WHY EVERY FACTOR COUNTS IN CONTAMINATION INVESTIGATIONS

Excellent Pharma Consulting

Jeanne Moldenhauer

Investigations of Environmental Monitoring Sampling Data

- Olden days approach to EM data

  Sterility and Endotoxin data passed, no impact on the batch release

  Changed around 1994 and issuance of the Sterile Process Validation for use in FDA Submissions

- As knowledge of the weaknesses of sterility testing became popular, the EM data became a “defacto sterility evaluation” by many regulators.
- Industry leaders and organizations have tried to debunk this approach, but it prevails in many regulators.
- We are going to look at a few Case Study observations and how they were applied in a regulatory inspection.

Case Study 1 – Environmental Isolates

- Contaminated Product was found at a Blood Center Dialysis Site (During Covid -19 times).
- Contamination events occurred periodically over about two years.
- Contaminant was identified using genomic methods by regulators.
- Both the Blood Center Dialysis Sites and a Manufacturing Site had a closely related isolate in their environmental monitoring samples.
- The manufacturing site isolate was not isolated at the time of manufacturing of the contaminated products and not in “close proximity to the event”.
- The site isolate was not found in the same filling/packaging line as the contaminated product. (found in a gowning room for another filling process)

- **What would you do? Would you have discarded the product?**

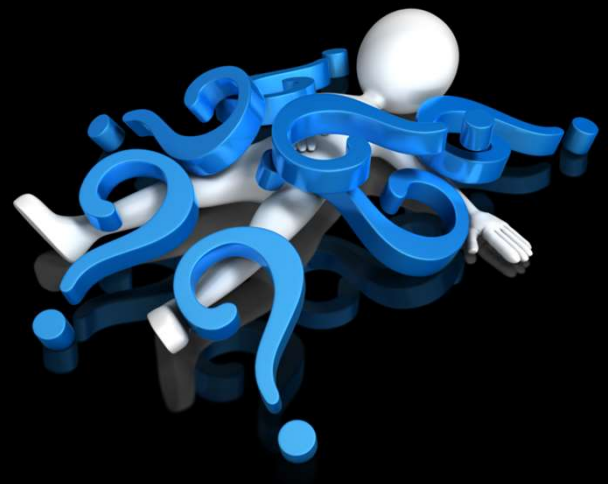
Case Study 1 – Environmental Isolates

- The identification of the contaminant was either *Acinetobacter calcoaceticus–baumannii* complex (ACBC) or *Staphylococcus saprophyticus.* Neither organism forms bacterial spores. Note: There was a third organism identified, but little follow-up on this organism in the investigation.
- The product is terminally sterilized using a validated steam sterilization cycle.
- The "contaminated product" was released using an FDA-Approved Parametric Release program.

- **Does this change your decision about release of the "contaminated" batches? Why or why not?**

Case Study 1 – Environmental Isolates

- If the “contaminated product” was considered polymicrobial (i.e., more than one contaminant) do you need to find all of the contaminating microorganisms at the manufacturing site to say it is the root cause?
- Does your opinion change if one of the organisms is a common contaminant like *Staphylococcus*?



Case Study 1 – Environmental Isolates

- Further review of the environmental monitoring data indicated that for the “genetically similar” contaminate found in the manufacturing site EM program, the count on the plate was 1 CFU.

- **Does that change your opinion on resolution of the EM data? Why or why not?**

Case Study 1 – Environmental Isolates

- Looking at the identification methods, in some cases, Whole Genome Sequencing (WGS) or Next Generation Sequencing (NGS) were used. In other cases, Multi-locus Sequence Typing (MLST) was used.
- MLST uses sequencing from 400-500 DNA base pair fragments of seven housekeeping genes to allow small variations within a species to be detected.
- What level of matching (e.g., 100%) would you expect to be the root cause of the contamination?

- **Does this change your decision regarding the EM contaminant and potential batch discard? Why or why not?**

A Regulatory Inspection – CDC/FDA

- All the case study examples come from data generated as part of a regulatory inspection conducted over several years.

- Kracalik, I., Kent, A. G., Villa, C. H., Gable, P., Annambhotla, P., McAllister, G....Basavaraju, S. V. (2023). Posttransfusion Sepsis Attributable to Bacterial Contamination in Platelet Collection Set Manufacturing Facility, United States. *Emerging Infectious Diseases*, *29*(10), 1979-1989. https://doi.org/10.3201/eid2910.230869.

- Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (I. Kracalik, A.G. Kent, P. Gable, P. Annambhotla, G. McAllister, J. Noble-Wang, A.L. Halpin, S.V. Basavaraju); Food and Drug Administration, Silver Spring, Maryland, USA (C.H. Villa, O. Illoh, A.F. Eder); University of California San Francisco School of Medicine, San Francisco, California, USA (D. Yokoe, C.R. Langelier); Utah Department of Health, Salt Lake City, Utah, USA (K. Oakeson)

The Article's Abstract

- "During May 2018–December 2022, we reviewed transfusion-transmitted sepsis cases in the United States attributable to polymicrobial contaminated apheresis platelet components, including *Acinetobacter calcoaceticus–baumannii* complex or *Staphylococcus saprophyticus* isolated from patients and components. Transfused platelet components underwent bacterial risk control strategies (primary culture, pathogen reduction or primary culture, and secondary rapid test) before transfusion. Environmental samples were collected from a platelet collection set manufacturing facility. Seven sepsis cases from 6 platelet donations from 6 different donors were identified in patients from 6 states; 3 patients died. Cultures identified *Acinetobacter calcoaceticus–baumannii* complex in 6 patients and 6 transfused platelets, *S. saprophyticus* in 4 patients and 4 transfused platelets. Whole-genome sequencing showed environmental isolates from the manufacturer were closely related genetically to patient and platelet isolates, indicating the manufacturer was the most probable source of recurrent polymicrobial contamination. Clinicians should maintain awareness of possible transfusion-transmitted sepsis even when using bacterial risk control strategies."

Figure 1

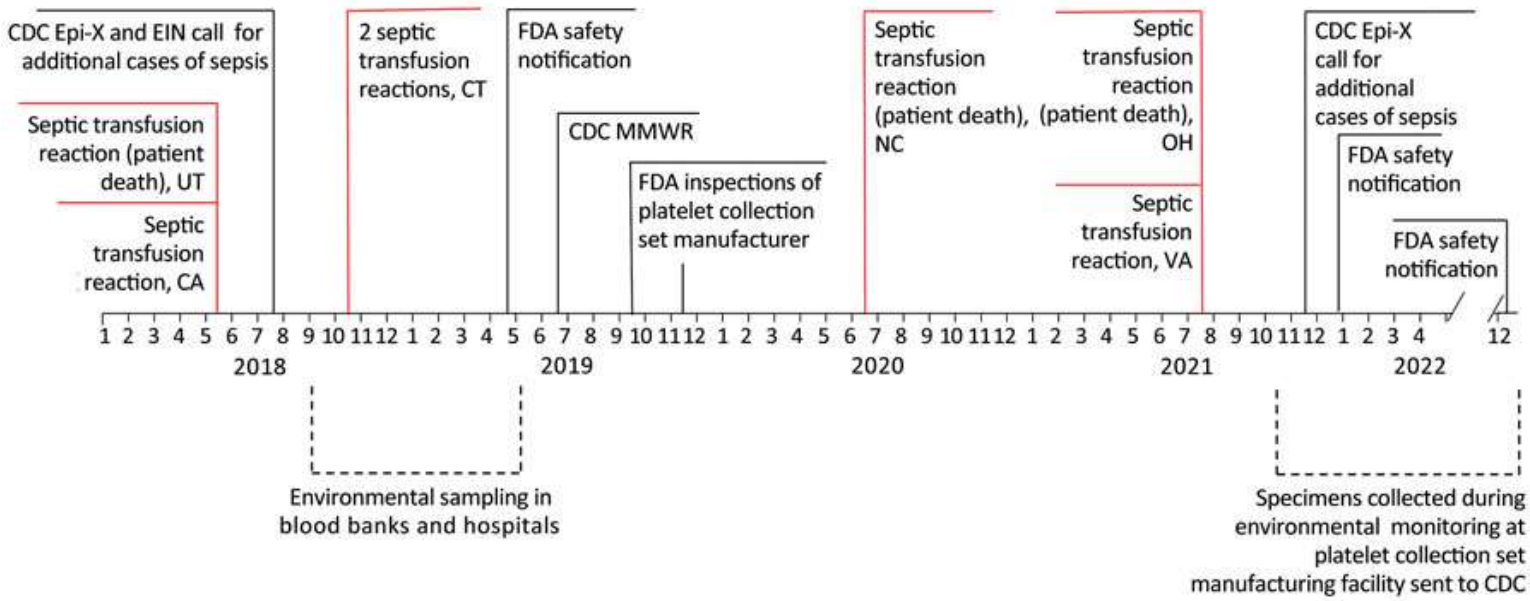


Figure 1. Investigation timeline of transfusion-transmitted sepsis cases and key events for bacterial contamination in platelet collection set manufacturing facilities, United States, 2018–2022. CDC, Centers for Disease Control and Prevention; EIN, Emerging Infections Network; Epi-X, Epidemic Information Exchange; FDA, Food and Drug Administration; MMWR, report published in Morbidity and Mortality Weekly Report (9).

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Why the Regulators' Believe This Was a Single Root Cause for the Contamination

- "Bacterial contamination of platelet components most commonly occurs during blood collection and typically involves either a single identified species of Gram-positive bacteria associated with normal skin microflora or, less commonly, Gram-negative bacteria from asymptomatic donor bacteremia. However, multiple episodes of polymicrobial contamination with identical bacterial species in platelet components across different states is exceedingly rare, suggesting a possible common source of contamination."

Risk Mitigation

- "Strategies to mitigate sepsis risk caused by bacterial contamination of platelets include bacterial cultures incubated before release for transfusion, secondary rapid testing after bacterial culture with a bacterial detection device, and pathogen reduction after platelet collection (*8*). In the United States, a pathogen-reduction device for platelets that uses synthetic psoralen and ultraviolet light to inactivate microorganisms was approved by the US Food and Drug Administration (FDA) in 2014 and adopted voluntarily by some blood establishments. In response to ongoing reports of transfusion-transmitted sepsis, FDA established regulations and recommendations in guidance during September 2019 to implement certain bacterial risk control strategies for platelets collected before October 1, 2021, including pathogen reduction, bacterial culture methods, and secondary rapid testing (*8*)."

The Investigation Conducted

- Triggered by: CDC and FDA received 4 reports of transfusion-transmitted sepsis attributable to platelet components collected by 1 blood establishment from 3 donors at separate collection facilities in 3 states.

- Preliminary whole-genome sequencing (WGS) showed that respective species isolates (from the contaminated units) were closely related genetically, suggesting a common source of contamination.

- April 16, 2019, FDA issued a safety communication, updated on December 2, 2021, and December 22, 2022, encouraging blood establishments and healthcare facilities to report platelets contaminated with ACBC or *S. saprophyticus* by submitting a MedWatch report or by directly contacting FDA Center for Biologics Evaluation and Research

The Investigation Continued

- As the investigation progressed, additional reports identified *L. adecarboxylata* as a platelet contaminant in combination with ACBC or *S. saprophyticus*. Beginning in May 2021, isolates identified during routine environmental sampling by a platelet collection set manufacturer were sent to CDC for testing. (to meet the requirements of public health surveillance as defined in 45 Code of Federal Regulations, part 46.102(l) (*2*). No institutional review board approval was needed.)
- Cases of transfusion-transmitted sepsis were identified through mandatory reporting of transfusion-related deaths to the FDA under 21 CFR 606.170(b) or voluntary reports to the CDC or FDA by US blood establishments, health departments, or healthcare facilities. We reviewed reports of transfusion-transmitted sepsis for case definition and imputability criteria contained within the National Healthcare Safety Network Hemovigilance Module protocol .
- Cases were included if identical bacterial species were isolated from a transfused patient and a transfused platelet component, and an implicated strain (ACBC or *S. saprophyticus*) was isolated from either a transfused patient or transfused platelet component.

The Investigation Continued

- Blood establishments or healthcare facilities voluntarily reported, to the FDA or CDC, platelets contaminated with ACBC or *S. saprophyticus* identified by primary bacterial culture screening before distribution. These contaminated units were not released for transfusion. (21 CFR 606.171).

Figure 2

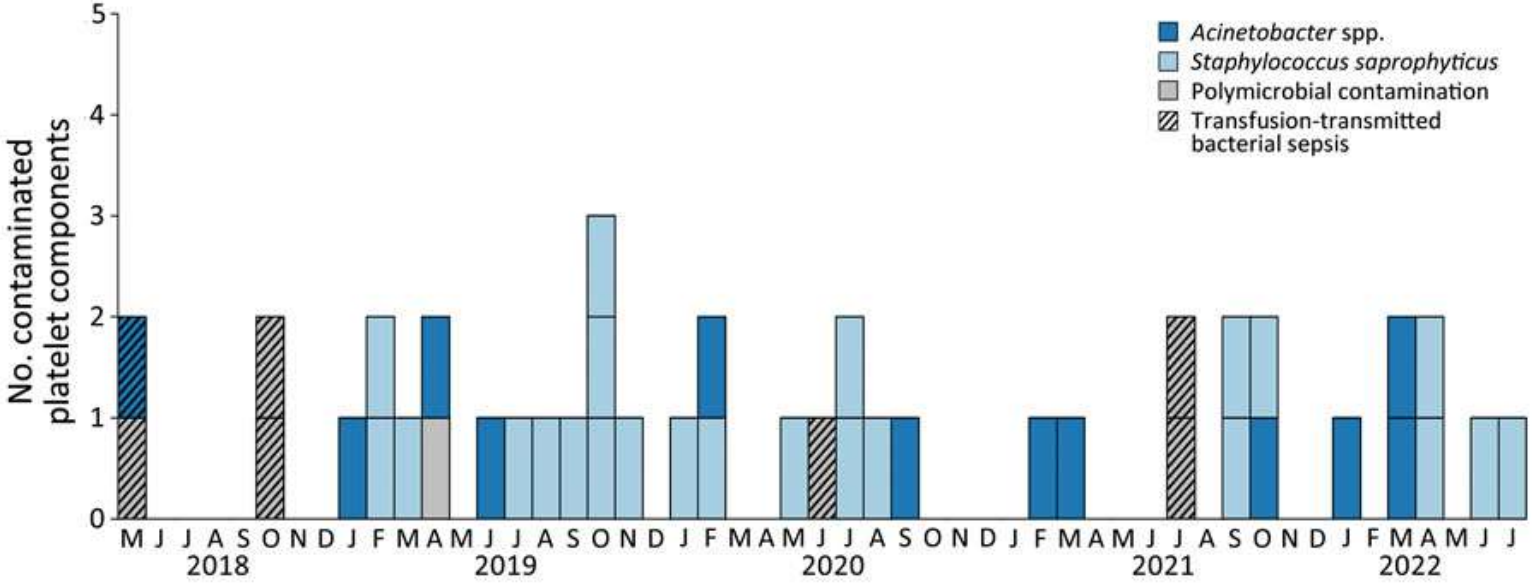


Figure 2. Platelet components contaminated with Acinetobacter spp. or Staphylococcus saprophyticus identified from cases of transfusion-transmitted bacterial sepsis or routine bacterial testing before transfusion, United States, 2018–2022.

Kracalik I, Kent AG, Villa CH, Gable P, Annambhotla P, McAllister G, et al. Posttransfusion Sepsis Attributable to Bacterial Contamination in Platelet Collection Set Manufacturing Facility, United States. Emerg Infect Dis. 2023;29(10):1979-1989. https://doi.org/10.3201/eid2910.230869

The Investigation Continues

- FDA/CDC/Health officials reviewed focused environmental surface sampling conducted by CDC and local and state health departments in blood establishments and healthcare facilities in 5 US states (California, Connecticut, Massachusetts, North Carolina, and Utah) from which platelet components were collected, or in hospitals in which cases of transfusion-transmitted sepsis were reported.
- Epidemiologic data collected during the investigation was used to identify potential reservoirs, and sampling locations, such as equipment used to store platelet components.

The Investigation Continues

- The manufacturer of the apheresis bags was required to send a subset of EM samples from two plants to CDC for analysis.
- The microbiological evaluation included WGS, if possible and other testing.
- One ACBC isolate cultured from the environment in a platelet collection set manufacturing facility underwent MLST sequence typing at an outside laboratory. Results were sent to CDC for additional analysis, but the isolate was not available for WGS.

Investigation Results

- During May 2018–November 2022, a total of 7 cases of platelet transfusion-transmitted sepsis were identified

  in patients from 6 states (California, Utah, Connecticut, North Carolina, Ohio,

  and Virginia)

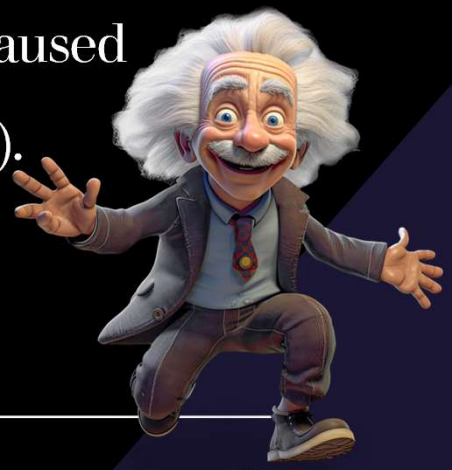
- Of the cases examined, 3 were identified as being transfusion-related fatalities under 21CFR 606.170(b); others were voluntarily reported by state health departments or blood establishments. Other than receipt of platelet

  transfusion, no commonalities were observed among persons

  who died.

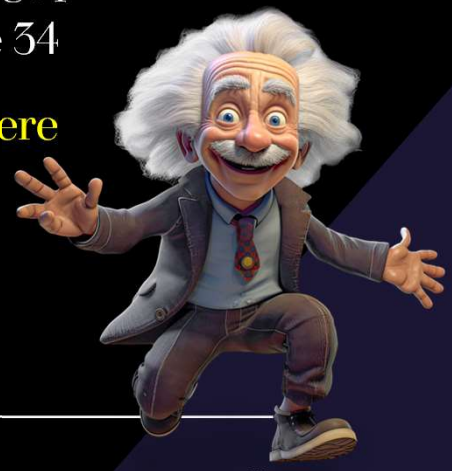
Investigation Results

- Platelets were collected from 6 different donors in 6 states, all by 1 blood establishment. Two of the implicated platelet units were from the same collection procedure. Disposition of co-components from the 7 cases of transfusion-transmitted sepsis included 3/7 (43%) platelet co-components transfused into 3 other patients without incident, as reported by blood establishments or transfusion services; 2/7 (14%) sequestered co-components that were culture negative; and 2/7 (14%) co-components that caused septic reactions were part of this investigation (patients C and D).



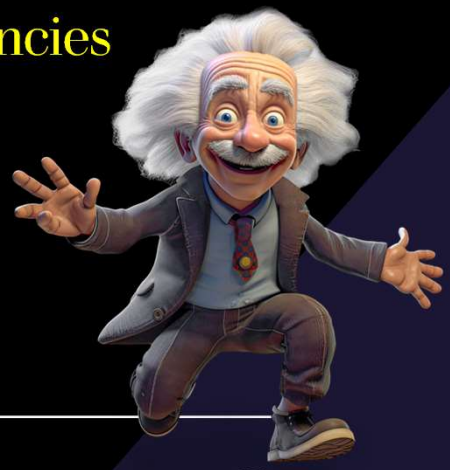
Investigation Results

- Blood establishments or transfusion services did not report additional details of their investigation into the co-component transfusions
- A total of 90 environmental samples were collected from blood establishment and healthcare facilities in 5 states (California, Connecticut, Massachusetts, North Carolina, and Utah) during May, June, and November 2018; February and May 2019; and July 2020.
- Of the 90 samples cultured, 29 (32%) yielded 34 implicated strain isolates. Recovery of isolates was primarily associated with samples taken from equipment used to store (e.g., platelet agitators) and transport platelet components (e.g., quality control cart). Of the 34 isolates, 19 (56%) were ACBC, 11 (32%) were *S. saprophyticus*, and 4 (12%) were *L. adecarboxylata*.



Investigation Results

- FDA inspected the manufacturing facilities in Puerto Rico and the Dominican Republic to assess the risk for a common source of contamination. As part of those activities, during October 2021–October 2022, additional culture and sequencing was performed on **74** environmental samples collected at the 2 manufacturing facilities yielding, 84 isolates: 35 *Acinetobacter* spp. and 49 *S. saprophyticus*.
- FDA inspections of the manufacturing facilities identified deficiencies related to environmental controls and the assurance of platelet collection set sterility.



Investigation Results

- A total of 191 isolates obtained over 4 years underwent WGS: 118 environmental isolates from healthcare facilities, blood establishments and 2 platelet collection set manufacturing facilities; 56 from posttransfusion patient blood. Sequencing and analysis showed that respective isolates of ACBC, *S. saprophyticus*, and *L. adecarboxylata* from different sources were closely related genetically and formed several closely related, respective outbreak clusters. Isolates from post transfusion patient blood, platelet components, and a platelet collection set manufacturing facility formed 3 distinct *S. saprophyticus* outbreak clusters.
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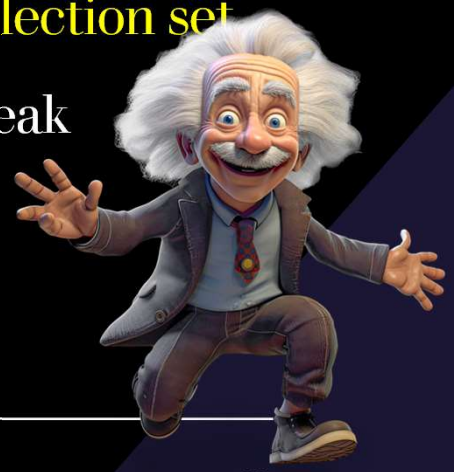

Investigation Results

- **One** ACBC isolate obtained in July **2022** from a platelet collection set manufacturer was the same potentially novel ACBC species in outbreak cluster 1 and was closely related by multilocus sequence typing, but was not available for WGS and analysis at CDC.
- In the NCBI, ACBC isolates from cluster 1, which included this potentially novel species, grouped distinctly from all other ACBC with available data. All outbreak clusters in ACBC and *S. saprophyticus* clustered apart from publicly available NCBI génomes.

Specific Concerns with the Report

- Why were the similar organisms found at the blood center and healthcare centers not considered in the potential root cause?

  -This occurred during Covid-19 when hospitals were understaffed.

  -The apheresis process is complicated and prone to contamination in handling.

  -The CDC reported a higher incidence of hospital acquired infections during the Covid-19 time period.

  - The timeline for the collection of the environmental samples and the manufacturing site is not explained, **nor is it relevant** to a contamination event years earlier.
  - The apheresis product is **terminally sterilized** at the manufacturing site in Puerto Rico with steam. After some additional handling in the Dominican Republic, it is subjected to **E-Beam** Sterilization. Prior to administration, it is subjected to a **pathogen reduction process**. What data is there to show that the contaminants could survive all these processes?

Considerations of Other Sources of Contamination

- Genetically **similar organisms** were found at the "set manufacturing site" and the various healthcare and blood banking facilities.

  No discussion is provided on the rationale for discrediting the contamination as coming from the blood banking and hospital locations, other than resistance

  *This occurred during the Covid-19 Pandemic. The CDC issued notices of shortages in staffing and associated issues in hospitals and healthcare locations during this time. They also indicated a **higher risk** of hospital acquired infections.*

- The ACBC organism is considered one of the **most adaptive organisms**.

Figure 3

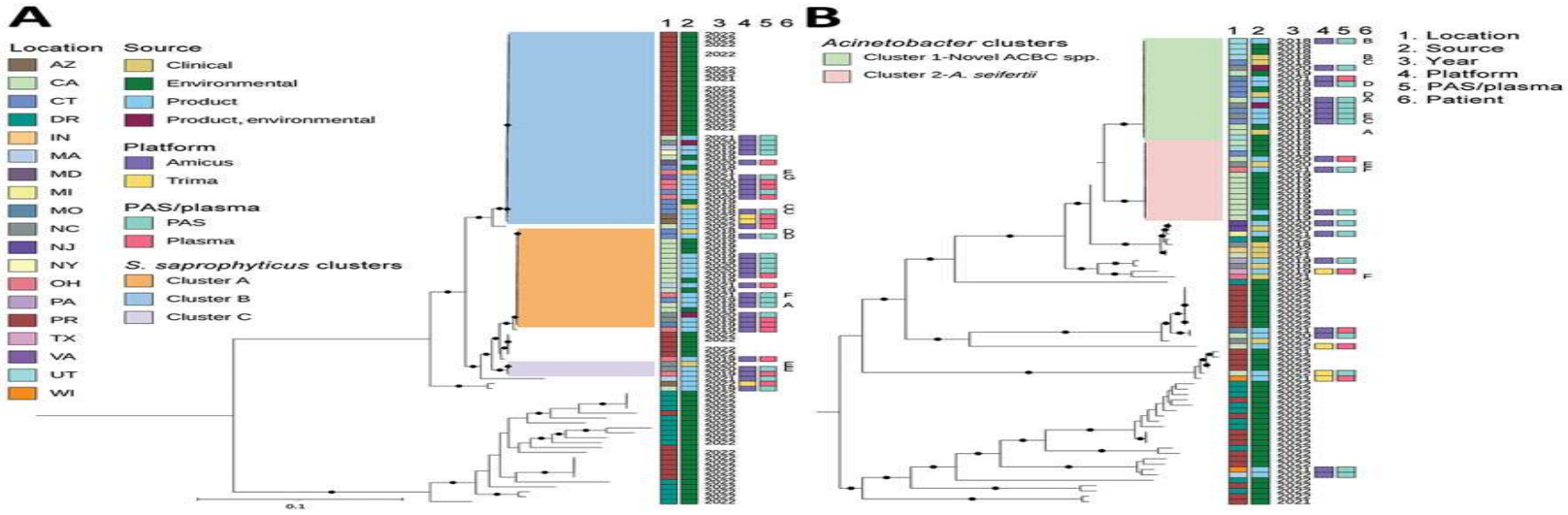

Figure 3. Whole-genome sequencing of Staphylococcus saprophyticus (A) and ACBC (B) isolates implicated in the bacterial contamination of platelet blood products, United States, 2018–2022. Maximum-likelihood phylogenies based on core genes were generated by using Roary (https://github.com/sanger-pathogens/Roary) and RaxML (https://cme.h-its.org); phylogenetic trees were midpoint rooted. Clusters were identified based on SNVPhyl (https://snvphyl.readthedocs.io) and highlighted if they included isolates linked to a sepsis transfusion case. Acinetobacter spp. isolates not falling in the ACBC were also included. Black circles on branches indicate 100% support for the branch of 100 bootstraps. US states are identified by 2-letter postal codes. Scale bars indicate nucleotide substitutions per site. ACBC, Acinetobacter calcoaceticus–baumannii complex; DR, Dominican Republic; PAS, platelet additive solution; PR, Puerto Rico.

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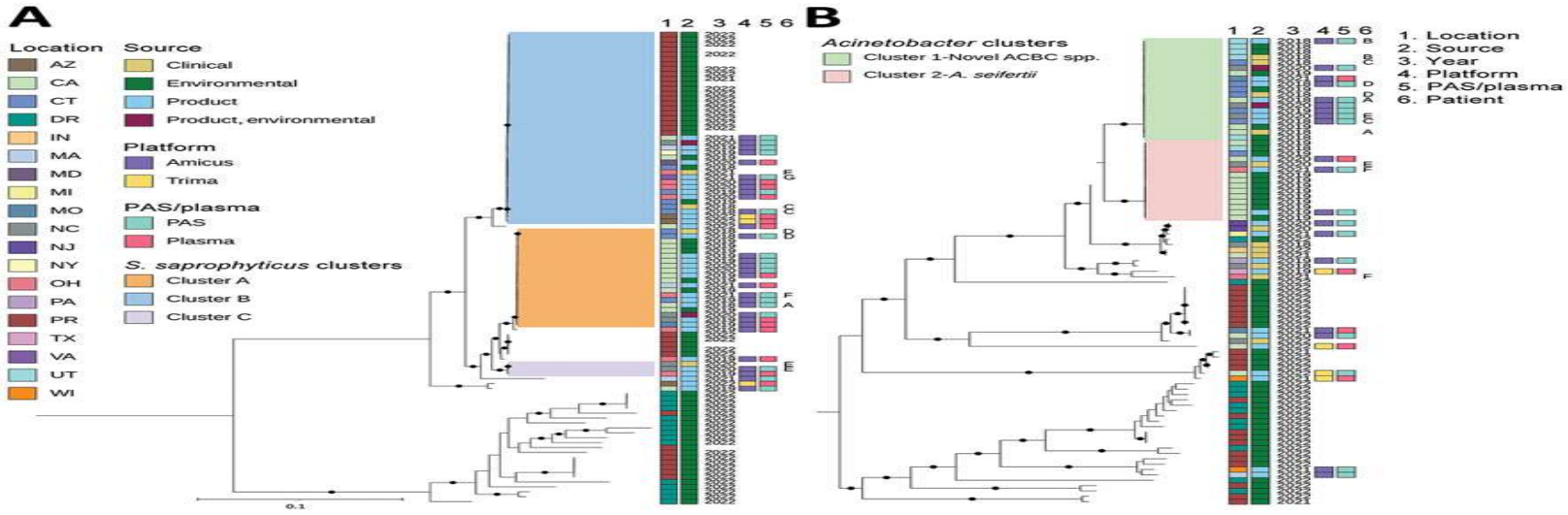
Specific Concerns with the Report

- No consideration was given **to how** the organism got from the gowning room of one fill line to the production area of another fill line.
- Data generated at the PDA's Aseptic Processing Training Class, shows the low likelihood of **1 cfu** resulting in contamination and growth in a container. In fact, studies in France showed is took about 10,000 cfu to result in a contaminated container.
- No consideration was given to the **adaptability** of the ACBC's ability to adapt is considered " unrivaled". (How do we know that adaptive clusters will be there months or years later?)

Figure 3



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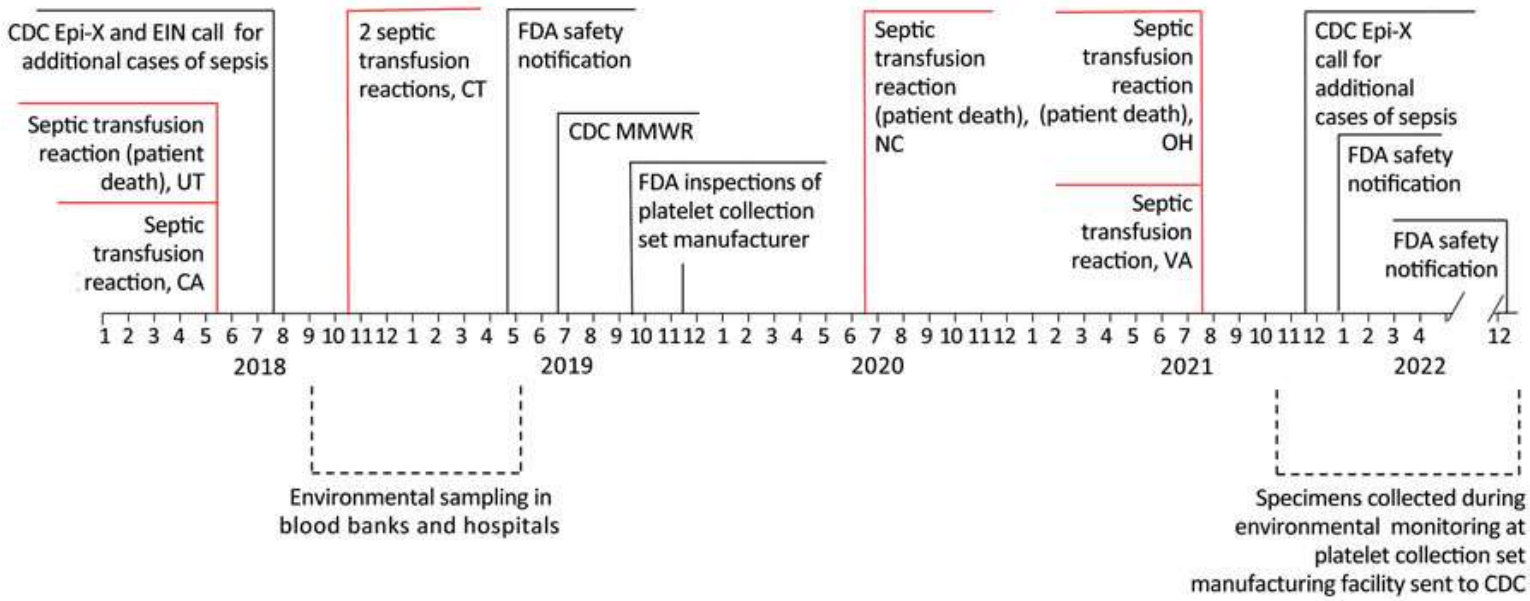
When an Organism is Implicated in a Contamination Event, is Timing Important?

- Looking back at figure 1, the EM samples collected by the Agency occurred in late 2021 and early 2022 – **Years after** the contaminated product resulted in patient reactions (2018 and 2019)
- This is where the “similar organism” that resulted in the root cause determination was found. (Remember, it was a count of 1 cfu)

Figure 1



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Effects of the Method of Sterilization

- The apheresis sets including solutions were terminally sterilized in a validated steam sterilizer.
- Parametric Release had been approved at this facility since 1983.
- After the terminal sterilization cycle, the sets were shipped to another location for some additional handling and then subjected to E-BEAM sterilization.
- Due to the high risk of contamination, a Pathogen Reduction process is also used at the blook banks/healthcare facilities prior to patient administration.

Microorganism Adaptability

- ACBC is considered one of the most adaptable organisms existing.

  Variety of ways to acquire or adapt, e.g., horizontal gene transfer or natural transformation (Castanheira, et al., 2023)
- Microbial resistance (what made it a "novel" contaminant) does not only come from the genome, e.g., plasmids may carry this trait.
- This creates a difficulty in comparing organisms over a long period of time.

When an Organism is Implicated in a Contamination Event, Is Concentration (Amount) of the Organism Detected Important?

- In this particular root cause investigation, the EM sampling for the "similar contaminant" was 1 EM sample with 1 cfu on the plate.

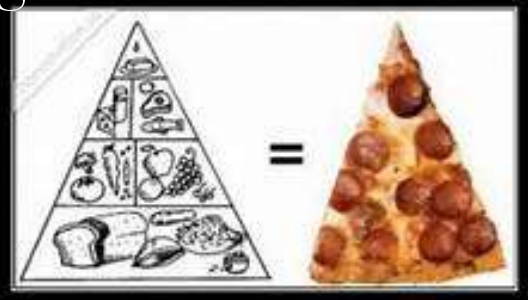
When Multiple Organisms are the Contaminants, Do You Have to Recover all of them at a Site to be the Root Cause?

- The article stipulated that the sepsis was claimed to be "polymicrobial"
- Finding "one" genetically similar organism at the site, if this sufficient to claim the site is the root cause?
- What if the only "genetically similar organism" is a typical human borne organism (e.g., *Staphylococcus saprophyticus*)?

How Close is Close Enough?

The report states that the ACBC organism was "similar to" the product contaminants.

- How close does it have to be to be a match?
- Can you look at identifications from multiple ID methods and say that it is "the same" organism?
- For some organisms, is Genus/Species enough?
- Etc.



How Come Some Co-Components Were Negative, if the Contamination Came from the Manufacturer?

- Depending upon the apheresis set, multiple bags are used

- The multiple bags can be used for different patients. (Co-Components of one set)

- Testing performed on co-components of “contaminated sets” (i.e., that resulted in sepsis) and were found negative for contamination

What Parts of the Manufacturing Process Need to be Considered?

- Regardless of the type of contamination event, it is generally expected that one reviews the entire process;
  - Receipt of Ingredients
  - Material transfer
  - Compounding
  - Formulation
  - Filling
  - Sterilization
  - Inspection
  - Packaging
  - Etc.

Use of Consultants

- It has become extremely common to include consultants in the review of your contamination control contaminants, especially in analyzing whether you found the “real root cause”, whether you need a “more convincing story”, or whether you are missing evaluations.

- Depending upon the regulatory agency involved, you may get a requirement to hire consultants (GMP trained) to aid in the investigation.

Personal Conclusions on the Investigation

- Finding a single “matching” contaminant after a long period of time alone may not be sufficient in determining the site is the root cause of the problem.
- It needs to be reasonable that the organism can survive the entire manufacturing process and has a route to get into the product.
- You cannot discredit three functioning sterilization processes used in the product, without showing that the organism has the ability to survive all three of these processes.

Specific Concerns with the Report

- If there are multiple contaminants, do you need to find them all at the source (root cause)?
- Can the organisms survive in the apheresis solution bags from manufacture to use?
- And so many more

Applicability

- Should you go ahead and change all your SOPs to do this type of investigation?
- Know your “enemy.”
- Consider how you “know” your investigation is complete (provide guidance in SOP)

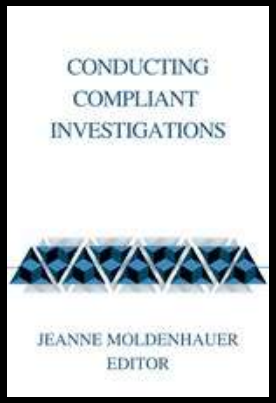
Use Quality Tools

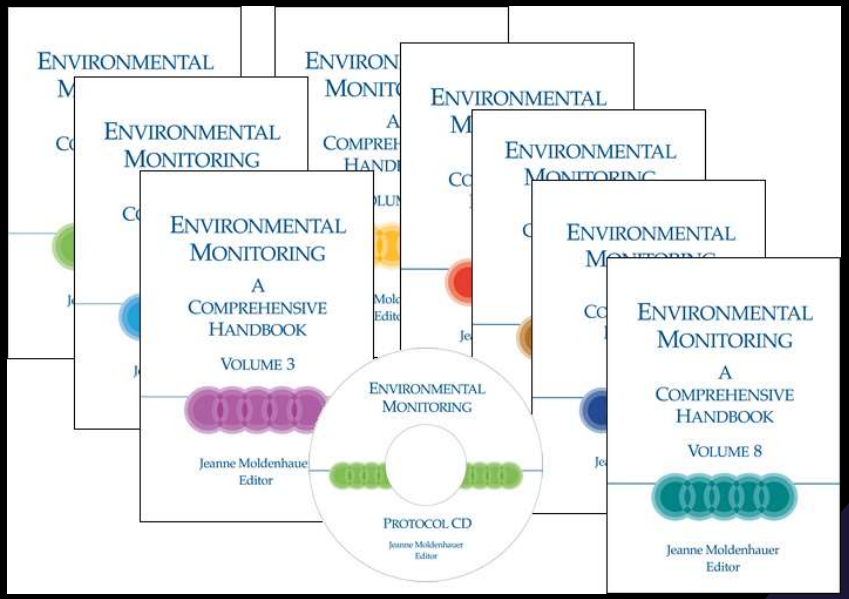
- There are a variety of Quality Tools available to use in conducting your investigations. Use them!

- Make sure you look at data for a sufficient period of time.

- Look for literature to cite (peer-reviewed journals) to support your claims.

Aids to Conducting Investigations






TO ACCOMPLISH GREAT THINGS,
WE MUST NOT ONLY ACT, BUT ALSO DREAM;
NOT ONLY PLAN, BUT ALSO BELIEVE.
—Anatole France
PresenterMedia


WE MAY ENCOUNTER
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—Maya Angelou
PresenterMedia

Lingering Questions?

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