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Environmental surveillance for *Salmonella enterica* in a veterinary teaching hospital

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Objective—To evaluate the extent of environmental contamination with *Salmonella enterica* in a veterinary teaching hospital.

Design—Longitudinal study.

Samples—Environmental samples obtained from 69 representative locations within a veterinary teaching hospital by use of a commercially available electrostatic wipe.

Procedure—Environmental samples were obtained for bacteriologic culture, and antimicrobial susceptibility testing was performed on each environmental isolate. Environmental isolates were compared with isolates obtained from animals during the same period to investigate potential sources of environmental contamination.

Results—54 *S enterica* isolates were recovered from 452 (11.9%) cultured environmental samples. Five different serotypes were recovered; the most common serotypes were *S* Newport and *S* Agona. Within the 5 serotypes recovered, 10 distinguishable phenotypes were identified by use of serotype and antimicrobial susceptibility patterns. Of the environmental isolates, 41 of 54 (75.9%) could be matched to phenotypes of isolates obtained from animal submissions in the month prior to collection of environmental samples.

Conclusions and Clinical Relevance—Results indicated that environments in veterinary hospitals can be frequently contaminated with *S enterica* near where infected animals are managed and fecal specimens containing *S enterica* are processed for culture in a diagnostic laboratory. Bacteriologic culture of environmental samples collected with electrostatic wipes is an effective means of detecting contamination in a veterinary hospital environment and may be beneficial as part of surveillance activities for other veterinary and animal-rearing facilities. (*J Am Vet Med Assoc* 2004;225:1344–1348)

Salmonella enterica is an important cause of disease in animals, and nosocomial epidemics have also been a cause of veterinary teaching hospital closures.[1-4] Documented risk factors for nosocomial *Salmonella* spp infection in animals include concurrent disease, antimicrobial treatment, stress, and use of common instruments such as thermometers.[1,2,5,6] Control of nosocomial infections is therefore an important biosecurity concern in veterinary hospitals. Not only can animals infected with *Salmonella* organisms serve as a source of exposure for other animals or humans, but the faculty, staff, students, and visitors also can traffic the bacteria throughout the veterinary hospital.[1,2,5] Use of electrostatic wipes for environmental sampling in a veterinary hospital is a simple and sensitive method for sampling large areas of the hospital. The purpose of the study reported here was to evaluate the extent of environmental contamination with *S enterica* in a veterinary teaching hospital.

Materials and Methods

Study design—Environmental samples were collected from the **James L. Voss Veterinary Teaching Hospital (JLV-VTH)** and the **Veterinary Diagnostic Laboratory (VDL)** at Colorado State University at approximately 2-week intervals from June 9 to August 19, 2002. Representative samples were obtained from the floor; hand contact surfaces such as doorknobs, handles, keyboards, telephones, and medical instruments; and selected sites throughout the JLV-VTH and VDL and were cultured for *S enterica*. *Salmonella enterica* isolates were further characterized by determining serotype and antimicrobial susceptibility. Isolates obtained from environmental samples were compared with isolates cultured from specimens (such as feces or tissues) submitted to the VDL from hospitalized animals and outside sources for the month prior to each sample date. Temporal and spatial relationships among isolates were subjectively evaluated by use of phenotypic characteristics as the basis for comparison.

Environmental sampling—Floors that were sampled in the small animal areas of the hospital, in the diagnostic laboratory, and on the second floor were waxed and sealed tile floors. Floors that were sampled in the large animal areas of the hospital were concrete. All tile floors are routinely sealed quarterly to semiannually. Cleaning protocols for the small animal facility include cleaning and disinfecting wards and the critical care unit

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twice per day and disinfecting animal examination and treatment rooms between animals and more thoroughly each night. All other areas of the small animal facility are cleaned nightly. Cleaning protocols for the large animal facility included cleaning and disinfecting contaminated areas as needed and nightly.

Sample site selection—A total of 452 samples were collected from 69 sites during the study. All areas used in animal management and for the VDL are located on the first floor of the facility (**Figure 1**), whereas the cafeteria, library, classrooms, and faculty offices are located on the second floor. Sites sampled for this study included general traffic areas (first floor hallways, small animal waiting area, large animal waiting area, first floor locker rooms, stairwells, second floor hallways, second floor locker rooms, bookstore, and the food animal classroom; 21 hand contact samples and 154 floor samples), central service areas (laundry, medical records, and radiology; 21 floor samples), VDL areas (laboratory hallway, gross pathology hallway, necropsy, virology laboratories, bacteriology laboratories, and the media preparation room; 7 hand contact samples and 72 floor samples), small animal patient management areas (small animal examination rooms, treatment rooms, critical care, anesthesia; 7 hand contact samples and 49 floor samples), small animal housing areas (7 hand contact samples and 21 floor samples), equine patient management areas (medicine service records rooms, surgery service records rooms, technician's office; 7 hand contact samples and 21 floor samples), food animal areas (reproduction laboratory, locker rooms, technicians' office, and records room; 9 hand contact samples and 35 floor samples), and isolation areas (equine isolation office, calf isolation housing, and treatment areas; 8 hand contact samples, 2 wall samples, and 11 floor samples). Sites were selected on the basis of traffic patterns for personnel and animals within the hospital and were intended to be representative of all animal management areas, support areas, and major personnel traffic areas.

Sample collection—Environmental samples were collected approximately biweekly throughout the hospital by use of a different commercially available electrostatic dust collection wipe[a] to sample each site. Sampling was performed in the morning on each sample date. Floor samples were collected by use of a commercial sweeper[a] to sample most of the floor surface area in designated sites. The sweeper was disinfected with 70% ethanol between uses and allowed to dry. Hand contact surfaces were sampled with the wipes by use of a gloved hand. After sampling, wipes were sealed into prelabeled sterile bags[b] by use of a gloved hand. Disposable latex gloves were worn when handling wipes, changing gloves between samples. Samples were immediately transported to the laboratory for processing.

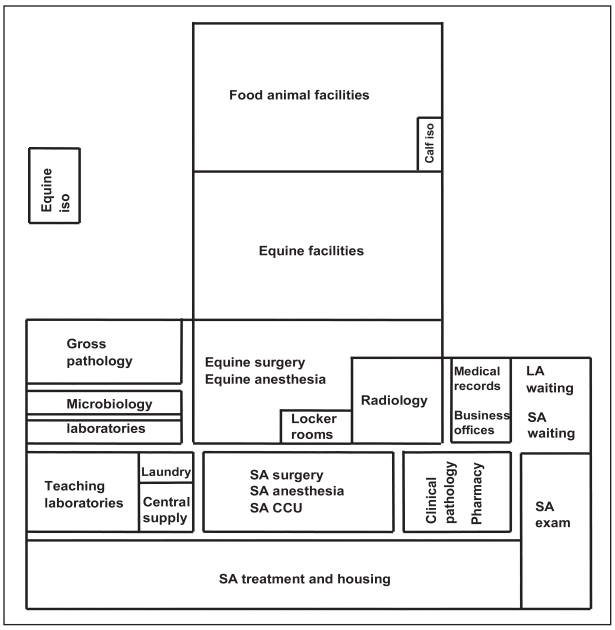

Figure 1—Schematic diagram of the first floor of the James L. Voss Veterinary Teaching Hospital. SA = Small animal. LA = Large animal. Iso = Isolation. CCU = Critical care unit.

Sample processing—Buffered peptone water[c] (90 mL) was added to the bags containing the electrostatic wipes, which were agitated in a paddle blender[d] for 30 seconds and incubated for 24 hours at 35°C. Enriched samples (1 mL) were passed to tetrathionate broth containing iodine[e] (9 mL) and incubated for 24 hours at 35°C. Samples were vortexed and passed to Rappaport-Vassiliadis R10 broth[f] (0.1 mL to 10 mL). After incubating 24 hours at 35°C, tubes were vortexed, and samples of both Rappaport-Vassiliadis R10 and tetrathionate broth were streaked for isolation on xylose-lysine-tergitrol[g] agar plates and incubated for 24 hours at 35°C.

Control samples—Several positive and negative control samples were processed with each replicate batch of environmental samples that was cultured. Electrostatic wipes that had been inoculated with cultured *S enterica* were used as positive control samples. New, unused electrostatic wipes were used as negative control samples. Control samples were processed by use of the same methods for culture and isolate identification as environmental samples.

Colony selection and biochemical confirmation—Three isolated colonies from the XLT-4 agar plates were selected on the basis of colony morphology typical of *Salmonella* spp (discrete, round, red colonies with a black center after 24 hours of incubation at 35°C). Each isolate was inoculated in triple sugar iron agar,[h] lysine iron agar,[i] and urea agar[j] slants and incubated at 35°C for 24 hours for biochemical confirmation.

Phenotype characterization—A phenotype was assigned to each environmental isolate on the basis of serogroup, serotype, and antimicrobial susceptibility. Biochemically confirmed *Salmonella* spp isolates were serogrouped by use of commercial polyvalent O antisera (groups A through I and Vi)[k] and individual O grouping antisera.[l] Samples that did not agglutinate were further characterized by use of a commercial identification system.[m] Colonies with positive results for agglutination with polyvalent antisera or identified as *Salmonella* spp by the commercial system were serotyped by the **National Veterinary Services Laboratory (NVSL)**, Ames, Iowa. Isolates were evaluated by characterizing antimicrobial susceptibility to a standardized panel of antimicrobial drugs (amikacin, amoxicillin-clavulanate, ampicillin, ceftiofur, cephalothin, chloramphenicol, enrofloxacin, gentamicin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole) by use of the Kirby-Bauer disk diffusion method. Assays were read on a commercial reading system.[n] Assays and interpretations were conducted in compliance with the NCCLS M31-A document.

Comparison of environmental isolates and those recovered from animal samples—*Salmonella enterica* isolates obtained from environmental samples were compared with isolates cultured from specimens submitted to the VDL from hospitalized animals and outside sources during the month prior to each sampling date. A majority of these specimens were fecal samples obtained from hospitalized large animal patients as part of an active surveillance program for detecting shedding of *Salmonella* spp; fecal samples were collected from all large animal inpatients on admission and then on Monday, Wednesday, and Friday throughout their hospitalization. Serotype and antimicrobial susceptibility patterns were used in combination to categorize all environmental isolates into 10 unique phenotypes (arbitrarily designated A through J). These unique pheno-

types were then compared with the laboratory database for *S enterica* isolates recovered from specimens submitted to the VDL for any matches with these unique phenotypes. Any matching isolates were further evaluated by use of temporal and spatial information recorded for animals in the hospital database (eg, location of stable or kennel, admission, and discharge dates).

Table 1—Results of bacteriologic culture of environmental samples for *Salmonella enterica* from various sites in the James L. Voss Veterinary Teaching Hospital (JLV-VTH) from June 9 to August 19, 2002.

	Culture results*						
Site	**Jun 9**	**Jun 23**	**Jul 12**	**Jul 19**	**Jul 21**	**Aug 6**	**Aug 19**
Laundry	(–)	(–)	(–)	(–)	(–)	(–)	C
Gross Path Hall	(–)	D	(–)	D	E	H	(–)
Path Classroom	(–)	(–)	(–)	E	D	(–)	A
Micro Hall	(–)	(–)	(–)	J	D	(–)	(–)
Virology Lab A	(–)	(–)	I	(–)	(–)	(–)	(–)
Virology Lab B	(–)	(–)	(–)	(–)	D	(–)	(–)
Bact Lab A	(–)	(–)	D	D	D	(–)	(–)
Bact Lab B	(–)	(–)	(–)	D	D	(–)	(–)
Bact Lab C	(–)	D	D	I	D	(–)	(–)
Bact Lab D	(–)	(–)	(–)	E	D	(–)	(–)
Media Prep Rm	(–)	(–)	(–)	(–)	(–)	G	(–)
Diag Lab –h	(–)	(–)	D	I	I	(–)	(–)
Eq Rec Rm A	(–)	(–)	F	(–)	A	A	(–)
Eq Office	(–)	(–)	(–)	(–)	(–)	A	(–)
Eq –h	(–)	(–)	(–)	(–)	(–)	A	A
Eq Iso Office	D	D	D	(–)	(–)	(–)	(–)
Eq Iso Office –h	(–)	(–)	(–)	(–)	(–)	D	(–)
FA Repro Lab	(–)	(–)	(–)	(–)	A	A	(–)
FA Locker Rm A	(–)	(–)	(–)	(–)	(–)	(–)	A
FA Locker Rm B	(–)	(–)	(–)	(–)	(–)	(–)	A
FA Office	(–)	(–)	(–)	(–)	(–)	(–)	A
FA Office –h	NS	NS	NS	NS	NS	A	A
FA Rec Rm	(–)	(–)	(–)	(–)	(–)	A	A
FA Rec Rm –h	NS	NS	NS	NS	NS	A	(–)
FA –h	(–)	(–)	(–)	(–)	A	NS	NS
Calf Iso A	NS	NS	NS	NS	NS	B	A
Calf Iso B	NS	NS	NS	NS	NS	(–)	A
Calf Iso A –h	NS	NS	NS	NS	NS	NS	A
Calf Iso B –h	NS	NS	NS	NS	NS	A	A
Total positive	**1**	**3**	**6**	**8**	**12**	**12**	**12**
Total samples	**63**	**63**	**63**	**63**	**63**	**68**	**69**

A total of 452 samples were collected from 69 sites; *S enterica* was recovered from 54 samples. Only results for sites from which *S enterica* was recovered are depicted.

*Letters refer to phenotype categories for isolates in Table 2.

(–) = Negative culture results. NS = Not sampled. Path = Pathology. Micro Hall = Microbiology laboratory hallway. Bact Lab = Bacteriology laboratory. Diag Lab = Diagnostic laboratory. h = Samples from hand contact surfaces, others are floor samples. Eq = Equine. Iso = Isolation. FA = Food animal. Rec = Records.

Results

Hospital population and laboratory submissions— There were 4,257 small animal admissions to the JLV-VTH, including 3,252 (76.3%) dogs and 818 (19.2%) cats, and 913 large animal admissions to the JLV-VTH, including 711 (77.8%) horses and 116 (12.7%) cattle. Of those admissions, 947 were small animals with at least 1 night of hospitalization, including 654 (69.0%) dogs and 235 (24.8%) cats, and 415 were large animals with at least 1 night of hospitalization, including 290 (69.8%) horses and 67 (16.1%) cattle. During this same time period, there were 4,787 submissions to the bacteriology section of the VDL. Of those, 1,242 (25.9%) were submitted for aerobic bacteriologic culture of feces. Routine patient surveillance performed during the study period identified higher than average rates of shedding among large animal patients at the JLV-VTH.

Samples—Sample sets were obtained on 7 occasions (June 9, June 23, July 12, July 19, July 21, August 6, and August 19, 2002). A total of 452 environmental samples were obtained, and *S enterica* was recovered from 54 (11.9%). Five serotypes were recovered. The most common serotype recovered was *S* Newport (n = 23), followed by serotypes Agona (20), Havana (5), and Typhimurium (5). The serotype of 1 sample was not identified (NVSL designation, rough 0:e,h-1,2). Within these 5 serotypes, there were 10 distinguishable phenotypes (**Table 1**). The most common phenotypes were A (n = 23) and D (19). Samples obtained from 16 areas had positive results for *S enterica* on > 1 occasion during the study period, primarily in the large animal hospital and VDL. The same phenotype of *S enterica* was recovered on > 1 occasion from 12 of these 16 areas, and the same phenotypes were also isolated from adjacent areas on multiple occasions. Multiple phenotypes were recovered at different times from 8 of these 16 areas, but most isolates differed only by susceptibility pattern, not serotype. Of the *S enterica* isolates recovered from environmental samples, 34 were recovered from samples obtained within the hospital and 20 were recovered from samples obtained in the VDL (**Table 2**). *Salmonella enterica* was

Table 2—Phenotypic characterization of 54 *S enterica* isolates identified on bacteriologic culture of environmental samples obtained from the JLV-VTH from June 9 to August 19, 2002.

Phenotype	Serotype	Amik	Amox	Amp	Cef	Ceph	Chlor	Enro	Gent	Strep	Sulfa	Tetra	TMS	No. of environmental isolates
A	Newport	S	R	R	R	R	R	S	S	R	R	R	S	22
B	Untypable	S	R	R	R	R	R	S	S	R	R	R	S	1
C	Newport	S	R	R	R	R	S	S	S	R	R	R	S	1
D	Agona	S	S	S	S	S	S	S	S	R	R	R	S	19
E	Havana	S	S	S	S	S	S	S	S	S	R	S	S	3
F	Havana	S	R	R	R	R	R	I	S	R	R	R	S	1
G	Typhimurium	S	I	R	S	S	R	S	S	R	R	R	S	1
H	Agona	S	S	S	S	S	S	S	S	I	R	R	S	1
I	Typhimurium	S	S	S	S	S	S	S	S	I	I	I	S	4
J	Havana	S	S	S	S	S	S	S	S	S	I	S	S	1

Amik = Amikacin. Amox = Amoxicillin-clavulanate. Amp = Ampicillin. Cef = Ceftiofur. Ceph = Cephalothin. Chlor = Chloramphenicol. Enro = Enrofloxacin. Gent = Gentamicin. Strep = Streptomycin. Sulfa = Sulfomethoxazole. Tetra = Tetracycline. TMS = Trimethoprim-sulfamethoxazole. S = Susceptible. I = Intermediate. R = Resistant. Untypable = National Veterinary Services Laboratory designation, rough 0:e,h-1,2.

Table 3—Comparison of *S enterica* isolates identified on bacteriologic culture of environmental samples obtained in the JLV-VTH and *S enterica* isolates cultured from specimens submitted to a veterinary diagnostic laboratory from hospitalized animals and outside sources during the month prior to each sampling date from June 9 to August 19, 2002.

					Isolates obtained from animal submissions during the month prior to environmental sampling date		
			No. of environmental isolates				
Sample dates	Environmental phenotypes	Serotype	Hospital	Laboratory	No. of isolates from hospitalized animals (all serotypes)	Isolate from hospitalized animal matched environmental phenotype	Isolate from outside submission matched environmental phenotype
Jun 9	D	Agona	1	none	21	Yes	No
Jun 23	D	Agona	2	1	34	Yes	No
Jul 12	D	Agona	1	3		Yes	No
	F	Havana	1	none	32	No	No
	I	Typhimurium	none	2		No	No
Jul 19	D	Agona	1	2		Yes	No
	E	Havana	1	1	31	No	No
	J	Typhimurium	none	2		Yes	Yes
	I	Agona	none	1		No	No
Jul 21	A	Newport	3	none		No	No
	D	Agona	1	6	24	Yes	No
	E	Havana	1	none		No	No
	I	Typhimurium	none	1		No	Yes
Aug 6	A	Newport	9	none		Yes	Yes
	B	Untypable	1	none		No	No
	D	Agona	1	none	39	Yes	No
	G	Typhimurium	none	1		No	No
	H	Agona	1	none		No	No
Aug 19	A	Newport	11	none	48	Yes	Yes
	C	Newport	1	none		No	No

NA = Not applicable.

recovered from all of the positive control samples and none of the negative control samples.

Comparison of environmental and diagnostic laboratory samples—Phenotypes A through J were compared with information regarding isolates recovered from hospitalized animals and outside laboratory submissions to the VDL for the month prior to each sampling date. On the basis of comparison of unique phenotypes, 41 of 54 (75.9%) environmental isolates collected with electrostatic wipes could be matched to phenotypes of isolates obtained from hospitalized animals or other specimens submitted to the VDL during the month prior to collection of the environmental samples (**Table 3**).

Discussion

Results of this study indicated that environments in veterinary hospitals can be frequently contaminated with *S enterica* near where infected animals are managed and specimens containing *S enterica* are processed for culture in a VDL. However, biosecurity procedures used in the JLV-VTH and VDL were apparently effective in limiting more extensive dissemination; *Salmonella* bacteria were recovered from the first floor of the JLV-VTH and VDL near where animals known to be shedding *S enterica* were managed and where diagnostic specimens containing *S enterica* were handled but were not recovered from environmental samples of more removed high-traffic areas such as the hallways on the first floor of the JLV-VTH near the small animal care areas or hallways on the second floor of the building near the cafeteria, library, bookstore, classrooms, and offices. The JLV-VTH uses rigorous biosecurity procedures, which include routine cleaning and disinfection and routine surveillance to ensure that procedures are being closely followed. Hygiene standards are high, as would be expected at any tertiary referral center. However, results indicated that environmental contamination can occur frequently at veterinary hospitals and diagnostic laboratories, even under optimal conditions.

Most of the environmental isolates could be phenotypically matched to isolates obtained from hospitalized animals or specimens submitted to the VDL from other sources, suggesting that most, if not all, environmental contamination in the JLV-VTH and VDL was disseminated from these sources. The longitudinal nature of this study helped to identify areas that might have been intermittently contaminated; however, samples were only obtained approximately biweekly, which would provide opportunities to miss identification of transient contamination. Environmental contamination was more common in areas used in the care of large animals, compared with areas used in the care of small animals. This would be expected considering differences in the prevalence of shedding among large and small animals.[7,8,o] However, continued environmental surveillance of these areas at the JLV-VTH beyond the study period has indicated that *Salmonella* contamination does occasionally occur in the small animal hospital with some regularity, especially in high-traffic areas such as reception areas.

Challenges to the biosecurity precautions at the JLV-

VTH and the VDL were reasonably rigorous during the study period. The study was performed in 2002 during warm summer months, which typically are associated with high rates of shedding of *S enterica* in animals.[9-11] In addition, the caseload at the JLV-VTH is typically highest during the summer months, especially in the large animal hospital. Routine patient surveillance performed during the study period identified higher than average rates of shedding among large animal patients at the JLV-VTH. In addition, infection and shedding of a multidrug resistant strain of *S* Newport, which had been associated with epidemics on animal premises throughout the country, was detected on the premises of several clients who routinely bring cattle to the JLV-VTH for treatment. Both of these factors probably contributed to apparent nosocomial infections among a small number of large animal patients during the study period. Combined, these factors indicate that the challenge to our biosecurity and cleaning protocols was rigorous, which would tend to promote environmental contamination at the JLV-VTH during the study.

Although detection of any contamination with *Salmonella* spp in the hospital environment is notable, repeated detection in the same areas is of particular concern. Isolates of the same phenotype were recovered from a few areas during this study, which may have been attributable to repeated contamination from a single source, such as an infected animal or ineffective cleaning and disinfection. Results of bacteriologic culture of environmental samples obtained from other areas were positive on > 1 occasion, but *Salmonella* isolates had different phenotypes, suggesting that environmental contamination may have been attributable to different sources.

The sampling and culture technique used in this study was originally developed by other investigators at Washington State University.[p] Although culture techniques for *Salmonella* spp are generally considered insensitive, direct comparison to other culture techniques used at the JLV-VTH and VDL suggests that the technique used in this study is more sensitive than others we have previously used for environmental cultures.[5] A culture technique that is highly sensitive is useful for this type of investigation, but the accompanying use of enrichment procedures does not permit quantitative evaluation of environmental contamination. As such, detection of *S enterica* in this study did not necessarily equate with exposure to doses sufficient for infection of veterinary patients or humans. Another sensitive detection technique that has been described for detection of *Salmonella* spp is the polymerase chain reaction assay.[6,12,13] Polymerase chain reaction assays are not routinely used at the JLV-VTH to detect *Salmonella* spp in environmental samples or fecal samples obtained from hospitalized animals because we believe that phenotypic and genotypic characterization of isolates is essential for appropriate interpretation of surveillance data, which generally is not possible when using polymerase chain reaction assays. Further, it is theoretically possible to detect DNA from bacteria killed through cleaning and disinfection, and these results would not be distinguishable from results of assays detecting live bacteria.

Results of the study reported here and subsequent use of this environmental sampling and culture technique have provided useful monitoring information for the biosecurity program at the JLV-VTH. Regular environmental monitoring by use of these procedures has been incorporated into the routine surveillance procedures for the JLV-VTH. Similar use may be beneficial as part of surveillance activities for other veterinary and animal-rearing facilities.

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[a]Swiffer, Proctor & Gamble, Cincinnati, Ohio.
[b]Whirl-pak, NASCO, Modesto, Calif.
[c]Buffered peptone water, Becton Dickinson and Co, Cockeysville, Md.
[d]Bag Mixer, Interscience, St Nom La Breteche, France.
[e]Tetrathionate, Becton Dickinson and Co, Sparks, Md.
[f]Rappaport, Remel, Lenexa, Kan.
[g]XLT-4, Hardy Diagnostics, Santa Maria, Calif.
[h]Triple sugar iron agar slants, Becton Dickinson and Co, Sparks, Md.
[i]Lysine iron agar slants, Becton Dickinson and Co, Sparks, Md.
[j]Urea agar slants, Becton Dickinson and Co, Sparks, Md.
[k]Polyvalent antisera, Becton Dickinson and Co, Sparks, Md.
[l]Grouping anitsera, Becton Dickinson and Co, Sparks, Md.
[m]Micro-ID, Remel, Lenexa, Kan.
[n]Biomic, Giles Scientific Inc, Santa Barbara, Calif.
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