Antimicrobial Resistance in California: Updates on Clinical and Public Health Laboratory Testing

November 29th, 2018

Healthcare-Associated Infections (HAI) Program
Microbial Diseases Laboratory
California Department of Public Health

Presenter	Objectives
Sam Horwich-Scholefield, MPH CIC	• Describe AR testing capabilities of clinical laboratories as reported via the National Healthcare Safety Network (NSHN)
Stephanie Abromaitis, PhD Peng Zhang, PhD	• Summarize results of the first year of phenotypic and molecular carbapenemase testing at MDL • Provide updates on upcoming testing capabilities at MDL
Matthew Sylvester, PhD	• Illustrate the use of Whole Genome Sequencing to assess relatedness of isolates to inform outbreak response

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National Healthcare Safety Network (NHSN) Annual Survey

- All facilities reporting to NHSN complete an annual survey to describe and evaluate hospital and laboratory practices.
  - Hospital characteristics
  - Infection prevention measures
  - Antimicrobial stewardship programs
  - Microbiology testing methods and practices
- Unless otherwise indicated, all results reported are from the 2017 NHSN Annual Survey

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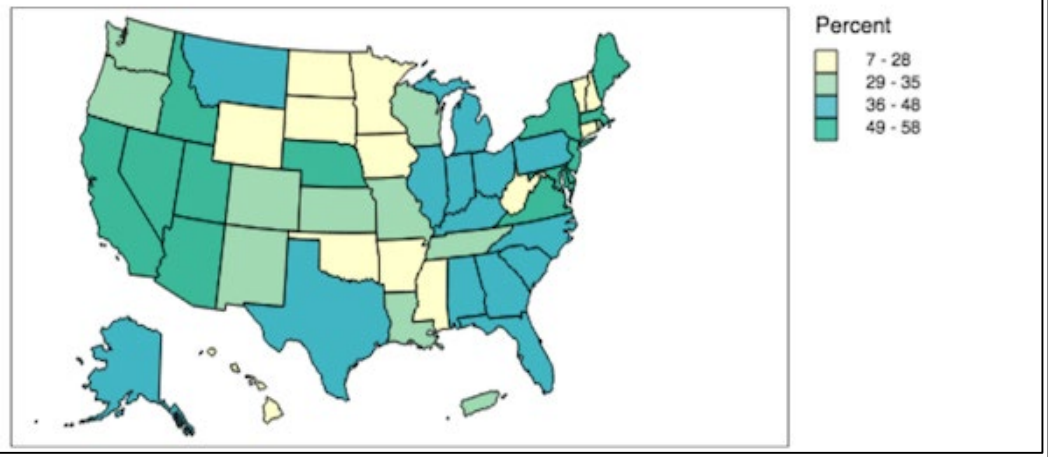
CDC Centers for Disease Control and Prevention

AR PATIENT SAFETY ATLAS

Antibiotic Stewardship in Acute Care Hospitals by State | 2014



Open Forum Infectious Diseases

BRIEF REPORT

Antifungal Susceptibility Testing Practices at Acute Care Hospitals Enrolled in the National Healthcare Safety Network, United States, 2011–2015

Snigdha Vallabhaneni,[1] Mathew Sapiano,[2] Lindsey M. Weiner,[2] Shawn R. Lockhart,[1] and Shelley Magill[2]

[1]Mycotic Diseases Branch and [2]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

Infection Control & Hospital Epidemiology (2018), **0**, 1–3
doi:10.1017/ice.2018.153

SHEA The Society for Healthcare Epidemiology of America

Concise Communication

Hospital microbiology laboratory practices for Enterobacteriaceae: Centers for Disease Control and Prevention National Healthcare Safety Network (NHSN) annual survey, 2015 and 2016

Alicia Shugart MA, Maroya Spalding Walters PhD, ScM, Lindsey M. Weiner MPH, David Lonsway MmedSc and Alexander J. Kallen MD, MPH

Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

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2017 NHSN Annual Survey Respondents, California (N=389)

Hospital Type	No.	Median Bed Size (IQR*)	Median ICU Bed Size (IQR*)
Community	269	156 (94-250)	18 (8-37)
Major Teaching	55	318 (226-450)	60 (32-90)
Critical Access	33	25 (16-25)	0 (0-4)
Long Term Acute Care	22	73 (54-95)	6 (4-6)
Pediatric	10	316 (80-356)	99 (30-146)

*Interquartile Range

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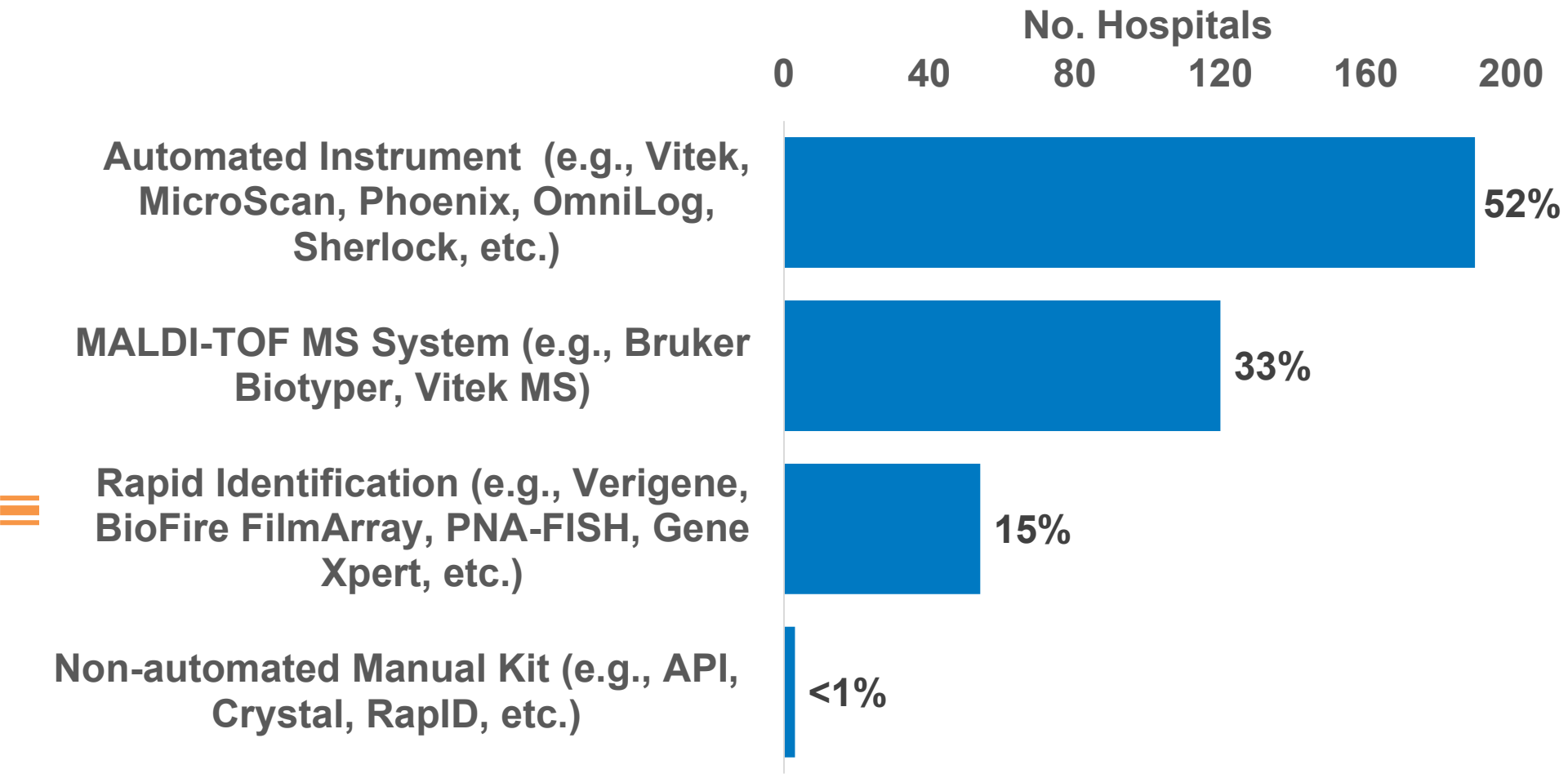
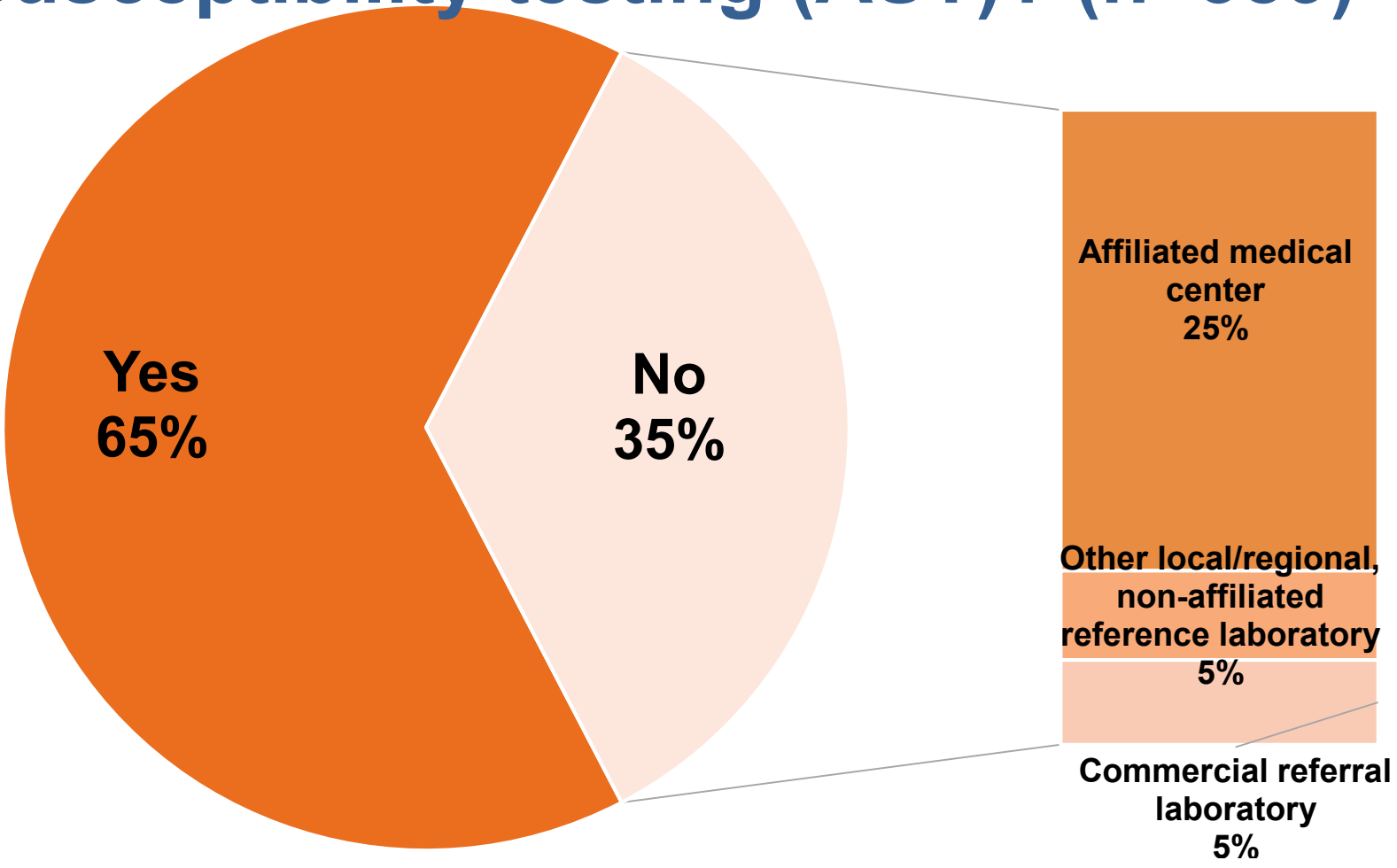
What is the primary or definitive method used to identify microbes from blood cultures? (n=367)

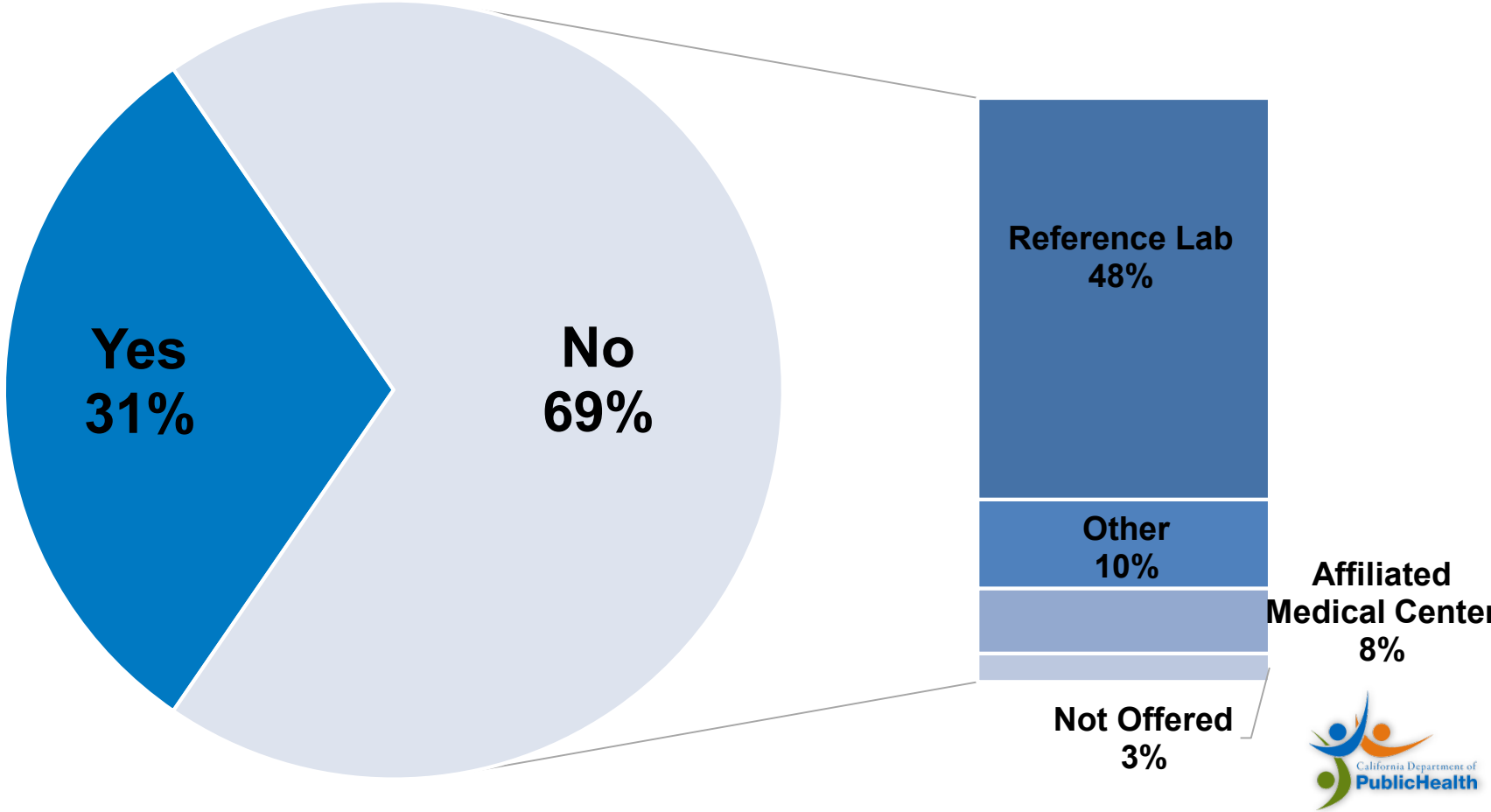


Does your facility have its own on-site laboratory that performs antimicrobial susceptibility testing (AST)? (n=389)

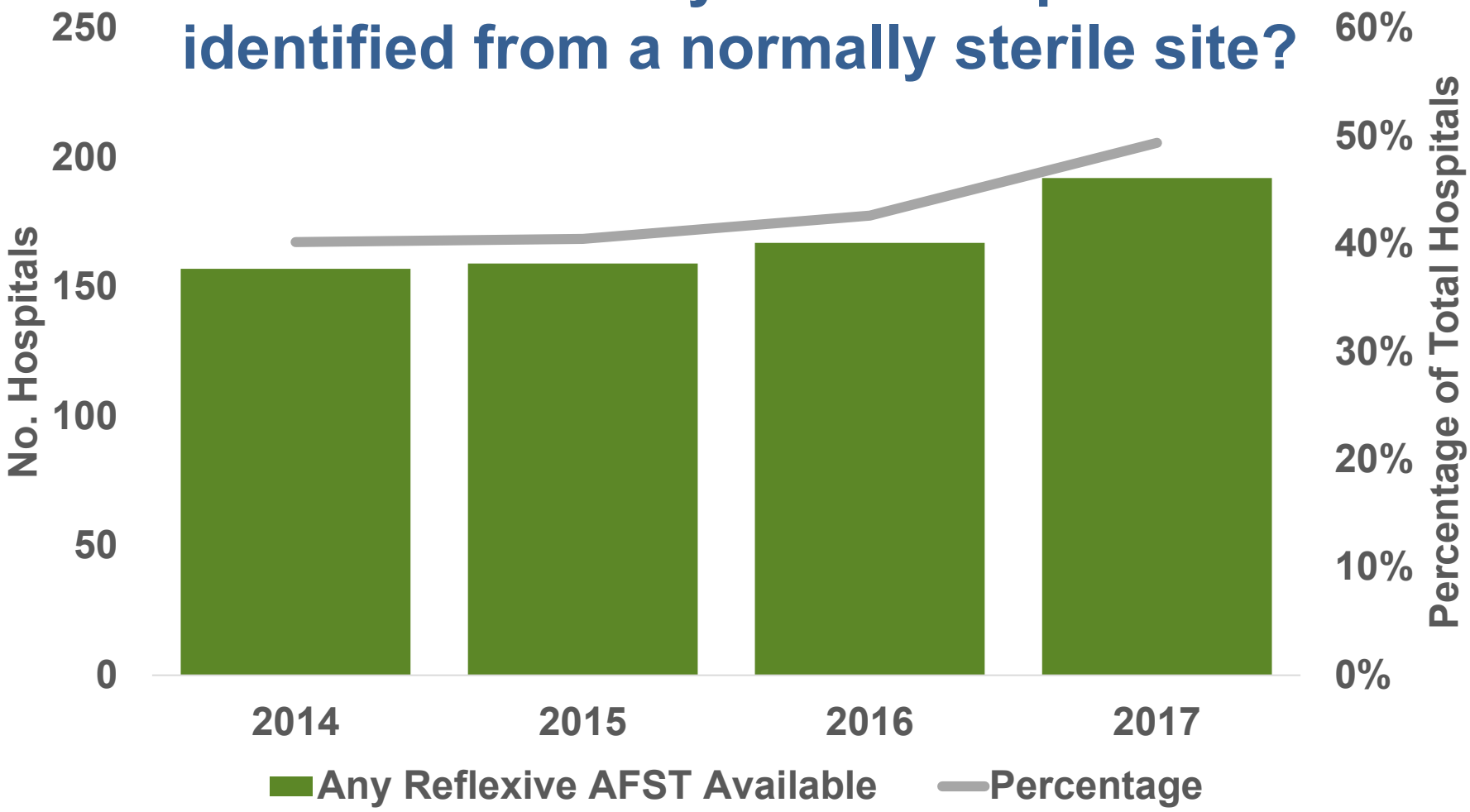


97% of hospitals reported using an Automated Testing Instrument

Does your facility have its own laboratory that performs antifungal susceptibility testing for *Candida* species? (n=389)



Is antifungal susceptibility testing performed automatically/reflexively without needing a specific order or request for susceptibility testing from the clinician for any *Candida* species identified from a normally sterile site?



Antibiotic Resistance Laboratory Network (ARLN) Antifungal Susceptibility Testing

- West Regional ARLN, located in Washington State, offers routine testing for antimicrobial resistance pathogens
  - Confirms *Candida* species identification using MALDI-TOF
  - Performs antifungal susceptibility testing

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Targeted Surveillance in California

- Enhanced testing for hard-to-detect pathogens
- ARLN supplies packaging materials, labels

Surveillance for:	Testing Performed at Washington State PHL
– Carbapenemase-producing *Acinetobacter* spp.	• ID (MALDI-TOF/Commercial methods) and AST • PCR for resistance mechanism
– mcr positive *E. coli* and *Klebsiella* spp.	• Colistin-susceptibility testing • PCR to detect mc-1/2
– *Candida auris* and multi-drug resistant *Candida* spp.	• Antifungal susceptibility testing and organism ID

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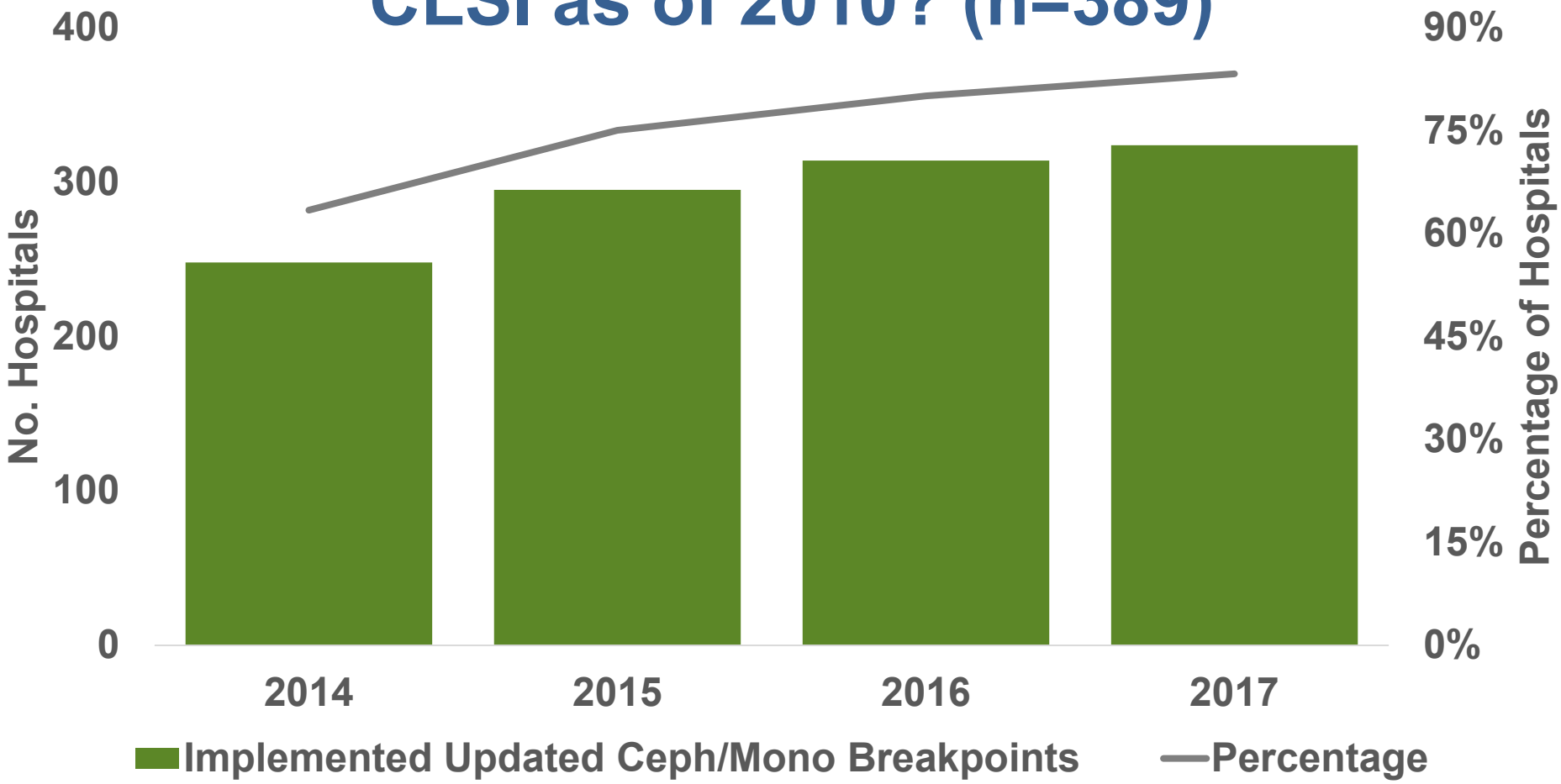
Enterobacteriaceae – Cephalosporin and Monobactam Breakpoints (MIC µg/ml) from 2009 to 2010

Agent	Old			Current		
	Susc	Int	Res	Susc	Int	Res
Cefazolin	≤8	16	≥32	≤1	2	≥4
Cefotaxime	≤8	16-32	≥64	≤1	2	≥4
Ceftriaxone	≤8	16-32	≥64	≤1	2	≥4
Ceftazidime	≤8	16	≥32	≤4	8	≥16
Cefepime*	≤8	16	≥32	≤2	4-8**	≥16
Aztreonam	≤8	16	≥32	≤4	8	≥16

*Cefepime breakpoints updated from CLSI M100-S23 (2013) to CLSI M100-S24 (2014)
**CLSI M100-S24 (2014) indicates cefepime breakpoints are Susceptible Dose Dependent (SDD)

Has the laboratory implemented the revised cephalosporin and monobactam breakpoints for Enterobacteriaceae recommended by CLSI as of 2010? (n=389)

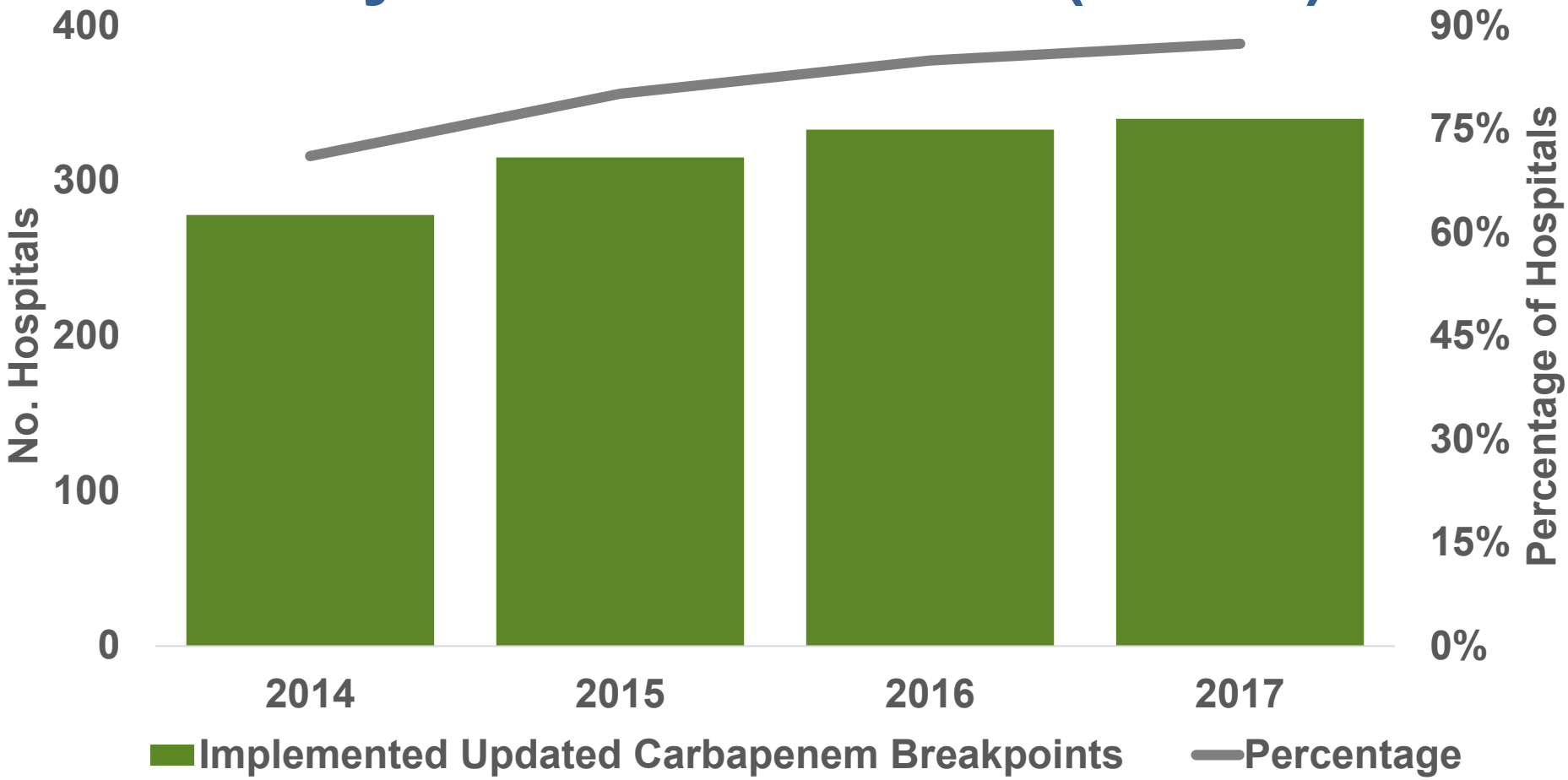


Enterobacteriaceae - Carbapenem Breakpoints (MIC µg/ml)[1]

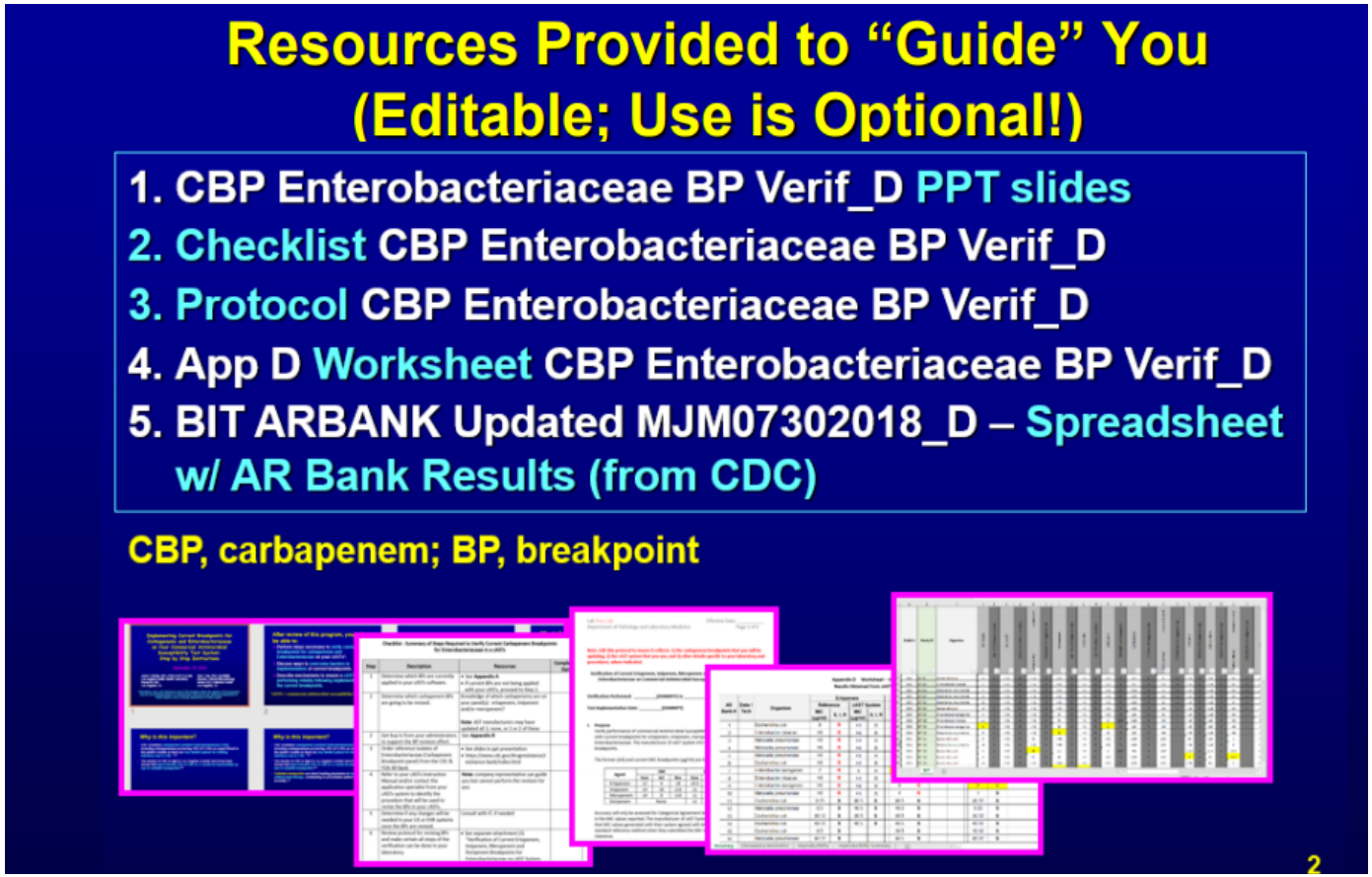
Agent	Old			Current		
	Susc	Int	Res	Susc	Int	Res
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4
Doripenem	none			≤1	2	≥4

[1] CLSI M100 28[h] ed; corresponding disk diffusion breakpoints also provided

Has the laboratory implemented the revised carbapenem breakpoints for Enterobacteriaceae recommended by CLSI as of 2010? (n=389)

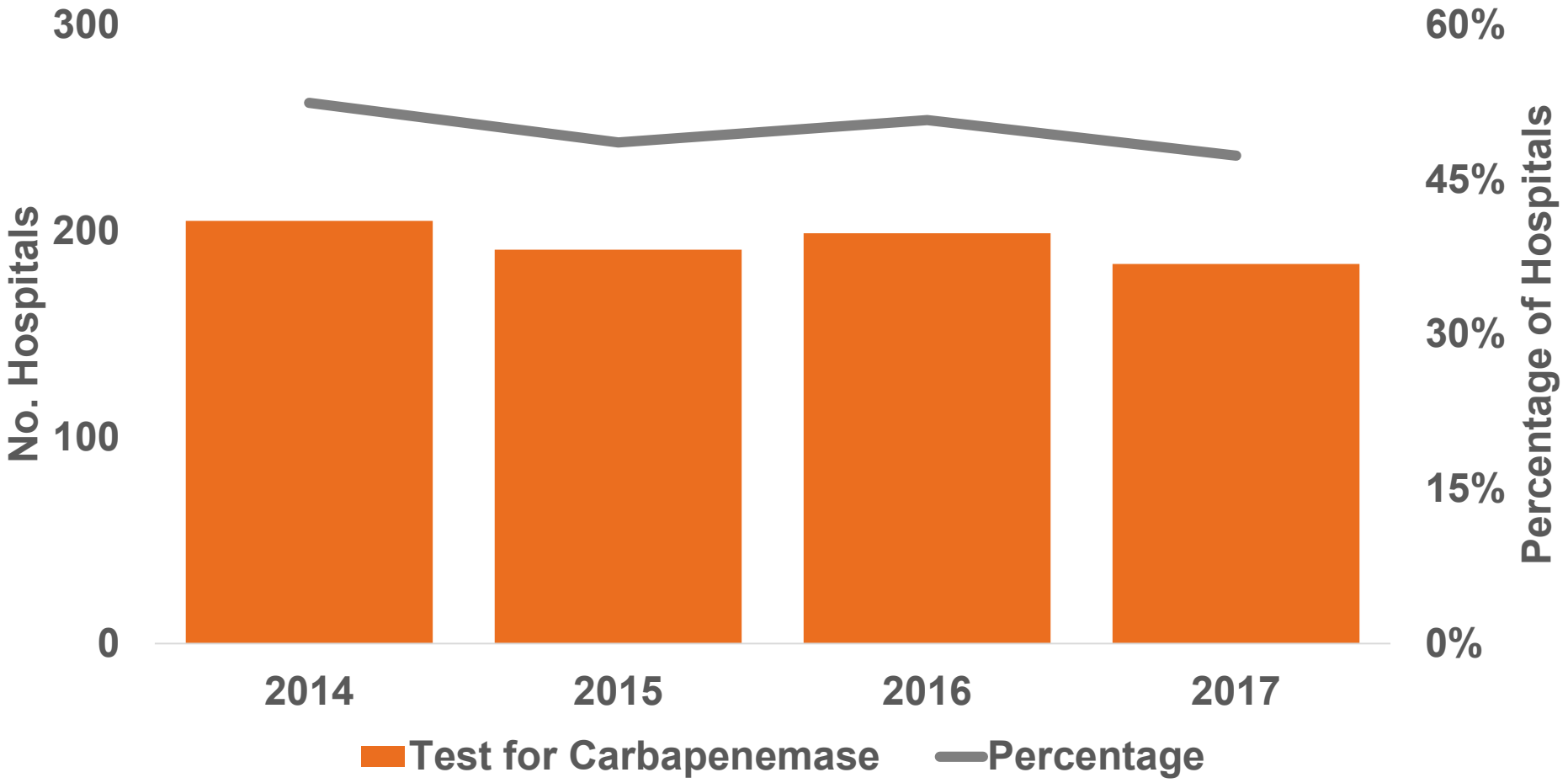


Webinar - *Implementing Current Breakpoints on Your AST System: Step by Step Instructions*

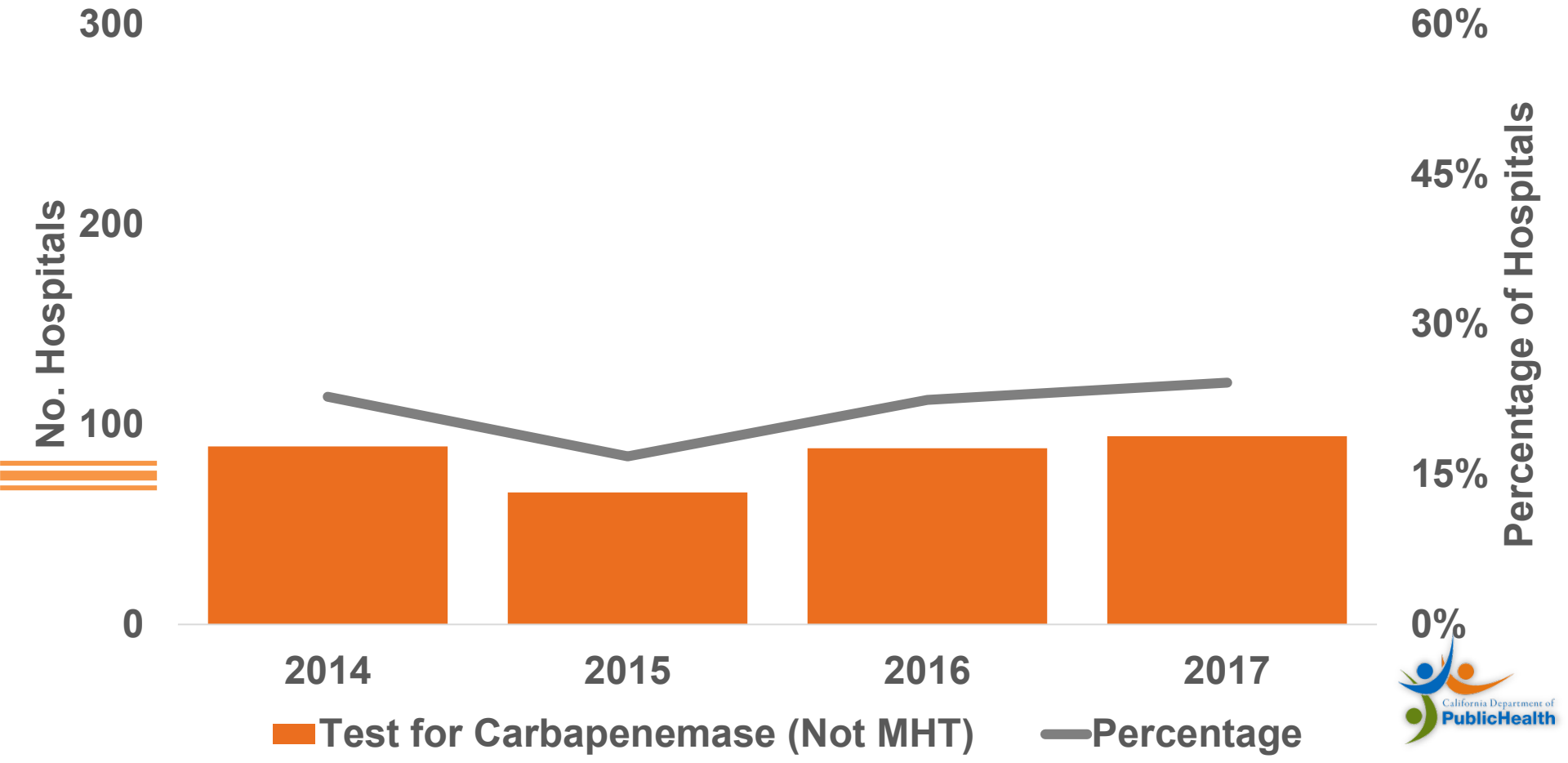


Webinar recording, slides, and supportive materials are available at: https://www.cdph.ca.gov/Programs/CHCQ/HAI/Pages/CA_ARLN.aspx

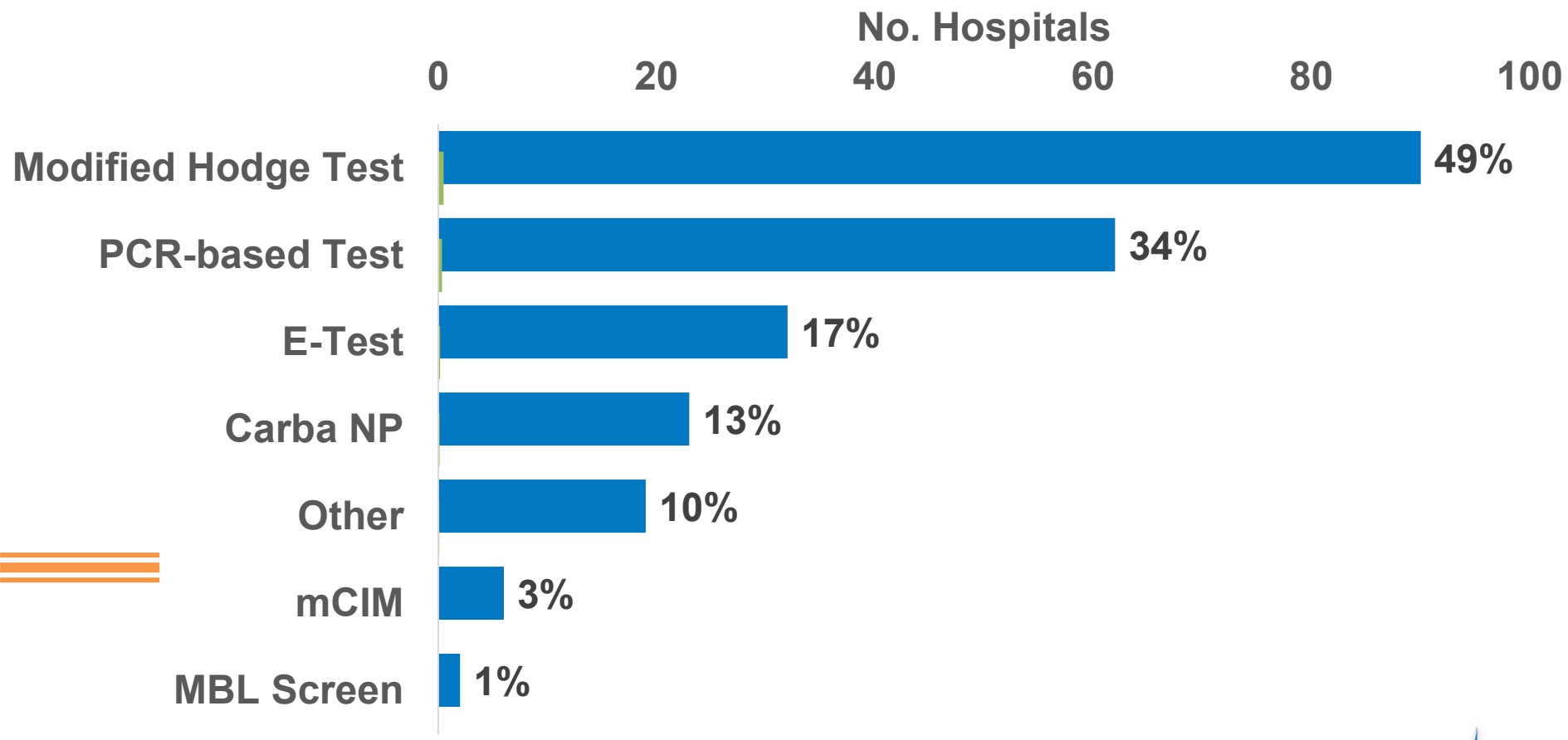
Does the laboratory perform a special test for presence of carbapenemase?



Does the laboratory perform a special test for presence of carbapenemase? (Modified Hodge Test excluded)



Carbapenemase Test Type (n=184)



Note some facilities indicated use of more than one test; sum is greater than 184

Carbapenemase Testing at MDL

- Beginning August 2017, CDPH Microbial Disease Laboratory (MDL) offers testing for
  - *Klebsiella* spp., *E. coli*, *Enterobacter* spp., or *Pseudomonas aeruginosa* resistant to at least one carbapenem.
    - Other species may be tested with prior consultation with HAI Program
  - Phenotypic (mCIM) and molecular (Carba-R) testing

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Carbapenemase Testing among Hospitals not Using Updated Breakpoints

- Hospitals using old breakpoints may use carbapenemase testing to identify epidemiologically concerning gram negative bacteria.
  - In 2016, 464 (44%) US hospitals using old breakpoints reported not performing carbapenemase testing.
  - In 2016, 29 (49%) California hospitals using old breakpoints reported not performing carbapenemase testing.

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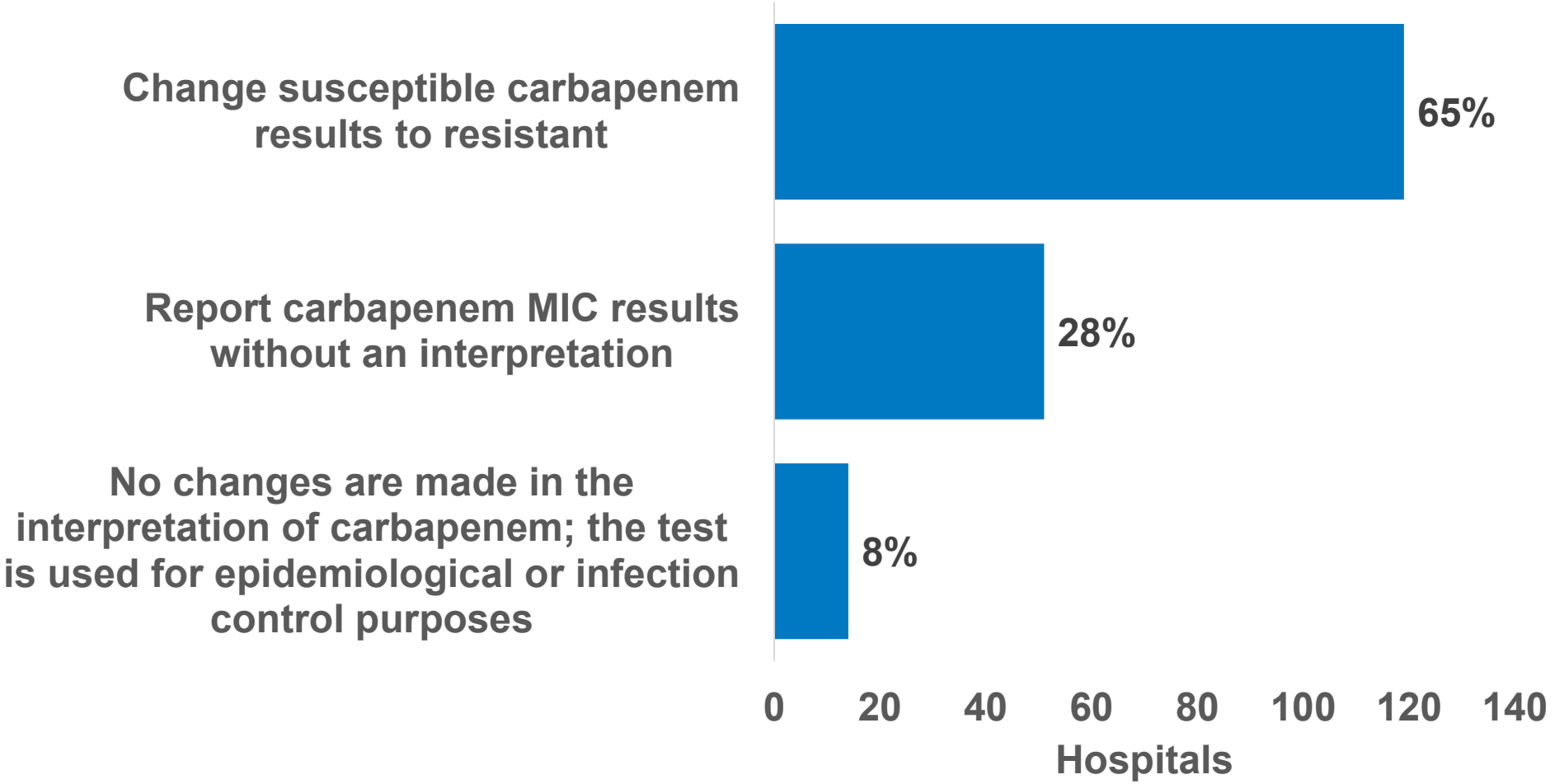
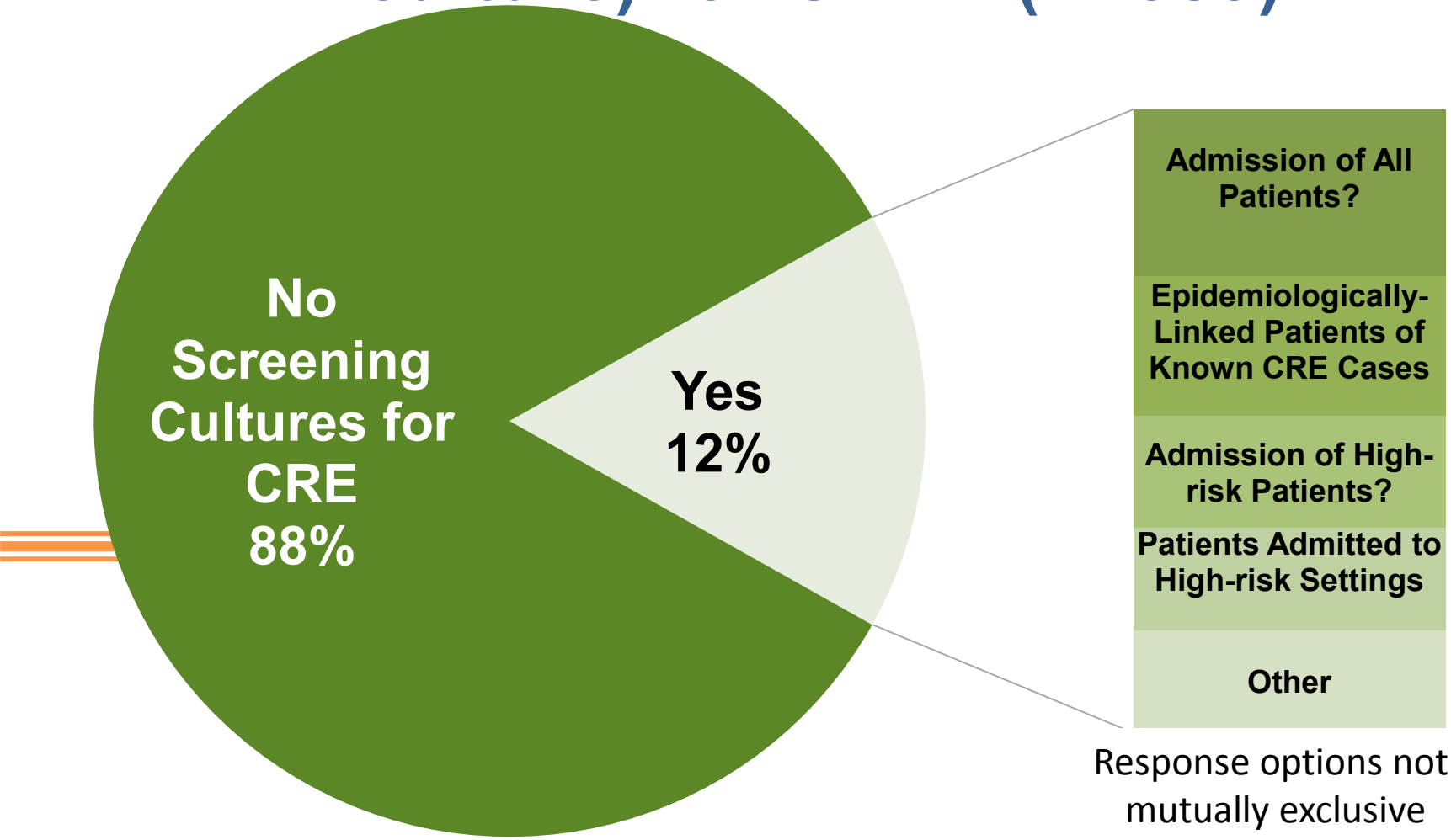
How does the laboratory report results if a carbapenemase is detected? (n=184)



Does the facility routinely perform screening testing (culture or non-culture) for CRE? (n=389)



Response options not mutually exclusive

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CRE Colonization Testing at ARLN

- West Regional ARLN offers CRE colonization when a patient/resident with CRE is identified
  - Epidemiologically linked to previously identified CRE case (roommate, residing on same unit, etc.)
  - Point prevalence survey when transmission suspected
- Contact HAI Program at HAIprogram@cdph.ca.gov to access free testing service

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CRE and Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) Testing at CDPH Microbial Diseases Laboratory (MDL)

August 2017 – October 2018

- **Stephanie Abromaitis, Ph.D. -** Foodborne & Waterborne Diseases Section
- **Peng Zhang, Ph.D. -** Bacterial Diseases Section
- **Matthew Sylvester, Ph.D. -** Core Laboratory

MDL Microbial Diseases Laboratory
Pathogen Experts Keeping California Safe

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CRE & Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) Testing at MDL

- In **2011** MDL began offering a lab-developed real time qPCR test to detect KPC

- In **2013** MDL began offering a lab-developed real time qPCR test to detect KPC and NDM

- In August **2017** MDL began offering:
  - Molecular CRE/CRPA testing: Cepheid Xpert® Carba-R
  - Phenotypic CRE/CRPA testing: Modified Carbapenem Inactivation Method (mCIM)

CRE & Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) Testing at MDL

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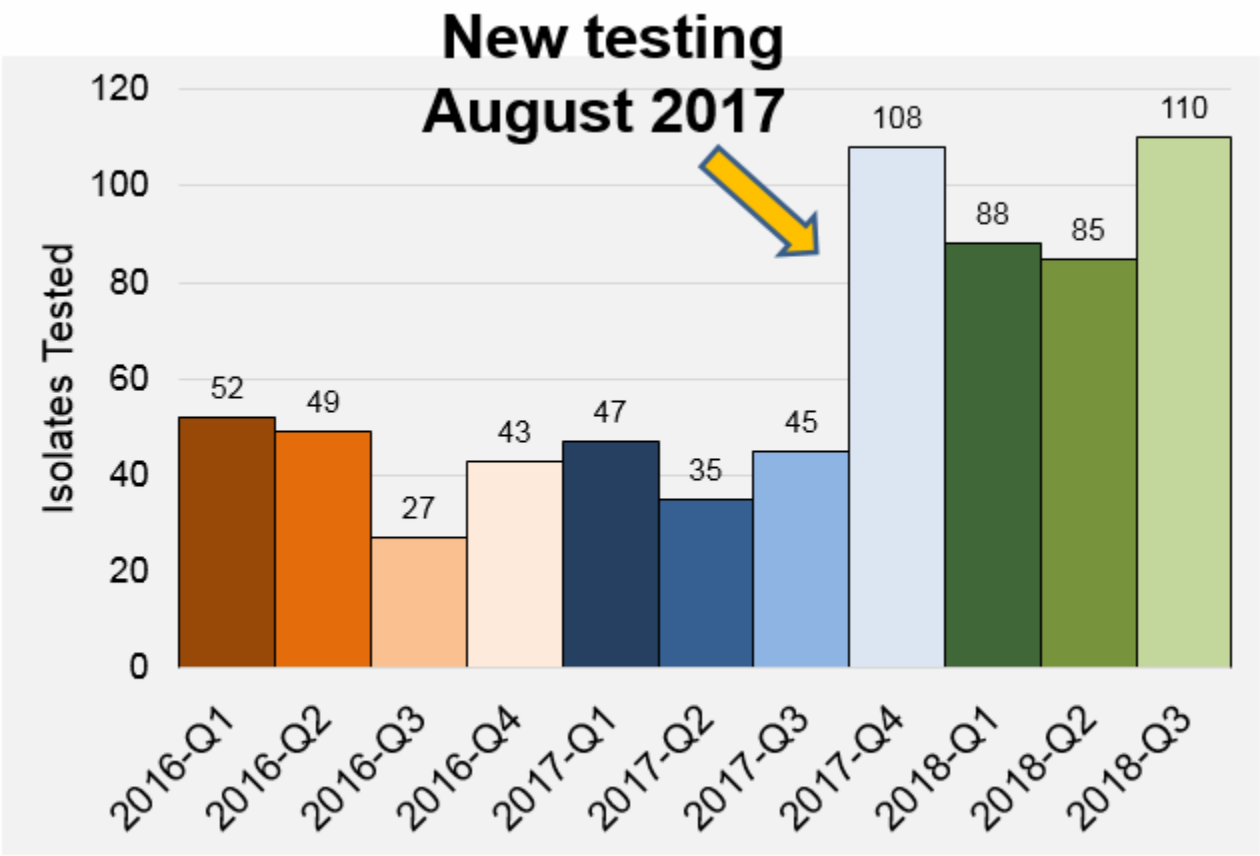
August 26, 2017 to October 31, 2018

CRE & CRPA Testing at CDPH-MDL



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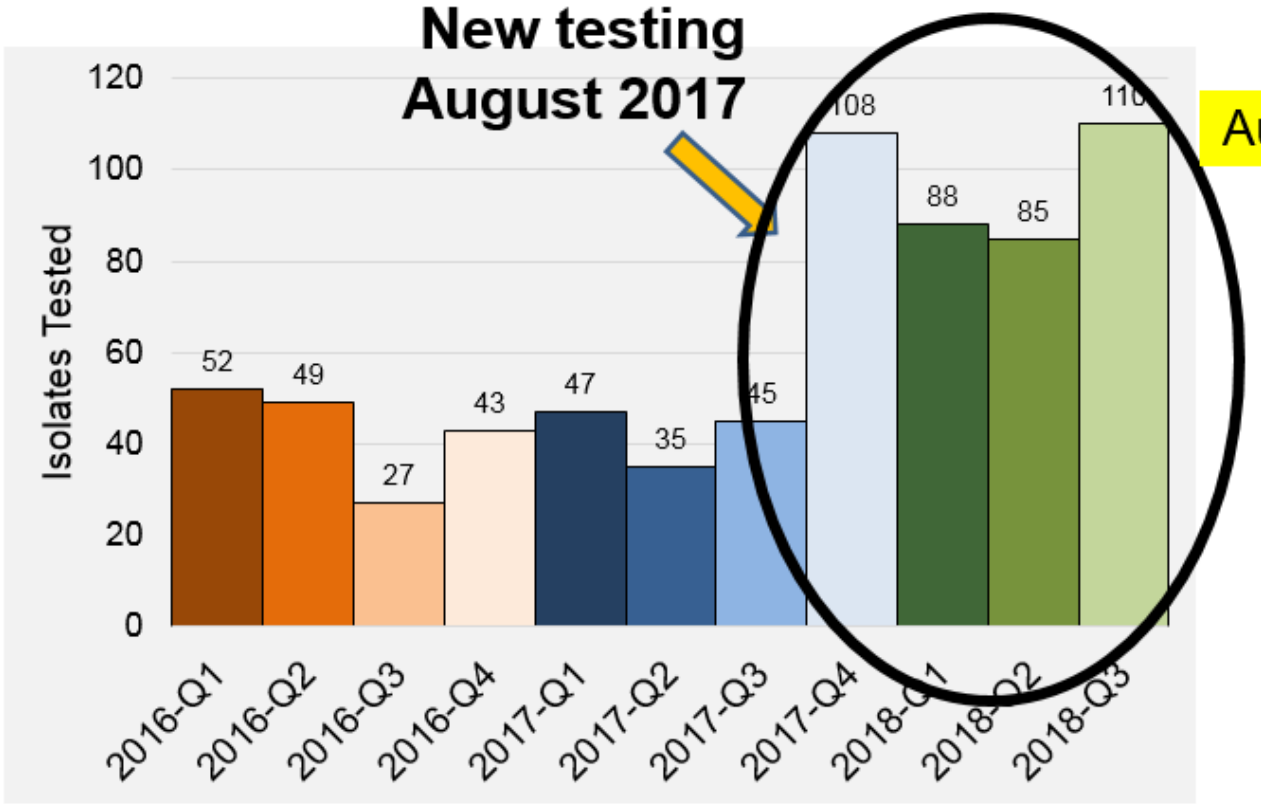
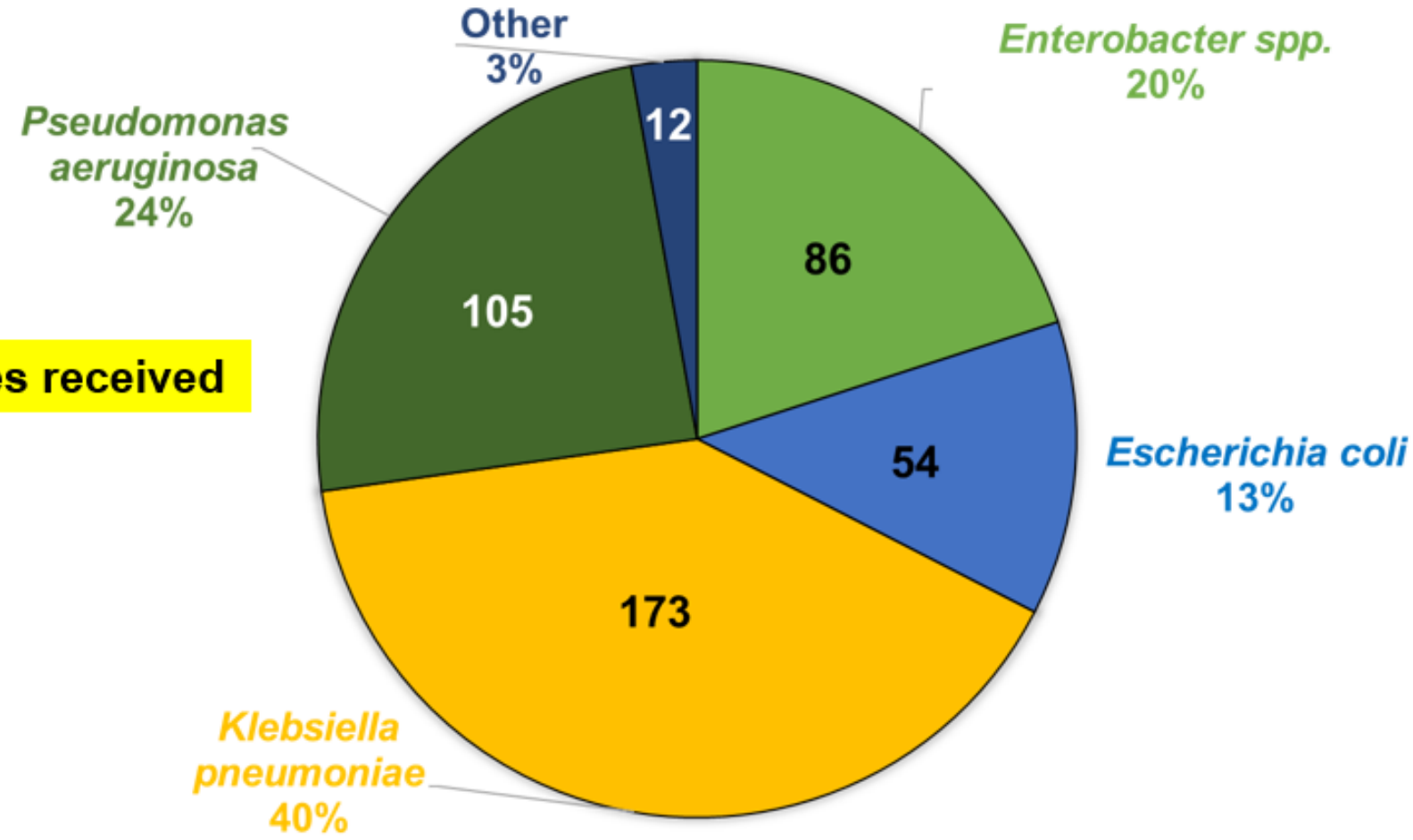
CRE & CRPA Testing at CDPH-MDL



Aug 2017 - Oct 2018

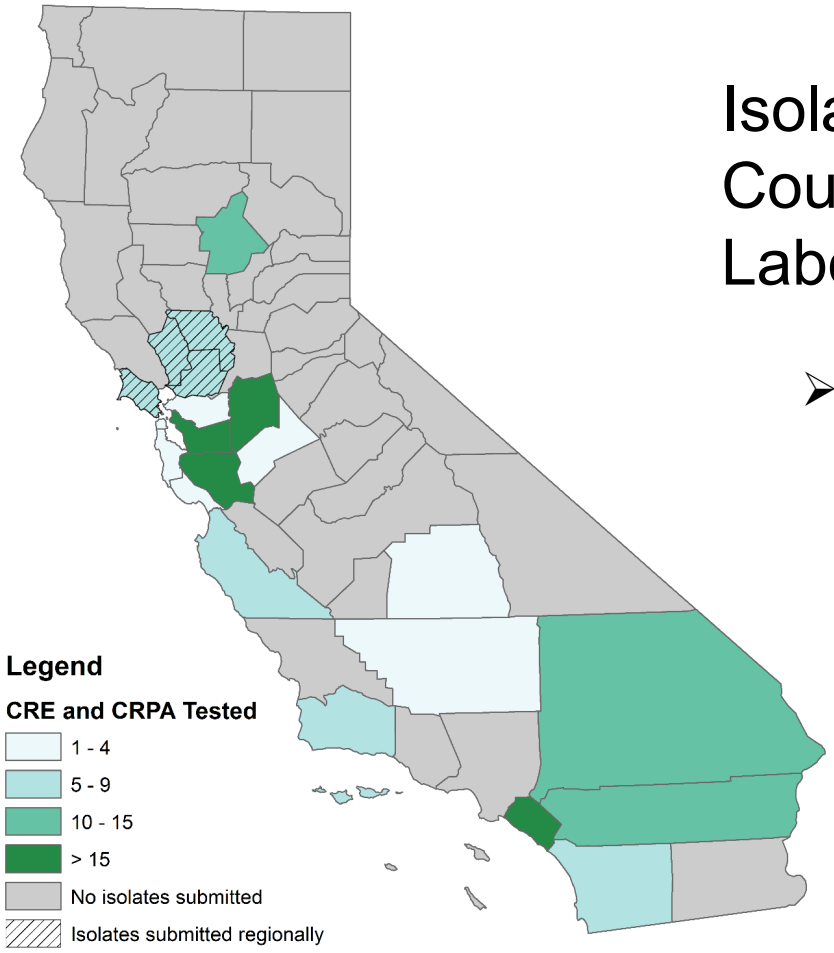
- **325** CRE
- **105** CRPA

Submissions by Organism



430 Isolates received

Submissions by County



Isolates were received from **18** County Public Health Laboratories

- 89% of total submission were from:
  - Alameda
  - Orange
  - San Joaquin
  - Santa Clara
  - Riverside
  - San Bernardino

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Submissions by County

Isolates received from **128** different healthcare facilities

Submitting County Public Health Lab (CPHL)	Origin Submitting Healthcare Facility
Orange	26
Alameda	33
San Joaquin	31
Santa Clara	8
Riverside	3
San Bernardino	4
Butte	4
Monterey	2
San Diego	1
Napa-Solano-Yolo-Marin	4
Santa Barbara	2
San Francisco	2
Stanislaus	2
Contra Costa	2
Kern	1
San Mateo	1
Santa Cruz	1
Tulare	1
Total	**128**

MDL CRE/CRPA Testing Workflow

Receive Isolate

Confirm Isolate ID

Rapid Molecular Testing

CRE/CRPA (+) Report

CRE/CRPA (-) Phenotypic Testing

MDL CRE/CRPA Molecular Testing

- **Xpert® Carba-R** FDA cleared test, approved June 2016
- The Xpert® Carba-R detects and differentiates gene sequences for the carbapenemase resistance genes
  - *bla*KPC (**KPC**)
  - *bla*NDM (**NDM**)
  - *bla*VIM (**VIM**)
  - *bla*IMP (**IMP**)
  - *bla*OXA-48 like (**OXA-48**)

MDL CRE/CRPA Molecular Testing

- There are multiple variants of each carbapenemase gene
- Not all variants of each of the “Big Five” carbapenemases are detected by the Xpert® Carba-R

MDL CRE/CRPA Molecular Testing

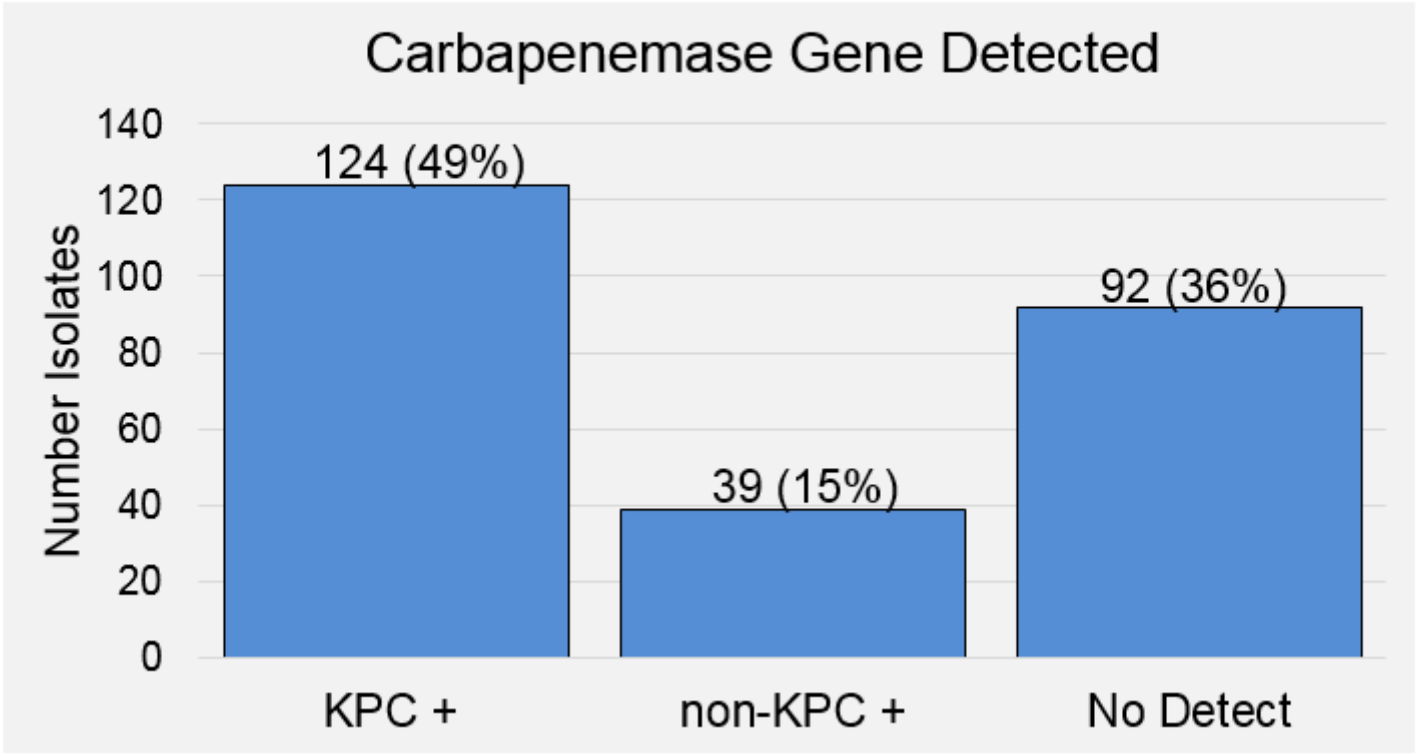
- There are multiple variants of each carbapenemase gene
- Not all variants of each of the "Big Five" carbapenemases are detected by the Xpert® Carba-R

Carbapenemase	Variants Detected by Xpert® Carba-R	Variants Not Detected by Xpert® Carba-R	Untested Variants (partial list)
IMP	IMP-1, 2, 6, 10, 11	IMP-7, 13, 14	IMP-3, 8, 9, 19, 20, 21, 22, 24, 25, 27, 30, 31, 33, 37, 40, 42

Adapted from Cepheid Xpert® Carba-R 510(k) Substantially Equivalent documents

MDL CRE Molecular Testing Results

255 CRE isolates were tested by Xpert® Carba-R



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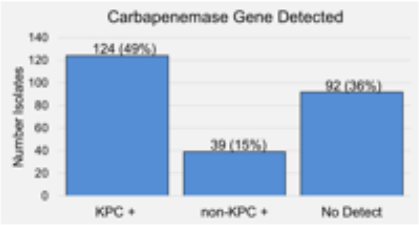
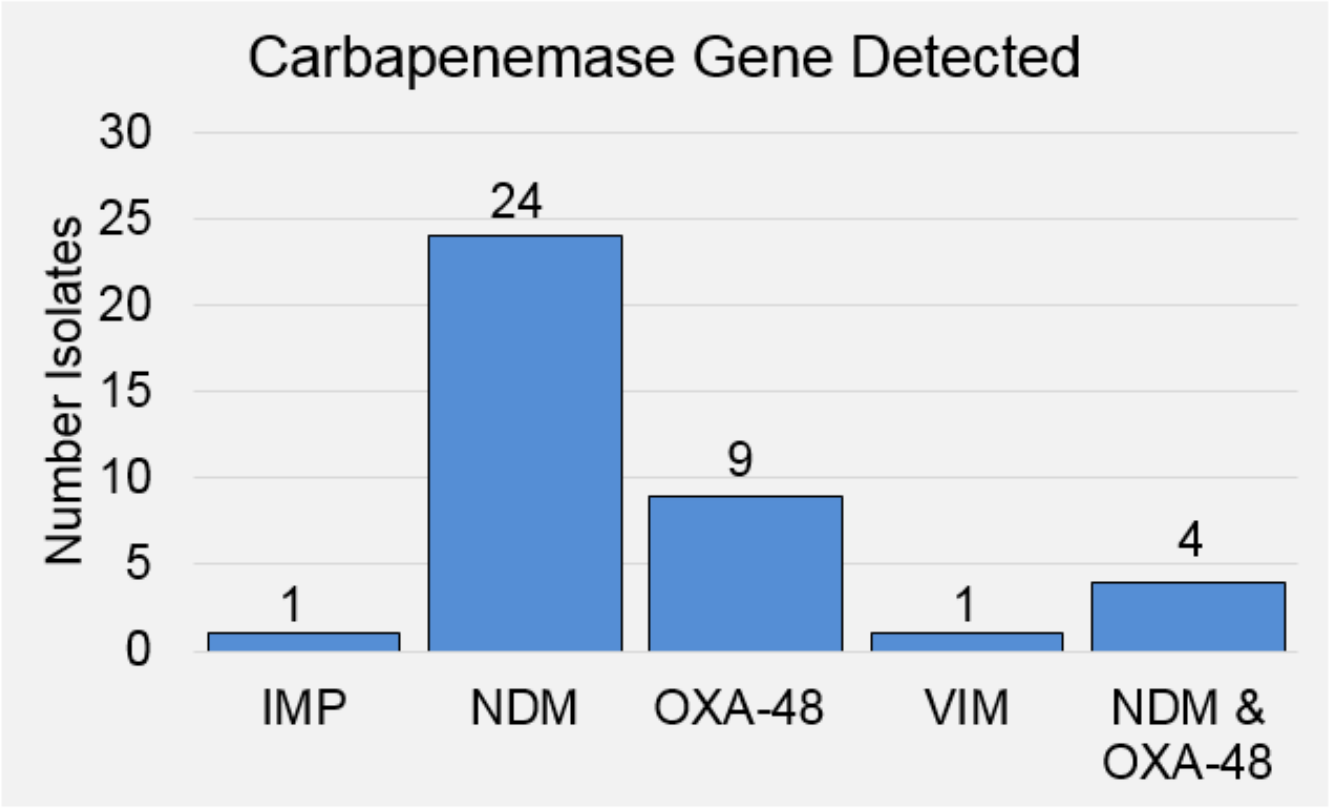
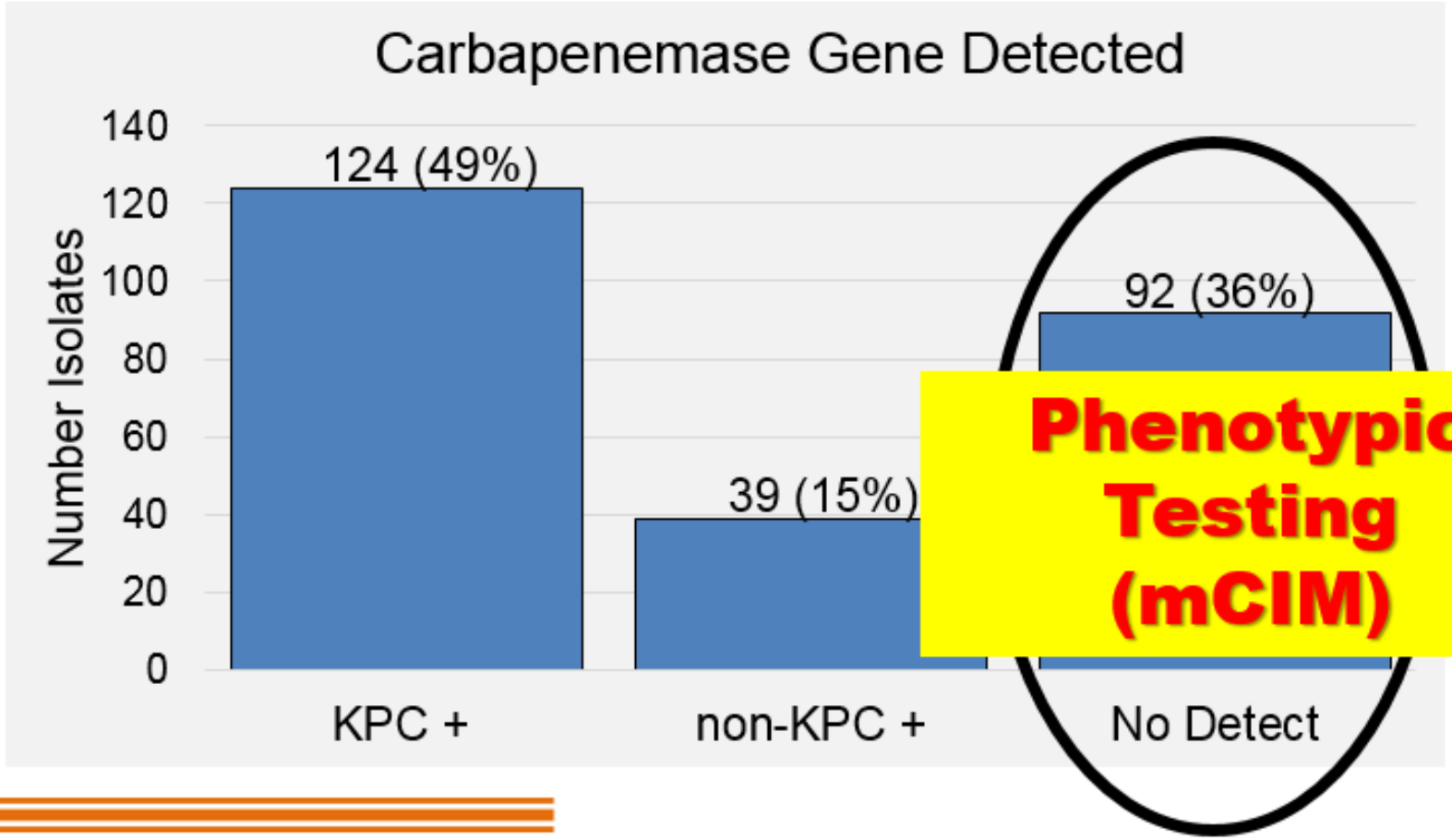
MDL CRE Molecular Testing Results



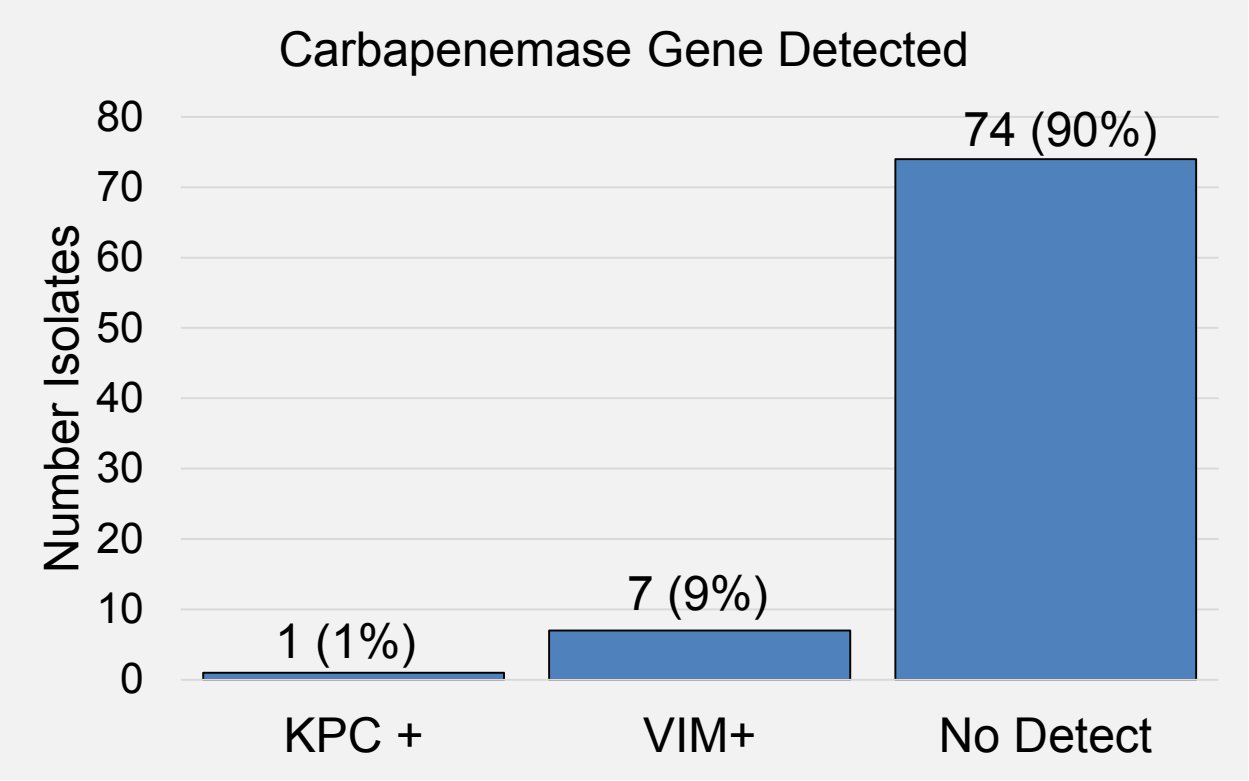


MDL CRE Molecular Testing Results



MDL CRPA Molecular Testing Results

82 CRPA isolates were tested by Xpert® Carba-R



MDL CRPA Molecular Testing Results

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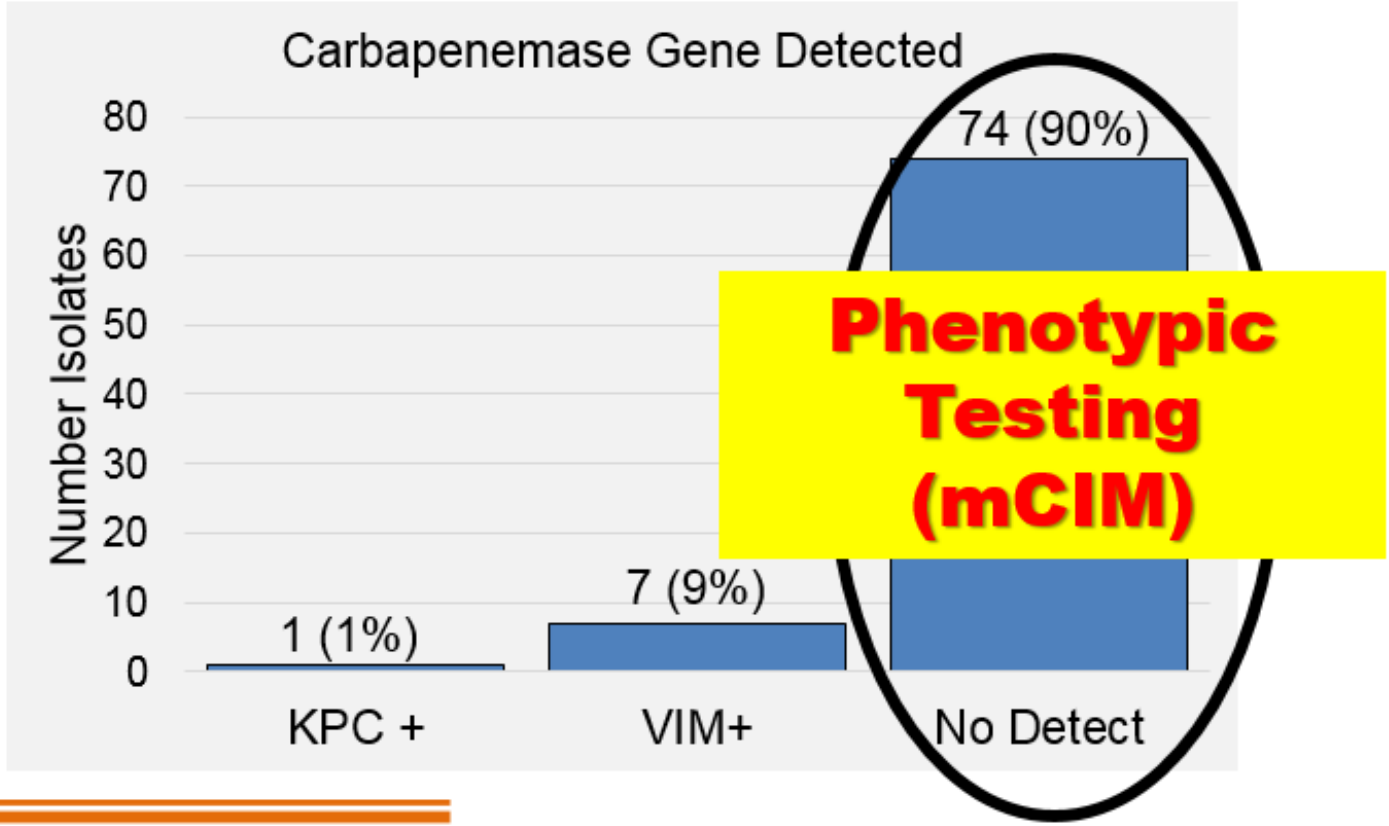


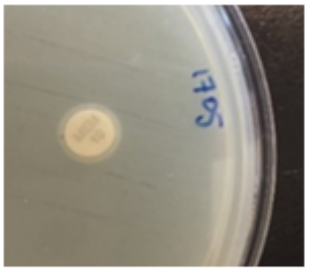
Phenotypic Testing for Carbapenemase Production

	Phenotypic Test Used for Epidemiological or Infection Control-Related Testing		
	Carba NP	**Modified Carbapenem Inactivation Method (mCIM)**	**EDTA-modified Carbapenem Inactivation Method (eCIM)**
Organisms	*Enterobacteriaceae* and *P. aeruginosa* that are not susceptible to one or more carbapenems	*Enterobacteriaceae* and *P. aeruginosa* that are not susceptible to one or more carbapenems	*Enterobacteriaceae* that are positive by mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary
Limitations	Special reagents are needed and certain carbapenemase types (eg, OXA-type) are not consistently detected	Requires overnight incubation	Requires overnight incubation and only valid when mCIM is positive

CLSI M100 28th ed.

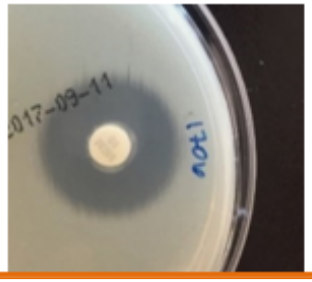
Phenotypic Testing for Carbapenemase Production Modified Carbapenem Inactivation Method - mCIM

- **How does mCIM work?**
  - Meropenem in a disk is inactivated (hydrolyzed) by the carbapenemase produced by bacteria in a bacterial suspension.
  - The inactivation of meropenem is determined by transferring and incubating the disk on a plate with meropenem-susceptible indicator *E. coli*.



➢ **Carbapenemase producer**

Meropenem in the disk is inactivated and allows indicator *E. coli* to grow. No zone or very small zone of inhibition around the disk.

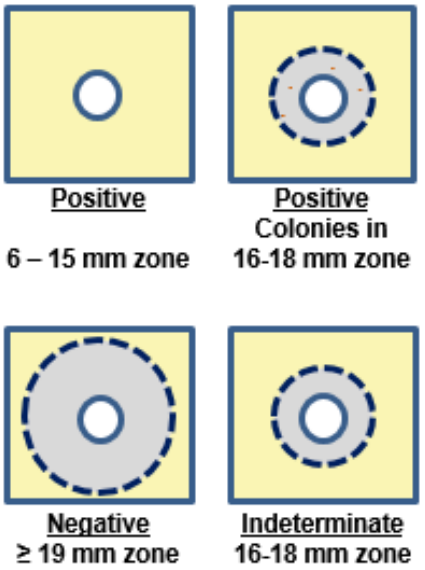


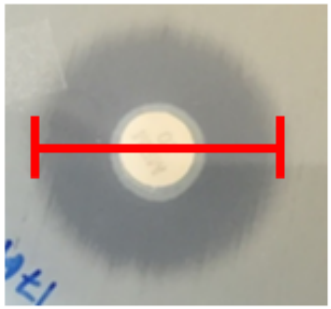
➢ **Non- carbapenemase producer**

Meropenem in the disk retains its activity and inhibits the growth of indicator *E. coli*. A zone of inhibition around the disk.

Phenotypic Testing for Carbapenemase Production Modified Carbapenem Inactivation Method - mCIM

- **How is mCIM Result Interpreted?**
  - **Carbapenemase positive**: zone 6-15 mm or presence of colonies within a 16-18 mm zone.
  - **Carbapenemase negative**: zone ≥19 mm.
  - **Indeterminate**: zone 16-18 mm.





CLSI M100 28th ed.

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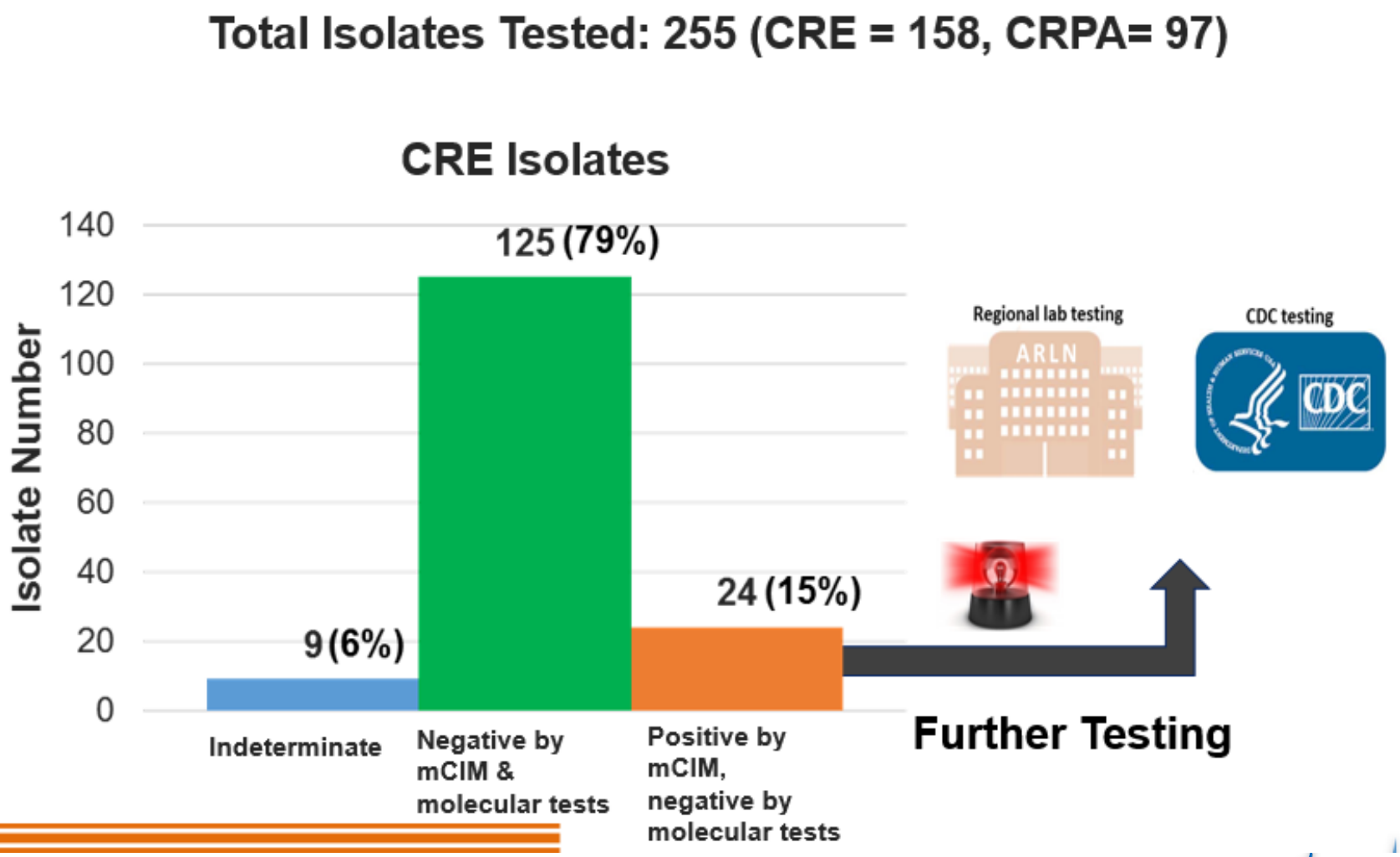
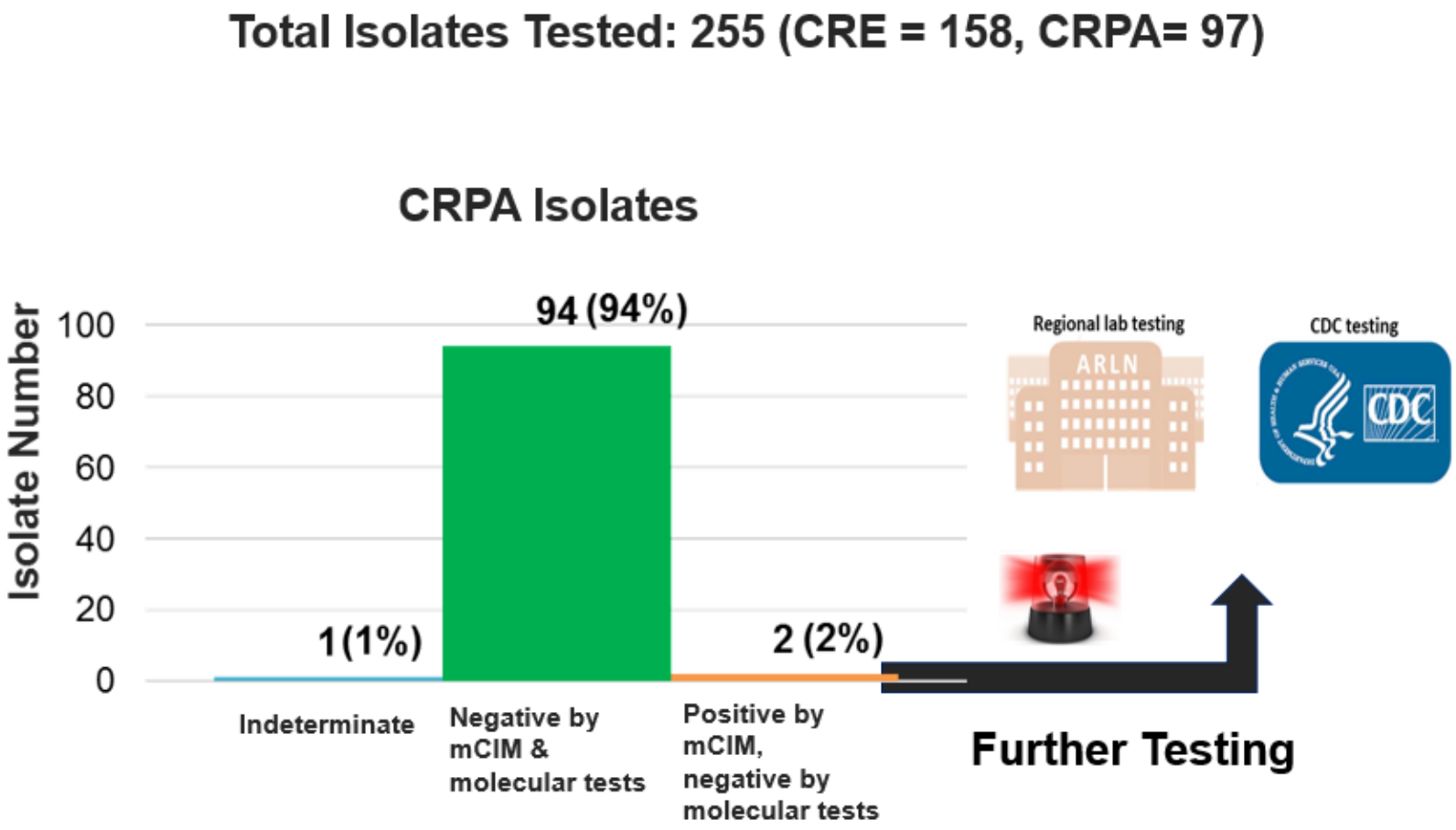
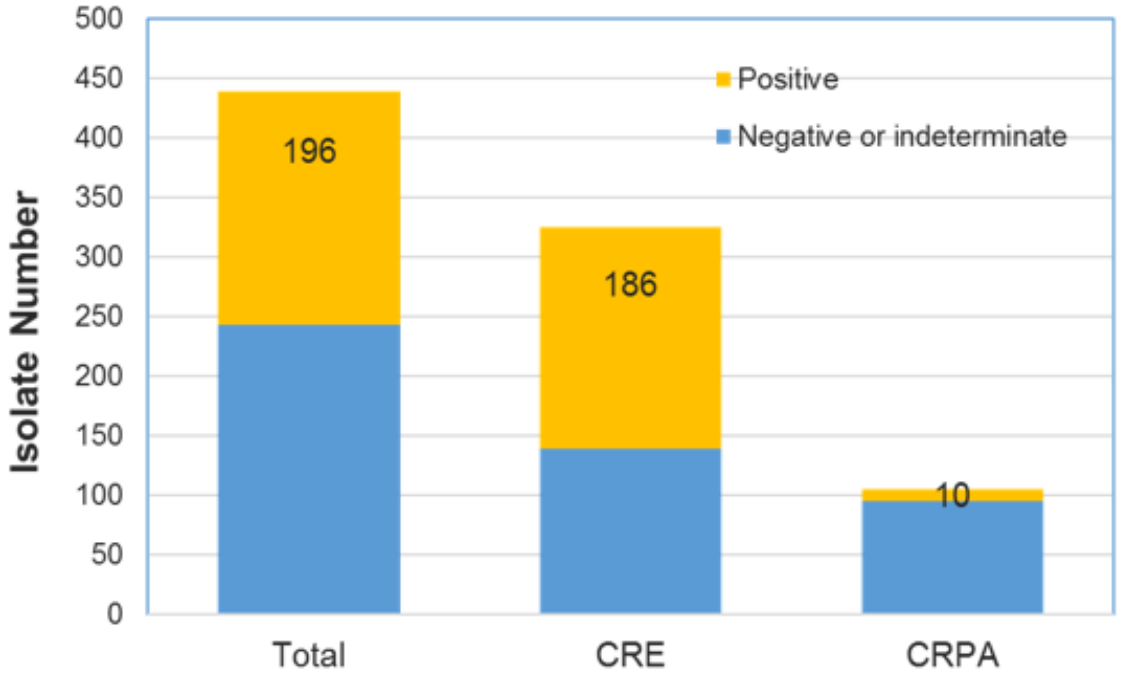
mCIM Testing on Carbapenemase Gene Undetected CRE/CRPA Isolates



mCIM Testing on Carbapenemase Gene Undetected CRE/CRPA Isolates



Summary of Carbapenem-Resistant Organisms Tested in MDL



- Total isolates tested: 430
  - 196 isolates (45.6%) are positive for carbapenemases
- Suspected CRE isolates tested: 325
  - 186 isolates (57.2%) are CP-CRE
- Suspected *P. aeruginosa* isolates tested: 105
  - 10 isolates (9.5%) are CP-CRPA

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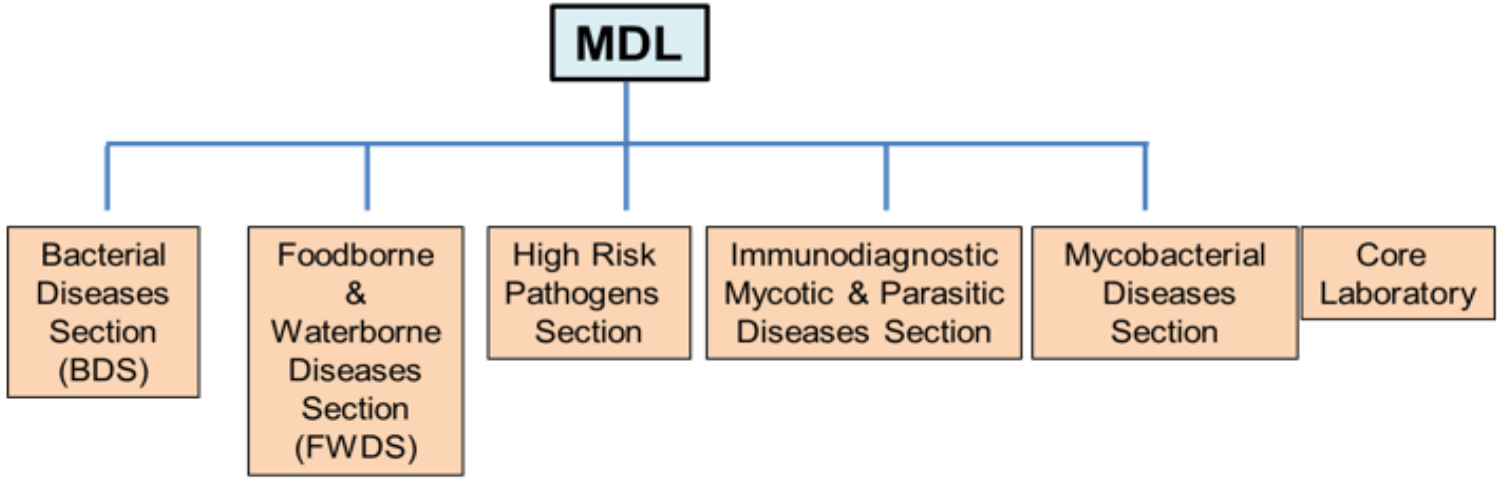
Suggestions and Reminders for Submission

- **Send isolates that are resistant to *at least* one carbapenem**
  - AST results must be included with the submission
- **Avoid sending multiple isolates from same patient collected on same day**
- **For isolates that have already been tested using molecular testing:**
  - Send those that have tested negative
  - Do not send positive isolates for confirmation without prior consultation with CDPH HAI Program and MDL
- **Make sure field for original submitting facility is complete**
- **MUST get prior approval from CDPH HAI Program for submission of organisms other than *Klebsiella* spp., *E. coli*, *Enterobacter* spp., and *P. aeruginosa* (HAIProgram@cdph.ca.gov)**
  - CDPH HAI Program may request additional epi information

Carbapenemase Testing at MDL/CDPH



Testing available	mCIM	Xpert® Carba-R, lab-developed qPCR				Bacterial WGS
Testing coming soon	Sensititre for CRE/CRPA					
Future	Lab-developed qPCR for colistin resistant genes					

Carbapenemase Gene Detection and Genetic Relatedness by Whole Genome Sequencing

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Pathogen Experts Keeping California Safe

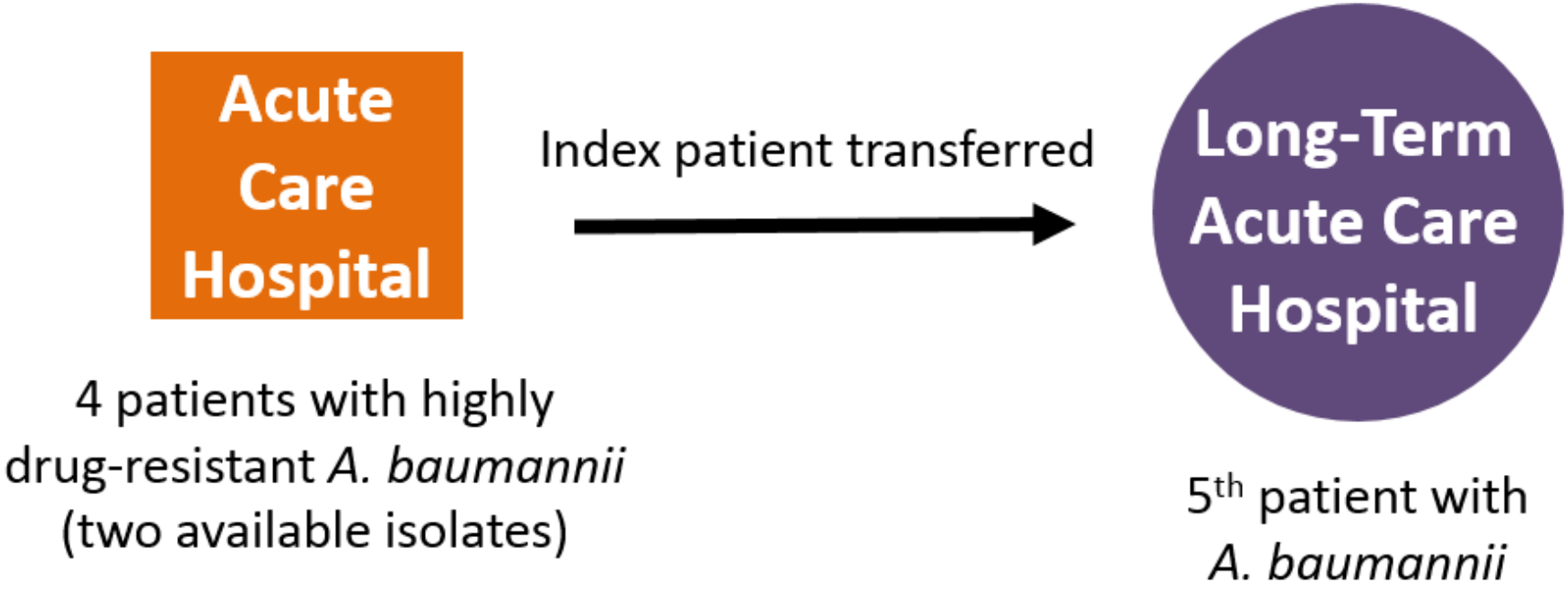
California Department of PublicHealth

Bacterial Whole Genome Sequencing and Carbapenemases

- MDL offers a CLIA-validated Whole Genome Sequencing (WGS) assay on the Illumina MiSeq sequencing platform

- This additional genetic testing may be useful for:
  - Species confirmation
  - Identification of antibiotic resistance genes
  - Establishing relatedness
  - *Multilocus sequence typing (MLST)*
  - *Virulence gene prediction*

Carbapenem-resistant *Acinetobacter baumannii* Outbreak in California



Species Confirmation with WGS

Center for Genomic Epidemiology

KmerFinder 3.0 results:

Template	Num	Score	Expected	Template length	query_coverage	Coverage	Depth
NZ_CP015483.1 Acinetobacter baumannii strain ORAB01, complete genome	1211	142489	0	148957	96.41	97.68	0.96

Species is confirmed to be *Acinetobacter baumanii*

Antibiotic Resistance Gene Prediction from WGS

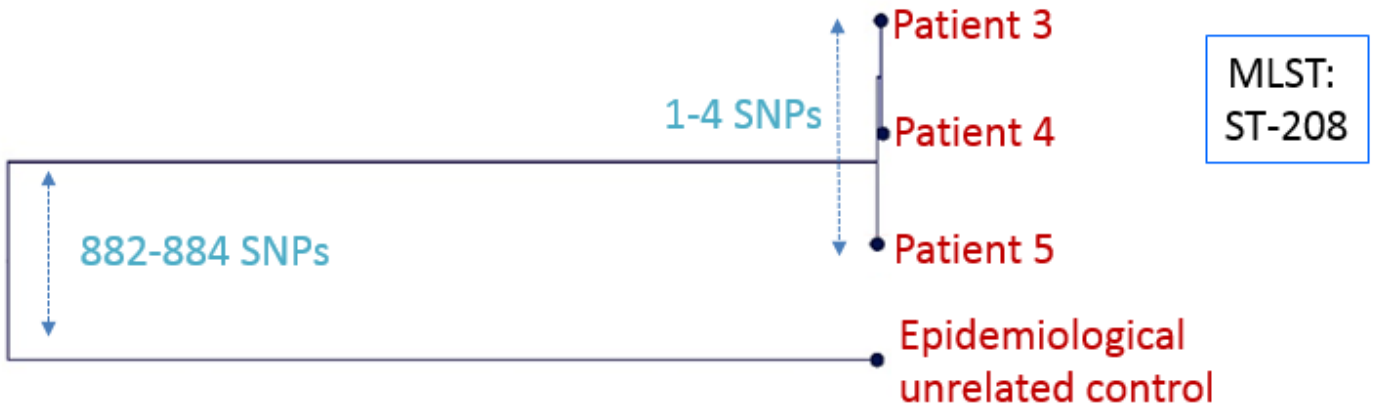
Center for Genomic Epidemiology

ResFinder-3.1 Server - Results

Beta-lactam					
Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype
blaOXA-66	100.00	825/825	M18C00240_S5_L001_R1_001_5_(paired)_trimmed_(paired)_contig_18	247314..248138	Beta-lactam resistance
blaADC-25	99.91	1152/1152	M18C00240_S5_L001_R1_001_5_(paired)_trimmed_(paired)_contig_19	307335..308486	Beta-lactam resistance
blaOXA-237	100.00	831/831	M18C00240_S5_L001_R1_001_5_(paired)_trimmed_(paired)_contig_25	79..909	Beta-lactam resistance

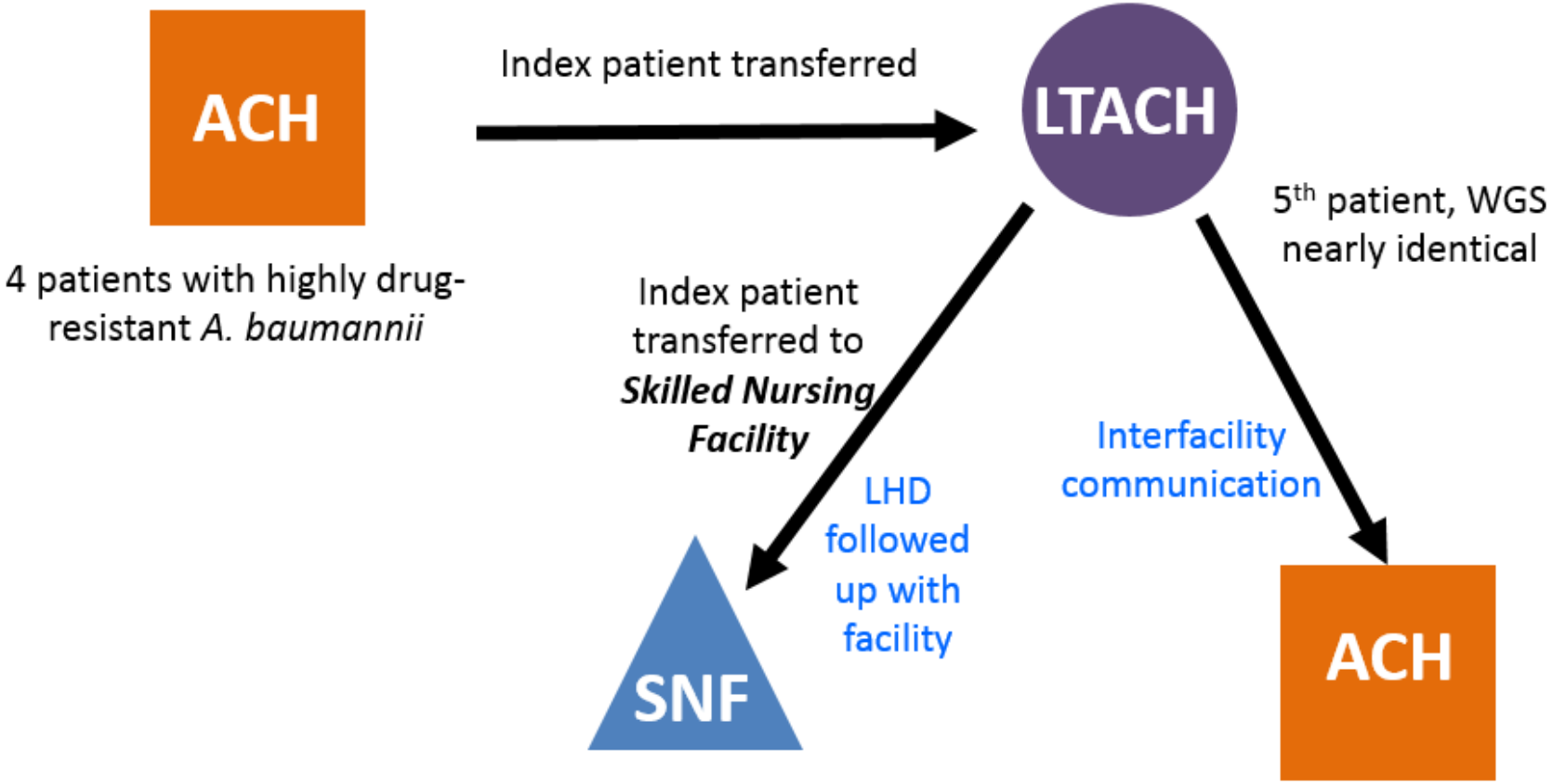
Gene encoding OXA-237 carbapenemase detected in a carbapenem-resistant *Acinetobacter baumanii*

Phylogenetic Analysis Supports Isolate Relatedness



- Sequencing helped to identify an outbreak of *A.baumanii* carrying a rare OXA-237 carbapenemase gene
- Closely-related isolates suggest a transmission route between facilities

Sequencing Informed Follow-up



Isolate Submission

Due to limited testing capacity, before sending isolates to CDPH MDL for whole genome sequencing, facilities or public health departments must obtain prior approval from the CDPH HAI Program by emailing HAIprogram@cdph.ca.gov. The HAI Program will request additional epidemiological information to determine if whole genome sequencing is feasible at that time.

Questions?

- Please type all questions into the chat box and the presenters will answer them.
- A copy of the slides and a recording of the webinar will be posted on the CDPH HAI Program website, and all webinar participants will be notified when they are available.
- For any questions about this presentation or ARLN Targeted Surveillance, please email HAIProgram@cdph.ca.gov.
- Sign up for the California AR Lab Network mailing list (https://www.surveymonkey.com/r/ARLabNetworkContact) for information on future webinars