Supplement to *Risk Factors for Severe Cutaneous Anthrax in a Retrospective Case Series and Use of a Clinical Algorithm to Identify Likely Meningitis and Evaluate Treatment Outcomes, Kyrgyz Republic, 2005-2012*

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Supplementary Text.   Methods for Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing is routinely performed on cultures positive for *Bacillus anthracis* from patients in Kyrgyz hospitals. The sensitivity of the pathogen to antimicrobial drugs was determined by the agar-based disk diffusion method using colonies typically from 16-18 hours agar cultures. A suspension of microbes was prepared from the grown colonies in a 0.9% sodium chloride solution. This suspension in a volume of 0.3 ml was then applied to the surface of the Mueller-Hinton or AGV nutrient medium and spread evenly with a spatula. Cups were incubated for 15 minutes at room temperature to absorb the liquid. The disks were placed with tweezers on the surface of the seeded nutrient medium equally distanced from one another, retreating about 2 cm from the wall of the dish (no more than 4 disks). They were kept for 15 minutes at room temperature, and then, were turned upside down and incubated at (36 ± 1) °C for 18-20 hours. The diameter of the zones of growth inhibition around the discs was measured, including the diameter of the disc itself, to the nearest 1 mm. Small colonies within the growth retardation zone were not counted. For blurry zone edges or for zones with double contours, the zone diameter was measured along the most distinct boundary.

Supplementary Table 1. Sensitivity, Specificity, and Likelihood Ratios of Clinical Algorithms for the Identification of Meningitis in Adults with Systemic Cutaneous Anthrax Based on a Dataset from the English-Language Literature, 1880-2018

	TP	FN	FP	TN	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	LR+ (95% CI)	LR- (95% CI)	Proportion of Unclassified Cases
Derivation Sample[a]									
1. Altered mental status	29	10	5	123	74 (61-88)	96 (93-99)	19.04 7.91-45.84)	0.27 (.16-.46)	0%
2a. At least two of the following: FV/38, N/V, SHA, ABP, AMS, MPE, RR≥30	33	6	16	82	85 (73-96)	84 (76-91)	5.18 (3.25-8.27)	0.18 (.09-.39)	17%
2b. At least three of the following: FV/38, N/V, SHA, ABP, AMS, MPE, RR≥30	17	16	4	92	52 (34-69)	96 (92-100)	12.36 (4.48-34.11)	0.51 (.36-.72)	
3a. At least two of the following: FV/38, N/V, SHA, AMS, MPE	33	6	14	113	85 (73-96)	89 (84-94)	7.68 (4.60-12.81)	0.17 (.08-.36)	18%
3b. At least three of the following: FV/38, N/V, SHA, AMS, MPE	14	25	3	125	36 (21-51)	98 (95-100)	15.32 (4.64-50.56)	0.66 (.52-.83)	
4a. At least two of the following: FV/38, N/V, SHA, AMS, MPE, RR≥30	33	6	16	82	85 (73-96)	84 (76-91)	5.18 (3.25-8.27)	0.18 (.09-.39)	18%
4b. At least three of the following: FV/38, N/V, SHA, AMS, MPE, RR≥30	16	17	3	93	48 (31-66)	97 (93-100)	15.52 (4.83-49.89)	0.53 (.38-.74)	
5a. At least one of the following: N/V, SHA, AMS, MPE	37	2	26	102	95 (88-100)	80 (73-87)	4.67 (3.29-6.63)	0.06 (.02-.25)	24%
5b. At least two of the following: N/V, SHA, AMS, MPE	20	19	3	125	51 (36-67)	98 (95-100)	21.88 (6.86-69.75)	0.50 (.36-.69)	
6a. At least one of the following: SHA, AMS, MPE	37	2	21	107	95 (88-100)	84 (77-90)	5.78 (3.88-8.61)	0.06 (.02-.24)	25%
6b. At least two of the following: SHA, AMS, MPE	14	25	3	125	36 (21-51)	98 (95-100)	15.32 (4.64-50.56)	0.66 (.52-.83)	
7a. At least one of the following: N/V, AMS, MPE	35	4	26	102	90 (80-99)	80 (73-87)	4.42 (3.09-6.33)	0.13 (.05-.33)	26%
7b. At least two of the following: N/V, AMS, MPE	17	22	0	128	44 (28-59)	100 (100-100)	112.88 (6.94-1835.35)[b]	0.56 (.43-.74)	
Validation sample[a]									
3a. At least two of the following: FV/38, N/V, SHA, AMS, MPE	9	4	5	23	69 (44-94)	82 (68-96)	3.88 (1.62-9.28)	0.37 (.16-.86)	20%
3b. At least three of the following: FV/38, N/V, SHA, AMS, MPE	4	9	2	26	31 (6-56)	93 (83-100)	4.31 (0.90-20.60)	0.75 (.51-1.09)	
Sensitivity analysis[a]									
3a. At least two of the following: FV/38, N/V, SHA, AMS, MPE	20	9	18	119	69 (52-86)	87 (81-93)	5.25 (3.20-8.61)	0.36 (.21-.62)	14%
3b. At least three of the following: FV/38, N/V, SHA, AMS, MPE	10	19	5	133	34 (17-52)	96 (93-99)	9.52 (3.51-25.77)	0.68 (.52-.89)	

Abbreviations: ABP, abdominal pain; AMS, altered mental status; CI, confidence interval; FN, false negative; FP, false positive; FV/38, fever (symptom) or documented temperature >38°C (adults) >38.5°C (children); LR, likelihood ratio; MPE, malignant pustule edema; N/V, nausea or vomiting; RR, respiratory rate; SHA, severe headache; TN, true negative; TP, true positive.

[a] All analyses exclude suspected meningitis cases. Derivation sample based on an 80% random sample of adults (aged ≥18 years) with systemic cutaneous anthrax, validation sample is the remaining 20% of adults with systemic cutaneous anthrax. Sensitivity analysis was based on the full adult systemic cutaneous anthrax dataset excluding those that died on day 1 of hospitalization.

[b] Continuity correction factor of 0.5 added to TP, FN, FP, and TN.

Supplementary Table 2.   Antimicrobial Resistance Testing by Severity and Meningitis Status Among Cutaneous Anthrax Patients with a Positive Culture, Kyrgyz Republic, 2005-2012

Characteristics	Overall (N=124) n (%)	Mild (N=90) n (%)	Moderate (N=23) n (%)	Severe (N=11) n (%)	Likely Nonmeningitis[a] (N=39) n (%)	Likely Meningitis[a] (N=22) n (%)
Amoxicillin						
Resistant	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate	9 (7.3)	7 (7.8)	1 (4.4)	1 (9.1)	3 (7.7)	2 (9.1)
Susceptible	115 (92.7)	83 (92.2)	22 (95.7)	10 (90.9)	36 (92.3)	20 (90.9)
Ampicillin						
Resistant	69 (55.7)	57 (63.3)	8 (34.8)	4 (36.4)	27 (69.2)	9 (40.9)
Intermediate	25 (20.2)	11 (12.2)	11 (47.8)	3 (27.3)	4 (10.3)	5 (22.7)
Susceptible	30 (24.2)	22 (24.4)	4 (17.4)	4 (36.4)	8 (20.5)	8 (36.4)
Cefalexin						
Resistant	2 (1.6)	2 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate	6 (4.8)	5 (5.6)	0 (0)	1 (9.1)	2 (5.1)	1 (4.5)
Susceptible	116 (93.6)	83 (92.2)	23 (100)	10 (90.9)	37 (94.9)	21 (95.5)
Chloramphenicol						
Resistant	24 (19.4)	16 (17.8)	6 (26.1)	2 (18.2)	6 (15.4)	7 (31.8)
Intermediate	22 (17.8)	13 (14.4)	7 (30.4)	2 (18.2)	3 (7.7)	7 (31.8)
Susceptible	78 (62.9)	61 (67.8)	10 (43.5)	7 (63.6)	30 (76.9)	8 (36.4)
Ciprofloxacin						
Resistant	2 (1.6)	2 (2.2)	0 (0)	0 (0)	1 (2.6)	0 (0)
Intermediate	7 (5.7)	6 (6.7)	1 (4.4)	0 (0)	2 (5.1)	1 (4.5)
Susceptible	115 (92.7)	82 (91.1)	22 (95.7)	11 (100)	36 (92.3)	21 (95.5)
Doxycycline						
Resistant	4 (3.2)	4 (4.4)	0 (0)	0 (0)	2 (5.1)	0 (0)
Intermediate	10 (8.1)	7 (7.8)	2 (8.7)	1 (9.1)	2 (5.1)	1 (4.5)
Susceptible	110 (88.7)	79 (87.8)	21 (91.3)	10 (90.9)	35 (89.7)	21 (95.5)
Gentamicin						
Resistant	26 (21.0)	14 (15.6)	8 (34.8)	4 (36.4)	7 (18.0)	5 (22.7)
Intermediate	37 (30.0)	27 (30.0)	8 (34.8)	2 (18.2)	5 (12.8)	10 (45.5)
Susceptible	61 (49.2)	49 (54.4)	7 (30.4)	5 (45.5)	27 (69.2)	7 (31.8)
Ofloxacin						
Resistant	1 (0.8)	1 (1.1)	0 (0)	0 (0)	1 (2.6)	0 (0)
Intermediate	11 (8.9)	2 (2.2)	6 (26.1)	3 (27.3)	0 (0)	4 (18.2)
Susceptible	112 (90.3)	87 (96.7)	17 (73.9)	8 (72.7)	38 (97.4)	18 (81.8)
Penicillin						
Resistant	14 (11.3)	10 (11.1)	3 (13.0)	1 (9.1)	5 (12.8)	3 (13.6)
Intermediate	6 (4.8)	4 (4.4)	2 (8.7)	0 (0)	4 (10.3)	0 (0)
Susceptible	104 (83.9)	76 (84.4)	18 (78.3)	10 (90.9)	30 (76.9)	19 (86.4)
Rifampicin						
Resistant	1 (0.8)	1 (1.1)	0 (0)	0 (0)	1 (2.6)	0 (0)
Intermediate	10 (8.1)	10 (11.1)	0 (0)	0 (0)	2 (5.1)	2 (9.1)
Susceptible	113 (91.1)	79 (87.8)	23 (100)	11 (100)	36 (92.3)	20 (90.9)
Streptomycin						
Resistant	16 (12.9)	13 (14.4)	3 (13.0)	0 (0)	4 (10.3)	2 (9.1)
Intermediate	35 (28.2)	12 (13.3)	16 (69.6)	7 (63.6)	4 (10.3)	10 (45.5)
Susceptible	73 (58.9)	65 (72.2)	4 (17.4)	4 (36.4)	31 (79.5)	10 (45.5)

Characteristics	Overall (N=124) n (%)	Mild (N=90) n (%)	Moderate (N=23) n (%)	Severe (N=11) n (%)	Likely Nonmeningitis[a] (N=39) n (%)	Likely Meningitis[a] (N=22) n (%)
Trimethoprim / Sulfamethoxazole						
Resistant	32 (25.8)	20 (22.2)	8 (34.8)	4 (36.4)	11 (28.2)	6 (27.3)
Intermediate	54 (43.6)	43 (47.8)	10 (43.5)	1 (9.1)	14 (35.9)	9 (40.9)
Susceptible	38 (30.7)	27 (30.0)	5 (21.7)	6 (54.6)	14 (35.9)	7 (31.8)

[a] The designations "likely nonmeningitis" and "likely meningitis" were applied based on a clinical algorithm that categorized patients based on signs and symptoms. Patients meeting ≥3 criteria were designated as "likely meningitis" while patients meeting ≤1 were designated "likely nonmeningitis."

Supplementary Table 3.   Antimicrobial Use in the Year Prior to Current Illness Hospitalization by Antimicrobial and Illness Type Among 230 Cutaneous Anthrax Patients, Kyrgyz Republic, 2005-2012

Illness Type	Ampicillin		Chloramphenicol		Gentamicin		Co-trimoxazole	
	n	(%)	n	(%)	n	(%)	n	(%)
Infected skin wound	52	(22.6)	67	(29.1)	43	(18.7)	18	(7.8)
Erysipelas	29	(12.6)	26	(11.3)	19	(8.3)	13	(5.7)
Fever, unknown origin	35	(15.2)	71	(30.9)	81	(35.2)	57	(24.8)
Sore throat	68	(29.6)	36	(15.7)	46	(20.0)	56	(24.3)
Cough	46	(20.0)	25	(10.9)	36	(15.7)	62	(27.0)
Overall	230	(100.0)	225	(97.8)	225	(97.8)	206	(89.6)

Supplementary Table 4.   Unadjusted and Age-Adjusted Odds Ratios for Patient Chronic Conditions and Social Histories by Likely Meningitis Status Among Cutaneous Anthrax Patients, Kyrgyz Republic, 2005-2012

Characteristics	Likely Nonmeningitis[a] (N=89) n (%)	Likely Meningitis[a] (N=37) n (%)	Unadjusted Odds Ratio (95% CI)	Age-Adjusted Odds Ratio (95% CI)
Chronic conditions				
Number of chronic conditions				
0	68 (76.4)	9 (24.3)	Reference	Reference
1	15 (16.9)	12 (32.4)	6.0 (2.2, 16.9)	6.1 (2.2, 17.3)
2	5 (5.6)	6 (16.2)	9.1 (2.3, 35.9)	9.8 (2.4, 40.9)
3	1 (1.1)	10 (27.0)	75.6 (8.6, 661.7)	91.2 (8.8, 947.8)
Social history				
Smoker (male)[b]				
Never	42 (53.8)	5 (15.6)	Reference	Reference
Former	2 (2.6)	7 (21.9)	29.4 (4.7, 182.3)	27.0 (4.0, 180.4)
Current	34 (43.6)	19 (59.4)	4.7 (1.6, 13.9)	4.7 (1.6, 14.0)
Alcohol use (male)[b]				
Never	55 (70.5)	18 (56.3)	Reference	Reference
Former	9 (11.5)	11 (34.4)	3.7 (1.3, 10.5)	3.3 (1.1, 10.1)
Current	14 (17.9)	3 (9.4)	0.7 (.2, 2.5)	0.6 (.2, 2.5)

[a] The designations "likely nonmeningitis" and "likely meningitis" were applied based on a clinical algorithm that categorized patients based on signs and symptoms. Patients meeting ≥3 criteria were designated as "likely meningitis" while patients meeting ≤1 were designated "likely nonmeningitis."

[b] All female patients reported being never smokers and never drinking alcohol, except 1 who reported being a current smoker and 1 who reported being a former drinker.

Supplementary Table 5.   Sensitivity Analysis of Patient Demographics, Presenting Signs and Symptoms, Laboratory Results and Treatments Using an Alternative Classification of Meningitis Status Among Cutaneous Anthrax Patients, Kyrgyz Republic, 2005-2012

Characteristics	Likely Nonmeningitis[a] (n=153) n (%)	Likely Meningitis[a] (n=8) n (%)	Odds Ratio (95% CI)	P Value
Demographics				
Age (years)				
< 18	2 (1.3)	0 (0)	…	…
18-49	130 (85.0)	6 (75.0)	Reference	…
≥ 50	21 (13.7)	2 (25.0)	2.11 (.2, 12.5)	.7
Male	137 (89.5)	6 (75.0)	0.4 (0.06, 1.9)	.2
Chronic conditions				
Obesity	24 (15.7)	5 (62.5)	9.0 (2.0, 40.0)	.004
Diabetes	0 (0)	4 (50.0)	155.2 (24.0, ∞)	<.0001
Hypertension	15 (9.8)	4 (50.0)	9.2 (2.1, 40.6)	.003
COPD	29 (19.0)	1 (12.5)	0.6 (0.07, 5.2)	.7
Number of chronic conditions				
0	108 (70.6)	2 (25.0)	Reference	…
1	26 (17.0)	1 (12.5)	2.1 (0.2, 23.8)	.6
2	14 (9.2)	2 (25.0)	7.7 (1.0, 59.2)	.05
3	5 (3.3)	3 (37.5)	32.4 (4.3, 239.7)	.0007
Social history				
Smoker (male)[b]				
Never	65 (47.5)	0 (0)	Reference	…
Former	10 (7.3)	2 (33.3)	14.1 (1.6, ∞)	.02
Current	62 (45.3)	3 (50.0)	3.9 (0.6, ∞)	.1
Alcohol use (male)[b]				
Never	85 (62.0)	3 (50.0)	Reference	…
Former	31 (22.6)	2 (33.3)	1.8 (0.3, 11.5)	.5
Current	21 (15.3)	1 (16.7)	1.3 (0.1, 13.6)	.8
Illness presentation				
Anthrax severity category				
Mild	124 (81.0)	0 (0)	Reference	…
Moderate	23 (15.0)	6 (75.0)	41.7 (7.6, ∞)	<.0001
Severe	6 (3.9)	2 (25.0)	41.9 (4.8, ∞)	.003
Fever or chills	52 (34.0)	7 (87.5)	13.6 (1.6, 113.4)	.02
Headache	30 (19.6)	8 (100)	43.3 (8.4, ∞)	<.0001
Severe headache	0 (0)	4 (50.0)	155.2 (24.0, ∞)	<.0001
Nausea or vomiting	12 (7.8)	5 (62.5)	18.5 (3.2, 134.0)	.0007
Confusion	0 (0)	2 (25.0)	51.7 (6.0, ∞)	.002
Malignant pustule edema	36 (23.5)	6 (75.0)	9.8 (1.9, 50.4)	.007
Measured fever[c]	21 (13.7)	8 (100)	65.3 (12.5, ∞)	<.0001
Heart rate per minute, mean (SD)	80.9 (5.5)	85.1 (8.9)	1.1 (1.0, 1.3)	.04
Tachycardia[d]	1 (0.7)	1 (12.5)	21.7 (1.2, 384.3)	.04
Respirations per minute, mean (SD)	17.6 (1.6)	18.6 (2.8)	1.4 (1.0, 2.0)	.09
Tachypnea[e]	4 (2.6)	2 (25.0)	12.4 (1.9, 81.6)	.009
Systolic hypertension[f]	33 (21.6)	4 (50.0)	3.6 (.9, 15.3)	.08
Diastolic hypertension[f]	11 (7.2)	3 (37.5)	7.7 (1.6, 36.8)	.01

Characteristics	Likely Nonmeningitis[a] (n=153) n (%)	Likely Meningitis[a] (n=8) n (%)	Odds Ratio (95% CI)	P Value
Laboratory results on admission				
White blood count x10^9/L, mean (SD)	7.0 (2.1)	9.0 (3.3)	1.4 (1.1, 1.9)	.02
Hemoglobin g/L, mean (SD)				
Male	128 (6.1)	132 (3.9)	1.1 (1.0, 1.4)	.1
Female	127 (9.1)	116 (9.9)	0.9 (.7, 1.1)	.2
Hematocrit, mean (SD)				
Male	0.44 (0.03)	0.44 (0.03)	0 (0, ∞)	.4
Female	0.44 (0.04)	0.41 (0.02)	0 (0, ∞)	.3
Platelets x10^9/L, mean (SD)	265 (51)	264 (60)	1.0 (1.0, 1.0)	.9
Lesion culture positive[g]	70 (48.6)	4 (50.0)	1.1 (.3, 4.4)	.9
Lesion Gram stain positive	76 (49.7)	3 (37.5)	0.9 (.2, 4.4)	.9
Blood culture positive[g]	6 (4.1)	3 (37.5)	14.0 (2.7, 72.8)	.002
Positive culture or Gram stain[g]	133 (86.9)	7 (87.5)	1.1 (.1, 9.0)	1.0
Positive culture, Gram stain, or anthraxin test[g]	141 (92.2)	7 (87.5)	0.6 (.1, 5.2)	.6
Treatment and Outcome				
Antimicrobial treatment combinations				
Monotherapy alone	139 (90.9)	5 (62.5)	Reference	…
Sequential monotherapy	8 (5.2)	0 (0)	2.7 (0, 16.3)	.8
Dual therapy alone	0 (0)	1 (12.5)	23.2 (1.2, ∞)	.04
Dual therapy followed by monotherapy	6 (3.9)	2 (25.0)	9.0 (.7, 71.6)	.09
Triple therapy	0 (0)	0 (0)	…	…
Fatal	0 (0)	0 (0)	…	…
Length of hospital stay in days, mean (SD)	17.1 (3.5)	21.1 (6.4)	1.2 (1.1, 1.4)	.008

[a] The designations "likely nonmeningitis" and "likely meningitis" were applied based on a clinical algorithm that categorized patients based on signs and symptoms. Patients meeting ≥3 criteria were designated as "likely meningitis" while patients meeting ≤1 were designated "likely nonmeningitis."

[b] All female patients reported being never smokers and never drinking alcohol except one who reported being a current smoker and one who reported being a former drinker.

[c] Measured fever: recorded temperature at admission >38°C for patients ≥18 years of age or >38.5°C for patients 13-17 years of age.

[d] Tachycardia: recorded heart rate at admission of ≥100 beats per minute for patients ≥18 years of age or >110 beats per minute for patients 13 to 17 years of age.

[e] Tachypnea: recorded respiratory rate at admission of >20 breaths per minute for patients ≥18 years of age or >14 breaths per minute for patients 13 to 17 years of age.

[f] Systolic and diastolic hypertension: recorded blood pressure at admission of systolic ≥130 mmHg and diastolic ≥90 mmHg, respectively.

[g] There were 6 patients with unspecified blood culture results, 7 patients with unspecified lesion culture results, and 1 patient with unspecified Anthraxin results.