California Antimicrobial Resistance (AR) Laboratory Network: *mcr-1* and Detecting Colistin Resistance

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California AR Lab Network Goals

- **Enhance situational awareness** of healthcare-associated AR pathogens by facilitating information and data sharing
- **Connect healthcare facilities and laboratories to additional laboratory testing resources** to enhance patient care and infection control activities
- **Strengthen collaboration** among clinical and public health laboratorians, infection control practitioners, and public health epidemiologists

Enhancing Situational Awareness through Data Sharing

- Quarterly reports
  - Carbapenem-Resistant Enterobacteriaceae
  - Multidrug Resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter* spp.
  - For labs that perform carbapenemase testing: numbers of carbapenemase-producing isolates, by specific carbapenemase
- Cumulative antibiograms

Sample Data Sharing Template

Organism	Total number of isolates tested*	Number of isolates resistant to at least one carbapenem**	Number of isolates identified as multidrug resistant***	Number of isolates identified as carbapenemase producing†
EXAMPLE	**18**	**6**	**N/A**	**5**
Klebsiella species				
E. coli				
Enterobacter species				
Acinetobacter baumanii				
Pseudomonas aeruginosa				

If applicable, please indicate the type of test your lab uses to determine carbapenemase production†† No Carbapenemase Test

California Department of PublicHealth

Connecting Facilities and Laboratories to Testing Resources

- Rapid CRE colonization testing of rectal & fecal swabs available through the CDC Antibiotic Resistance Laboratory Network (ARLN)
  - CRE colonization testing can be coordinated via your local health department and HAI Program
- *Candida auris* identification available through CDPH Microbial Diseases Laboratory (MDL)
  - For questions about fungal diagnostic testing services available at MDL, contact linlin.li@cdph.ca.gov

Connecting Facilities with Resources

- As part of CDC ARLN, CDPH will recruit laboratories to participate in sentinel surveillance for carbapenemase-producing *Acinetobacter* and *mcr-1*
  - Carbapenem-resistant *Acinetobacter*
  - *E. coli* or *Klebsiella spp.* that are:
    - susceptible to all carbapenems
    - resistant to 3rd generation cephalosporins
    - positive test result for ESBL production, if available

Strengthening Collaboration

Healthcare Providers and Laboratories	Local Public Health Laboratories	CDPH Microbial Diseases Laboratory	CDC ARLN (Washington State)
Engage local & state health department to understand when and where to submit isolates or specimens	CRE characterization (e.g. carbapenemase testing)		CRE colonization testing of rectal & fecal swab specimens
	Genetic relatedness testing (e.g., whole genome sequencing)		Sentinel surveillance projects
Report outbreaks/clusters to local public health & L&C District Office		Characterization of novel/unusual resistance (e.g., novel carbapenemases, *mcr*-1 and other *mcr* variants, suspected *Candida auris*)	

Other California AR Lab Network Activities

- CDPH HAI Program California AR Lab Network website will be updated soon

- Facilities can sign up for California AR Lab Network updates using our online form

mcr-1 and Detecting Colistin Resistance: Objectives

- Understand the epidemiology of the *mcr-1* gene
- Describe methods to detect colistin resistance
- Discuss the role of the California AR Lab Network in *mcr-1* surveillance

Guest Presenters

- **Alex Kallen, MD, MPH, FACP**

  Lead, Antimicrobial Resistance and Emerging Pathogens Team

  Prevention and Response Branch

  Division of Healthcare Quality Promotion, CDC
- **Romney Humphries PhD, D(ABMM), M (ASCP)CM**

  Section Chief of Clinical Microbiology

  Assistant Professor

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  David Geffen School of Medicine at UCLA

Update on colistin-resistance mediated by *mcr*-1

Alex Kallen, MD. MPH, FACP

Lead

Antimicrobial Resistance and Hospital Infection Prevention Team

Prevention and Response Branch, Division of Healthcare Quality Promotion

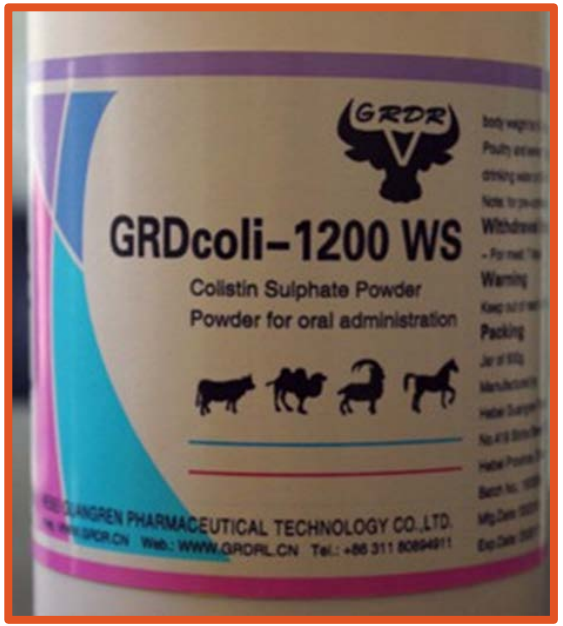
March 21, 2017

Objectives

- Describe mcr-1 resistance and why it is potentially important
- Describe epidemiology of mcr-1 in the U.S.
- Describe efforts in U.S. to detect and respond to *mcr-1*

Colistin (polymyxin E)

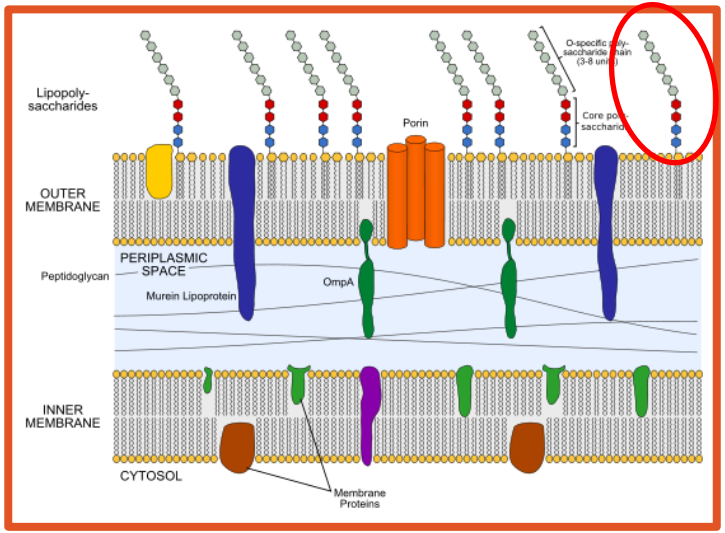
- Polymyxin class of antibiotics
- Antibiotic used to treat serious, highly resistant infections
  - Broad activity against gram negative bacteria
  - Associated with toxicities
  - Available in U.S. in topical and IV formulations
  - Used elsewhere orally for selective digestive decontamination
- Used widely in veterinary medicine outside the U.S.



www.alibaba.com

Colistin Resistance

- Chromosomal resistance mechanisms well-documented
  - Colistin binds lipopolysaccharide
  - ~11% of ESBLs tested at CDC have colistin MIC ≥4 µg/ml



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Colistin Susceptibility Testing

- Multiple methodological issues and technical challenges
  - No FDA-cleared automated testing methods
  - E-test underestimates MIC by 1-2 doubling dilutions
  - Disk diffusion does not work due to poor diffusion
- ASM 2016: Laboratories that choose to test for colistin susceptibilities for clinical decisions should use broth microdilution
  - Vast majority of clinical labs in U.S. do not have this capacity
  - Might need to have reference labs perform this testing

Identifying Isolates for *mcr-1* Screening

- MicroScan ID/AST panel has colistin well (4 µg/ml) for identification
  - 2 *mcr-1 E. coli* isolates in CDC/FDA AR Bank (MIC = 4 µg/ml)
  - Panel accurately identified colistin R in both isolates across 3 replicates per isolate and 2 inoculation methods*
  - Could be useful for surveillance purposes for identifying *mcr-1*
  - Cannot be used for clinical purposes
- Gradient diffusion method (e.g. E-test)
  - Issue with false susceptible results (very major errors)
  - Can be only be used for surveillance purposes and has limited sensitivity

Plasmid-mediated colistin resistance (*mcr-1* gene)

- First report of plasmid-mediated colistin resistance November 2015 (China)
  - Found in (*E. coli*):
    - 78/523 (15%) raw meat samples
    - 166/804 (21%) animal samples
    - 16/1322 (1%) human healthcare samples

Liu YY et al. *Lancet Infect Dis.* 2016; 16(2):161-8.
Skov RL, Monnet DL. *Euro Surveil.* 2016; 21(9)

Why is mcr-1 potentially important?

- Plasmid-mediated with high propensity for spread
- Although many isolates to date are treatable with other antibiotics, has potential to add colistin-resistance to isolates with high levels of resistance
  - Further limits or eliminates treatment options

Emergence of *mcr-1*

- Since initial report, found globally
  - >20 countries and 6 continents
  - Food animals, meat, vegetables, surface water
  - Ill patients, asymptomatically colonized patients
- Multiple species: *E. coli, K. pneumoniae, Salmonella enterica, Shigella sonnei*
- Earliest isolates identified from 1980s (chickens, *E. coli*, China)
- Earliest human isolate from 2008 (*Shigella sonnei*, Vietnam)

Molecular features of *mcr-1*

- Highly transmissible
  - Stably maintained in absence of polymyxin drug pressure
  - Potential for movement and rapid spread through epidemic clones
- Increased colistin MICs 8 to 16-fold
  - Typical MICs 4 to 8 µg/ml
- Other variant, *mcr-2*, identified in Belgium
  - 77% homology with *mcr-1*
  - In Belgium, more common than *mcr-1*
  - Similar colistin MIC increase as *mcr-1*

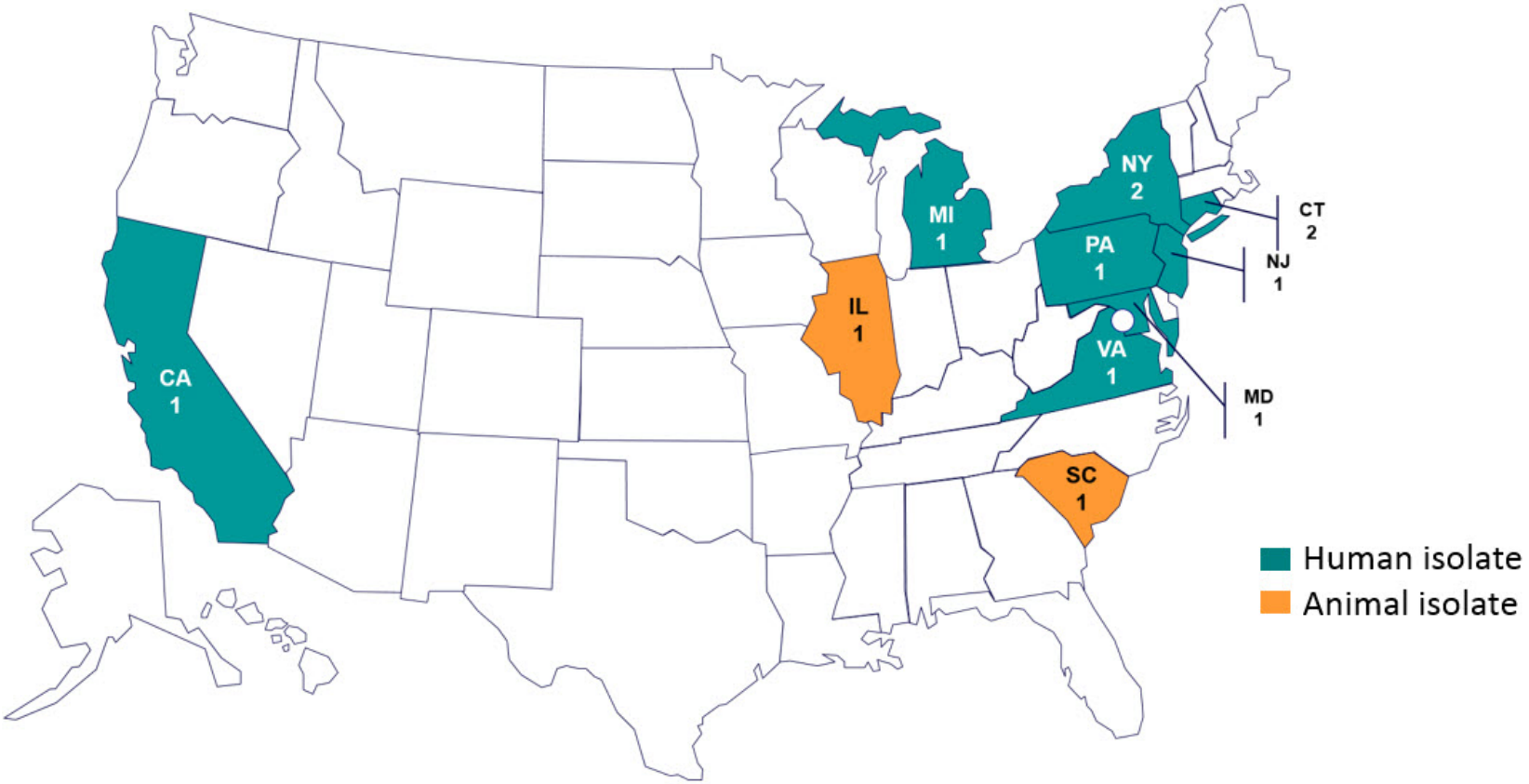
Surveillance for *mcr-1* in the U.S.

- Retrospective surveillance
  - U.S. Government
    - NARMS, DHQP reference and surveillance isolates, WRAIR MRSN
  - Academia and private labs: SENTRY, Rutgers
- Prospective surveillance
  - CDC HAN, June 2013: Send Enterobacteriaceae with colistin MIC ≥4 µg/ml to CDC for mechanism testing
  - ARLN: Regional lab testing for *mcr-1*

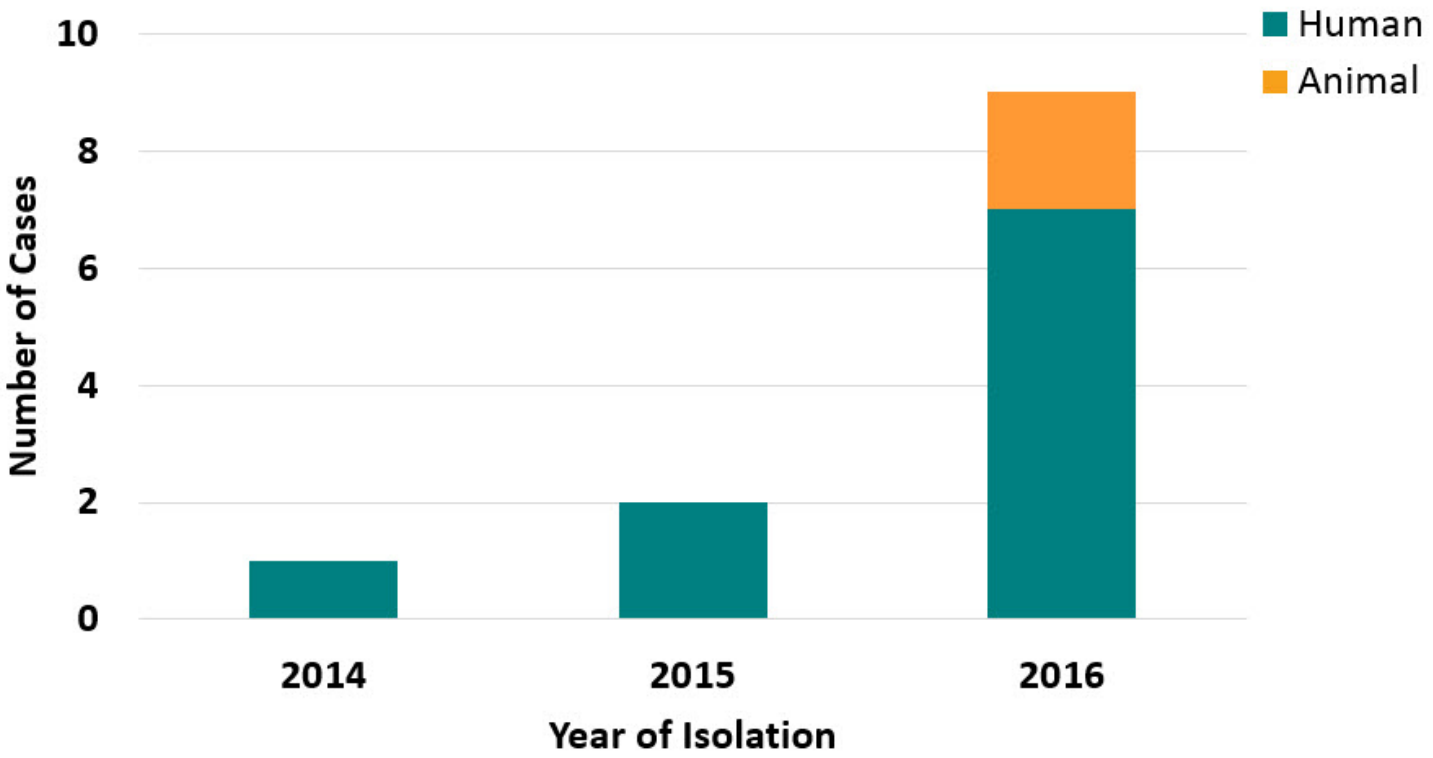
mcr-1 in the U.S.

- 12 reports since February 10, 2017
  - 10 human isolates
  - 2 porcine isolates collected at slaughter
- Human isolates identified through
  - Retrospective surveillance (n=3)
  - Routine whole genome sequencing (n=3)
  - Prospective surveillance (n=4)
- Animal isolates identified through USDA prospective surveillance

mcr-1 Cases by Location, as of February 10, 2017, n=12



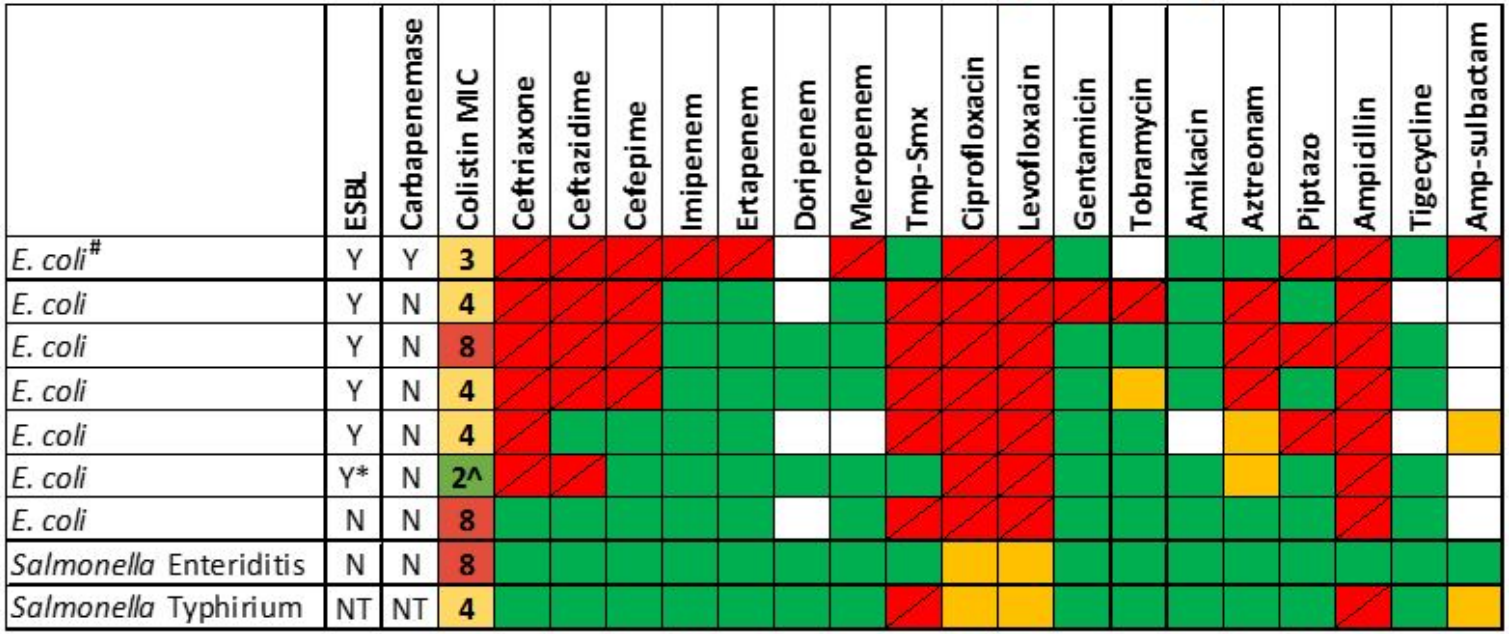
mcr-1 Cases by Year, as of February 10, 2017, n=12



mcr-1 Isolate Characteristics

- Human isolates
  - 8 *E. coli*
    - 6 ESBLs
    - 1 carbapenemase-producer: NDM
    - 2 neither
  - 2 *Salmonella*
    - 1 MDR Typhimurium
    - 1 susceptible Enteritidis
- Animal isolates: *E. coli*
- Colistin MICs ranged from 2-8 µg/ml (mode: 4 µg/ml)

mcr-1 Isolate Susceptibilities, Among Isolates Characterized Prior to December 31, 2017, N=9



	ESBL	Carbapenemase	Colistin MIC	Ceftriaxone	Ceftazidime	Cefepime	Imipenem	Ertapenem	Doripenem	Meropenem	Tmp-Smx	Ciprofloxacin	Levofloxacin	Gentamicin	Tobramycin	Amikacin	Aztreonam	Piptazo	Ampicillin	Tigecycline	Amp-sulbactam
E. coli[#]	Y	Y	3	R	R	R	R	R	NT	R	S	R	R	S	NT	S	S	R	R	S	R
E. coli	Y	N	4	R	R	R	S	S	NT	S	R	R	R	R	R	S	R	S	R	NT	NT
E. coli	Y	N	8	R	R	R	S	S	S	S	R	R	R	S	S	S	R	R	R	S	NT
E. coli	Y	N	4	R	R	R	S	S	S	S	R	R	R	S	I	S	R	S	R	S	NT
E. coli	Y	N	4	R	S	S	S	S	NT	NT	R	R	R	S	S	NT	I	R	R	NT	I
E. coli	Y*	N	2^	R	R	S	S	S	S	S	S	R	R	S	S	S	I	S	R	S	NT
E. coli	N	N	8	S	S	S	S	S	NT	S	R	R	R	S	S	S	S	S	R	S	NT
Salmonella Enteriditis	N	N	8	S	S	S	S	S	S	S	S	I	I	S	S	S	S	S	S	S	S
Salmonella Typhirium	NT	NT	4	S	S	S	S	S	S	S	R	I	I	S	S	S	S	S	R	S	I

[#] **E-test for Colitin; MicroScan for all others**

* AmpC

^ Polymyxin B MIC = 4

S Susceptible

I Intermediate

R Resistant

NT Not tested

mcr-1 Patient Demographics and Risk Factors

Patient Characteristic	Number of Patients N=10
Median age in years, Range	53.5 (2-76)
Female	6
Travel outside of U.S. in 6 months prior	7
Any hospitalization in 6 months prior	5
Hospitalization outside of U.S.	1

mcr-1 Public Health Investigations: Containment

- Objectives: Identify risk factors and assess transmission
  - Prevent transmission in settings where other highly-resistant organisms are common
- Investigation components
  - Epi investigation to identify risk factors and contacts
    - Chart review
    - Patient interview
  - Screening cultures of contacts
  - Prospective surveillance

Results: Screening

- To date:
  - 26 household contacts screened – all negative
  - 8 Healthcare contacts screened - all negative
  - 85 HCP screened – all negative
- Duration of carriage
  - 6/10 rescreened – 2 positive
    - Patient 1 positive at 1 month and 2 months, first negative at 3 months
    - Patient 7 positive at 1.5 months, awaiting additional cultures

Recommendations – HAN June 13, 2016

- **Infection Prevention:** Healthcare providers should follow Standard and Contact precautions
- **Laboratory Testing:** Enterobacteriaceae isolates with a minimum inhibitory concentration (MIC) to colistin of 4 µg/ml or higher should be tested for confirmation and the presence of *mcr-1*
  - Exceptions: intrinsic resistance (*Morganella*, etc.), *Enterobacter spp.*
  - Testing when needed for clinical care
- **Validation of Laboratory Testing:** CDC is making test-bacteria with elevated colistin MICs, available through the FDA-CDC AR Bacteria Isolate Bank
- **Environmental Cleaning**: Healthcare facilities should ensure rooms where patients with antibiotic-resistant infections have been placed receive thorough daily and terminal cleaning
- **Report isolates to Public Health**

Summary

- Most colistin resistance is not caused by *mcr-1*
- *mcr-1* appears to be a highly mobile resistance element that has the potential to add to resistance among already highly resistant Gram-negative bacilli
- Appears to be uncommon in the United States thus far
  - Prevalence requires further evaluation
- Isolates identified in the U.S. have been identified primarily among people with travel outside the U.S.
  - Importance of assessing these exposures at admission
- Colonization does not appear to be persistent
- Limitations to colisitin susceptibility testing makes identifying these isolates challenging

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Colistin Susceptibility Testing Options

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CLSI-EUCAST Joint Working Group Testing Recommendations – 2016

1. Reference testing of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. is by the ISO-standard broth microdilution method (20776-1). Note:
    a. Cation-adjusted Mueller-Hinton Broth is used
    b. No additives may be included in any part of the testing process (in particular, no polysorbate-80 or other surfactants)
    c. Trays must be made of plain polystyrene and not treated in any way before use
    d. Sulphate salts of polymyxins must be used (the methanesulfonate derivative of colistin must not be used - it is an inactive pro-drug that breaks down slowly in solution)

2. Susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion, cannot be recommended until historical data have been reviewed or new study data have been generated. Work on these methods is ongoing.

http://www.eucast.org/ast_of_bacteria/guidance_documents/#c8577

Colistin / Polymyxin B

2017 CLSI Breakpoints / ECV

Organism	MIC (µg/ml)			Zone (mm)		
	Susc	Int	Res	Susc	Int	Res
Acinetobacter spp.	≤2	-	≥4	none		
Pseudomonas aeruginosa	≤2	-	≥4	none		
Enterobacteriaceae	Insufficient clinical and PK/PD data to set "breakpoint"					

Organism	ECV (µg/ml)	
	WT	NWT
Enterobacteriaceae	≤2	≥4

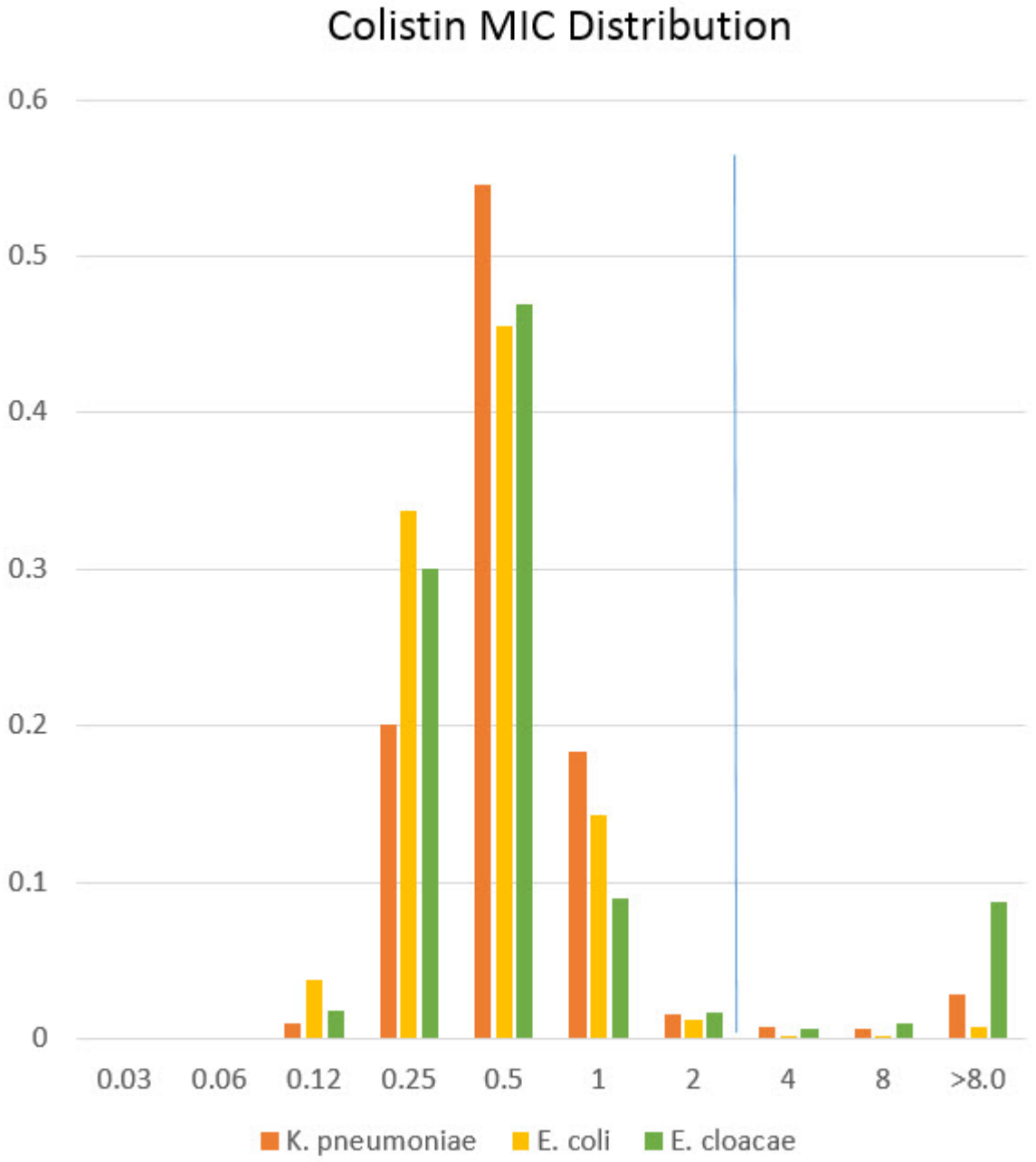
NOT a clinical breakpoint!!

- **Deleted breakpoints for "non-Enterobacteriaceae"**

CLSI M100 27th ed.

What is an ECV?

- Epidemiological cut-off value
- Differentiates isolates, based on MIC, into wild-type (“normal”) and non-wild-type (“not normal”)
  - NWT = acquired or mutational resistance mechanism
- NOT a clinical breakpoint – but most would not use if “NWT”



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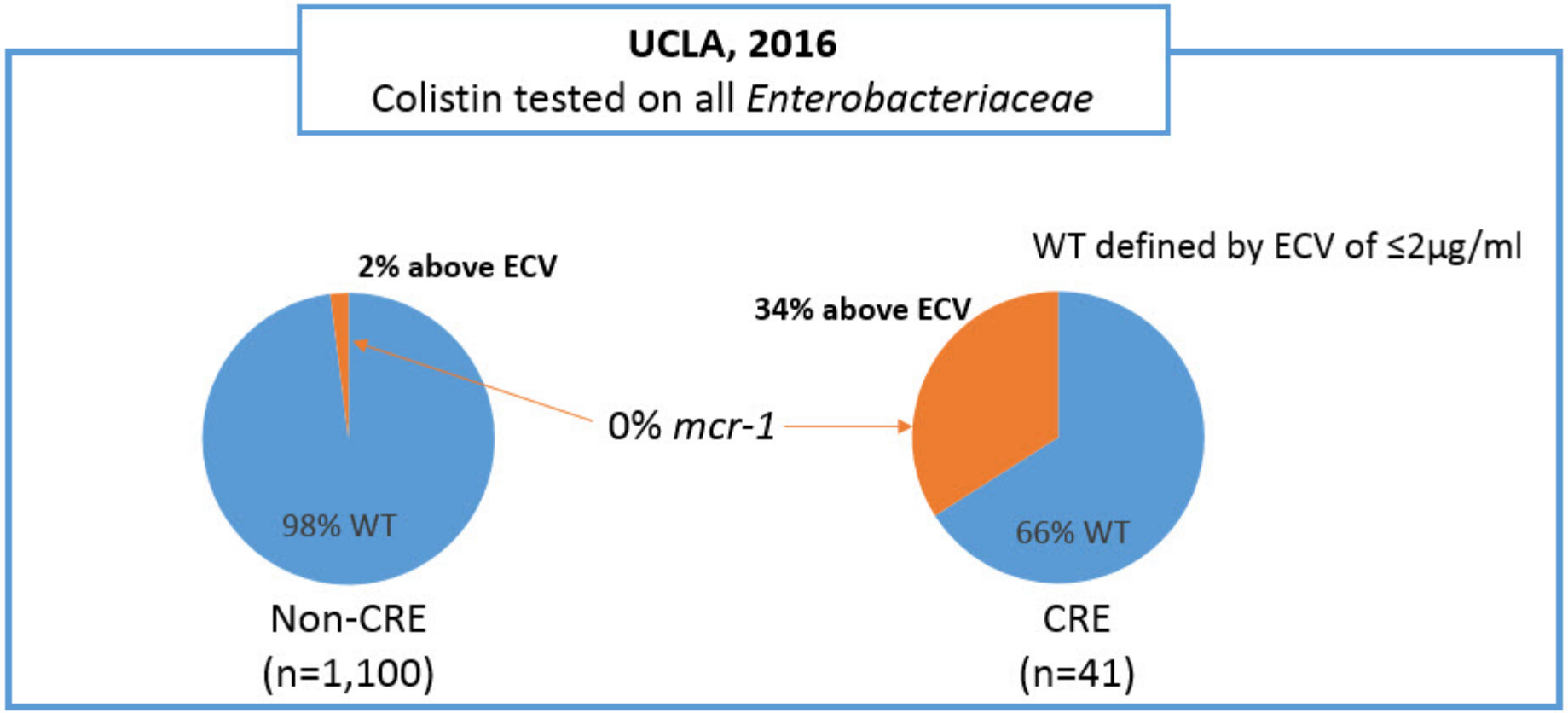
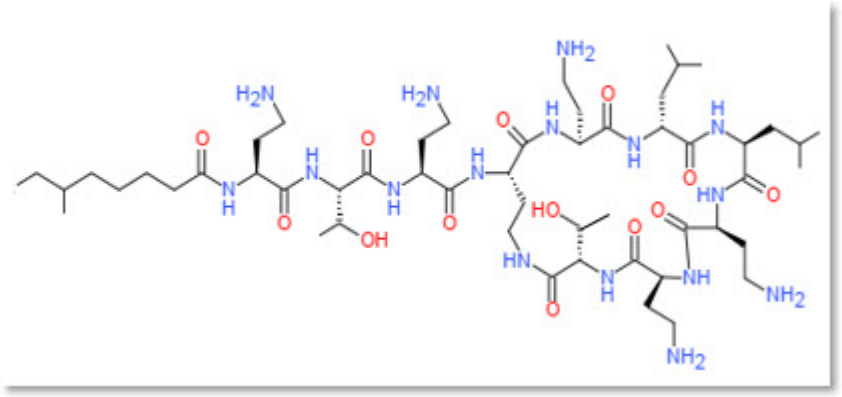
What are the resistance mechanisms?

- *mcr-1* & *mcr-2 – plasmid mediated, transmissible*
- Other mutations that lead to modification of LP



Use of surfactant for Colistin BMD

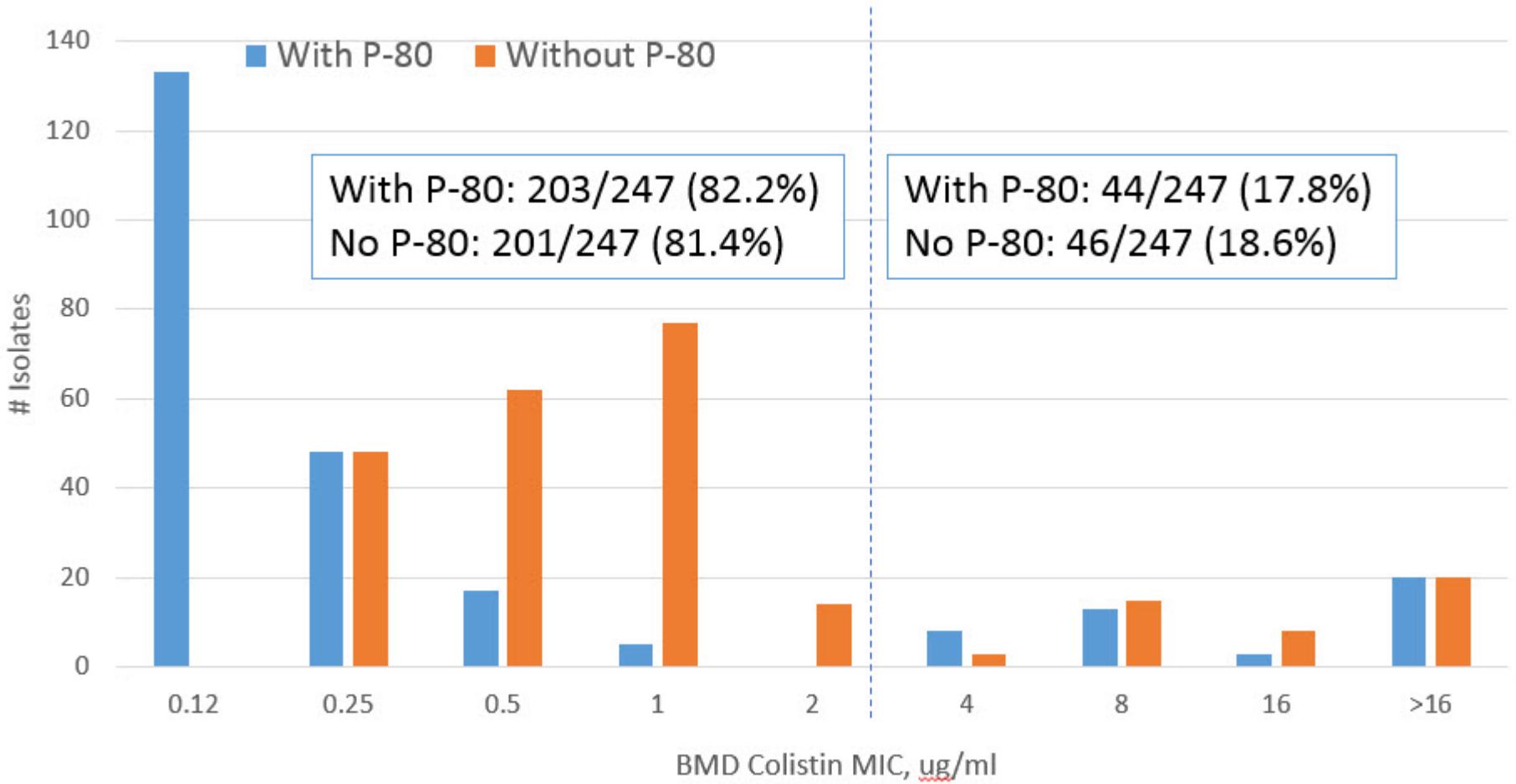
- colistin ‘sticks’ to polystyrene (tubes, microdilution trays)
  - 0.5 µg/ml colistin well shown to only have 0.0375 µg/ml (7.5%) nominal colistin[1]
- Many reference laboratories used surfactant (e.g., polysorbate 80) in inoculating water for BMD
- 0.002% P-80 vs. no P-80 → lower MIC (mostly at MIC below 2.0 µg/ml)[2]
  - Synergist activity of colistin on outer membrane & P-80 on inner?
  - Disruption of electrostatic adsorption to panel?



[1] Karvanen 2011. ICAAC Abstr: D-690

[2] Hindler 2013 JCM 15:1678; Sader 2012 DMID 74:412

Effect of P-80 on colistin MIC



N=247 GNR (63 *E. coli*, 61 *Klebsiella,* 60 *A. baumannii*, 63 *P. aeruginosa*)
Sader et al 2012 DMID 74:412

When is testing needed?

- Activity vs. most (but not) all MDR *P. aeruginosa, A. baumannii*, and CRE
- *Proteus/Providencia, Serratia, Burkholderia cepacia* – intrinsically resistant
- Increasing colistin use → emerging resistance
- Most labs cannot test routinely and do so on request only

Commercially Available Tests for Colistin

Test Method	Manufacturer	Regulatory Status	Notes
Disk	BD	RUO	Not recommended by CLSI/EUCAST Must confirm "S"
Etest	bioMerieux	RUO	Not recommended by CLSI/EUCAST
Sensititre Broth Microdilution	ThermoFisher	RUO	Broth microdilution Custom panel
MicroScan colistin well on dried Gram negative ID panel	BeckMan Coulter	RUO for AST	Some prelim data to suggest may work for all but *A. baumannii*

FDA cleared test unlikely (for now) …. No FDA breakpoint (colistin) or sponsor

Disk

M100S 26th Edition Table 2B-1 *Pseudomonas aeruginosa*

LIPOPEPTIDES								
O	Colistin	10 µg	~~≥11~~		~~≤10~~	≤2	4	≥8
O	Polymyxin B	~~300 units~~	≥12	–	~~≤11~~	≤2	4	≥8

Disk breakpoints will be removed from CLSI M100S 27th Edition Standard

Why?

- Very poor performance documented to date (23% VME in one study[1])
- Very difficult to differentiate “S” from “R” zone sizes

1 Lo-Ten-Foe. 2007. AAC 51:3726

Colistin (10 µg) Disk Diffusion for 4 isolates of MDR *P. aeruginosa*

10 mm (R)

(R)

BMD, ≤0.25 µg/mL (S)

µg/mL (S)

11 mm

BMD, ≤0.25

13 mm (S)

(S)

BMD, >4 µg/mL (R)

µg/mL (S)

13 mm

BMD, ≤0.25

Jerke et al 2016 CMN 38:69

Colistin Etest Studies (select)

Organism	Ref.	Results	Reference
A. baumannii (N=115) 22-R	BMD	CA: 98.2%; EA: 16.5% 1.7% VME; 0.0% ME "Etest OK for category calls for A. baumannii"	Arroyo 2005. JCM.43:903.
A. baumannii (N=58) 0-R P. aeruginosa (N=47) 15-R	AD	A. baumannii – 0.0 % VME; 1.9% ME P. aeruginosa – 11% VME; 30% ME "Etest unreliable for *P. aeruginosa*"	Tan 2007. CMI. 14:539.
P. aeruginosa (N=64) 12-R	AD	8.3% VME; 50% mE "Etest unreliable"	Goldstein. 2007. JAC 59:1039.
A. baumannii (N=27) 8-R P. aeruginosa (N=60) 9-R K. pneumoniae (N=20) 8-R	BMD	A. baumannii – 50 % VME P. aeruginosa – 0% VME 7.8% ME K. pneumoniae – 25% VME "isolates with high MIC (>8 µg/mL), have low Etest MICs"	Hindler. 2013. JCM. 15:1678

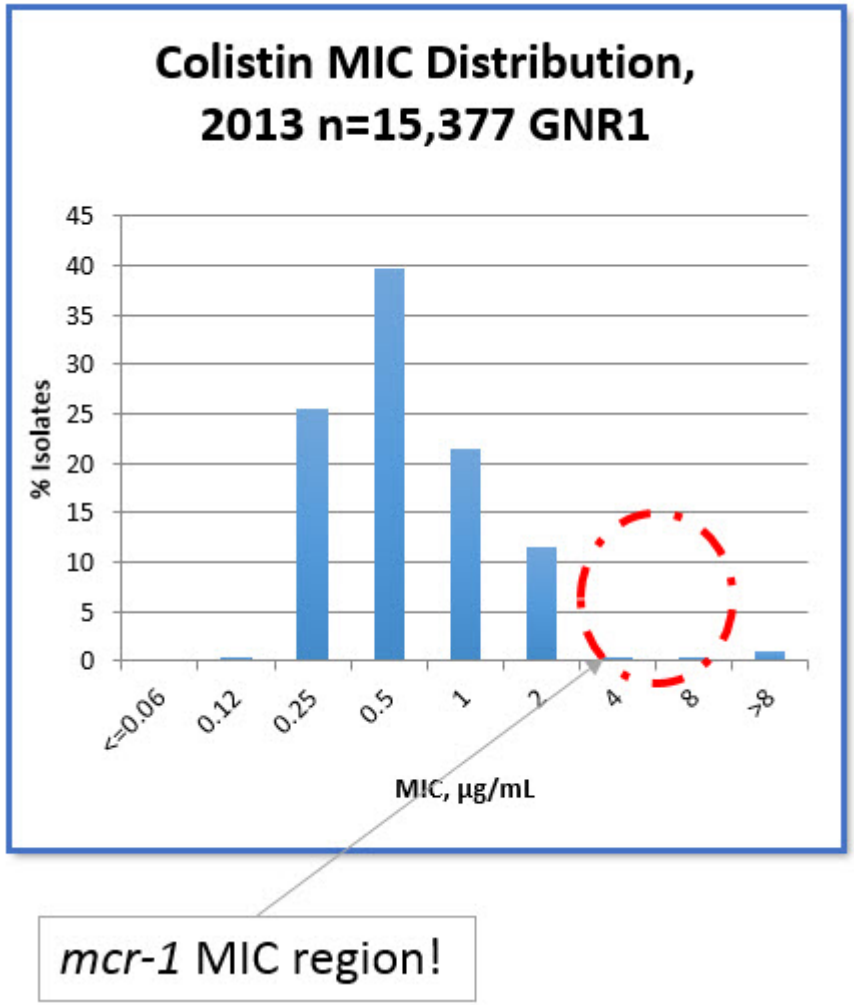
BMD, broth microdilution; AD, agar dilution. VME, very major error (false-S), ME, major error (false-R)

Sensititre

- RUO GNX2F & GNX3F panels
- 0.25 – 4.0 µg/ml colistin & polymyxin B wells

- One study[2]: 3/10 VME (30%) and 2/40 ME (5%) vs. BMD
  - All 3 VME were MIC of 2 µg/mL by Sensititre, 4 µg/ml by BMD
  - ME were MIC of 4µg/mL by Sensititre, 2 µg/ml by BMD

- Prudent practice: use caution if MIC is 2 – 4 µg/ml



1 Sader 2015 DMID 83: 379
2 Hindler 2013 JCM 15:1678

MicroScan Colistin Well

- NOT FDA-cleared for this indication (but used x-US)

Modification of an FDA-cleared test (RUO?)

- MUST do a verification study!
- Disclaimers to results?
- How about QC?

K. pneumoniae (n=14) MIC >4 µg/mL
E. cloacae (n=6)
Results: 40/40 (100%) correlated with BMD

What else?

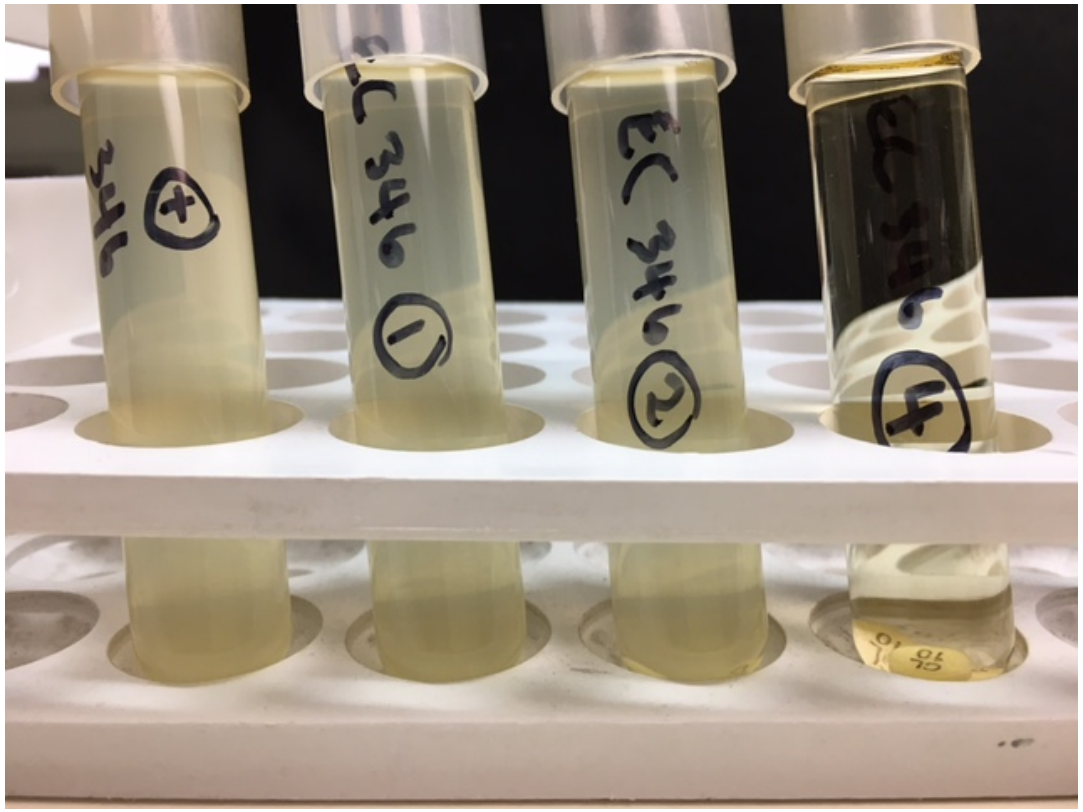
- CLSI investigating two methods:

1) Agar screen plate
2) Disk-elution method

    uses disk added to CA-MHB

    Add disks

    - 1 disk in 10 mL CA-MHB = 1 ug/mL
    - 2 disks in 10 mL CA-MHB = 2 ug/mL



mcr-1 positive E. coli, MIC = 4 ug/mL

How about polymyxin B?

- Much less data in literature on performance of AST methods
- Prudent to test agent to be used
  - No known resistance mechanisms that affect colistin & not polymyxin B (or vice versa)
  - Cross-susceptibility likely
  - To date, not enough data (“R” isolates) to determine if CLSI criteria can be met
  - Differences observed: methodological
    - Both are mixtures of 8-11 related compounds
    - >70% colistin A + colistin B OR polymyxin B1 + B2
    - Lot-to-lot differences in ratio

Jerke et al 2016 CMN 38:69

So: what should we do?

Test	Notes
Disk / Etest	Do not use
Sensititre / MicroScan RUO	- Perform verification - Make sure QC is in control - Report with disclaimer - Consider sending isolates with MIC 2-4 µg/ml (Sensititre) for confirmation/ use caution
BMD	- Reference method - Do not use surfactant - Make sure QC is in control - Difficult to do in-house
Reference laboratories	- Call them to find out method! - If BMD (incl. Sensititre) → OK - If Etest / disk → not OK
Soon (next year?)	- Agar screen plate, Disk-elution method

Questions

The HAI Program is available for consultation. Contact us by email: HAIProgram@cdph.ca.gov