Northeast Region Antibiotic Resistance Laboratory Network Newsletter

Volume 1 • Issue 1 • December 2020

AR Lab Network All Region Meeting

December 7-9, 2020

In lieu of the in-person regional meetings cancelled due to the SARS-CoV-2 pandemic, there will be a virtual meeting targeted for the first week of December.

This meeting will be held daily for 3-4 hours. As in past meetings, the goal will be to:

- facilitate information sharing between jurisdictions,
- strengthen the relationship between HAI coordinators and laboratory staff
- and find potential solutions to common barriers.

You will need to have an account before attempting to register. If you do not have an account, below is the link in which you can setup your account.

Create An Account Link

A Look Back at **AR LAB NETWORK** Regional Meetings

2017 AR Lab Network 2-Day Regional Laboratory Training Participants

2018 AR Lab Network Regional Meeting Participants

Have an idea, articles or poster to be included in our next newsletter? email Shirley.Kelly-Parson@health.ny.gov

2019 AR Lab Network Regional Meeting Participants

2019 AR Lab Network Regional Meeting Clinical Lab Participants

2019 *Candida auris* Workshop

NEW STAFF

JUNE CHAN, PHD – APHL AR FELLOW

After majoring in microbiology at the University of Maryland, June has continued to study pathogenic microorganisms in a variety of contexts — pursuing research on methicillin-resistant *Staphylococcus aureus* (MRSA) at the National Institutes of Health and studying aspects of the gut microbiome and its potential impacts on colorectal cancer at Johns Hopkins University. As an APHL Fellow at the Wadsworth Center, June carries out routine testing to detect and monitor emerging carbapenem resistance found in clinical isolates and colonization screenings. June is also conducting applied research to improve molecular diagnostics for carbapenem-resistant organisms (CROs), to understand the spread of CROs better, and to establish CRO prevalence in high-risk populations (e.g., solid organ transplant recipients). From March to June 2020, June volunteered in the COVID-19 surge capacity response at the New York State Department of Health, participating in all aspects of the Virology Section molecular testing workflow and in the Diagnostic Immunology immunoassay used to test the serum of potential convalescent plasma donors for SARS-CoV-2 antibodies.

CATHARINE PRUSSING, MPH, PHD – APHL BIOINFORMATIC FELLOW

Kate received a master's degree in Infectious Disease Epidemiology from Johns Hopkins University and completed an Applied Epidemiology fellowship at the NYC Department of Health and Mental Hygiene. She obtained her Ph.D. from the University at Albany Department of Biomedical Sciences in the laboratory of Dr. Jan Conn, where she investigated the effect of anthropogenic environmental modifications on the biting behavior, population genetics and ecology of the south American malaria vector *Nyssorhynchus darlingi*. Kate's fellowship project looks at the relatedness of bacterial plasmids carrying antibiotic resistance genes using long-read sequencing data and was a joint project of the Bioinformatics Core and Bacteriology Laboratory.

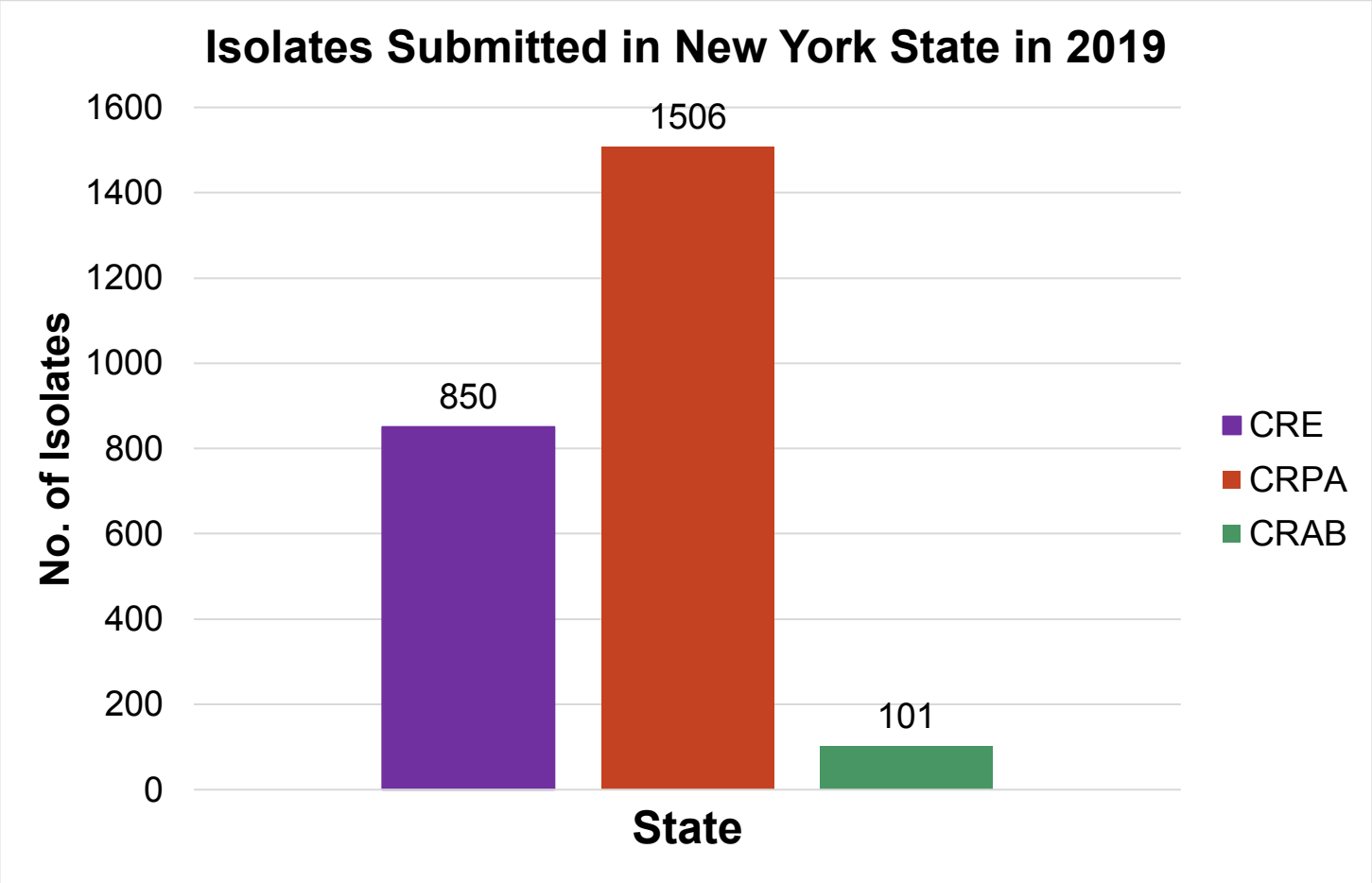
SHANNON KILBURN – AR/EPI LIASION

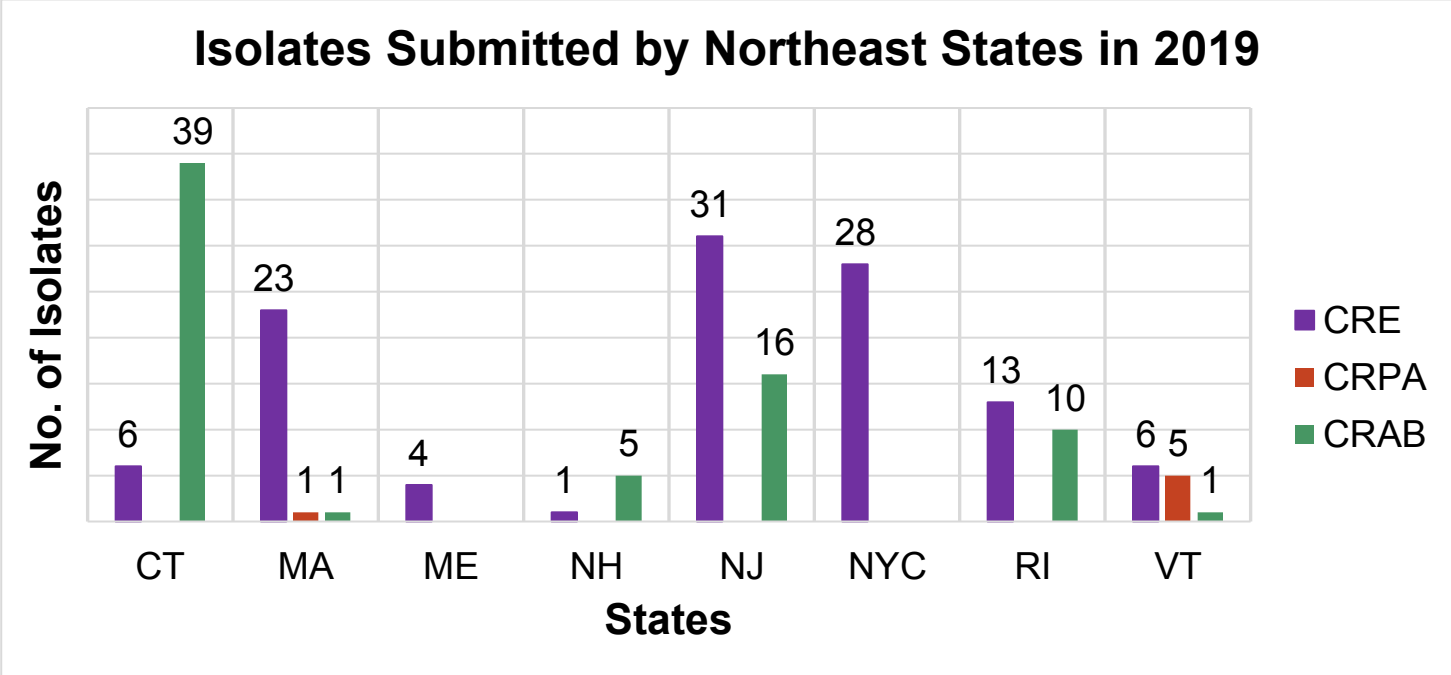
Shannon graduated from the University at Albany School of Public Health in 2019 with a Master's in Public Health, Epidemiology concentration. In addition, she has a first degree in Biological Sciences. While a public health student, she had the opportunity to gain professional public health experience through different capacities in different divisions at the New York State Department of Health. She worked part-time as a Graduate Assistant in Office of Public Health Practice, where she assisted with and completed various projects. Her first internship was the Summer of 2018 at the AIDS Institute where she conducted an epidemiological study using New York State HIV surveillance data. Shannon's second internship was at the Division of Epidemiology, January to May 2019, where she assisted with the investigation of Blastomycosis in Eastern Upstate New York and conducted a study using the data that was gathered. Shannon joined the AR Lab at Wadsworth Center as a Research Scientist/Epidemiologist in February 2020.

KELLI HAGER, MPH – APHL AR FELLOW

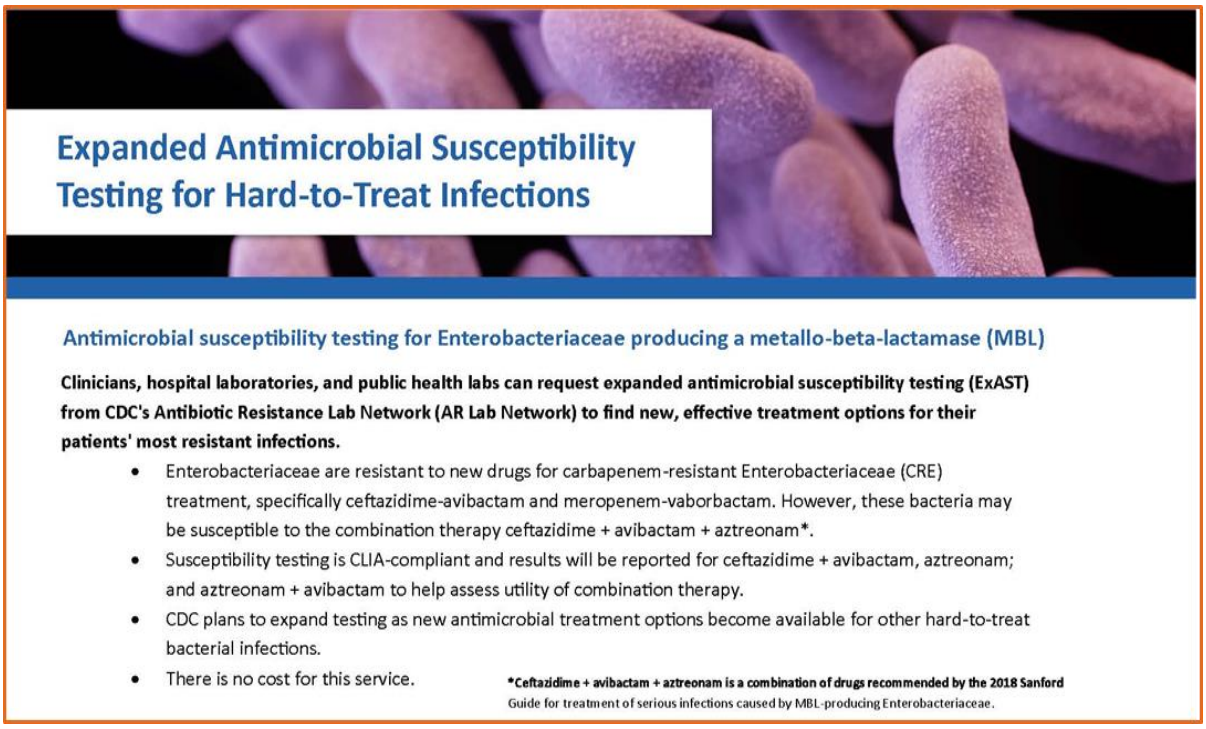
Kelli Hager discovered her passion for laboratory sciences while working for IDEXX Laboratories where she served as the Immunology Safety Advisor and Quality Control Manager. She left IDEXX to pursue a Master's in Public Health with a concentration in Infectious Disease and Vaccinology at UC Berkeley. Her graduate thesis was to develop and validate a quantitative reverse transcriptase-PCR assay for the detection of pyrethroid resistance in West Nile vectors. This assay was used to map pyrethroid resistance in Alameda County, California and found that even though ACMAD applied less than 10 ounces of adulticides to the County, resistance still remained. Kelli believes that the more we know about the resistance mechanisms of emerging pathogens, the more equipped we will be to develop therapeutics and prevention strategies against them. We are excited to have Kelli in the AR Lab Network as an antimicrobial resistance fellow.

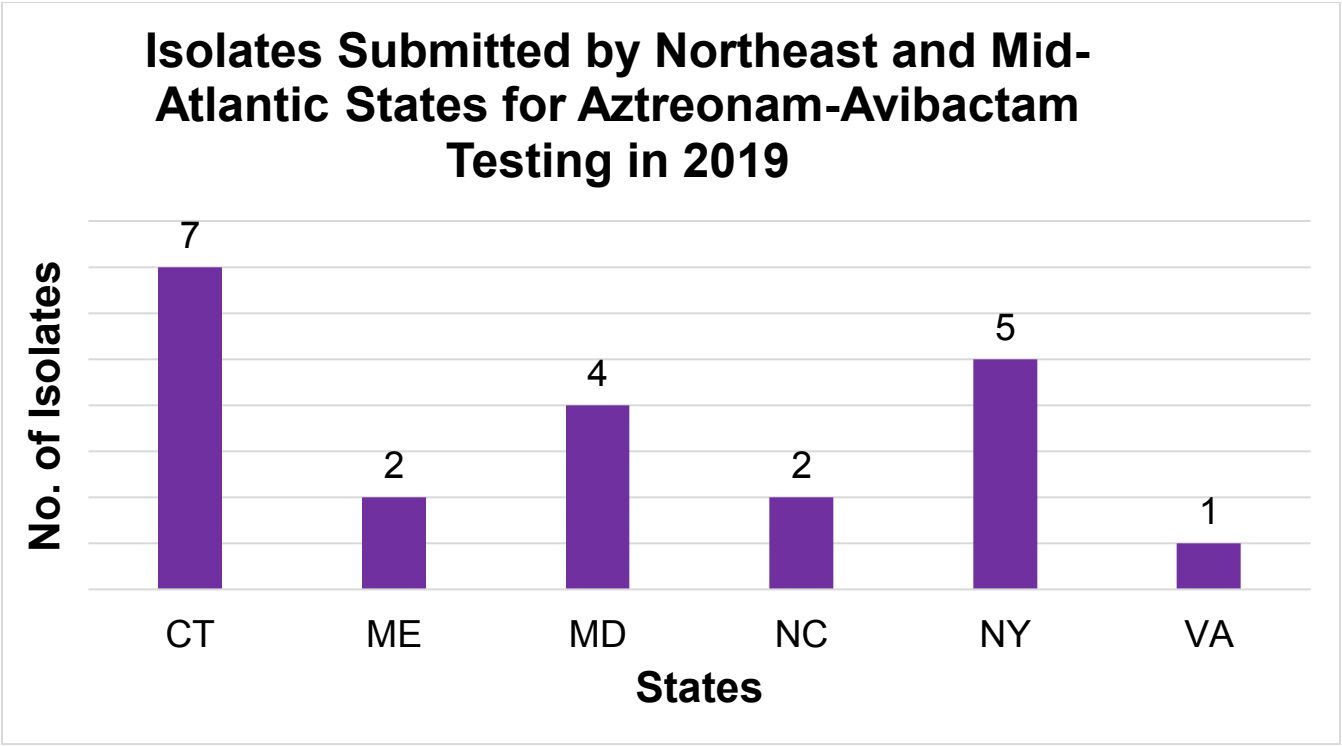
CARBAPENEM RESISTANT ORGANISM TESTING DATA



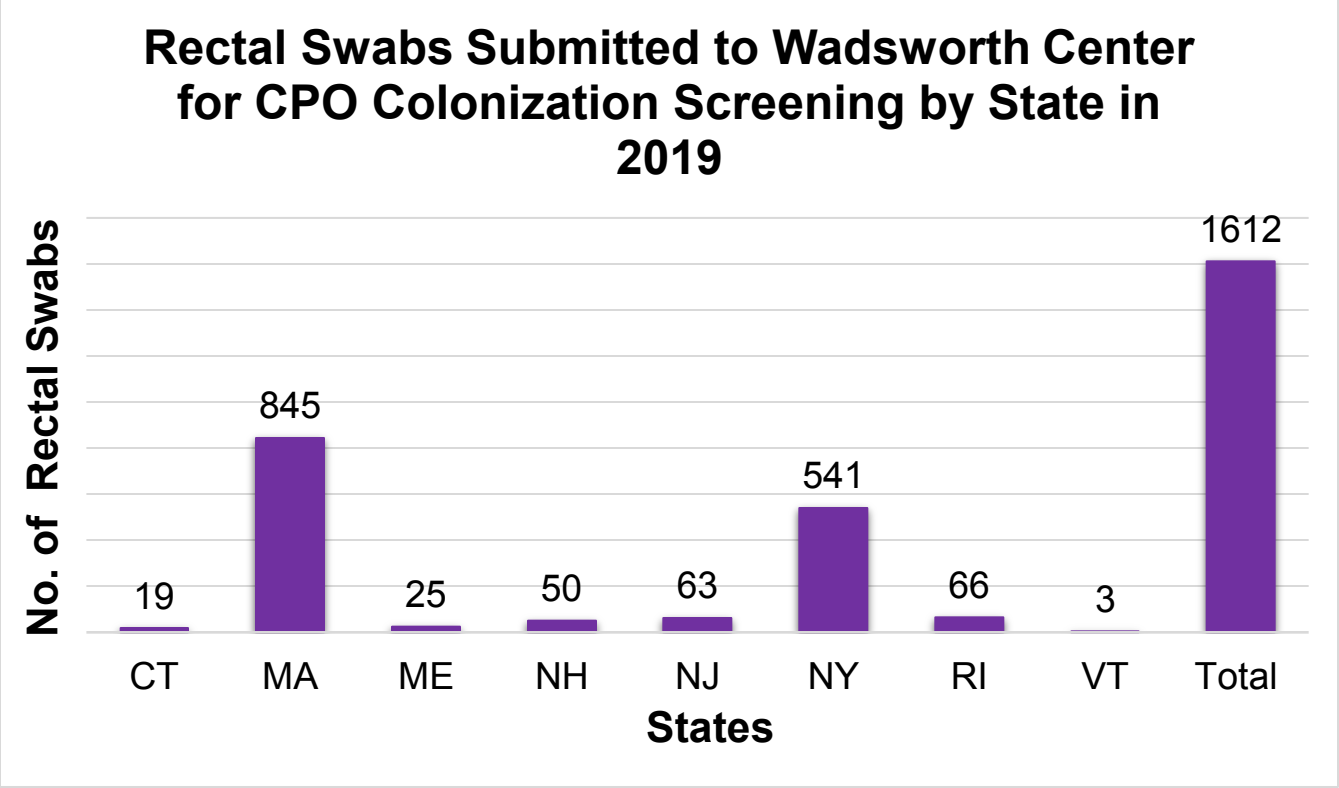


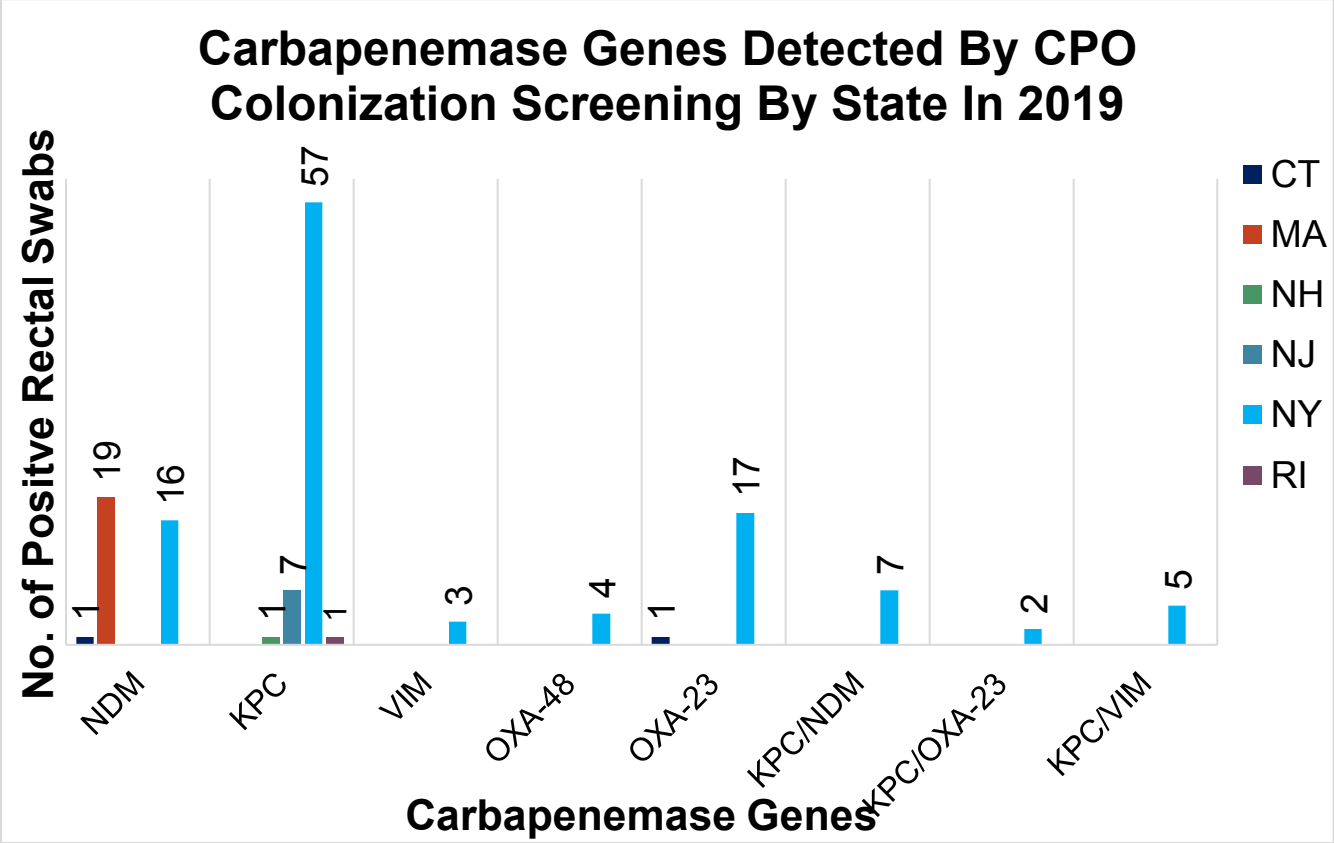
ISOLATES SUBMITTED BY NORTHEAST AND MID-ATLANTIC STATES FOR AZTREONAM-AVIBACTAM TESTING IN 2019





CARBAPENEMASE COLONIZATION SCREENING DATA





CANDIDA AURIS PUBLICATIONS

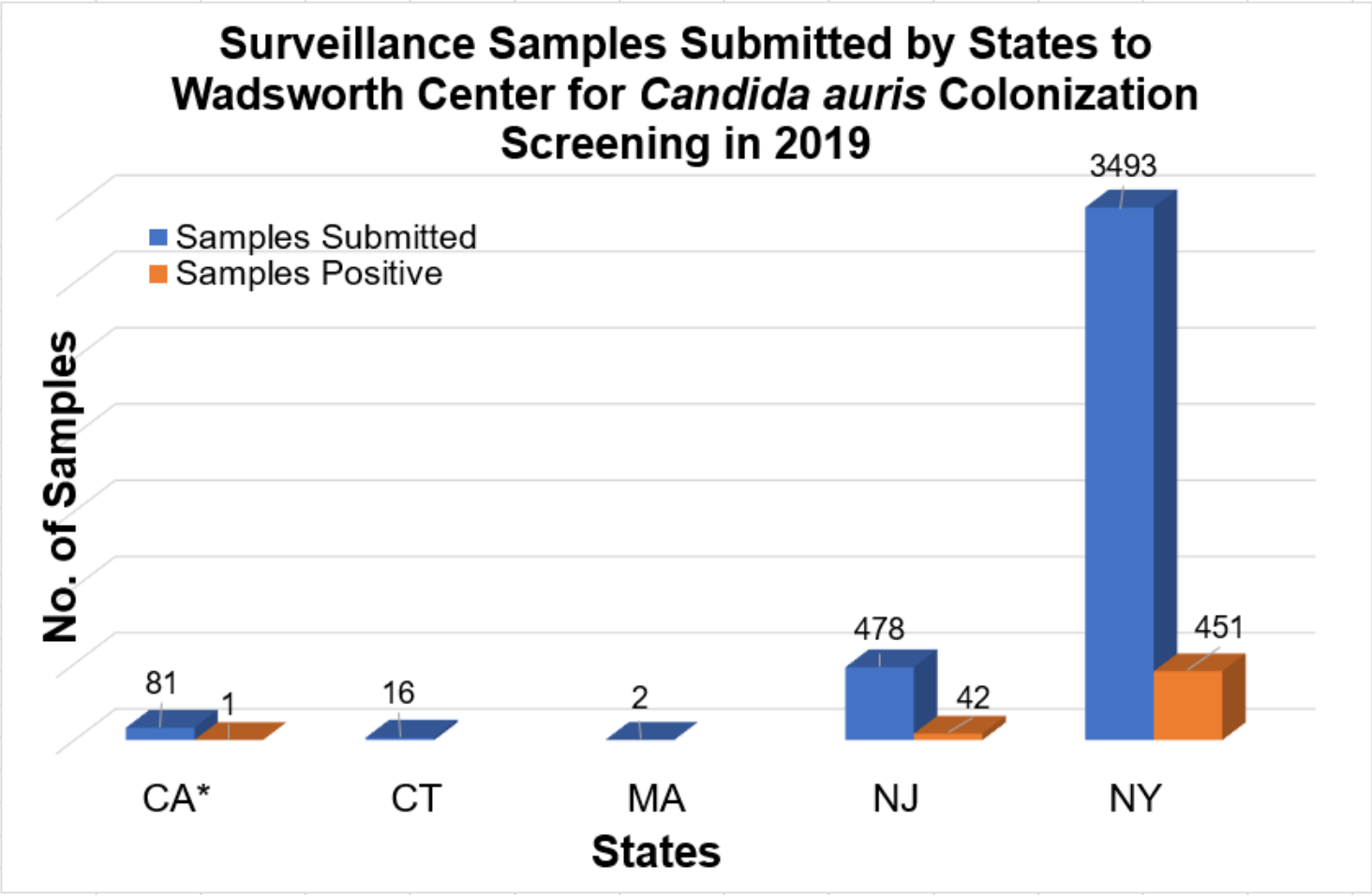
1. Zhu Y, Kilburn S, Kapoor M, Chaturvedi S, Shaw KJ, Chaturvedi V. *In Vitro* Activity of Manogepix against Multidrug-Resistant and Panresistant Candida auris from the New York Outbreak. Antimicrob Agents Chemother. 2020 Oct 20;64(11). doi: 10.1128/AAC.01124-20. Print 2020 Oct 20. PubMed PMID: 32839219.

2. Rossow J, Ostrowsky B, Adams E, Greenko J, McDonald R, Vallabhaneni S, Forsberg K, Perez S, Lucas T, Alroy K, Slifka KJ, Walters M, Jackson BR, Quinn M, Chaturvedi S, Blog D. Factors associated with Candida auris colonization and transmission in skilled nursing facilities with ventilator units, New York, 2016-2018. Clin Infect Dis. 2020 Sep 28;. doi: 10.1093/cid/ciaa1462. [Epub ahead of print] PubMed PMID: 32984882.

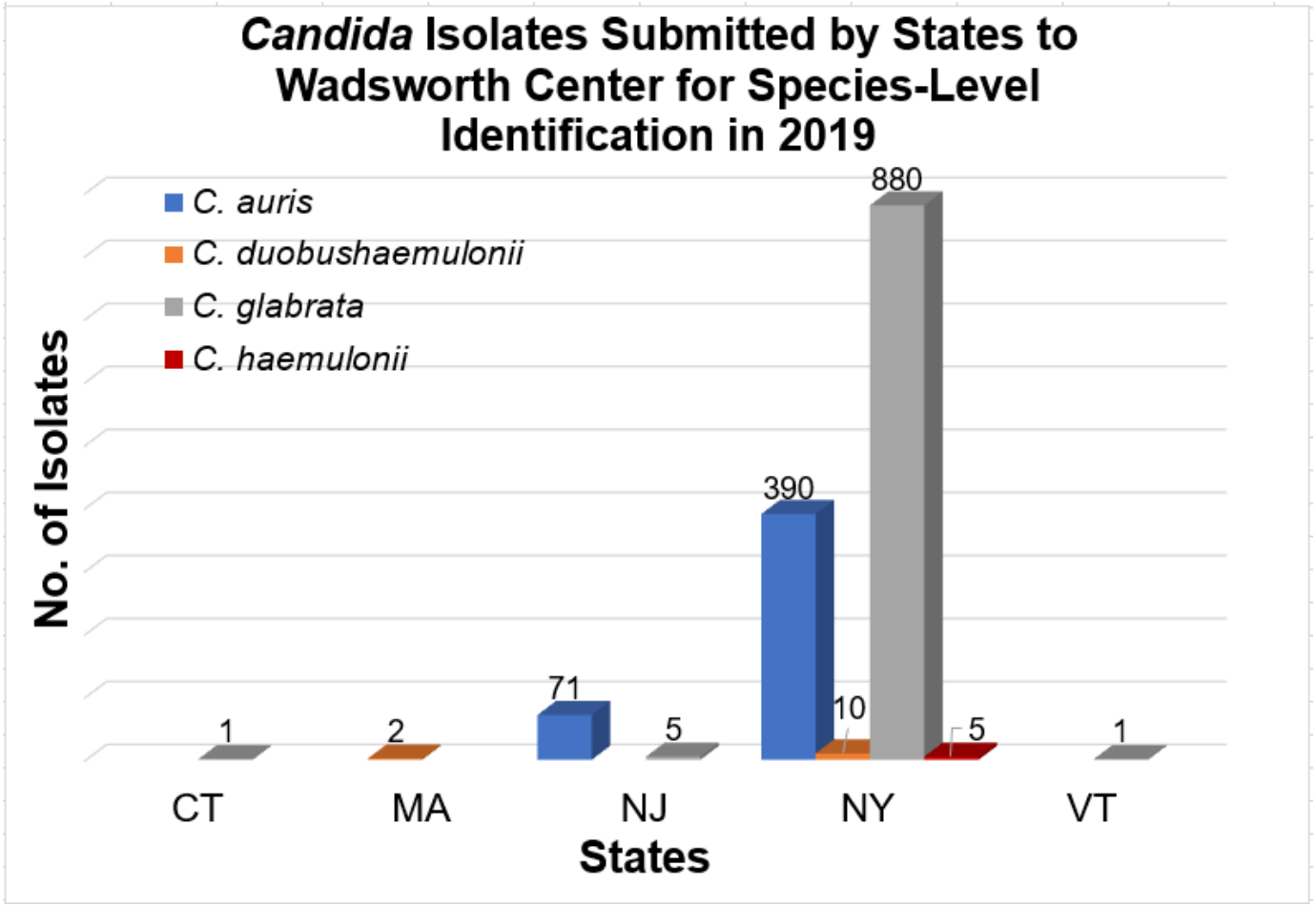
3. O"Brien B, Liang J, Chaturvedi S, Jacobs JL, Chaturvedi V. Pan-resistant Candida auris: New York subcluster susceptible to antifungal combinations. Lancet Microbe. 2020 August; 1(5):e193-e194.

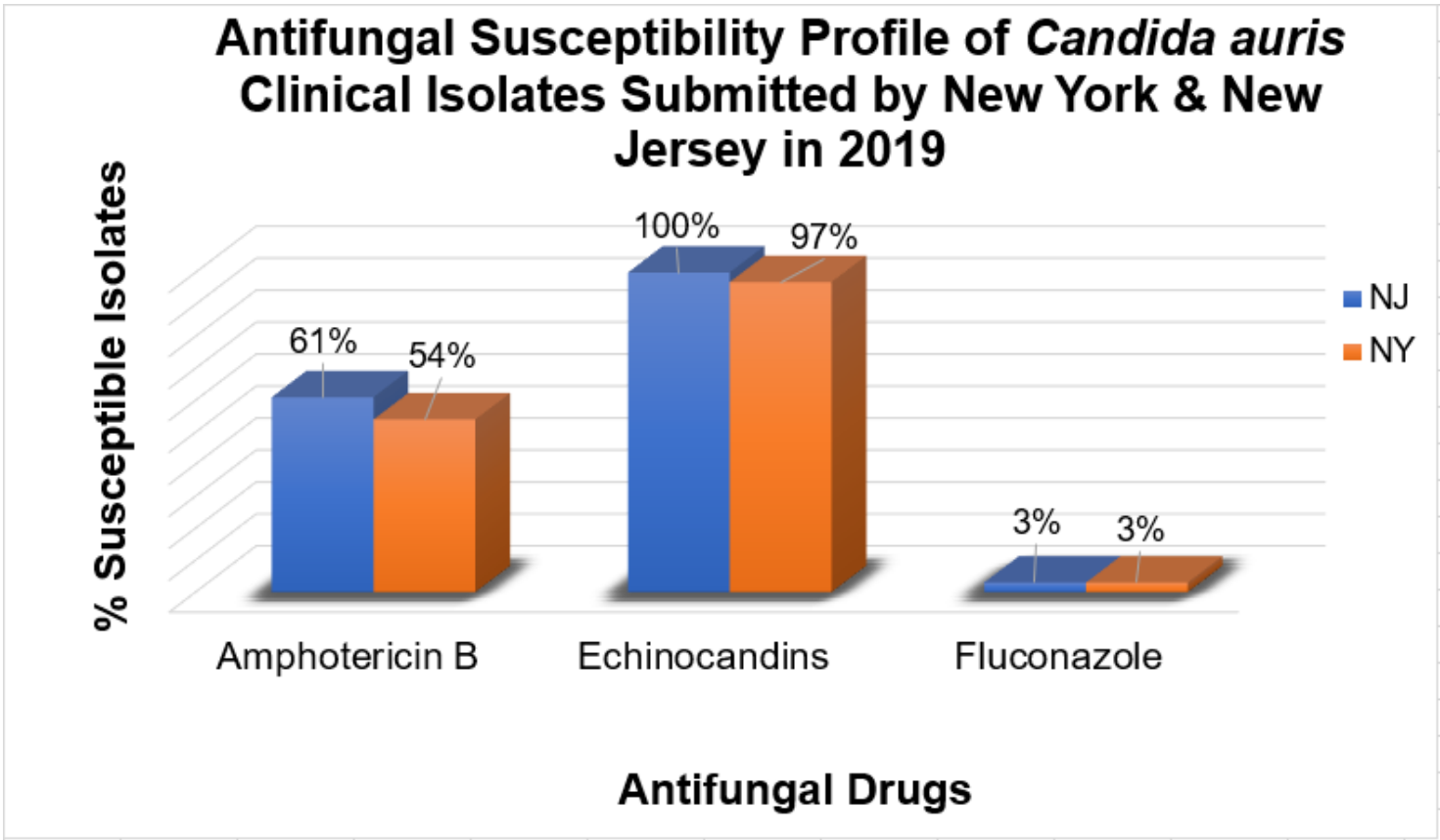
4. Zhu Y, O'Brien B, Leach L, Clarke A, Bates M, Adams E, Ostrowsky B, Quinn M, Dufort E, Southwick K, Erazo R, Haley VB, Bucher C, Chaturvedi V, Limberger RJ, Blog D, Lutterloh E, Chaturvedi S. Laboratory Analysis of an Outbreak of Candida auris in New York from 2016 to 2018: Impact and Lessons Learned. J Clin Microbiol. 2020 Mar 25;58(4). doi: 10.1128/JCM.01503-19. Print 2020 Mar 25. PubMed PMID: 31852764; PubMed Central PMCID: PMC7098748.

CANDIDA AURIS DATA



*CA Samples were tested as part of surge capacity.


Candida Isolates Submitted by States to Wadsworth Center for Species-Level Identification in 2019
C. auris
C. duobushaemulonii
C. glabrata
C. haemulonii
No. of Isolates
1
2
71
5
390
10
880
5
1
CT
MA
NJ
NY
VT
States


Antifungal Susceptibility Profile of *Candida auris* Clinical Isolates Submitted by New York & New Jersey in 2019
% Susceptible Isolates
61%
54%
100%
97%
3%
3%
NJ
NY
Amphotericin B
Echinocandins
Fluconazole
Antifungal Drugs

Pan-resistant *Candida auris*: New York subcluster susceptible to antifungal combinations

Recently we reported the emergence of pan-resistance in *Candida auris* from New York.[1] Since 2016, New York hospitals and health-care facilities have faced the highest number of clinical cases and surveillance cases of *C auris* in the USA.[2] Effective strategies for the prevention, control, and treatment of *C auris* are still being developed; however, the development of strategies could be complicated by the observed pan-resistance. A conceptual framework supports using drug combinations to combat the threat of antimicrobial resistance.[3] Accordingly, we studied strains of pan-resistant *C auris* to find out whether they are susceptible to combinations of current antifungal drugs and what genetic features distinguish pan-resistant *C auris* found in New York. Details of the methods are in the appendix (pp 2–5). Four pan-resistant *C auris* strains were 100% inhibited in vitro by combinations of two antifungal drugs using fixed concentrations achievable in vivo. Expectedly, flucytosine combinations with either amphotericin B, azoles, or echinocandins were the most effective (figure, A; appendix pp 8, 12). Time-kill analysis showed that every two-drug combination caused a reduction in growth greater than 2 $\log_{10}$ relative to the same drugs used separately, which is suggestive of fungicidal action (figure, B; appendix pp 9, 13). These results are consistent with our recent publication on the efficacy of antifungal combinations for New York *C auris* strains with various multidrug-resistance patterns (appendix p 25). On the basis of a comparative genomic analysis we found four pan-resistant *C auris* strains with mutations in 11 gene targets associated with major antifungal drugs (appendix pp 18–24).

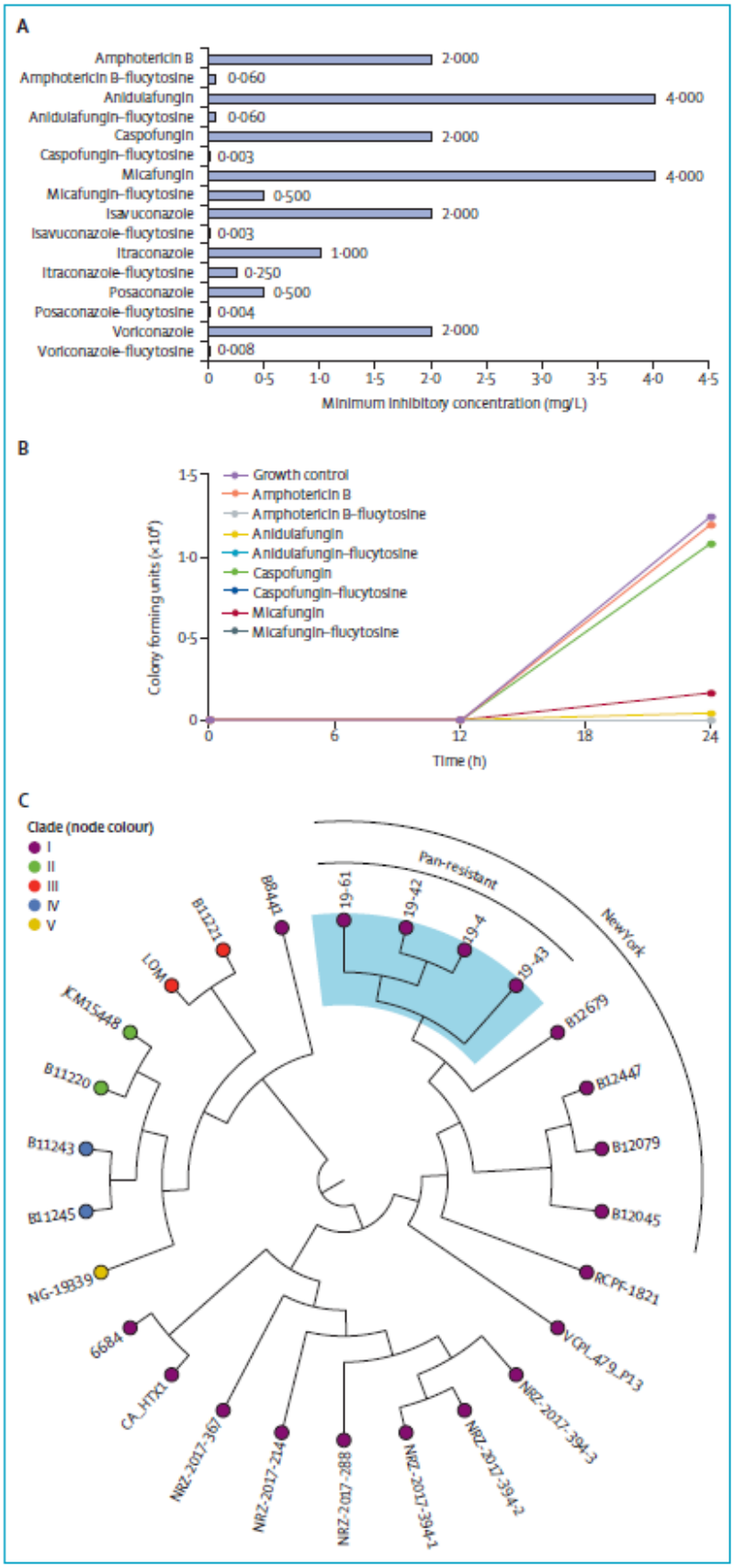


Figure: Characterisation of pan-resistant *Candida auris*
(A) Pan-resistant *C auris* susceptible to two-drug combinations flucytosine and amphotericin B, azole or echinocandins, representative data for *C auris* 19–4.
(B) Time-kill curve with amphotericin B, or echinocandins alone, or in combination with flucytosine, representative data for *C auris* 19–43.
(C) Four pan-resistant *C auris* strains, distinct sub-cluster among New York strains. Neighbour joining tree derived from whole genome assemblies of strains representing all known clades.

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See Online for appendix

These findings are similar to other reports[4,5] for drug-resistant *C auris* strains. All four pan-resistant strains constituted a distinct subcluster among New York strains (figure, C; appendix pp 16, 17). Two different non-synonymous mutations in the predicted sequence of the FKS1 protein were observed. *C auris* strains 19–4 and 19–61 showed FKS1 Ser635Pro, whereas *C auris* strains 19–42 and 19–43 showed FKS1 Ser635Tyr (appendix p 19). These mutations are in a known hotspot of *FKS1*, a glucan synthase gene, and the target of echinocandin antifungal drugs. Finally, pan-resistance appears to exact a fitness cost in at least two *C auris* strains (19–42 and 19–43), which showed an extended lag growth phase (appendix p 14) and high resistance to caspofungin (>16 mg/L). Further results are presented in the appendix (pp 2–25). Our findings suggest that pan-resistant *C auris* strains remain susceptible to antifungal combinations, which might help to expand the therapeutic options. Genetic analysis suggests that ongoing mutations occurring in response to antifungal drug pressure are the probable drivers of emerging pan-resistance seen in the New York *C auris* strains.

We declare no competing interests.

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New York State Department of Health, Wadsworth Center, Albany, NY 12203, USA (BO, JL, SC, VC); and QIAGEN Digital Insights, Redwood City, CA, USA (JLJ)

1 Ostrowsky B, Greenko J, Adams E, et al. *Candida auris* isolates resistant to three classes of antifungal medications - New York, 2019. *MMWR Morb Mortal Wkly Rep* 2020; **69:** 6–9.

2 Zhu Y, O'Brien B, Leach L, et al. Laboratory analysis of an outbreak of *Candida auris* in New York from 2016 to 2018: impact and lessons learned. *J Clin Microbiol* 2020; **58:** e01503–19.

3 Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nat Rev Microbiol* 2019; **17:** 141–55.

4 Rhodes J, Abdolrasouli A, Farrer RA, et al. Genomic epidemiology of the UK outbreak of the emerging human fungal pathogen *Candida auris*. *Emerg Microbes Infect* 2018; **7:** 43.

5 Chow NA, Muñoz JF, Gade L, et al. Tracing the evolutionary history and global expansion of *Candida auris* using population genomic analyses. *MBio* 2020; **11:** e03364–19.

PROJECT HIGHLIGHTS FROM FELLOWS

June Chan, PhD Transplant Surveillance Project - In recent years, passive reporting to the CDC has identified carbapenemase-producing carbapenem-resistant organisms (CPOs) among organ transplant recipients. This may represent an emerging source of spread. Rectal swabs were collected from solid organ transplant (SOT) recipients receiving inpatient care, across five academic hospitals. Testing at the Wadsworth Center with commercial and lab-developed assays found that 8% (7 of 92) of SOT recipients were positive for carbapenemase genes. Additional surveillance in areas with varied CPO epidemiology will inform whether SOT recipients should be routinely screened for CPOs.

Pilot Surveillance for Carbapenemase-producing Carbapenem-resistant Organisms Among Hospitalized Solid Organ Transplant Recipients

June L. Chan[1], Elizabeth Nazarian[1], Kimberlee A. Musser[1], Emily A. Snavely[1], Monica Fung[2], Sarah B. Doernberg[2], Stephanie Pouch[3], Surbhi Leekha[4], Judith A. Anesi[5], Rosy Priya Kodiyanplakkal[6], Sarah E. Turbett[7], Maroya Spalding Walters[8], Lauren Epstein[8]
[1]Wadsworth Center, NYSDOH, Albany, NY [2]University of California San Francisco, San Francisco, CA [3]Emory University School of Medicine, Atlanta, GA [4]University of Maryland Medical Center, Baltimore, MD [5]University of Pennsylvania, Philadelphia, PA [6]Weill Cornell Medicine, New York, NY
[7]Massachusetts General Hospital, Boston, MA [8]Centers for Disease Control and Prevention, Atlanta, GA

CP-CRO Screening Locations: September 2019 to June 2020

Figure 1. Locations of hospitals in pilot SOT recipient CP-CRO screening. From September 2019 to June 2020, five academic hospitals conducted colonization screening point prevalence surveys (PPS) on units where SOT recipients received inpatient care. Each facility conducted two PPS, approximately one month apart. Rectal swabs were collected from all consenting patients, regardless of transplant status; 154 patients were sampled. Intensive care units (ICUs) were excluded. *: Denotes hospital conducting PPS Positive specimens were only detected from Facilities A and B. **: Denotes a single hospital that conducted 1 PPS.

Positive Swab Culture & Downstream Analyses

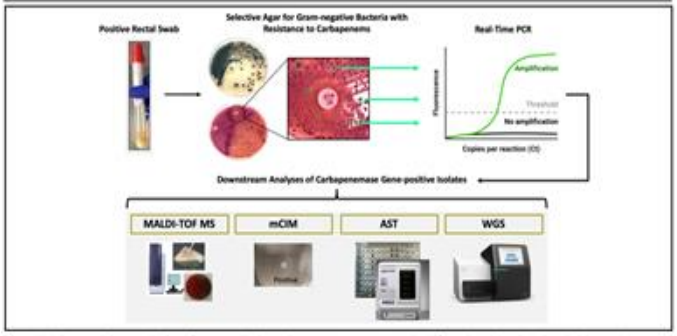

Figure 3. Culture and characterization of recovered isolates. From a positive specimen, the 2nd rectal swab is cultured on CHROMagar™ mSuperCARBA™ agar and MacConkey agar (with an ertapenem and meropenem antibiotic disc) to recover Gram-negative bacteria resistant to carbapenems. Colony isolates are screened by PCR for carbapenemase gene confirmation. The isolate is then identified with MALDI-TOF mass spectrometry and whole-genome sequencing. The modified carbapenem inactivation method (mCIM) is performed to assess carbapenemase production from the isolate. Broth microdilution antimicrobial susceptibility testing (AST) is performed with the Thermo Scientific™ Sensititre™ GNX2F AST Plate to assess isolate drug susceptibilities to 21 antimicrobial compounds, including the carbapenems.

Results

TABLE 1: Characteristics of Carbapenemase-producing Carbapenem-resistant Organism (CP-CRO) Pilot Surveillance Participants

	Solid Organ Transplant Recipients (N=92)
Age (median years, range)	57 (18-77)
Male	51 (55%)
Transplant organ[1]	
kidney	44 (48%)
liver	39 (42%)
pancreas	9 (10%)
lung	6 (7%)
Patients with carbapenemase genes detected	7 (8%)
Detected by CARBA-R: bla_{KPC}	4 (57%)
Organisms[2]	Enterobacter cloacae complex, Klebsiella pneumoniae, Klebsiella oxytoca
Detected by CARBA-R: bla_{NDM}	1 (14%)
Organisms[2]	Klebsiella oxytoca
Detected by LDT: bla_{OXA-23}	2 (29%)
Organisms[2]	not recovered
Time from transplantation to point prevalence survey (PPS) date among all solid organ transplant (SOT) recipients, N = 92, median days (range)[3]	40 (0-7151)
Time from transplantation to PPS date among SOT recipients with carbapenemase genes detected, N = 7, median days (range)[3]	108 (4-1529)

8% (7 of 92) of SOT recipients were positive for carbapenemase genes

[1] Some patients received dual SOTs

[2] Carbapenemase genes detected & associated organisms

[3] Time interval = # of days from SOT to specimen collection for screening

Table 1. Characteristics of surveillance participants who were SOT recipients. Five carbapenemase gene-positive patients were detected by the Carba-R® Assay: 4 bla_{KPC} and 1 bla_{NDM} (blue). Two additional positive patients were detected by the LDT: 2 bla_{OXA-23} (green).

TABLE 2: Patient Demographics, Culture and Phenotypic Testing Results from Carbapenemase Gene Positive Specimens

Patient	Carbapenemase Gene	Organism	Sex	Age	Interval	mCIM	Doripenem	Ertapenem	Imipenem	Meropenem
SOT[a] (liver)	bla_{KPC}	Klebsiella oxytoca	M	52	12	POS	R	R	R	R
SOT[a] (kidney)	bla_{KPC}	Enterobacter cloacae complex[c]	F	47	323	POS	S	R	I	S
	bla_{KPC}	Klebsiella pneumoniae[c]				POS	I	R	R	I
SOT[a] (liver)	bla_{KPC}	Klebsiella oxytoca	M	66	33	POS	S	S	I	S
SOT[a] (liver)	bla_{NDM}	not recovered	F	36	108					
SOT[a] (liver)	bla_{KPC}	not recovered	F	48	185					
SOT[a] (liver)	bla_{NDM}	not recovered	M	45	1529					
SOT[a] (kidney)	bla_{OXA-23}	not recovered	M	52	4					
non-transplant[b]	bla_{OXA-23}	not recovered	M	63						
non-transplant[b]	bla_{KPC}	Citrobacter farmeri	M	67		POS	R	R	R	R

[a] Individuals received treatment at Facility A

[b] Individuals received treatment at Facility B

3% (2 of 62) of non-transplant patients were positive for carbapenemase genes

Interval = # of days from SOT to specimen collection for screening

[c] Organisms were cultured from the same patient

Table 2. Patient demographics, culture and phenotypic testing results. Patients receiving treatment at Facility A (blue) or Facility B (green). POS = mCIM-positive test result with a <6mm meropenem inhibition zone. S, I, R = Susceptible, Intermediate, or Resistant AST result to respective carbapenem antibiotics.

Conclusions

- Carbapenemase genes were detected in **8%** (7 of 92) of SOT recipients
- CP-CRO colonization by *Enterobacteriaceae* was confirmed in **4%** (4 of 92) of SOT recipients
- Carbapenemase genes were detected in **3%** (2 of 62) of non-transplant patients
- All cultured isolates produced functional carbapenemases and exhibited non-susceptibility to at least one carbapenem tested
- We did not identify carbapenemase gene-positive patients in 3 out of 5 of the participating hospitals

References

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2. Moreno Camacho A, Ruiz Camps I. Nosocomial infection in patients receiving a solid organ transplant or haematopoietic stem cell transplant. *Enferm Infecc Microbiol Clin.* 2014;32(6):386-395.
3. Fishman JA, Grossi PA. Donor-derived infection--the challenge for transplant safety. *Nat Rev Nephrol.* 2014;10(11):663-672.
4. Pouch SM, Satlin MJ. Carbapenem-resistant Enterobacteriaceae in special populations: Solid organ transplant recipients, stem cell transplant recipients, and patients with hematologic malignancies. *Virulence.* 2017;8(4):391-402.

Kate Prussing, PhD.

Using Long read sequencing to better understand plasmid transfer across antimicrobial resistant bacterial species in healthcare associated infections.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 19 August 2020
doi: 10.3389/fmicb.2020.02007

Nanopore MinION Sequencing Reveals Possible Transfer of *bla*$_{KPC-2}$ Plasmid Across Bacterial Species in Two Healthcare Facilities

Catharine Prussing[1]*, Emily A. Snavely[1†], Navjot Singh[1], Pascal Lapierre[1], Erica Lasek-Nesselquist[1], Kara Mitchell[1], Wolfgang Haas[1], Rita Owsiak[2], Elizabeth Nazarian[1] and Kimberlee A. Musser[1]

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Carbapenemase-producing *Enterobacteriaceae* are a major threat to global public health. *Klebsiella pneumoniae* carbapenemase (KPC) is the most commonly identified carbapenemase in the United States and is frequently found on mobile genetic elements including plasmids, which can be horizontally transmitted between bacteria of the same or different species. Here we describe the results of an epidemiological investigation of KPC-producing bacteria at two healthcare facilities. Using a combination of short-read and long-read whole-genome sequencing, we identified an identical 44 kilobase plasmid carrying the *bla*$_{KPC-2}$ gene in four bacterial isolates belonging to three different species (*Citrobacter freundii*, *Klebsiella pneumoniae*, and *Escherichia coli*). The isolates in this investigation were collected from patients who were epidemiologically linked in a region in which KPC was uncommon, suggesting that the antibiotic resistance plasmid was transmitted between these bacterial species. This investigation highlights the importance of long-read sequencing in investigating the relatedness of bacterial plasmids, and in elucidating potential plasmid-mediated outbreaks caused by antibiotic resistant bacteria.

Keywords: carbapenem-resistant enterobacteriaceae, *klebsiella* pneumoniae carbapenemase, horizontal gene transfer, plasmids, long-read sequencing, hybrid genome assembly, molecular epidemiology

INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) are an urgent global health threat, and have been categorized by the World Health Organization (World Health Organization [WHO], 2017) and the United States Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention [CDC], 2019a) as top priorities for research, drug discovery, surveillance, and control. CRE that produce carbapenemases are particularly concerning epidemiologically because carbapenemase genes can be transferred among bacteria via mobile genetic elements, including plasmids (Bonomo et al., 2017). In the United States, the most commonly identified carbapenemase is *Klebsiella pneumoniae* carbapenemase (KPC), which has become endemic in parts of the country since it was first described in 1996 (Woodworth et al., 2018; Castanheira et al., 2019).

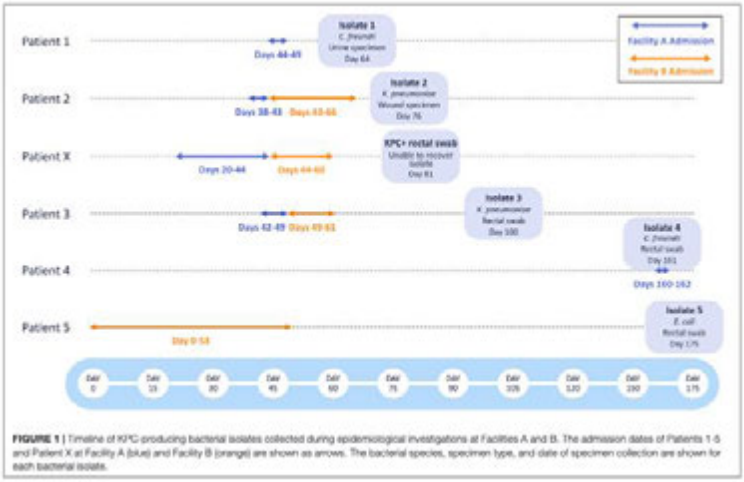
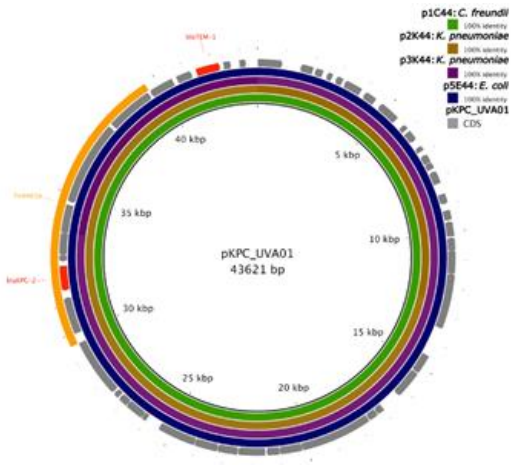


FIGURE 1 | Timeline of KPC-producing bacterial isolates collected during epidemiological investigations at Facilities A and B. The admission dates of Patients 1-5 and Patient X at Facility A (blue) and Facility B (orange) are shown as arrows. The bacterial species, specimen type, and date of specimen collection are shown for each bacterial isolate.



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Submit your ideas, posters and/or articles to be included in our next newsletter to:

Shirley Kelly-Parson
AR Program Assistant
Shirley.Kelly-Parson@health.ny.gov

read all about it…

- **National Action Plan for Combating Antibiotic-Resistant Bacteria, 2020-2025**
- **Massachusetts uses antibiograms to monitor statewide changes in drug resistance.**
- **Early in the pandemic were antibiotics prescribed too often?** *(US News & World Reports)*
- **While the world is gripped by COVID-19, another devastating health threat is building—this one from bacteria** *(Business Insider)*
- **COVID-19 and antibiotic Rx; California antibiotic stewardship mandate; AMR Action Fund critique (CIDRAP)**
- **"Superbugs" far greater risk than COVID in Pacific, scientist warns** *(Microsoft News- MSN UK)*
- **First report of an E. coli isolate co-harboring two different mcr genes** *(Dovepress)*
- **How Covid-19 might affect antimicrobial stewardship programs** *(Infection Control Today)*

Apply to Host an APHL AR Lab Network Fellow

State and local public health laboratories interested in applying to host an AR Lab Fellow can find additional information on the host laboratory instructions and application page. Application period ends Feb 28, 2021.

Apply to Host an APHL-CDC COVID-19 Laboratory Associate

Does your laboratory need additional personnel to assist with the COVID-19 pandemic response? Consider hosting an APHL-CDC COVID-19 Laboratory Associate! Associates will be available for temporary (through June 2021), full-time assignments and can fill a variety of critical roles at all levels of the laboratory.

Learn more and apply to host a COVID-19 Laboratory Associate

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email Shirley.Kelly-Parson@health.ny.gov

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