Foundational Training for the Entry-level Clinical Microbiologist Part Two: CLSI M35-A2 Document for Same-day Identification of Bacteria and Yeast

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The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

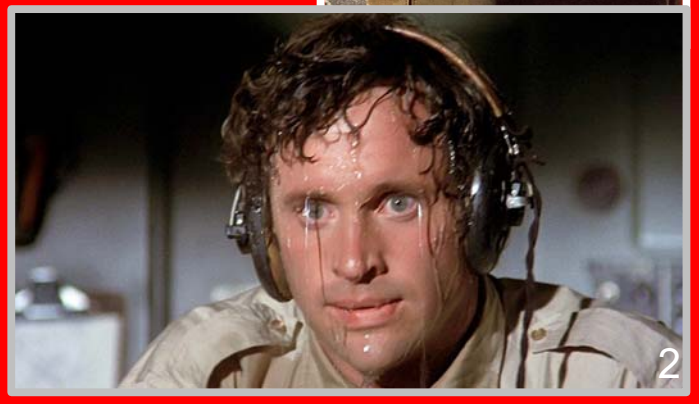
WHY WE'RE HERE

I. Erin Bowles

II. Sean McGee Hicks

III. Provide foundational training in entry-level clinical microbiology laboratory skills

IV. Extrapolate these skills toward understanding of CLSI M35-A2



OBJECTIVES

1. Apply basic bench-level skills, along with spot and rapid identification reagents, to the abbreviated identification of commonly-encountered patient isolates;

2. Recognize the utility of the CLSI M35-A2 guideline as an adjunct to routine operations in the clinical microbiology laboratory; and,

3. Recognize important limitations within the CLSI M35-A2 guideline for certain organism groupings

Life Without MALDI

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Introduction to CLSI M35-A2

IMPETUS

- We've been doing this for years (odor, spot testing, *et cetera*); CLSI sought to standardize the practice
- Cost savings, patient benefits

Study	Turnaround Time *P*	Length of Stay *P* [4]	Mortality Index *P* [4]	Average Cost *P* [4]	Notes
Doern *et al.*[1]	< 0.0005	NS[5]	< 0.02	0.01	Rapid MicroScan product
Barenfanger *et al.*[2]	0.001[4]	0.006	0.45	0.04	VITEK product
Kerremans *et al.*[3]	< 0.0001	ND[6]	0.21	ND	VITEK products

[1]J Clin Microbiol 32:1757-1762; 1994
[2]J Clin Microbiol 37:1415-1418; 1999
[3]J Antimicrob Chemother 61:428-435; 2008

WARNINGS

- "Isolates to be tested should match the criteria required for proper identification."

  CLSI M35-A2; 2008

- Sniffing/wafting <u>can</u> be dangerous

  Once mold colony is ruled out, opening of plates from non-invasive sources (i.e., urine, sputum) is common & relatively safe

  P. aeruginosa, *H. influenzae*, *Eikenella*, *S. anginosus* group odor can be detected by opened plates "not sniffed purposely"

SOME FINE PRINT

- Isolates conforming to reactions described in M35-A2 identify organism with >95% accuracy; identification can be reported without qualification
- "Confirmation by additional procedures is unnecessary for many of the species described in this document."

CLSI M35-A2; 2008

- Lack of a positive result does not rule out the identification of an isolate; just signifies need for additional testing

STOCK UP

Bile solubility reagent
Rapid spot CAMP
H_2O_2 (aerobes)
H_2O_2 (anaerobes)
H_2O_2 (*N. gonorrhoeae*)
Germ tube
Rapid hippurate hydrolysis
Spot indole

Indoxyl acetate
MUG test
Spot oxidase
δ-aminolevulinic acid
Rapid trehalose
Urea/phenylalanine deaminase
PYR, LAP
Latex agglutination (*S. aureus*)

CATALASE

- Touch center of isolated colony with stick

  Transfer to clean glass slide
  Place drop of hydrogen peroxide onto cell paste

- “Immediate” bubbling (< 20 seconds) is positive
- No non-platinum loops; don’t grab blood agar
- If slow-growing isolate on blood agar (no growth MacConkey) or small GNR, perform test in biological safety cabinet

SPOT INDOLE

- 5% (w/v) *p*-dimethylaminobenzaldehyde
  1% paradimethylaminocinnamaldehyde in HCl (N)
- Moisten piece of filter paper with reagent

  Rub portion of colony from blood agar onto paper
  Growth medium must contain sufficient tryptophan
- Pigment formation (< 20 seconds) is positive
- Cannot use media containing dyes
  Cannot use Mueller-Hinton or high-glucose agar
  Detectable indole diffuses to adjacent colonies
  within 5 mm (false-positive results)

SPOT OXIDASE

- 1% tetramethyl-*p*-phenylenediamine dihydrochloride
- Moisten piece of filter paper with reagent

  Rub portion of colony from blood agar onto paper
  Use wooden stick or platinum bacteriological loop

- Blue/purple pigment (< 10 seconds) is positive
- Cannot use MacConkey or other purple agar
  Nickel-based alloy wires containing chromium or iron may yield false-positive results upon organism transfer

M35-A2 Rapid Identification

Campylobacter jejuni/coli

- Presumptive identification

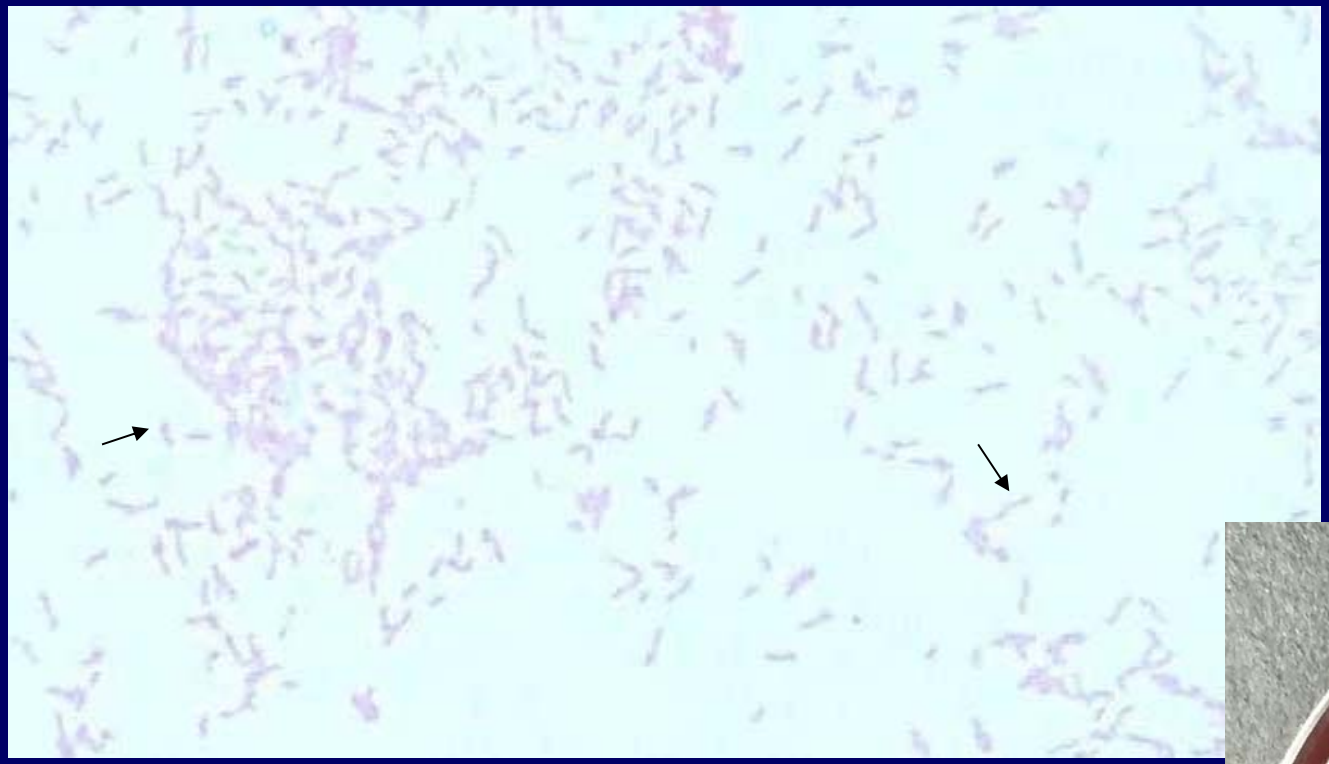
  Gram-negative bacilli (gull wing) Oxidase-positive
  Darting motility Catalase-positive

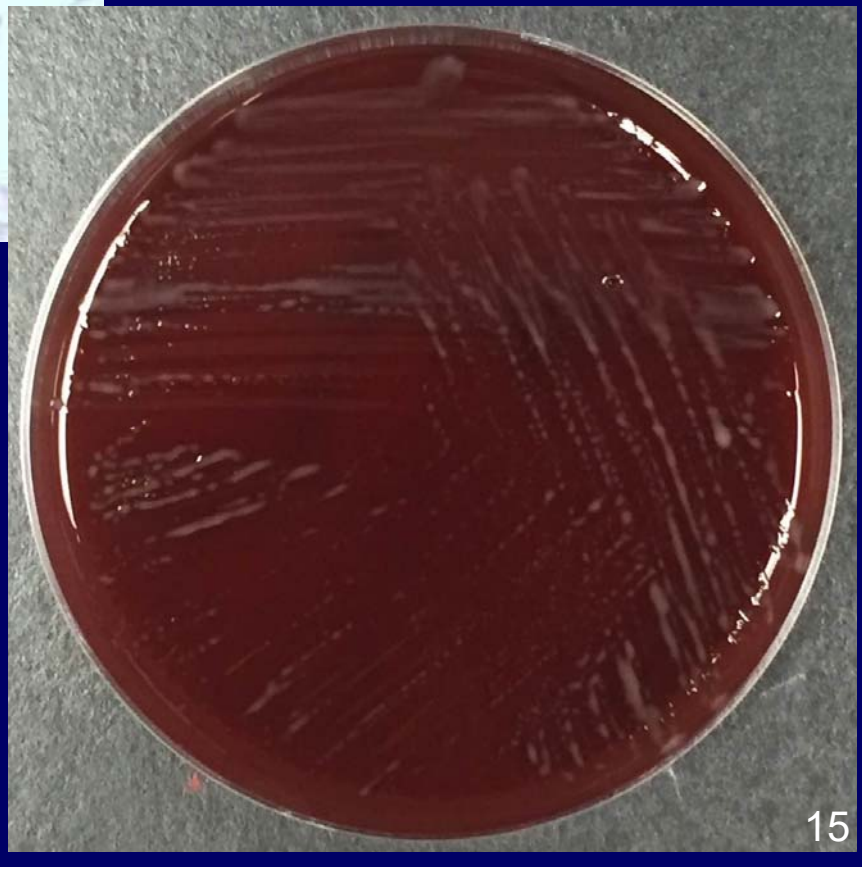
- Additional tests for definitive identification

  Hippurate-positive *Campylobacter jejuni*
  Indoxyl acetate-positive *Campylobacter jejuni/coli*

- Notes

  Isolated colonies on *Campylobacter*-selective medium incubated in microaerophilic 42ºC





Streptococcus pneumoniae

- Presumptive identification

  GPC (lancet-shaped in pairs) Catalase-negative
  α-hemolysis on blood agar

- Additional tests for definitive identification

  Bile solubility-positive

- Notes

  ~1% of *S. pneumoniae* with typical colony morphology may not be bile soluble

Viridans group *Streptococcus*

- Presumptive identification

  GPC (pairs, chains) Catalase-negative
  Non-hemolytic or α-hemolysis

- Additional tests for definitive identification

  PYR-negative LAP-positive
  Bile solubility-negative if α-hemolytic

- Notes

  Aerococcus urinae: cocci in clusters/tetrads
  Pediococcus spp.: cocci in clusters/tetrads

Aerococcus viridans

- Presumptive identification

  GPC (tetrads, clusters)
  α-hemolytic
  Catalase-negative

- Additional tests for definitive identification

  PYR-positive
  LAP-negative

- Notes


Intermission

Streptococcus pyogenes

- Presumptive identification

  GPC (pairs, chains) Catalase-negative
  CFU (>0.5 mm diameter); sharp β-hemolysis

- Additional tests for definitive identification

  PYR-positive OR
  Lancefield group A by latex agglutination

- Notes

  Careful observation of size and hemolysis, as *Enterococcus* spp. can demonstrate β-hemolysis

Streptococcus agalactiae

- Presumptive identification

  GPC (pairs, chains) Catalase-negative
  Small zone of β-hemolysis on blood agar

- Additional tests for definitive identification

  Hippurate-positive OR CAMP-positive
  OR Lancefield group B via latex agglutination

- Notes

  Hippurate method not to be used on non-hemolytic colonies

Streptococcus agalactiae

- Presumptive identification

  GPC (pairs, chains) Catalase-negative
  Small zone of β-hemolysis on blood agar

- Additional tests for definitive identification

  Hippurate-positive OR CAMP-positive
  OR Lancefield group B via latex agglutination

- Note

LIMITATIONS/additional factors

β-hemolytic *Enterococcus* spp. can be hippurate-positive

Many viridans group streptococci and non-hemolytic *S. agalactiae* are hippurate-positive

Enterococcus spp.

- Presumptive identification

  GPC prs, chains (no clusters) Catalase-negative
  Non-β-hemolytic on blood agar (>1 mm diameter)

- Additional tests for definitive identification

  PYR-positive

- Notes

  Demonstrate an LAP-positive reaction with α-hemolytic *Enterococcus* spp. (improved specificity)

Streptococcus anginosus group

- Presumptive identification

  GPC (pairs, chains) Catalase-negative
  CFU (<0.5 mm diameter); variable hemolysis

- Additional tests for definitive identification

  Odor of butterscotch or vanilla OR
  Lancefield group F by latex agglutination

- Notes

  May be Lancefield group A, C, F, or G by latex agglutination

Staphylococcus aureus

- Presumptive identification

  Catalase-positive
  Tube/slide coagulase- or latex agglutination-positive

- Additional tests for definitive identification

  Typically β-hemolytic colonies on blood agar

- Notes

  Tube coagulase required if non-hemolytic isolates from urine

Staphylococcus aureus

- Presumptive identification

  Catalase-positive
  Tube/slide coagulase- or latex agglutination-positive

- Additional tests for definitive identification

  Typically β-hemolytic colonies on blood agar

- Not

LIMITATIONS/additional factors

S. schleiferi and *S. lugdunensis* may be slide coagulase-positive (clumpy, rather than complete agglutination); aforementioned species PYR-positive

S. saprophyticus may yield positive latex agglutination results

Staphylococcus lugdunensis

- Presumptive identification

  Gram-positive cocci
  Tube coagulase-negative
  Catalase-positive

- Additional tests for definitive identification

  PYR-positive (deep ruby red);
  Polymyxin B-resistant; ornithine-positive

- Notes

  May be slide coagulase-positive or clumpy

Listeria monocytogenes

- Presumptive identification

  Small Gram-positive bacillus
  Usually tumbling motility
  Catalase-positive
  Small β-hemolysis

- Additional tests for definitive identification

  Hippurate-positive

- Notes

  Isolate should be from blood culture or CSF culture
  Listeria can be non-motile after 35-37ºC incubation

Listeria monocytogenes

- Presumptive identification

  Small Gram-positive bacillus
  Usually tumbling motility
  Catalase-positive
  Small β-hemolysis

- Additional tests for definitive identification

  Hippurate-positive

- Not

LIMITATIONS/additional factors

Presence of catalase is not a required factor for identification in an otherwise typical isolate

Species confirmation not necessary; 95% of clinical isolates *monocytogenes*

Escherichia coli

- Presumptive identification

  Gram-negative bacilli via Gram or growth on selective medium

  Oxidase-negative
  Indole-positive

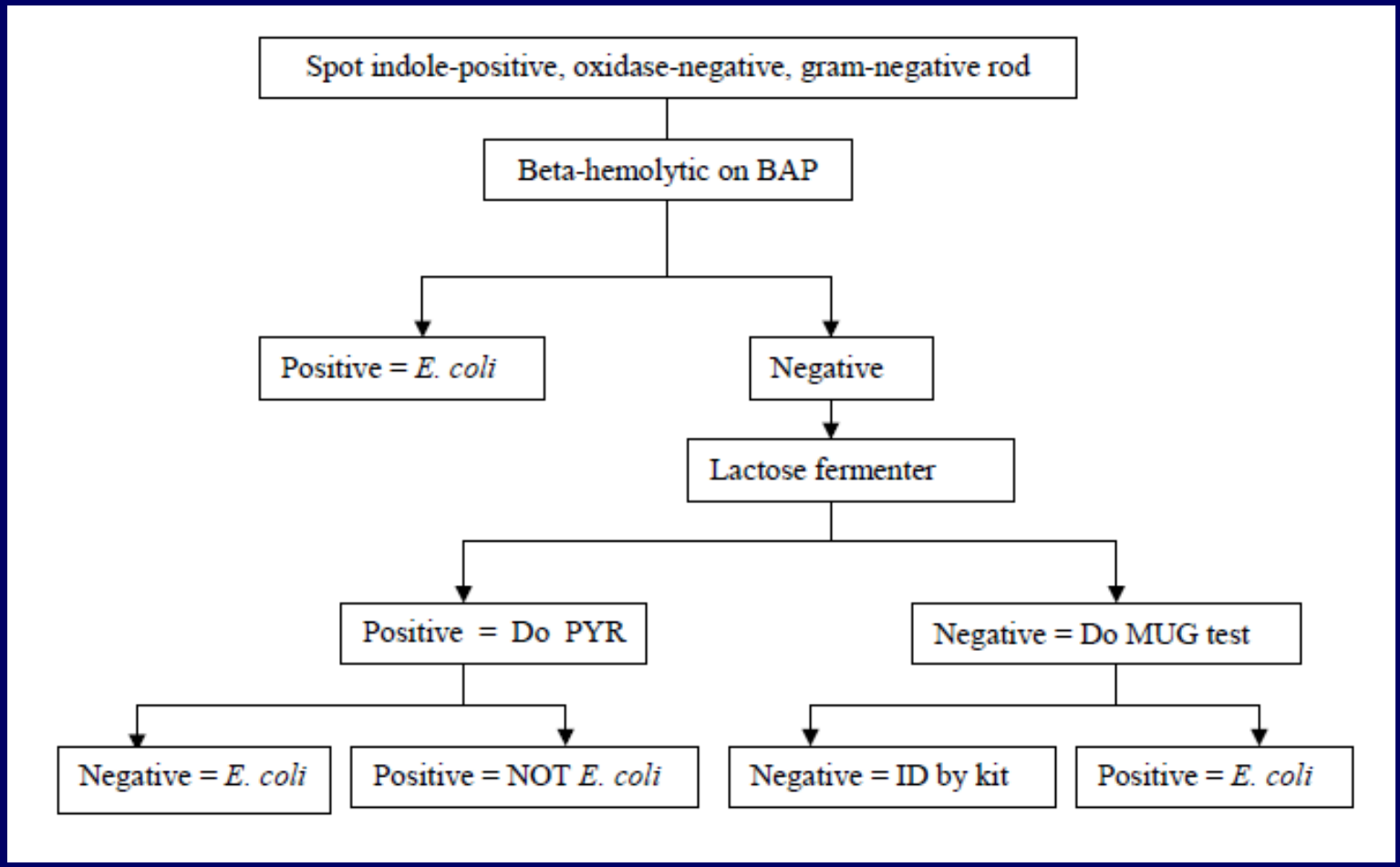
- Additional tests for definitive identification

  Hemolytic OR lactose-positive AND PYR-negative
  OR lactose-negative AND MUG-positive

- Notes

  Isolate must be growing as large colonies
  Isolate cannot be derived from gastrointestinal source

Escherichia coli



CLSI M35-A2; 2008

Escherichia coli

- Presumptive identification

  Gram-negative bacilli via Gram or growth on selective medium

  Oxidase-negative
  Indole-positive

- Additional tests for definitive identification

  Hemolytic OR lactose-positive AND PYR-negative
  OR lactose-negative AND MUG-positive

- Not

LIMITATIONS/additional factors

Occasional *Shigella* spp. can be indole-positive and MUG-positive; therefore, no rapid identification of *E. coli* from blood, fecal, GI sources

Positive MUG test may be preliminary rule-out test for O157:H7

e

Proteus mirabilis

- Presumptive identification

  Spreading (sic) colony Indole-negative

- Additional tests for definitive identification

  None, if susceptible to ampicillin

- Notes (if resistant to ampicillin)

Proteus spp.	Ornithine decarboxylase	Maltose fermentation
P. mirabilis	positive	negative
P. penneri	negative	positive

Proteus mirabilis

- Presumptive identification

  Spreading (sic) colony Indole-negative

- Additional tests for definitive identification

  None, if susceptible to ampicillin

- [illegible]

LIMITATIONS/additional factors

Instead of pursuing maltose or ornithine decarboxylase testing, one could report ampicillin-resistant (indole-negative) Proteae as "indole-negative *Proteus*" or *P. mirabilis*/*P. penneri*

Proteus vulgaris

- Presumptive identification

  Spreading (sic) colony Indole-positive

- Additional tests for definitive identification

- Notes

Pseudomonas aeruginosa

- Presumptive identification

  Indole-negative Oxidase-positive
  Metallic/pearlescent, rough, pigmented, mucoid

- Additional tests for definitive identification

  Grape-like odor; corn tortilla

- Notes

  Most often strongly β-hemolytic on blood agar

Pseudomonas aeruginosa

- Presumptive identification

  Indole-negative Oxidase-positive
  Metallic/pearlescent, rough, pigmented, mucoid

- Additional tests for definitive identification

  Grape-like odor; corn tortilla

- Not

LIMITATIONS/additional factors

Rare *Aeromonas* spp. may resemble *P. aeruginosa*, but can be differentiated by positive spot indole test

Burkholderia cepacia isolates from CF patients can resemble *P. aeruginosa*

Haemophilus influenzae

- Presumptive identification

  Gram-negative coccobacillus
  Good growth on chocolate; not blood, MacConkey

- Additional tests for definitive identification

  δ-aminolevulinic acid-negative

- Notes

  Satellitism on blood agar around *Staphylococcus* streak separates *Haemophilus* from *Brucella* and *Francisella*

Haemophilus influenzae

- Presumptive identification

  Gram-negative coccobacillus
  Good growth on chocolate; not blood, MacConkey

- Additional tests for definitive identification

  δ-aminolevulinic acid-negative

LIMITATIONS/additional factors

Rapid identification algorithm applies to CSF and respiratory isolates

Haemophilus haemolyticus cannot be differentiated from *H. influenzae*, with exception of hemolysis characterization on horse blood agar

OVERALL UTILITY I

	n	Ampicillin/Sulbactam	Piperacillin/Tazobactam	Imipenem	Meropenem	Aztreonam	Cefazolin	Ceftriaxone	Cefepime	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Trimethoprim/ Sulfamethoxazole
Escherichia coli	1595	58	96		100	96	89	95	96	93	100	80	80	79
Klebsiella pneumoniae	323	85	96		99	93	90	94	97	98	99	96	96	93
Klebsiella oxytoca	71	49	80		100	97	49	83	92	96	100	85	86	92
Enterobacter cloacae	89		87		97	90		88	98	98	100	94	94	86
Enterobacter aerogenes	38		71		100	84		87	97	100	100	89	89	97
Citrobacter freundii	30		93		100	93		93	100	97	100	96	97	90
Proteus mirabilis	155	84	99		100	100	87	100	100	95	100	81	85	81
Serratia marcescens	34				100	100		100	100	100	100	91	91	100
														Tobra
Pseudomonas aeruginosa	186		89	82	86	67			83	91		87	80	98
Organisms with extended-spectrum beta-lactamases (ESBL): 98														
Haemophilus influenzae beta-lactamase rate: 35.1%*														

OVERALL UTILITY II

	n	Penicillin	Methicillin	Erythromycin	Clindamycin	Vancomycin	Levofloxacin	Nitrofurantoin	Trimethoprim/sulfa	Tetracycline
methicillin-resistant *S. aureus* (45% MRSA rate)	255	0	0	10	69	100	41		100	91
methicillin-susceptible *S. aureus*	314	11	100	69	86	100	89		99	97
coagulase-negative *Staphylococcus*	221	13	48	28	50	100	65		66	91
Enterococcus species*	247-321	71				89		92		
viridans group *Streptococcus***	166	67		56	88	100				
*Streptococcus agalactiae***	167	100		39	54	100	98			
Beta-hemolytic streptococci other than group B**	40	100		83	85	100	100			
*Streptococcus pneumoniae***	81	PCN:	70.3% susceptible			Levofloxacin:		100.0% susceptible		
			18.5% intermediate					0.0% intermediate		
			11.1% resistant					0.0% resistant		

Acknowledgments

Jim Hermanson

Kacy Fisher

Joshua Kropp

Alana Sterkel, Ph.D.

David Uselman

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