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Beware of Unusual Organisms Masquerading as Skin Contaminants

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1. Introduction

Sepsis or bacterial blood stream infection is diagnosed by culturing blood to see what organisms grow. However, the growth of an organism in a blood culture does not always mean it is the cause of a disease. Some of blood cultures become positive because the needle that punctures the skin becomes contaminated with organisms from the patient's skin. skin and transfers them into the blood culture bottles where they grow. This gives an erroneous impression that the organisms were in the blood, causing disease, rather than on or in the skin.

These false-positive blood cultures, also known as contaminated blood cultures, represent a serious health care problem. The misperception that a patient is bacteremic may prolong hospitalization, increase cost, and lead to serious side effects including drug toxicity and *Clostridium difficile* infection. Use of antibiotics also contributes to the selection for antibiotic resistance. The presence of multi-drug resistant (MDR) bacteria may impact other patients by increasing the chance of hospital-acquired infections. To avoid these undesirable outcomes, the Clinical Laboratory Standards Institute (CLSI; Wayne, Pennsylvania) has promulgated guidelines for the identification and quantification of "contaminated cultures." A single blood culture growing one of the following skin organisms is presumed to be contaminated: coagulase-negative staphylococci (CNS), *Corynebacterium species*, *Bacillus species* (not *anthraxis*), *Micrococcus species*, *Streptococcus viridans* (alpha hemolytic streptococci), and *Propionibacterium acnes*. These are normal skin flora and may be in adenexa below the surface of the skin and thus not subject to removal, even by careful skin preparation. Thus some contaminated cultures are inevitable. Scrupulous attention to cleaning the venipuncture site and barrier protection may contribute to reduced rates of contamination. The nature of the cleaning agent and the time of skin contact are believed to be important. Whether the personnel who draw blood cultures are trained phlebotomists as

opposed to nurses or medical residents has been shown to make several percentage points of difference in the percentage of contaminated cultures. It is generally accepted that the target percentage of contaminated blood cultures should be less than 3%. However, many facilities, particularly busy emergency departments (EDs) do not achieve this goal, while other sites have a contamination rate closer to 1%. In many situations, the percentage of positive cultures that are interpreted as contaminants ranges from 25 to 50% of the total number of positive cultures. Further complicating the situation is that in very busy EDs, a second blood culture may not be drawn, and, even if the organism is not a contaminant, it would be classified as such because it could only grow in the single blood culture set. In addition, with the increase in immune-compromised patients, true infections with skin organisms have markedly increased. Both of these observations have lead to increased emphasis on the importance of drawing two blood cultures sets per episode of blood culture collection.

Modern blood culture instrumentation functions optimally with 10 ml of blood per culture bottle, or 20 ml of blood per culture set. This volume should contribute to the detection of low level bacteremias, but it is often difficult to obtain as well, because chronically ill persons, such as renal dialysis, cancer chemotherapy or bone marrow transplant patients may have compromised veins rendering difficult to impossible the collection of two culture sets, each containing an adequate volume of blood [1]. This may lead to false negativity of blood cultures. For a more complete evaluation and extensive references, see the excellent review articles by Hall and Lyman, [2]. and by Weinstein [3].

The Microbiology Division of Detroit Medical Center (DMC) University Laboratories serves the nine hospitals of the DMC (approximately 2000 beds), including two large general hospitals and others specializing in pediatrics, cancer (with a large bone marrow transplant center), acute trauma, rehabilitation, tertiary care, and surgery. In addition, microbiology receives about 50 percent of its specimens from physicians' offices and outpatient clinics, including large adult and pediatric HIV clinics, and serves as the primary or reference laboratory for two specialty hospitals. We receive approximately 80,000 blood culture sets annually. Our combined contaminated culture rate is approximately 3.7% overall. Thus we deal with over 3000 contaminated cultures per year. Not included in this number are those cultures that, although initially apparently filling the criteria for a contaminated culture, have a similar organism appearing in another blood culture within a 7-day period; these are recategorized as true infections.

To avoid working up contaminating organisms, we have established the following guidelines: for organisms that grow in a single blood culture set within seven days, and that appear, based on spot testing, as shown in Table 1, to be *Corynebacteria*, CNS, or alpha (not *Streptococcus pneumoniae* or *S. bovis*) or non-hemolytic streptococci: we do not perform either a complete identification or susceptibility testing. Due to the way we routinely process *Micrococcus sp.*, *Bacillus sp.* not *cereus* or *anthracis*, *Propionibacterium acnes*, and *Clostridium sp.* not *tetani*, they are not subjected to special limited workup as potential "contaminated cultures."

Organism Group	Tests to Identify as Potential Contaminants
Coagulase Negative Staphylococcus	Non-hemolytic, Gram positive cocci in clusters, catalase +, slide and tube coagulase -, pyrrolidiny l arylamidase -, bile esculin -
<i>Streptococcus viridans</i> group	Gram negative cocci in pairs and chains, alpha hemolytic, catalase -, not bile soluble
<i>Streptococcus species</i> , no further identification	Non-hemolytic, Gram + cocci in pairs and chains, catalase -, pyrrolidiny l arylamidase -
<i>Corynebacterium species</i>	Gram positive bacilli of correct morphology, catalase +, non-motile, non-hemolytic

Table 1. Spot tests used to identify potential blood culture contaminants in our laboratory if present in only one blood culture set per 7-day period

We call the Gram stain results of positive blood cultures on each patient to the clinician. If a physician follows up on the culture and finds that the laboratory regards an organism that he considers to be the etiologic agent of disease to be a presumptive contaminant, a call to the laboratory will lead to definitive identification and susceptibility testing. Or if a similar organism grows within 7 days of the original isolate, our laboratory automatically identifies and antibiotic susceptibility tests both organisms. Our clinicians are accustomed to this practice, which has been in place for more than 20 years, and are quick to call the laboratory if they feel that a full workup is warranted.

Our laboratory has detected a number of organisms that at first appeared to be typical contaminants. On further investigation, stimulated by physician request, or subsequent isolation of the same organism, they have proven to be unusual isolates. This chapter describes four of these cases, three of which have been previously published [4, 5, 6]. It describes the methodologies we used to arrive at a definitive identification, and indicates how we came to realize that they were not “typical skin contaminants.” This chapter does not highlight the detection of the usual skin flora that appear to be true etiologic agents of disease, although these are far more common. We use the unusual isolates to illustrate laboratory and clinical procedures that are important when the laboratory limits workup of presumed contaminated cultures.

2. Methods

Blood for the detection of infecting organisms is cultured in aerobic and anaerobic bottles of the BACTEC 9240 blood culture system (Becton Dickinson, Deerfield, IL, USA), according to the manufacturer's instructions. When the instrument flags a blood culture bottle as positive, a Gram stain is done and the fluid is subcultured to aerobic and anaerobic agar plates. We use PNA-FISH (AdvanDx, Woburn, Massachusetts) to identify fully within one hour those blood culture organisms that exhibit Gram stain morphology compatible with *Staphylococcus aureus*, or with *Enterococcus species*. Our laboratory uses the MicroScan WalkAway 9600 (WalkAway; Siemens, Deerfield, Illinois) for the organism identification and susceptibility testing reported here. When indicated, we use the RapID Str (Remel, Lenexa, KS), and the API Coryne Strip (bioMérieux, Durham, North Carolina), for organism identification. We also utilize E strips (AB Biodisk, Solna, Sweden) for susceptibility testing

as needed. We follow CLSI recommendations for susceptibility testing and result interpretation when recommendations are available (Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Information Supplement. CLSI document M100-S20 (ISBN 1-56238-716-2). Clinical and Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, Pennsylvania 19087-1898 USA, and Clinical Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. CLSI document M45-A [ISBN 1-56238-607-7]. Wayne, Pennsylvania.). Ancillary tests whose use is reported in this chapter include leucine amino peptidase (LAP; LAP Disk, Remel), pyrrolidiny arylamidase (PYR; Identicult-AE-PYR; PML Microbiologicals, Tualatin, Oregon), and the Staphyloslide latex agglutination test (BBL, Lawrence, Kansas). Organism identification by molecular techniques involved amplification and sequencing of 16S rDNA using the MicroSEQ Microbial Identification System as instructed by the manufacturer (Applied Biosystems, Carlsbad, California). Sequence data were analyzed using BLAST at the NCBI web site or the MicroSEQ ID 16S rRNA 500 v2.2 Library.

2.1. Case 1: Organism masquerading as a viridans streptococcus [4]

Case Report: On day 23 after an autologous hemopoietic stem cell transplant for acute lymphocytic leukemia, a 34 y/o man was admitted through the emergency department (ED) with fevers, neutropenia and graft failure. A single blood culture was drawn, and he was begun on empiric vancomycin (VAN) and aztreonam for neutropenic fever. Both the aerobic and anaerobic bottles from that blood culture turned positive on hospital day 3. The organism was initially thought to be a viridans streptococcus. Due to the failure of the recent, apparently successful stem cell transplant, the clinician felt that the patient had a *bone fide* infection. At his request, the organism was tested by MicroScan (PC-20 panel), which showed the organism to be VAN resistant. E-test (AB Biodisc, Solna, SW) revealed Daptomycin (DAP) susceptibility and patient was switched to DAP. Blood cultures drawn on hospital day 3 turned positive on hospital day 6 for the organism, and also for *Escherichia coli* and *Enterococcus faecalis*. Imipenem was added and the patient improved rapidly. After a second bone marrow transplant, he did well.

Laboratory investigation. Both the aerobic and anaerobic blood culture bottles of the single blood culture drawn in the ED, grew gram positive cocci/coccobacilli in pairs and chains at 12 and 24 hours respectively. The bottles were subcultured to standard aerobic and anaerobic media. After 2 days, pinpoint, α -hemolytic colonies grew both aerobically and anaerobically. The organism was catalase negative and was initially thought to be a viridans streptococcus. The next day susceptibility testing (MS PC-20 panel) performed at physician request showed the organism to be VAN R but gave no identification. The patient was switched to DAP, as described above.

The organism was tested on the RapID™ Str panel which identified it as *Streptococcus intermedius*, *Listeria monocytogenes* or *Pediococcus pentosaceus*, depending on what assumptions were made about ambiguous biochemicals. MicroSeq sequencing and BLAST analysis of the first 500 bases of the 16S rDNA gene revealed the highest identity between

our isolate and *Weissella confusa* and *W. cibaria*. We next sequenced the entire 16S rDNA gene. The 1493 base-long sequence showed 100% identity to the sequence of *W. confusa* strain Inje LM S-338 (1492/1493; DQ321751.1).

Approximately 6 months later, a similar VAN-R organism with a slightly different susceptibility profile was isolated from multiple blood cultures of a patient in the burn unit at another of our component hospitals. An alert technologist recognized the biochemical profile from the MicroScan, and sent it for rDNA sequencing to confirm its identity as *W. confusa*. *W. confusa* has been found in fermented foods and has also been reported as a cause of sepsis in 5 other patients [7, 8, 9]. These human cases, like ours, were VAN-R.

Commentary: The MicroScan does not have *W. confusa* in its data base, and thus could not be expected to identify the organism. However, the biochemical reactions were clear cut and allowed recognition of the subsequent patient's isolate. In contrast, the RapID Str panel, which has *W. confusa* in its data base, provided several erroneous identifications, all at high probability, depending on what assumptions were made about ambiguous biochemical reactions. Fortunately our experienced laboratorians realized that the α -hemolysis, the Gram stain morphology, and the VAN resistance rendered each of these identifications unlikely. Molecular identification was definitive.

2.2. Case 2: Organism masquerading as a coagulase-negative *Staphylococcus*

Case Report: A 45 year old female with congestive heart failure and a long history of end stage renal failure, on dialysis, was admitted through the ED for chest pain and back pain. She was afebrile. A myocardial infarction was ruled out. On hospital day 2 she developed a fever to 102F and two blood cultures were drawn. The initial culture bottle turned positive within 24 hr and the fluid was positive for Gram positive cocci in clusters. PNA-FISH analysis suggested that it was not *S. aureus*. On culture, it first appeared to be a CNS, and thus a presumed skin contaminant. However the second culture turned positive later that day and grew a similar organism, leading to a full workup. Despite negative PNA-FISH and coagulase tests, MicroScan identified the organism as a methicillin-sensitive *S. aureus*. VAN was administered after her dialysis, and her fever resolved within 24 hr. Follow up blood cultures were negative.

Laboratory investigation: The isolate grew in both the aerobic and anaerobic bottles of the both blood culture sets within 24-36 hrs. Staining revealed Gram-positive cocci in clusters. PNA-FISH analyses, performed on the fluid withdrawn from the culture bottles, were negative for *S. aureus*. Both the tube and slide coagulase tests were negative, suggesting coagulase-negative *Staphylococcus* species. However, when the organism was inoculated into the MicroScan panel, it was identified as *S. aureus*. Further, the Staphyloslide latex agglutination test, which detects both clumping factor (the cause of positive slide coagulase tests) and staphylococcal protein A, was positive as would be expected with *S. aureus*. We presume the Staphyloslide detected protein A, since the slide coagulase was negative. The organism utilized mannitol in the presence of salt, again suggesting *S. aureus*. Isolates from all 4 bottles had identical colony morphologies and identical MICs (in $\mu\text{g/ml}$) including:

Oxicillin 0.5, VAN 1, Erythromycin >4 (Resistant) and Clindamycin resistant by the D test for inducible resistance. 16S rDNA sequencing and BLAST analysis identified the organism as *S. pseudolugdenensis/pettenkorferi*.

Commentary: This isolate was identified with >90% confidence as *S. aureus* by the MicroScan, and its positive reactions on mannitol salt and on Staphyloslide latex agglutination appeared to confirm its identity as *S. aureus*. However, the organism's failure to react with the PNA-FISH *S. aureus* probe and its lack of slide and tube coagulase activity were strongly opposed to this diagnosis. As stated above, we presume that the Staphyloslide latex agglutination test reacted with staphylococcal protein A, which is supposed to be adequate to identify *S. aureus*. 16S rRNA sequencing and BLAST analysis identified the organism as *S. pseudolugdenensis/pettenkorferi*, each of which has been reported to be the etiologic agent of sepsis in some cases, and a presumed skin contaminant in others [10, 11]. The organism seems more likely to represent *S. pettenkorferi*, as *S. pseudolugdenensis* is reported to be pyrrolidonyl arylamidase positive and our organism was not. Had we not performed the PNA-FISH analysis on this organism, we might have called it a very rare *S. aureus* that was negative for both slide and tube coagulase. Because of the general susceptibility of the organism, this would not have affected the antibiotic susceptibility result interpretation; but such may not always be the case as CLSI recommends different susceptibility breakpoints for *S. aureus* and CNS. They further recommend that the *S. aureus* susceptibility interpretations be used for *S. lugdenensis*. Whether this should apply to some of the other CNS, and if so to which ones, remain to be determined. Fortunately the patient responded rapidly to VAN therapy. The identity of the organism was not known until after she had been discharged. We had not previously seen such an organism, but over the last several years, other patients, not all of them on dialysis, were found to have infections by similar organisms.

2.3. Case 3: Organism masquerading as a *Corynebacterium* [5]

Case Report: A 20-year-old female with relapsed T precursor cell leukemia received an allogeneic peripheral blood stem cell transplant from an unmatched male donor (C and DQ mismatch). She engrafted on post-transplant day (PTD) 9, and her maximum neutrophil count was $3.0 \times 10^9/L$. Transplant complications included hyperacute graft-versus-host disease (GVHD) of the skin, chemotherapy-related mucositis, and neutropenic fever. Therapy included tacrolimus, methylprednisolone, and mycophenolate mofetil.

On PTD 61, she developed abdominal cramping, nausea, vomiting, and diarrhea. Colonoscopy showed diffuse erythema and extensive colitis with numerous erosions. Biopsy confirmed Stage III GVHD. She received intravenous (IV) piperacillin/tazobactam for 14 days for a febrile episode that developed after the endoscopy; blood cultures showed no growth. After 3 days of IV antibiotic therapy, another febrile episode occurred, associated with a blood culture positive for coagulase-negative staphylococcus and *P. acnes*. Therapy with IV vancomycin was added. Within 48 h, after the antibiotics were discontinued, she developed a low-grade fever, and her absolute neutrophil count (ANC) decreased

markedly. Therapy with mycophenolate mofetil was discontinued, and filgrastim treatment was begun to raise her ANC.

Two blood cultures drawn over several days were positive for a Gram positive rod which appeared to be a *Corynebacterium* species. VAN was restarted. One negative blood culture was followed by several more that were positive for the same organism. One of the blood cultures was also positive for *Candida glabrata*. Therapy with IV micafungin was added. The Hickman catheter, suspected to be the source of the bacteremia/fungemia was removed and the catheter tip grew the bacterium. Because it initially grew in two blood cultures, the presumptive *Corynebacterium* was identified and susceptibility tested. Based on the susceptibility results, VAN was discontinued, and IV ampicillin, 2 g every 6 hr was administered. The patient became afebrile within 24 hr, and her ANC stabilized at $2 \times 10^9/L$.

Laboratory Investigation: The organism from the first positive blood culture grew as small, grey, non-hemolytic colonies that were catalase positive, leading to a presumptive identification of *Corynebacterium* sp. Another culture, drawn before the initiation of VAN therapy, was also positive with the same organism. The growth of the organism in multiple blood cultures, and a call to the laboratory by the clinician requesting that the initial isolate be worked up due to the decrease in the patient's ANC, lead to organism identification and susceptibility testing. API Coryne Strips identified all isolates as *Listeria grayi*. Susceptibility testing suggested that it was resistant to VAN (MIC: $\geq 32 \mu\text{g/ml}$) by E Test, but susceptible to ampicillin (MIC 0.5 mg/ml). After the presumptive source of infection was removed and ampicillin was started, she rapidly became afebrile.

Commentary. *L. grayi* is one of the presumed "non-pathogenic" *Listeria*, although it has been reported as the cause of bacteremia in other immune-compromised hosts [12, 13]. It lacks the beta hemolysin that is a presumed factor in the pathogenesis of the more virulent *L. monocytogenes*. Susceptibility of *L. grayi* to VAN has not been investigated. Although no interpretive criteria for MICs for this rare organism exist, a VAN MIC of $\geq 32 \mu\text{g/ml}$ is interpreted as resistant for all organisms which do have such criteria. The ampicillin MIC would be interpreted as susceptible for such organisms. Both interpretations were presumed to apply to this organism, and the assumption were apparently validated by the patient's response to therapy.

2.4. Case 4: Organism masquerading as a *Corynebacterium* [6]

Case Report: A 39-year-old African-American male, known to be HIV positive, with a recent CD-4 count of 33 cells/mm³, presented to the ED complaining of epigastric and left upper quadrant pain. He reported allergy to trimethoprim sulfamethoxazole (TMS) and to most beta-lactam antibiotics, although not to cefotetan, which he had received successfully before. He had previously been treated for *Cryptococcus neoformans* meningoencephalitis and for *Toxoplasma gondii* infections of the central nervous system. He reported compliance with the dapsone prescribed for *Pneumocystis* prophylaxis, but only occasionally took his antiretroviral therapy. His temperature was 104.4F and heart rate was 129 beats/min. His neutrophil count was 8600 cells/mm³. No biochemical abnormalities were detected. Two blood cultures were drawn, and cefotetan and gentamycin were started for a presumed

intra-abdominal abscess. Chest roentgenograms were unremarkable. CT scan of the abdomen showed hepatomegaly and calcification of the pancreas. Both adrenal glands were enlarged with heterogeneous attenuation, consistent with abscesses. The kidneys were enlarged with a 2.5 x 3 cm area of attenuation in the right kidney. On hospital day 2, a CT-guided aspiration of an adrenal abscess yielded 15 cc of purulent fluid. Stain of the fluid was interpreted as showing Gram-positive cocci in chains. Antibiotics were continued.

On hospital day 4, growth was detected in the both aerobic bottles of the blood cultures drawn on day 1. Gram stain revealed positive rods. At this time the patient developed electrolyte abnormalities suggesting adrenal insufficiency and a cosyntropin stimulation test showed a blunted response with aldosterone levels < 1 ng/ml.

On hospital day 5 the patient developed hypotension, hypoglycemia, and hyperkalemia: hydrocortisone and fludrocortisone were administered. On hospital day 6, antibiotic susceptibility results of the blood isolate became available. Because of the reported allergy to TMS, doxycycline was added. By the next day (hospital day 7), the patient was afebrile, and felt well. For this reason, even when told that the organism was likely a *Nocardia*, the clinicians elected to continue doxycycline.

On hospital day 22 (day 16 of doxycycline) fever and abdominal pain recurred and the patient reported a headache. Ataxia and grand mal seizures occurred. Blood cultures were drawn. An MRI of the patient's brain revealed numerous small lesions that had not been there a year previously. After questioning about the history of his TMS allergy, which was vague, the patient was started on TMS, imipenem, amikacin, and ciprofloxacin. Other antibiotics were discontinued. Within days, the patient's fever, abdominal pain, and neurologic abnormalities had resolved. He was alive and well 18 months later.

Laboratory Investigation: On hospital day 5, the blood and chocolate agar plates inoculated on day 4 from the positive blood culture bottles grew tiny, beige, non-hemolytic, catalase-positive colonies, suggestive of *Corynebacterium* sp.. These were used to inoculate a MicroScan panel and an API Coryne strip. The next day, the MicroScan panel and the Coryne strip gave no identification but MicroScan MICs were available. Using the CLSI interpretations for Gram positive organisms other than *Streptococcus pneumoniae*, the organism was resistant to ampicillin and gentamicin and susceptible to TMS and to tetracycline (≤ 2 µg/ml). On hospital day 7, the adrenal culture became positive with numerous beige, catalase positive colonies growing on the blood agar plate. When staining revealed Gram positive rods, the original Gram stain was reviewed. The organisms previously thought to be Gram-positive cocci in chains were recognized as beaded, Gram positive rods, a classic finding on Gram stains of *Nocardia*. Examination of the now two-day-old plates from the positive blood cultures revealed that the colonies were larger, irregular and darker with a strong odor of wet dirt, also characteristic of *Nocardia*. The organisms were partially but not fully acid fast. The clinicians were notified that the organism was likely a *Nocardia* species. Biochemical testing was begun and the genus was confirmed based on lysozyme resistance and urease positivity.

On hospital day 8, the MicroScan MIC from the adrenal isolate yielded an MIC for tetracycline of 8 µg/ml, the implications of which were unclear. *Nocardia* generally grow too

slowly for MIC determinations, and the technique has not been validated for these organisms. No *Nocardia* grew on his respiratory culture.

Blood cultures drawn when the patient's fever recurred and he developed seizures also grew beige, catalase-positive colonies with a wet dirt odor, and the Gram stain was positive for beaded Gram positive rods. At the request of the clinicians, it was tested for tetracycline susceptibility and the tetracycline MIC was $\geq 128 \mu\text{l}$.

The organism was identified as a member of the *Nocardia asteroides* complex based on its inability to hydrolyze xanthine, tyrosine, or casein within 21 days. 16S rDNA sequencing revealed that the organism was *N. farcinica*.

Commentary: This patient with advanced HIV disease had a complicated course of infection with *N. farcinica*, a virulent member of the *N. asteroides* complex. His therapy was complicated by the initial misinterpretation of the Gram stain morphology, a recurrent problem with *Nocardia* which is frequently beaded, and by the subsequent impression that this rapidly growing organism was a *Corynebacterium*: it grew so rapidly that the possibility of its being *Nocardia* was not considered initially. The hypothesis that the blood isolate should be classified as a skin contaminant that might not be worked up was eliminated when we called the Gram stain result from the first positive blood culture to the clinician who was emphatic about the need to work up the organism, but realization of the genus of the organism and of the fact that the isolates from the adrenals and the blood were identical took several more days.

Unlike our patient's isolates, *Nocardia* usually grow too slowly to be susceptibility tested by MIC, even by accident. The cumbersome agar dilution technique, rather than determination of MICs, is the standard for susceptibility testing of *Nocardia*, so most infections are treated empirically. Before the organism was identified but after the MIC to tetracycline was determined to be very low, this patient was treated with doxycycline and responded dramatically.. Although the initial isolate appeared susceptible, the tetracycline MIC of the organism that appeared after relapse was determined at clinician request: it had increased dramatically into a range that would routinely be interpreted as resistant in other organisms.

Nocardia is still a common cause of death in AIDS patients, but in the U. S. its incidence was low, even prior to the era of highly active antiretroviral therapy, probably since TMS, given to many patients with advanced HIV disease for *Pneumocystis* prophylaxis, is also active against *Nocardia* [14]. Because of his presumed allergy to TMS, this patient received dapsone prophylaxis which does not affect *Nocardia*. Most common infections with *Nocardia* are respiratory, but infections at other body sites in the absence of pulmonary infection are known [14]. The brain is probably the most common extrapulmonary site of infection, with single and multiple abscesses reported. Our patient represents the first case of bilateral adrenal abscesses with adrenal insufficiency which is thought to require more than 90% destruction of the adrenal tissue. Renal abscess is also unusual. Our patient also had positive blood cultures, which are very rare although hematogenous spread of the organism from the lung to

extrapulmonary sites is the postulated route of dissemination. Two cases of *Nocardia* bacteremia prior to our patient did have pulmonary disease [15, 16]. However our patient did not, so the source for his initial bacteremia is unclear. Our patient's disease symptoms paralleled the presence, resolution and recurrence of his bacteremia.

3. Discussion

Each year, our laboratory receives approximately 80,000 blood culture sets. On average, about 3.7%, or approximately 3000 of these meet the criteria for contaminated cultures. Some isolates, such as the ones described here meet the initial criteria, but, because of growth in multiple cultures, they are removed from the list and are not represented above. Others are worked up at physician request, and are removed from the list if the organism identification changes. Appropriate classification of and reduced work up of contaminated blood cultures is very important: it saves technologist time, and avoids unnecessary laboratory expenses. It prevents providing a report to the treating physician that could lead to the use of unnecessary antibiotics, and ultimately to harm to the patient due to drug side effects or to the development of antibiotic-associated *Clostridium difficile* infections. It may result in a patient being discharged from the hospital sooner since a diagnosis of sepsis is avoided. It helps retard the selection for drug-resistant bacteria. However, it is important to realize that while some organisms at first appear to be contaminants, they are actually etiologic agents of disease. Most such bacteria are actually skin organisms, often infecting immune-compromised hosts. This chapter highlights the isolation of four unusual organisms that also appeared at first to be contaminants but later proved to be unusual and significant etiologic agents of disease. Three of the four reports have been published previously [4, 5, 6]. However, it has a number of other messages as well.

Our data emphasizes the importance of drawing two blood culture sets at the outset of a new episode of presumed sepsis. Failure to draw the second culture could lead to the failure to work up an organism that is actually the etiologic agent of disease, but appears to be a contaminant found in only one culture.

A related issue is that the organisms discussed here are Gram-positive and most are VAN-resistant. All the isolates on the list of usual skin contaminants are Gram positive, so this is not surprising. The inclusion of VAN in many empiric regimens for presumed sepsis may predispose the at risk patient to subsequent infections with VAN-resistant organisms. It is clear that VAN cannot be assumed to be the drug of choice for treatment of all Gram positive organisms. In one of the cases presented here, those in the laboratory recognized the importance of the infective organism because it was VAN resistant. Only one blood culture was drawn in the ED, and VAN was part of the empiric antibiotic cocktail started for febrile neutropenia or presumed sepsis. The treatment failure caused by the organism's resistance to VAN lead to the collection of additional cultures, and the same organism grew again. Full work up of the organism was begun, and it was soon recognized that the organism was an unusual isolate, and not the skin organism suggested by the spot testing. Infections with organisms that were susceptible to VAN might have resolved and been missed in this scenario.

Our results also stress the importance of close interactions between the clinicians and laboratory personnel. Workup of several of the organisms reported here was begun at clinician request in the absence of a second blood culture set, or before the second blood culture set had turned positive, because the clinicians were convinced that the patient was septic. They did not want a delay in the proper identification and susceptibility testing of the infecting organism, regardless of the presumptive identification. Such interactions result in good, cost-effective patient care.

It should be noted that such procedures can be a double-edged sword. Clinicians need to be aware that the laboratory limits the work-up of presumed contaminants and intervene quickly as needed. Microbiology laboratory professionals need to be alert to the fact that since the number of immune-compromised patients has increased, the potential for actual infections by normal skin flora is also increased. Furthermore, although in the majority of cases it is good medical practice to not work up the contaminated blood cultures, the clinical microbiologist must keep a high index of suspicion for unusual, low-prevalence pathogens that can resemble routine causes of contaminated blood cultures.

Identification of most of the organisms discussed here was accomplished or confirmed by molecular techniques. At one time organism identification by polymerase chain reaction and DNA sequencing (using 16S rDNA as a target, for example) was regarded as esoteric and prohibitively expensive. Now such testing is offered by many major hospital systems and reference laboratories. Compared to the expense of having laboratory personnel expend significant time and supplies in identifying atypical organisms, molecular identification, whether performed in house or by a reference laboratory, is often more rapid and financially prudent [17].

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