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# Detection and Identification of Bacterial Contamination in Blood Samples from Cancer Patients

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## Abstract

### Title

Bacterial detection in cancer samples.

### Background

Bacterial contamination follow up is an integral part of quality control for blood sample handling and processing. Blood sample contamination is promoted by inappropriate blood drawing practices or laboratory environments. This study investigated the presence of Gram-positive bacteria in the skin microenvironment that are capable of causing serious infections in cancer patients. Moreover, this study examined whether such contamination is caused by a lack of appropriate disinfection during blood collection, which may facilitate entry of skin bacteria into the bloodstream, or by downstream mishandling of samples by laboratory staff before or during analysis. Alternatively, blood contamination may result from an undiagnosed infection, such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* or *Streptococcus pneumoniae*.

### Methods and findings

The bacteria described above have been detected by polymerase chain reaction (P.C.R). Our results revealed that 82% of examined samples were contaminated by *Staphylococcus aureus* prior to any further blood processing.

### Conclusion

The presence of *Staphylococcus aureus* before any blood processing indicating bacterial contamination results from incorrect venipuncture practices or the

presence of an underlying infection that has yet to be diagnosed.

**Keywords** Bacterial contamination; PCR; Gram+ bacteria; Cancer patients; Blood samples

## Introduction

Bacterial contamination of blood samples resulting from improper blood drawing technique or mishandling of samples by laboratory staff is a frequent problem in diagnostic laboratories. Factors potentially leading to bacterial growth include poor hand hygiene, lack of glove sterilization, insufficient skin disinfection or skin core removal of collected blood during phlebotomy. Gram-positive (Gram+) bacteria account for at least half of all documented microbiological infections in cancer patients [1]. Skin bacteria, such as *Staphylococcus epidermidis* (*S. epidermidis*), are the most frequent source of contamination. While these organisms do not typically grow at low temperatures (1-6°C), they survive and readily multiply when plated and stored at room temperature (20-24°C) [2]. Skin bacteria, such as *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*) and *S. epidermidis*, can contaminate blood samples. Moreover, bacteria frequently found in mammalian respiratory tracts, such as *Streptococcus pneumoniae* (*S. pneumoniae*), have also been reported to cause frequent infections in cancer patients [3]. Immunosuppression induced by the underlying cancer or its attendant therapy, such as neutropenia and breakdown of mucosal barriers during long-term vascular catheter placement or graft versus host disease, can synergize to render cancer patients particularly susceptible to Gram+ infections.

In this study, presence of the aforementioned skin and respiratory bacteria was investigated by polymerase chain reaction (PCR). To determine whether specimen contamination was caused by the physician during blood collection or by the laboratory environment, blood sampled directly from the blood collection tube was cultured in chocolate blood agar prior to DNA isolation. From the same collection tube, DNA was also isolated from plasma to assess staff handling. Bacterial presence

or absence was determined by PCR amplification of species-specific genomic regions.

## Material and Methods

### Sample collection

Blood samples from patients diagnosed with cancer were collected in sterilized 50-ml Falcon conical tubes (Corning, NY, USA) containing 7 ml of 0.02 M ethylenediaminetetraacetic acid (EDTA; Duchefa Biochemie B.V., Haarlem, The Netherlands) as an anticoagulant. Total volume of collected blood was 20 ml. Samples were cultivated on a roller for 30 minutes and then sent to the laboratory for analysis. Time of sample transit to the laboratory did not exceed 72 hours. Samples were received both from USA and Europe.

### Examination for bacterial contamination

For detection of contamination, 50 µl was collected from all patient samples and placed on petri dishes containing blood agar base (70133; Sigma-Aldrich, St. Louis, MO, USA), prepared according to the manufacturer's instructions. Samples were incubated for 48 h at 37°C and then examined for contamination by determining the presence of bacterial colonies. Twenty-eight contaminated samples were randomly selected, and their DNA was isolated and analyzed by PCR of both colonies and serum.

### DNA isolation

One bacterial colony was isolated from contaminated samples and resuspended in 20 mg/ml of lysozyme solution. Plasma from the same samples was isolated, centrifuged, and resuspended in lysozyme solution. All samples were subsequently incubated for

2 hours at 37°C. DNA extraction was carried out using a QIAamp DNA Mini Kit (51306; Qiagen, Hilden, Germany) following the protocol for Gram+ bacteria. Samples were then evaluated spectrophotometrically.

### PCR amplification

Primers used for amplification of bacterial DNA were designed according to the literature (Table 1). Primers for *S. aureus* amplified an approximately 270-bp DNA fragment of the nuc A gene, which encodes nuclease A [4]. For *S. pyogenes*, primers amplified a 407-bp DNA fragment from the putative transcriptional regulator gene spy1258 [5]. For *S. pneumoniae*, primers amplified the capsulation locus cps; whereas, for *S. epidermidis*, the putative transcriptional regulator gene serp0107 was amplified [6]. One positive (commercial DNA for each bacterium) and two negative (no template, reference genomic DNA) controls were used for each reaction. PCR was performed in a total volume of 25 µl. The reaction mixture was composed of 0.5 units of Taq DNA polymerase (M8305; Promega, Madison, WI, USA), 1X PCR buffer, 0.4 mM primers, 2 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, and ddH<sub>2</sub>O. The cycling program consisted of one cycle at 95°C for 2 min; 35 cycles of 94°C for 15 s, 62.6°C for 20 s, and 72°C for 30 s; and one cycle at 72°C for 2 min. To validate the quality of PCR products, an extra PCR reaction was performed on all samples using universal primers for 16S rRNA. Primers used were based on the literature [7]. After the completion of all cycles, 2 µl of 6X DNA loading buffer (9156; Takara, Tokyo, Japan) was added to each tube and the resultant products were examined by 2% agarose gel electrophoresis in the presence of Midori Green (MG04; Nippon Genetics, Tokyo, Japan). A 100-bp ladder (MWD100; Nippon Genetics) was used to estimate the size of PCR products.

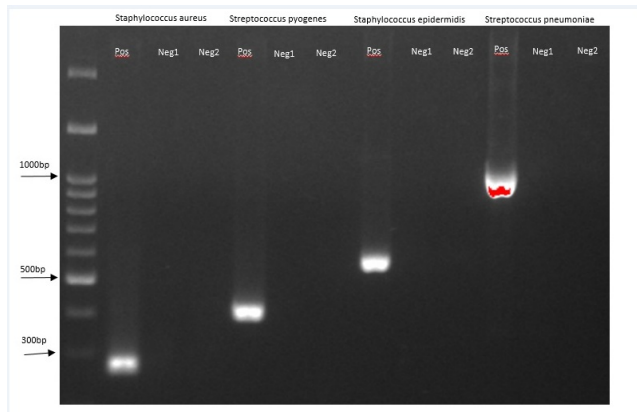
**Table 1:** Bacterial primers and amplified loci.

| Organisms                         | Amplified gene | primers   | length |
|-----------------------------------|----------------|---|--------|
| <i>Staphylococcus aureus</i>      | nuc A          | 5'-GCGATTGATGGTGATACGGTI-3'<br>5'GCCAAGCCTTGACGAACATAAGC-3' | 270bp  |
| <i>Staphylococcus epidermidis</i> | serp0107       | 5'-TTGAGCTTGTCATTGGTTTCG-3'<br>5'-TGTAGAGGTTGCACGTCGAG-3'   | 581bp  |
| <i>Streptococcus pyogenes</i>     | Spy1258        | 5'-AAAGACCGCCTTAACCACT-3'<br>5'-TGGCAAGGTAACTTCTAAAGCA-3'   | 407bp  |
| <i>Streptococcus pneumoniae</i>   | Cps2           | 5'GCAATGCCAGACAGTAACCTCTAT3' 5'CCT GCC TGC AAGTCT TGA TT-3' | 1061bp |
| Universal primers                 | 16S rRNA       | 5'-AGGAGGTGATCCAACCGCA-3'<br>5'-AACTGGAGGAAGGTGGGGAT-3'     | 351bp  |

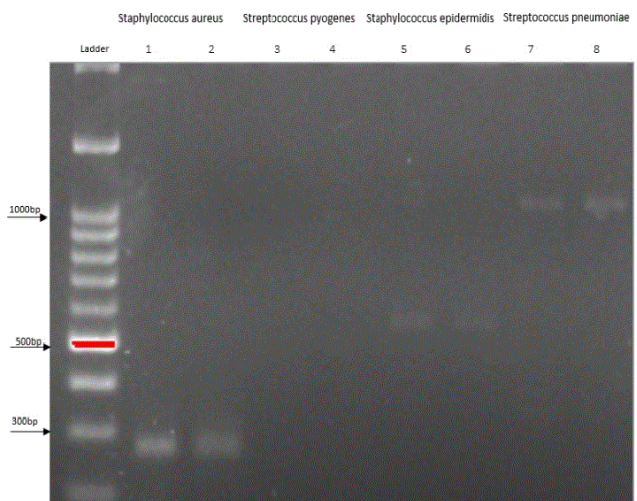
## Results

Based on the results of electrophoresis, all samples positive for at least one bacterium yielded an appropriate band for 16S rRNA (351 bp), indicating good quality of extracted DNA. Among paired plasma-colony samples, 23 were contaminated with *S. aureus*, 12 with *S. epidermidis*, 12 with *S. pneumoniae*, and two with *S. pyogenes* (one for both colony and plasma, and one only for the colony) (Table 2). Some of these samples were

contaminated with more than one bacterial strain (Figures 1 and 2). All expected bands were detected in positive controls, while bands were absent in negative controls. Faint bands appeared for *Staphylococcus aureus*, *Streptococcus epidermidis* and *Streptococcus pneumoniae*.



**Figure 1** Some of these samples were contaminated with more than one bacterial strain.



**Figure 2** Some of these samples were contaminated with more than one bacterial strain.

**Table 2** Percentage of samples contaminated with each bacterium.

| Species                          | Plasma samples | Samples from petri dish colonies | Plasma/Colonies positive |
|----------------------------------|----------------|----------------------------------|--------------------------|
| <i>Staphylococcus aureus</i>     | 82%            | 82%                              | 100%                     |
| <i>Streptococcus epidermidis</i> | 43%            | 43%                              | 100%                     |
| <i>Staphylococcus pyogenes</i>   | 3.5%           | 7%                               | 50%                      |
| <i>Streptococcus pneumoniae</i>  | 43%            | 43%                              | 100%                     |

## Discussion

Blood culture is perhaps one of the most important laboratory procedures. However, contamination of blood samples with

bacteria primarily derived from skin continues to be a frequent problem. Failure of the health worker to use strict aseptic technique when obtaining the blood specimen, or inappropriate sample handling by laboratory staff can lead to this problem. Alternatively, bacterial presence within samples may be independent of blood collection and handling, instead resulting from an underlying patient infection with the same bacteria. In this study, the presence of four Gram+ bacteria was investigated by PCR in blood samples collected from cancer patients.

Frequently found in the mammalian respiratory tract, *S. pneumoniae* is a part of the normal flora in small densities, however, when its population becomes too large, it essentially becomes pathogenic. Commonly found in pairs (diplococci), but also in isolation and short chains, *S. pneumoniae* are slightly pointed, cocci-shaped Gram+ bacteria characterized by a polysaccharide capsule that completely encapsulates cells.

*S. pyogenes* is a free-living organism, however, its ecological niche appears to be quite narrow: its only known biological host is human and, like many other streptococci, it lacks an environmental reservoir of known importance. Thus, direct human-to-human transmission, which mostly occurs via respiratory droplets or skin contact, is critical for its maintenance. Streptococcal organisms tend to reside extracellularly, but can also be found within mammalian host cells of the respiratory tract [8,9]. *S. pyogenes* is capable of causing a large variety of infections [10-18].

*S. aureus* is one of the most common hospital-acquired pathogens. As a normal inhabitant of skin and nasal mucous membranes of healthy humans that may only survive on dry skin, *S. aureus* is infectious to both animals and humans. It can be spread through contaminated surfaces, air and human contact. Approximately 30% of the normal healthy population is affected by *S. aureus*, as it asymptotically colonizes the skin of human hosts. Although, host colonization can be benign, a puncture or break in the skin can prompt this bacterium to enter through the wound and cause infection. As *S. aureus* are spherical, Gram+, and immobile, they form grape-like clusters [19].

Usually originating from disease, *S. epidermidis* is a Gram+ staphylococcus that lives on human skin and mucosa. As one of the five most frequent causes of nosocomial infections, *S. epidermidis* is often compared with *S. aureus*; together, the two genus-sharing pathogens cause over one million serious hospital-acquired infections each year. *S. epidermidis* is the dominant staphylococcus species on skin, while *S. aureus* lives mostly on mucosal surfaces; however, when *S. aureus* is present, *S. epidermidis* decreases drastically. Multiple *S. epidermidis* strains from the nares of healthy adults show that many types of this particular organism exist within a single individual. Notably, the ability to produce slime allows *S. epidermidis* to adhere to biomaterials.

In this study, blood samples were received at the laboratory from USA and European countries; many samples were found to be contaminated. To define when the contamination may have occurred, a small volume of each blood sample was placed on blood agar dishes and incubated to investigate the presence of



bacteria. Twenty-eight contaminated samples were randomly chosen and plasma from the corresponding blood-containing tube was isolated for investigation of bacterial presence. Results of PCR performed for both colonies and plasma indicated the most frequent contamination (82%) was caused by *S. aureus*, while 42% of samples were contaminated with *S. pneumoniae* and *S. epidermidis*. Additionally, one sample was contaminated with *S. pyogenes* on the petri dish, but not in the corresponding plasma.

A systematic analysis of bacteremia studies among cancer patients found that *S. aureus* accounted for 1.3-12% of all cases [20]. Malignancy is also a risk factor for invasive disease secondary to *S. pneumoniae*, with patients exhibiting active leukemia, lymphoma or myeloma, or those having undergone stem cell transplantation, having the highest incidence [21]. While pneumonia is the most common source of *S. pneumoniae* bacteremia in cancer patients, other sources, including occult, have also been reported [22,23]. Although most invasive infections arising from *S. pneumoniae* in cancer patients are caused by serotypes found in major pneumococcal vaccines, being immune compromised is also a risk factor for invasive pneumococcal disease caused by non-vaccine covered serotypes [24].

While there are many ways to differentiate true bacteremia from blood culture contamination, such as microbe identification and corroboration of clinical and laboratory data, we cannot be certain of the source of contamination because the patient's clinical condition and appropriateness of the laboratory environment where blood handling took place are unknown to us. Although, according to Weinstein's study of 843 positive blood samples, certain organisms like *S. pneumoniae* and *S. aureus* almost always represent true bacteremia in a significant proportion of cases, in contrast to organisms like coagulase-negative staphylococci, viridans group streptococci and enterococci [25]. In fact, coagulase-negative staphylococci are the most common blood culture contaminant, typically representing 70-80% of all contaminated blood cultures [26,27].

## Conclusion

The presence of identical bacterial strains in both colonies and plasma further suggests that examined samples arrived at the laboratory contaminated. However, the fact that many cancer patients are immunosuppressed and susceptible to the bacteria we investigated makes it more difficult to determine the contamination source. In addition, a lack of information about the clinical condition of patients, blood drawing procedure and laboratory environment in which blood drawing was performed prevents us from making a clear deduction. Therefore, to determine the actual source of contamination, additional studies must be performed with an increased number of samples and improved patient information.

## Conflicts of interest

The authors declare no conflict of interest

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