7300	30 (30)	−0.0090

*Cohen kappa *P*>.10 for all cases (the observed agreement between bone and nonbone specimens is no better than would be expected by chance alone). Kappa values were not calculated for organisms isolated from fewer than 5 bone specimens.

†Patients with polymicrobial bone infections are represented only once: 2 with *S aureus* (1 also had *E faecalis* and 1 *A baumannii*) and 1 with *Streptococcus agalactie* who also had *P aeruginosa*.

‡Unclassified microorganisms.

osteomyelitis was confirmed in 38 cases, and 1 with "normal" histopathologic bone findings had methicillin-susceptible *S aureus* grow in bone and nonbone cultures (concordant) after 4 months of purulent discharge from a sinus tract related to surgical correction of an open fracture of the left tibia.

COMMENT

This prospective study, assessing the validity of specimens other than bone to establish the cause of COM, was motivated by the poor prognosis of patients with post-
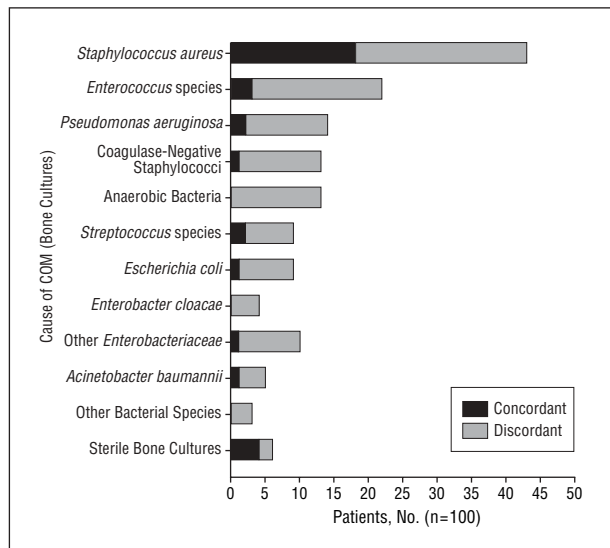
Figure. Causes of chronic osteomyelitis (COM) based on bone cultures from 100 patients. Black bars represent the number of patients with identical isolates from bone and nonbone specimens (concordant cultures); frequencies add to more than 100 because 65% had polymicrobial bone infection.

Table 3. Microbiological Concordance of Nonbone With Bone Specimens to Identify the Cause of Chronic Osteomyelitis in 100 Patients

Nonbone (New Method)	Bone (Gold Standard)		Total
	Cause Identified	Cause Not Identified	
Cause identified	26	2	**28**
Cause not identified	68	4	**72**
Total	**94**	**6**	**100**

Table 4. Concordance Between Bone and Nonbone Specimens to Identify the Cause of COM in Different Subsets of Patients

Patient Subset	Patients, No.	Concordant Cultures, No. (%)	Cohen Kappa ± SE	P Value*
Staphylococcus aureus	43	18 (42)	−0.585 ± 0.0666	>.99
Other cause	57	12 (21)	−0.043 ± 0.0527	>.99
Hematogenous COM	7	4 (57)	−0.272 ± 0.2303	>.99
Local spread COM	93	26 (28)	0.0087 ± 0.0265	>.99
Sequestra specimens	13	2 (15)	0.0137 ± 0.0193	>.99
Other bone specimens	87	28 (32)	−0.015 ± 0.0371	>.99
Sinus tracts	17	6 (35)	0.0507 ± 0.0534	>.99
Other nonbone specimens	83	24 (29)	−0.021 ± 0.0377	>.99
Incision				
Intact skin	35	10 (29)	−0.035 ± 0.0617	>.99
Infected skin	65	20 (31)	0.0047 ± 0.0372	>.99

Abbreviation: COM, chronic osteomyelitis.

*P>.05 implies that the Cohen kappa value is not statistically different from 0, and that kappa values not different from 0 indicate lack of concordance.

Table 5. Frequency of Monomicrobial and Polymicrobial Bone Cultures by Incised Skin Condition in 100 Patients With COM

Incision Group	Patients, No.	Cultures, No. (%)		P Value
		Monomicrobial	Polymicrobial	
Intact skin	35	23 (66)	12 (34)	.91
Infected skin	65	42 (65)	23 (35)	

Abbreviation: COM, chronic osteomyelitis.

traumatic COM reported by our orthopedic surgeons. Before 1998, antibiotic therapy of COM at our institution was based on the microorganisms isolated from nonbone specimens, and the surgeons cited at least 3 studies supporting their approach.[11-13] Although back then we had no data, clinical observation suggested an incurable disease because most patients treated under such an approach experienced a relapse.

In view of the situation, members of our group reviewed the evidence available and conducted a retrospective study of 50 patients seen at the Section of Infectious Diseases between February 1998 and August 2001.[7] Our results confirmed the most important conclusions of Mackowiak et al[6] and contradicted the findings of the more recent articles, but that study had some design problems, including its retrospective nature. Mackowiak et al had concluded that nonbone specimens did not predict the species of bacteria actually involved with bone infection and that every effort should be made to culture the bone, with 1 exception: *S aureus* appeared correctly predicted in 78% of patients by swab cultures of the sinus tracts associated with COM.[6] In the last 10 years, 3 other independent studies concluded that any nonbone specimen was as good as the bone to establish the cause of COM, including all possible microorgan-

isms.[11-13] Today, infectious diseases textbooks recognize the work of Mackowiak et al, but instead of highlighting their most valuable contribution emphasize that nonbone specimens are good for diagnosis of *S aureus* COM.[21]

Our group's retrospective findings with 50 patients[7] contradicted the conclusion of Mackowiak et al[6] regarding *S aureus* COM: only 38% of nonbone specimens were concordant with bone cultures. The present study, including twice the number of patients in a prospective design that allowed control of confounding variables, also found a low level of concordance for *S aureus* (42%). If the analysis is repeated discriminating polymicrobial from monomicrobial COM involving *S aureus*, concordance drops to 13% for the first and increases to 57% for the second type of bone infection. Such level of diagnostic accuracy for monomicrobial staphylococcal osteomyelitis is still too low to use as a foundation for the prolonged and difficult treatment of this expensive disease.

The Cohen kappa tests applied to these data are conclusive about the lack of concordance between nonbone and bone cultures for etiologic diagnosis of COM independent of the organisms involved, patient subgroup, or

specimen type. Our results regarding *S aureus* markedly differ from those in previous reports for at least 2 reasons: first, we collected data in a prospective manner and under a strict surgical protocol that prevented contamination of bone specimens with organisms colonizing adjacent soft tissue; second, we considered discordant *S aureus* isolates with different susceptibility patterns. In fact, general concordance for *S aureus* would rise to 81.4% if all isolates were taken into account independently of their antibiograms, but almost half of the patients (17 of 35) would have received inappropriate treatment if it were guided by nonbone cultures: 7 had *S aureus* differing in susceptibility from *S aureus* in the bone, and 10 had identical *S aureus* in both specimens but different copathogens in the bone.

The data also support the conclusion that bone biopsies are highly productive (94%) and not affected by the conditions of overlying soft tissue as long as the procedure is performed under surgical protocols designed to minimize the risk of contamination. This is important because many times the surgeon has only 1 opportunity for bone sampling, precisely at the time of debridement of colonized soft tissue surrounding the infected bone. Also, in contrast to common belief, sequestrum findings were not more concordant with those of nonbone specimens than bone or bone marrow specimens, and swab cultures from sinus tracts were no better at predicting COM cause than any other nonbone specimen. The importance of anaerobic cultures of the bone, which were isolated from 14% of our patients, cannot be overemphasized.[22]

In conclusion, only bone cultures should be used to guide antibiotic treatment in patients with COM, including those infected by *S aureus.* Choosing nonbone cultures for this purpose will lead to incorrect etiologic COM diagnosis and, therefore, inappropriate therapy.

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