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Major article

A cluster of gram-negative bloodstream infections in Connecticut hemodialysis patients associated with contaminated wall boxes and prime buckets

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Dialysis-related infections

Background: Maintenance hemodialysis (HD) patients are at increased risk of bloodstream infections (BSI). We investigated a cluster of *Delftia acidovorans* infections among patients undergoing HD at an outpatient unit (Facility A).

Methods: A case was defined as a Facility A HD patient with ≥1 culture positive for *D acidovorans* between February 1 and April 30, 2018. An investigation included review of patient records, facility policies, practice observations, and environmental cultures.

Results: The cluster included 2 patients with confirmed *D acidovorans* BSI. Both patients had recently been dialyzed at Station #2, where a wall box culture yielded *D acidovorans*. One patient also had a BSI due to *Enterobacter asburiae*, which was recovered from several other wall boxes and saline prime buckets (SPB). Observations revealed leakage of wastewater from wall boxes onto the floor, and that SPBs were not always disinfected and dried appropriately before reuse. Multiple deficiencies in hand hygiene and station disinfection were observed. No deficiencies in water treatment practices were identified, and water cultures were negative for the observed pathogens.

Conclusions: The cluster of *D acidovorans* infections was most likely due to indirect exposures to contaminated wall boxes and possibly SPBs due to poor hand hygiene and station disinfection.

Approximately 495,000 individuals receive maintenance hemodialysis (HD) for end-stage renal disease annually in the United States.[1] These patients are at high risk for infection because of impaired immune defenses, and the need for routine vascular access required for HD. For example, the National Healthcare Safety Network (NHSN) Dialysis Event Surveillance Report for 2014 noted that 6,005 outpatient HD facilities reported 29,516 bloodstream infections (BSIs).[2] The most frequently reported BSI pathogens are gram-positive organisms (62%), while gram-negative BSIs are less commonly reported (12.5%).[2] Clusters of gram-negative BSIs have been attributed to water sources including inadequate disinfection of water treatment or distribution systems,[3-5] errors in dialyzer reprocessing,[6-9] improperly handled medications,[10,11] hemodialysis equipment,[12-16] and set-up procedures.[17] A novel source of transmission was recently linked to dialysis effluent drains located in HD station wall boxes, which contain connections that supply dialysis machines with reverse-osmosis water, bicarbonate and acid solutions, and have a drain for spent dialysate.[18]

In June 2018, routine surveillance of BSI data reported to the Center for Disease Control and Prevention (CDC) NHSN program identified a cluster of 2 unusual infections caused by *Delftia acidovorans*. CDC notified an outpatient HD facility in Connecticut (Facility A) of the 2 cases that occurred among patients receiving care there. CDC's report to Facility A was based on the fact that *D acidovorans*, formerly known as *Comamonas acidovorans* or *Pseudomonas acidovorans*, has rarely been reported as a cause of infections in HD patients.[19]

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Facility A subsequently requested assistance from the Connecticut Department of Health (CT DPH). This study describes the investigation of the cluster of BSIs caused by *D acidovorans* and other gram-negative bacteria that occurred during a 3-month period among patients receiving care at Facility A. Our findings indicate that the unusual cluster of gram-negative BSIs was most likely due to contamination of dialysis station wall boxes and saline prime buckets.

METHODS

Case definition and setting

A confirmed case was defined as an episode of BSI caused by *D acidovorans* in a patient undergoing maintenance HD at Facility A during the period February 1 to April 30, 2018. A possible case was defined as a positive urine culture due to *D acidovorans*, in a patient without a recent history of urinary tract instrumentation or signs of infection at other body sites, which was suggestive of a transient bacteremia. Patients were considered to have a subsequent BSI if 1 or more different bacteria were recovered from blood cultures obtained > 2 weeks after a previous episode. Facility A is an outpatient dialysis center that has 19 HD stations and provided maintenance HD treatment to ~ 115 patients each month. The facility operates 3 shifts per day for 6 days each week.

Case finding and review

To search for additional case patients, results of all blood culture specimens reported to Facility A by reference laboratories and hospital laboratories for both Facility A's outpatient and hospitalized HD patients were reviewed retrospectively from February 1, 2018 to June 15, 2018, then prospectively through January 2019. The reference and hospital laboratories were asked to make the urine *D acidovorans* urinary isolate and any BSI isolates from affected patients that had been saved available to the investigating team for further analysis. In addition, 2017-2018 NHSN outpatient dialysis event surveillance data reported by Facility A were reviewed. A review of all 2017-2018 NHSN pathogen data reported by all CT HD facilities were reviewed for the isolation and reporting of *D acidovorans*.

Facility A and hospital case patient medical records were examined for patient demographics, medical history, dialysis records and clinical course. For the 2-week period prior to the date of a gram-negative BSI case, dialysis-specific records of cases were reviewed and dates of dialysis, dialysis shift, dialysis station number, wall box number, dialysis machine used, type of dialyzer, parenteral medications received during the dialysis session, recent antibiotic use, symptoms, and names of all dialysis staff per session were recorded on a standardized form and entered into an Excel database (Microsoft Excel 2013).

Facility observations

During the initial site visit, the CT DPH investigation team conducted a comprehensive review of dialyzer reprocessing history, the dialysate distribution system, infection control policies and procedures, procedures for maintenance and disinfection of the water distribution and treatment system and of dialysis machines (CWP 100, Model WRO H, *GAMBRO Healthcare, Lakewood, CO*), practices for performing cultures of the water system and dialysate, and monthly culture results. The team also observed the connections in the wall boxes, which are recessed into the wall behind each dialysis machine. Observations of infection control practices included: setup, initiation, and termination of dialysis via central venous catheter (CVC) and via arteriovenous (AV) graft or fistula; procedures for CVC connection, maintenance, and manipulation; dialysis machine and station disinfection, injectable medication preparation and administration, dialysis circuit priming procedures, general environmental disinfection; and hand hygiene.

Environmental sampling and laboratory testing

With environmental sampling guidance and laboratory support from CDC, environmental samples were taken of several sites including wall box drains and effluent spigots, saline prime buckets, dialysis machines, tap water from several sinks, reverse osmosis (RO) water used to make dialysate, countertops and medication preparation area. One-liter samples of city water were collected from taps at individual sinks. Environmental surface samples were collected using 3M Sponge-Sticks and swabs. The environmental specimens were sent to the CDC for culture. The identity of organisms isolated from environmental samples and available case patient isolates was confirmed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Pulse-field gel electrophoresis (PFGE) was performed on *Burkholderia cepacia complex (B pyrocinnia*) recovered from blood cultures from Patient 1 during and after the outbreak period, and on *Acinetobacter baumannii* isolates recovered from a countertop and from the blood of a non-case patient after the outbreak period. Isolates with a ≥90% similarity in PFGE band patterns were considered closely related. Heterotrophic plate counts of the tap water and RO water were performed as described by the America Water Works Association (AWWA) Standard Methods for the Examination of Water and Wastewater.[20]

RESULTS

Description of cases

Onset of 2 confirmed cases and 1 possible case of *D acidovorans* BSI occurred in the period from February 8 through April 30, 2018. One confirmed case (Patient 1) had 2 subsequent BSIs due to other gram-negative bacteria despite having his HD catheter exchanged after each BSI episode. *Enterobacter asburiae* and *Klebsiella oxytoca*, were recovered from the second BSI episode, and *Chryseobacterium indologenes* and *Burkholderia cepacia* were recovered from the third BSI episode (Fig 1). The other confirmed case (Patient 2), who had refused to have his HD catheter exchanged after the *D acidovorans* BSI, had a subsequent episode of BSI due to *D acidovorans* and *Stenotrophomonas maltophilia* (Fig 1). Patient 3, who had a urine culture positive for *D acidovorans* (10,000-50,000 CFU) and *S maltophilia* (10,000-50,000 CFU), was considered a possible BSI case because there was no recent history of an indwelling urinary catheter or urinary tract instrumentation, suggestive of a transient bacteremia with seeding of the urinary tract.

Characteristics of the 3 case patients are listed in Table 1. The most common clinical manifestations of BSI were chills and hypotension. The 2 patients with BSIs were hospitalized on multiple occasions.

During the 2-week period prior to an infection event, the percent of dialysis sessions during which patients were cared for by ≥ 3 caregivers/session was as follows: Patient 1: 89.5%, Patient 2: 85.7%, and Patient 3: 85.7%. In the 2-week period before each patient's positive *Delftia* culture, the 3 case patients shared 5 different caregivers. Expanded case-finding efforts, including a review of 2017-2018 NHSN dialysis pathogen data reported by CT HD facilities did not identify additional *D acidovorans* cases.

Environmental sampling and laboratory testing results

Seven patient isolates and 54 environmental samples were submitted to CDC for culture, identification and molecular typing. The 7 patient isolates were as follows. From Patient 1: 1 blood culture

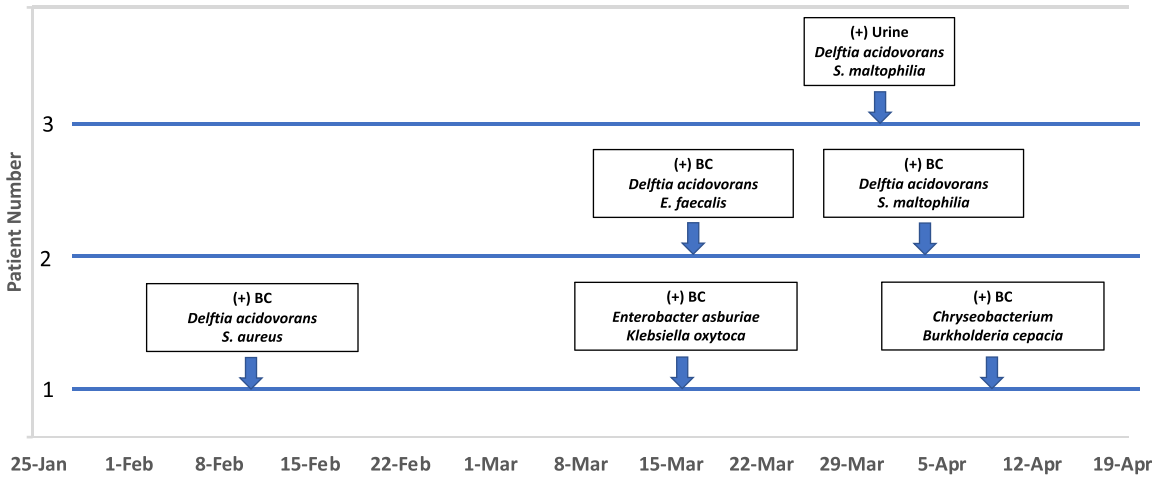

Fig 1. Timeline of positive blood cultures and urine culture from 3 case patients. (Figure 1 is 1.5- or 2-column fitting image.)

with *Chryseobacterium indologenes*, 2 catheter tip isolates with *Burkholderia cepacia* complex, and 2 blood isolates with *Burkholderia cepacia* complex and *Burkholderia pyrocinnia*; from Patient 2: a blood culture positive for *Enterococcus faecalis*, and from a non-case patient who was bacteremic in late June, several months after the outbreak period: an *Acinetobacter nosocomialis* blood culture isolate.

The 54 environmental samples submitted to CDC were from selected wall box drains (N = 7) and spigots (N = 7), saline prime buckets before (N = 7) and after (N = 2) cleaning, hollow handles of saline prime buckets before (N = 6) and after (N = 2) cleaning, water samples from dirty sinks (N = 2), reverse osmosis (RO) water (N = 2), swab samples from RO hoses (N = 3), countertop next to dirty sink (N = 1), medication preparation countertop (N = 2), machine soft touch keypad (N = 9), machine keyboard (N = 3), and a swab of Phoenix pH meter. Multiple gram-negative bacilli were isolated from the environmental cultures of the station wall box spigots, wall box drains and the inside and handle of the saline prime buckets (Table 2).

D acidovorans was isolated from the wall box spigot of Station #2 where both confirmed case patients had recently been dialyzed. Patient 1 had been dialyzed at Station #2 2 days before for his blood culture yielded *D acidovorans*. Patient 2 was dialyzed at Station #2 14, 11, 9, and 7 days earlier and on the day his blood culture grew *D acidovorans. E asburiae* was recovered from the wall box spigots and drains of Stations #12 and #14, saline prime bucket handles used at Stations #12 and #13, and from blood cultures of Patient 1 (Fig 1), who had been dialyzed at station 13 five days before the date of his *E asburiae* BSI. *D acidovorans* and *E asburiae* isolates from case patients were not available for molecular typing.

S maltophilia, which was isolated along with *D acidovorans* from a blood culture obtained from case patient 2 and a urine culture from Patient 3, was recovered from Station #3 wall box drain, as well as the countertop of the dirty sink. None of the case patient treatment sessions recorded during the 2-week period before infection onset occurred at Station #3. Two reverse-osmosis water samples were negative for marker organisms when cultured in July 2018. No *Burkholderia* spp. were recovered from environmental specimens tested.

The PFGE results revealed that the 2 catheter tip isolates obtained in March and April represented the same strain (albeit 1 lane was faint), and that they were unrelated to the 2 blood culture isolates obtained in August 2018. Figure 2 shows the PFGE results for 1 catheter tip isolate and the 2 blood culture isolates from case Patient 1.

A nosocomialis was found on the countertop of the sink designated for dirty items. The countertop isolate was unrelated to the patient *A nosocomialis* isolate, with greater than 7 band differences in their PFGE patterns (data not shown).

Facility observations and review of practices

During the direct observations of facility practices, investigators noted splashing of dialysate waste fluid from all 19 wall box connections, with water damage noted in 1 wall box housing, and large air gaps between the wall box spigots and drains that permitted the splashing of dialysate waste fluid (Fig 3 and Fig S1 video provided in Supplementary Material).

When questioned on disinfection procedures for cleaning the wall boxes, facility staff and administrators indicated that the wall boxes were never cleaned or disinfected. Multiple infection control lapses were observed during cleaning and disinfection of the HD station including: not applying an adequate amount of disinfectant to surfaces, not wiping all appropriate surfaces, a lack of knowledge regarding disinfectant contact times, and not removing station items before disinfection. In addition, investigators observed difficulties in disinfection of saline prime buckets due to the inability of disinfectant wipes to reach into the narrow hollow handle (see Supplementary Fig S2). Occasional rinsing of saline prime buckets with tap water after disinfection was observed. Deficiencies in hand hygiene

Table 1
Characteristics of case patients

Patient	Age*	Sex	Access type	Dialysis shift	Dialysis stations before† BSI	Underlying conditions possibly affecting risk of gram-negative infection	Years on dialysis	Duration of current access
1	35	M	PC	TTS-3	2, 7, 10, 11,13	Membranoproliferative glomerulonephritis	0.33	5 months
2	50	M	PC	TTS-3	1, 2, 6,	Type 1 diabetes mellitus	> 1.5	4 months
3	65	M	AV fistula	MWF-3	3, 7, 8, 11, 12, 15, 18	Pyelonephritis, hydronephrosis, recurrent UTIs, nephrolithiasis	> 2.5	26 months

AV, arteriovenous; *MWF-3*, third shift on Monday, Wednesday & Friday; *PC*, Permacath; *TTS-3*, third shift on Tuesday, Thursday & Saturday; *UTI*, urinary tract infection.
*Age rounded to nearest half-decade.
†In 2 weeks before positive Delftia culture.

Table 2
Microorganisms recovered from environmental cultures obtained at Facility A on August 2, 2018, by source

Station number	Source	Organism (s)
1	Prime bucket - inside	*Cryptococcus diffluens*
1	Prime bucket – handle	*Enterobacter kobei*
2*	Wall box spigot	*Delftia acidovorans*
2*	Wall box drain	*Enterobacter kobei*
3	Wall box drain	*Stenotrophomonas maltophilia*
3	Wall box spigot	*Pseudomonas aeruginosa*
7	Wall box spigot	*Enterobacter kobei, Escherichia coli*
7	Wall box drain	*Enterobacter kobei, E coli, Morganella morganii*
10	Wall box spigot	*Enterobacter kobei, E coli*
10	Wall box drain	*Enterobacter kobei, E coli*
12	Wall box spigot	*Enterobacter asburiae, Enterobacter albertii*
12	Wall box drain	*Enterobacter asburiae, E coli*
12	Prime bucket – handle	*Enterobacter asburiae*
13	Prime bucket – handle	*Enterobacter asburiae*
14	Wall box spigot	*Enterobacter asburiae*
14	Wall box drain	*Enterobacter asburiae, Enterobacter kobei*
	Countertop at dirty sink	*Acinetobacter nosocomialis, Stenotrophomonas maltophilia*

*Station at which patients 1 and 2 were dialyzed.

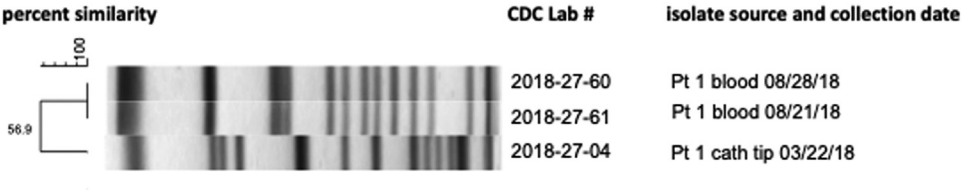

Fig 2. PFGE dendrogram of *Burkholderia cepacia* complex isolates collected from Patient 1 in March, and after the outbreak period in August 2018. (Figure is 1.5-column fitting image.)

practices included the use of soap and water as the primary method of hand hygiene rather than the use of alcohol-based hand rub, and when alcohol-based hand rub was used, inadequate amounts of hand rub were often applied, resulting in rubbing hands together for less than 15 seconds (sometimes as short as 5 seconds). A number of deficiencies regarding catheter management were observed. During AV fistula cannulation, chlorhexidine gluconate swabs were used to scrub the skin for only 10 seconds instead of 30 seconds. In some instances, the same swab was used for each cannulation instead of using a separate swab for each cannulation site. During AV fistula decannulation, hand hygiene was not always performed before donning clean gloves before starting the procedure, and some staff did not scrub the hub for a full minute. During dialysis catheter disconnection, the antiseptic on the hub was allowed to dry for only a few seconds prior to capping. The team noted that the medication prep area was close to the sink and medication contamination could possibly result from water splashes. No deficiencies in water treatment processes were identified during the investigation. Facility A has never had a dialyzer reuse program.

Fig 3. Wall box with white spigot and drain on right side of wall box. In the air space between spigot and drain, spent dialysate can be seen splashing into wall box next to the drain (arrow). (Figure is 1.5 or 2-column fitting image. Should be printed in color in print.)

Infection control and prevention measures

On September 26 and 27, 2018, CT DPH conducted a mandatory training for all Facility A staff to review investigation findings and to review basic infection control practices and CDC recommendations for the dialysis setting.[21] CT DPH advised the facility to implement the following recommendations:

- Implement daily disinfection with 1:100 bleach solution of the wall boxes including disinfection of the wall box spigots with a cotton swab soaked in the bleach solution
- Develop specific cleaning and disinfection procedures for used saline prime buckets, including:
  - soaking of the saline prime buckets in the 1:100 bleach solution
  - not rinsing saline prime buckets with tap water after disinfection, and complete air drying of saline prime buckets
  - the rotation of saline prime buckets between patients to allow for adequate time to disinfect and dry the saline prime buckets
- Reduce the air gap between spigot and drain of spent dialysate effluent to decrease splash

- Establish policies and procedures for preparing, maintaining and discarding bleach solutions for disinfection
- Develop policy and training programs for consistent cleaning and disinfection of the patient environment, including station, machines and wall boxes
- Develop specific cleaning and disinfection procedures for other reusable equipment (clamps, blood pressure cuffs, etc.); develop a system to document cleaning and disinfection after each dialysis session in the patient's medical record
- Draw blood cultures from peripheral veins as well as from central catheters
- Improve aseptic technique related to CVC access, care, and maintenance
- Improve hand hygiene with a particular emphasis on hand hygiene after wall box contact

A formal report outlining the DPH recommendations was sent under separate cover. A formal report entitled " Recommendations for the Development of Administrative Protocols and Procedures for Cleaning & Disinfection of Out-Patient Hemodialysis Facilities in the State of CT" was developed and distributed to the Facility A and to other HD facilities during CT DPH trainings.

DISCUSSION

The present study yielded several new findings in addition to confirming the findings of earlier investigations.[14,18,22-24] To the best of our knowledge, this represents the first reported cluster of *D acidovorans* infections among HD patients. CDC's detection of this unusual cluster of *D acidovorans* infections, and their detection and investigation of an earlier multicenter outbreak of gram-negative BSIs among HD patients that implicated wall boxes as the source of infection, illustrate the value of ongoing review of data reported to the NHSN Dialysis Event Surveillance Program.[18] Routine monitoring of bacteremia cases on an ongoing basis by facilities will improve timely detection of a small number of cases caused by an unusual organism, which may represent an outbreak. Although cultures of 2 RO water samples, swabs from 3 RO hose connections and 2 cultures of tap water failed to yield *D acidovorans* or other environmental gram-negative bacilli, it is possible that contaminated city water or RO water served as a source of wall box and saline prime bucket contamination. Failure to recover *D acidovorans* from tap or RO samples may have been due to insufficient sampling, or collection of water samples 4 months after the outbreak period.

The present investigation provided additional evidence that contaminated wall boxes can serve as a source of gram-negative BSIs in HD patients. Although environmental samples were obtained several months after the BSIs occurred, the ability of *D acidovorans* to produce biofilm and its relative resistance to chlorine suggest that the organism was likely present in the wall box when BSIs occurred.[25-28] Of interest, a recent investigation of a pseudo-outbreak of *D acidovorans* not associated with any infections identified city water as a source of biofilm formation in portable RO machines.[25] Also, *E asburiae*, which to our knowledge has not been reported as a cause of BSI in HD patients, caused a BSI in Patient 1 and was recovered from wall boxes and saline prime bucket handles at 2 stations. The *Burkholderia* strain that caused Patient 1's BSI in March was different than the strain that caused another BSI episode in Patient 1 after the outbreak period in August (not shown in Fig 1), based on PFGE findings. *Burkholderia* was not recovered from any environmental cultures, and the source from which Patient 1 acquired the organism was not identified.

Recurrent BSIs in Patients 1 and 2 were likely related to several risk factors. Both Patients 1 and 2 had long-term CVCs, which represent a major risk factor for BSIs among patients on maintenance hemodialysis.[16,18,29] Lapses in aseptic technique by staff during catheter care may also have put the 2 patients at increased risk.[29] Both Patients 1 and 2 often failed to follow recommended practices in managing their CVCs, and Patient 2 refused to have his CVC exhanged when recommended. Other risk factors included having dialysis during later shifts during the day, and exposure to 3 or more staff during a dialysis session.[7,18] Perhaps the recent use of human factors engineering analyses in hemodialysis units will identify opportunities to modify workflow and staffing practices that can reduce the risk of infection among hemodialysis patients.[30]

Our findings were similar to those of Novosad et al.,[18] which included: wall boxes were not perceived by staff as a contaminated area and large visible air gaps with fluid splashing were observed at each wall box. Poor hand hygiene practices and many lapses in station disinfection were observed in both the earlier CDC investigation and during the present study. In both outbreaks, it appears likely that splashing of wall box waste effluent and lack of cleaning and disinfection of the wall boxes created a reservoir for healthcare personnel to contaminate hands, dialysis machine surfaces and equipment, and patient vascular accesses. Based on the findings reported by Novosad et al.[18] the CDC now explicitly recommends that wall boxes be routinely disinfected.[31]

Another interesting finding of our investigation was the recovery of the *E asburiae* from the hollow handles of 2 saline prime buckets, but not from the main compartment of the saline prime buckets at stations #12 and #13. The finding is consistent with observations that disinfection of the hollow handles of saline prime buckets can be difficult. Inadequate disinfection of saline prime buckets has been identified as a potential contributing risk factor in a number of other outbreaks,[22-24] and during evaluation of outpatient HD units performed under the auspices of the CDC's Infection Control Assessment and Response (ICAR) program.[32-34] However, the microorganism responsible for such outbreaks has been recovered from saline prime buckets on only a few occasions.[14,18,28] Our finding provides additional suggestive evidence that inadequately disinfected saline prime buckets may serve as a source of infection in HD patients, highlights the need to assure that saline prime buckets with hollow handles are adequately disinfected between patients, and perhaps identifies a need to consider redesign of saline prime buckets. Transmission of organisms contaminating saline prime buckets may result if dialysis tubing and connectors come into direct contact with moist surfaces of saline prime buckets that have not been adequately disinfected.[14,28]

Interventions to mitigate the infection control breaches were started shortly after the initial site visit. They included reeducation of staff on infection control practices as outlined by CDC.[21] This investigation also provided an opportunity to provide education statewide on routine disinfection of station wall boxes and the importance of adherence to basic infection control practices in the dialysis setting.

Our study has a number of limitations. We were unable to demonstrate with absolute certainty that wall boxes were a source of infection because most patient isolates were not available for molecular typing and comparison with environmental isolates. However, the fact that *D acidovorans* and *E asburiae* have not previously been recovered from environmental sources in HD units suggests that isolates recovered from affected patients and environmental cultures represented the same strains. Also, the small number of case patients affected our ability to conduct a case-control study to identify other potential risk factors. Internal components of dialysis machines were not cultured because no deficiencies in water treatment and machine disinfection protocols were noted.

In summary, a cluster of unusual gram-negative BSIs was most likely due to exposure to contaminated wall boxes, and perhaps to inadequately disinfected saline prime buckets. Poor hand hygiene and inadequate station disinfection probably led to the transmission of pathogens to patients. New recommendations for routine disinfection of the station wall boxes should be incorporated in all dialysis facility

station disinfection policies.[31] And efforts to improve wall box design should be pursued.[18,30] The need to routinely disinfect saline prime buckets warrants continued emphasis, and additional investigations to determine if hollow saline prime bucket handles represent an independent infection risk should be considered.

Acknowledgments

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DISCLAIMER

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the CDC.

PRIOR PRESENTATION

Preliminary results of the investigation were presented in part at 2019 IDWeek Conference, October 5, 2019, Washington, DC.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2022.08.007.

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