In vitro evaluation of three bacterial culture systems for the recovery of Escherichia coli from equine blood

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Objective—To evaluate the effectiveness of a commercial conventional blood culture system (BCS), a commercial resin-containing BCS, and a commercial lysis-centrifugation–based BCS for the recovery of Escherichia coli from equine blood samples inoculated with that organism.

Sample Population—Samples of blood obtained from a clinically normal horse that were inoculated with E coli.

Procedures—Blood samples were aseptically collected and inoculated with an E coli specimen (50 CFUs/mL) that had been previously isolated from a foal with sepsis. Subsequently, samples were spiked with gentamicin at a concentration of 30 μg/mL, and 10 mL of each mixture was inoculated into 1 bottle or tube of each BCS. Samples were processed and incubated according to the manufacturer’s guidelines and inoculated onto 5% sheep blood agar plates. Plated samples were examined macroscopically at regular intervals for as long as 72 hours. Detection of E coli and time to detection were recorded for each medium.

Results—Detection frequency of E coli was significantly greater by use of the resin-containing BCS (7/23 bottles) than that achieved by use of the conventional BCS (7/23 bottles) or the lysis-centrifugation–based BCS (0/10 tubes). Mean detection time (6 hours after plating) did not differ between the BCS with conventional medium and the BCS with resin-containing medium.

Conclusions and Clinical Relevance—Results suggest that a BCS with resin-containing medium may provide clinical benefit in the successful recovery of E coli from the blood of foals with sepsis that have been previously administered gentamicin. (Am J Vet Res 2006;67:2025–2029)

Sepsis in neonates is a common cause of illness and death in newborn foals.1 In affected foals, rapid and reliable detection and subsequent identification of blood-borne microorganisms remain critical so that the appropriate treatment can be provided. Bacterial culture of blood is the current gold-standard test with which to diagnose sepsis in foals. However, in critically ill neonates with suspected bacteremia, antimicrobials are often administered before the collection of blood samples for culture, thereby masking the etiologic agent and complicating effective treatment. Therefore, improvement in the bacterial yield of cultured blood samples in this set of patients would facilitate more rational antimicrobial treatment.

Several methods have been designed to overcome prior antimicrobial administration that might inhibit isolation of pathogens via bacterial culture of blood. These methods include dilution of the blood sample in culture medium, addition of antimicrobial-inactivating enzymes to the culture, and a lysis-centrifugation method.2,3 In addition, results of a clinical study in humans have indicated that addition of nonionic adsorbing resins and cationic exchange resins (to adsorb antimicrobials when present) to the medium can shorten detection time and increase the number of positive results obtained via bacterial culture of blood samples collected from humans receiving antimicrobials. A limited amount of in vitro work has also confirmed the benefits of resin-containing systems.3 However, findings of other clinical studies have questioned the ability of resins to neutralize commonly used antimicrobials.

The optimal use of BCSs to determine sepsis in foals has not been investigated to our knowledge. In particular, the sensitivity of resin-containing media has not specifically been evaluated among equine neonates that have been administered antimicrobials and then evaluated at a referral hospital. Therefore, the purpose of the study reported here was to evaluate the effectiveness of a conventional commercial BCS, a resin-containing commercial BCS, and a lysis-centrifugation–based commercial BCS for the recovery of Escherichia coli from equine blood samples inoculated with that organism. Effectiveness was assessed in terms of culture sensitivity (for detection of pathogens and contaminants) and time to microbial detection. We hypothesized that resin-containing medium would improve detection of blood-borne bacteria in blood samples collected from foals that were already receiving antimicrobial treatment. However, testing this hypothesis in critically ill neonates in a clinical setting would require the inclusion of multiple variables in the study design. For instance, the rate of shedding of the...
pathogen into the bloodstream and the timing and rate of infusion of antimicrobials may be factors influencing the recovery of organisms via bacterial culture of blood. Therefore, to test our hypothesis under controlled conditions, we designed an in vitro model to simulate conditions of bacteremia. This model consisted of spiking blood samples collected from a healthy horse with 1 bacterial species and 1 type of antimicrobial drug. An *E. coli* isolate was chosen for this investigation because that organism represents the most common pathogen isolated from foals with sepsis. Likewise, gentamicin was chosen because it is one of the most common antimicrobial drugs used by field practitioners to treat foals with suspected sepsis before referral to a veterinary hospital.

**Materials and Methods**

**Blood specimens**—Blood samples for culture were obtained from 1 horse (a 7-year-old Standardbred mare) that belonged to the blood donor herd of the Veterinary Medical Teaching Hospital, School of Veterinary Medicine at the University of California, Davis. A complete physical examination and CBC were performed at the beginning of the study to determine the horse's health status. Blood was aseptically collected from a jugular vein by use of a catheter, immediately transferred into tubes containing sodium citrate, and stored at 4°C until use (within 6 hours of collection). Preliminary studies were performed to exclude the interference of sodium citrate on bacterial growth or antimicrobial activity in the various culture media used in this study. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California.

**Bacterial isolate**—The *E. coli* isolate used was a specimen that had been previously isolated from a foal with sepsis that had been admitted to the Lucy Whittier Neonatal Intensive Care Unit; the isolate was sensitive to gentamicin. The organism was grown overnight on brain-heart infusion medium before use. Turbidity of the culture preparation was then adjusted to match a McFarland 0.5 standard. Thereafter, serial dilutions up to a final dilution of 1:1,000 were made with sterile saline (0.9% NaCl) solution. A sample of this final dilution was used as the *E. coli* inoculum and the volume of the sample adjusted to achieve a final concentration of 50 CFUs/mL of blood. This concentration reflects the reported mean concentration of bacteria in blood of human infants with bacteremia.

**Experimental procedure**—All procedures were performed in an air flow chamber to minimize contamination. First, whole blood was inoculated with *E. coli* at a final concentration of 50 CFUs/mL. After vortexing, gentamicin was added to a final concentration of 30 μg/mL, and the mixture was agitated for 5 minutes. This concentration reflects the maximal clinically achievable concentration in foals at 0.5 to 3 hours after administration of a single dose of 6.6 mg of gentamicin/kg. The drug had been previously diluted in sterile water to a concentration of 1 mg/mL. The supernatant was discarded, and a 1-mL aliquot of the remaining sediment was equally divided among 3 blood agar plates and then incubated at 37°C with 5% CO₂. All conventional BCS bottles were incubated in a stationary position at 37°C for 24 hours without prior processing. Thereafter, a sample of each bottle was transferred by use of a sterile loop onto 3 blood agar plates as a terminal subculture and incubated at 37°C with 5% CO₂. All plated samples were examined macroscopically for growth at 6, 12, 24, 36, 48, 60, and 72 hours, and time to first appearance of colonies was recorded. Twenty-three bottles of the conventional BCS, 23 bottles of the resin-containing commercial BCS, and 10 tubes of the lysis-centrifugation–based BCS were spiked with the *E. coli*-gentamicin mixture following the aforementioned procedure.

Once bacterial growth was detected, the microorganism was identified to confirm it as the original contamination. The CFUs of pathogens per milliliter of blood recovered from any plate were determined by use of Gram stain and standard microbiologic procedures after further subculture on MacConkey and blood agar plates.

Detection of bacteria and time to that detection were recorded for each BCS. The CFUs of pathogens per milliliter of blood recovered from any plate were determined by use of Gram stain and standard microbiologic procedures after further subculture on MacConkey and blood agar plates.

**Results**

**Effect of sodium citrate on bacterial growth**—Samples of fresh blood and blood with sodium citrate were inoculated with *E. coli* (30 CFUs/mL) with or without addition of gentamicin (30 μg/mL). The same protocol of incubation described in the experimental procedure was followed for all blood combinations. Experiments were performed in duplicate for each combination and BCS. Growth of *E. coli* was observed at 6 hours after plating of all samples inoculated with *E. coli* in the absence of gentamicin. For those blood samples that were spiked with both *E. coli* and gentamicin, only those inoculated into the bottles containing resins yielded growth of *E. coli* (at 6 hours). The number of CFUs per milliliter of blood recovered from plates that yielded growth could not be determined because of excessive bacterial growth, regardless of the presence or absence of sodium citrate.

**Data and statistical analyses**—The study was based on a balanced factorial design. Three parameters were determined and compared among all culture combinations: bacterial recovery rates for each BCS (No. of culture-positive BCS/wall inoculated BCS expressed as a percentage), time to detection (expressed as hours from plating), and pathogen load in plate (defined as No. of CFUs per plate). Data were analyzed by use of χ² analysis and the Fisher exact test; analyses were performed with computer software. Significance was set at a value of *P* ≤ 0.05.

**Culture of bacterial-antimicrobial combinations**—The frequency of *E. coli* detection in the resin-containing BCS (14/23 [61%] bottles) was significantly (*P* ≤ 0.05) greater than the detection frequencies in the conventional BCS (7/23 [30%] bottles) and the lysis-centrifugation–based BCS (0/10 [0%] tubes). There was no significant difference in detection frequency between the latter 2 BCSs. For all cultures that yielded positive results
(by use of the conventional and resin-containing BCSs), growth was first observed at 6 hours after plating. Therefore, mean detection time did not differ between the BCS with conventional medium and the BCS with resin-containing medium. The number of CFUs per milliliter of blood recovered from any plate that yielded growth could not be enumerated because bacterial growth was too abundant. Finally, discrete, small colonies of contaminants (1 to 3 colonies/plate) were identified after 60 hours of incubation on some plates prepared from resin-containing BCS bottles (3/23 bottles; 3/69 plates) and after 36 hours of incubation on some plates prepared from the lysis-centrifugation–based BCS tubes (3/10 tubes; 3/30 plates). These colonies were comprised of either gram-positive cocci or rods.

Control cultures—Growth of E. coli was observed at 6 hours after plating of all bacteria control specimens irrespective of the BCS, whereas no growth was observed from the blood and antimicrobial control specimens after 72 hours of incubation.

Discussion

The main objective of the present study was to evaluate the recovery rate of E. coli from equine blood samples inoculated with that organism that were cultured in BCSs containing a conventional broth medium or a resin-containing broth medium or in a lysis-centrifugation–based BCS. The results of the study have indicated that the resin-containing BCS provides enhanced detection of E. coli from gentamicin-spiked, E. coli-inoculated equine blood specimens, compared with detection achieved by use of the conventional BCS or the lysis-centrifugation–based BCS. This is in accordance with previous studies3,14-27 in which bacterial recovery from blood samples obtained from humans with sepsis who had received antimicrobial treatment was increased by use of resin-containing media, compared with use of conventional media. Results of a limited number of in vitro antimicrobial-neutralizing studies8,9,24 have also confirmed the ability of resin-containing BCSs to remove antimicrobial agents (including aminoglycosides, cephalosporins, and penicillins) from blood samples. Furthermore, resin-containing medium improved recovery of microorganisms from blood samples collected from patients who were not receiving antimicrobial drugs, which suggests that resins adsorb other microbial inhibitors present in human blood.30 Of the 3 BCSs evaluated in our study, the resin-containing BCS appeared to be the only system to actively remove or inactivate antimicrobial agents by lysing leukocytes that contain viable bacteria via the mechanical action of resin beads, especially when culture vials are agitated in orbital shakers during incubation. Other data have indicated that addition of resins in broth media significantly shortens the time to detection of culture growth, compared with time to detection in standard broth media.31 In the present study, there was no difference in time to detection among cultures that yielded positive results; growth was first observed at 6 hours after plating in all instances. Earlier (< 6 hours) and more frequent assessments might have resulted in detection of different growth rates between the conventional and resin-containing BCSs. Additionally, serial dilutions would have allowed quantification of colonies for comparison among culture media.

In contrast, E. coli could not be recovered by use of the lysis-centrifugation–based BCS when blood samples were spiked with gentamicin. Several clinical studies3,32 in humans have suggested that the lysis-centrifugation–based BCS is more efficient than non–resin-containing BCSs for detection of bacteria; compared with the latter BCSs, detection of clinically important organisms has been reported to be 15% to 18% greater by use of the lysis-centrifugation–based BCS.3 The superior performance of the lysis-centrifugation–based BCS in those studies was attributed to the presence of saponin in the medium, which aids lysis of phagocytic cells with centrifugation, and to removal of organisms from natural inhibitory factors and from antimicrobials in blood.32 Lysis of blood cells may increase the detection of organisms that would otherwise be sequestered in or damaged by those cells. Although there was no significant difference in the rate of bacterial recovery between the lysis-centrifugation–based BCS and the conventional BCS, the fact that no culture growth was obtained by use of the former suggests that the lysis-centrifugation–based BCS may not offer any advantage, compared with use of conventional medium. Inclusion of a higher number of lysis-centrifugation–based BCS tube replicates in the present study might have allowed detection of a significant difference in recovery rate between that system and the conventional BCS. Alternatively, use of the entire volume (2 mL) of the sediment obtained after centrifugation of human blood samples inoculated with E. coli and spiked with different antimicrobials revealed that use of standard broth medium with resins resulted in the greatest recovery (100%), compared with the recovery achieved by use of the lysis-centrifugation–based BCS (68%) or standard broth medium (14%).

The significantly higher capacity of the resin-containing medium to recover E. coli in our study may reflect the ability of strong cationic-exchange resins to bind to positively charged antimicrobials, such as aminoglycosides, and the ability of polymeric adsorbing resins to bind to the hydrophobic regions of antimicrobial agents.9 In addition, various blood constituents, such as phospholipids and sterols of cell membranes, and soluble intracellular proteins can also bind to resins, thereby disturbing the action of antimicrobials. Moreover, resins may increase the recovery of organisms by lysing leukocytes that contain viable bacteria via the mechanical action of resin beads, especially when culture vials are agitated in orbital shakers during incubation. Other data have indicated that addition of resins in broth media significantly shortens the time to detection of culture growth, compared with time to detection in standard broth media.31 In the present study, there was no difference in time to detection among cultures that yielded positive results; growth was first observed at 6 hours after plating in all instances. Earlier (< 6 hours) and more frequent assessments might have resulted in detection of different growth rates between the conventional and resin-containing BCSs. Additionally, serial dilutions would have allowed quantification of colonies for comparison among culture media.
tion might have resulted in a better recovery rate. It is interesting that formation of blood clots in the sediment was commonly encountered during the process of plating, even after blood had been mixed with sodium citrate as an anticoagulant; thus, homogeneous distribution of the sample onto the agar plates was prevented. Clot formation might have also affected detection of bacterial colonies. In addition, a higher concentration of sodium polyanetholsulfonate, which acts as an anticoagulant and inactivates bacterial components and aminoglycosides, in the lysis-centrifugation–based BCS (0.96% vs 0.05% in the conventional BCS) did not seem to have a beneficial effect on the performance of the lysis-centrifugation–based BCS. The conventional BCS may have yielded bacterial growth, whereas the lysis-centrifugation–based BCS did not, because the former includes a 24-hour period of incubation of samples in enriched broth before plating. The large volume of enriched medium (70 mL) allows proliferation of inoculated bacteria and maximizes seeding of bacteria onto the agar plates. This may explain why positive culture results were obtained with the conventional BCS only when 100-μL samples were used for plating.

To our knowledge, no studies have been performed to evaluate the efficacy of different culture media on bacterial recovery from blood samples collected from critically ill neonatal foals. In foals that have received inappropriate empirical antimicrobial treatment before blood is collected, detection of bacteria may be prevented or delayed. Multiple alternatives to overcome false-negative culture results have been developed in human medicine, including broth dilution of blood, removal of organisms via membrane filtration, antimicrobial inactivation by penicillinase, or incorporation of resins into broth culture media. Clinical studies involving blood samples collected from foals with sepsis are necessary to determine whether a good correlation exists between rates of bacterial isolation from cultures of blood inoculated with microorganisms experimentally and blood specimens obtained from ill neonates. In the present study, many of the factors that might be encountered in a clinical study were controlled, and recovery of only 1 organism and use of 1 antimicrobial drug at a specific concentration were evaluated. Furthermore, mixing of bacteria and antimicrobial was performed immediately before inoculation of blood into the different media. The performance of different BCSs is influenced by the volume of the blood sample, blood-to-broth ratio, amount and rate of the antimicrobial drug removal by the system, kinetics of bacterial killing by the antimicrobial agent, duration of incubation and final subculture, and susceptibility of the bacteria to the antimicrobial agent. In addition, other factors intrinsic to the patient’s status, such as the rate of shedding of the microorganism into the bloodstream (transient vs continuous), type of organism (ie, rate of killing by the antimicrobial agent), and timing and rate of infusion of antimicrobial that determine its concentration in blood at the time of sample collection, may be determining factors in the success of bacterial recovery from blood samples. To mimic a clinical situation, we used the microorganism that is most frequently isolated from foals with septicemia and an antimicrobial drug commonly used for the prevention of septicemia caused by enteric pathogens.

Our data have suggested that a BCS with broth medium containing resins is more efficient in the recovery of Escherichia coli from equine blood samples inoculated with that organism and spiked with gentamicin, compared with recovery achieved by use of a BCS with conventional broth medium or a lysis-centrifugation–based BCS. Although clinical studies evaluating the efficacy of the 3 systems are warranted, we suggest that use of resin-containing medium may be of benefit in clinical practice for the evaluation of blood samples from neonatal foals that have received antimicrobials. We also suggest that the in vitro experimental procedures established for the present study may be used to evaluate the use of these and other BCSs to recover other pathogens from blood samples.

References