

Advanced Combination Antimicrobial Resistance Testing for Hard-to-Treat Carbapenem-Resistant Enterobacterales Carrying Metallo-β-lactamases Offers Additional Treatment Options

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Overview

- According to the Infectious Diseases Society of America 2024 guidance, ceftazidime-avibactam (TAZ-AVI) in combination with aztreonam (ATM) is the suggested treatment option for infections caused by carbapenem-resistant, metallo-β-lactamase (MBL) - producing Enterobacterales. This group of bacteria includes gram-negative bacteria that are some of the most common causes of infection including *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* species (formerly reported as *Enterobacteriaceae*). Aztreonam-avibactam (ATM-AVI) is a promising antibiotic combination in phase 3 clinical trials awaiting FDA approval for commercial use.
- Wadsworth Center (WC) serves as the Northeast Regional Laboratory and is one of 7 Antimicrobial Resistance (AR) Lab Network Regional Laboratories offering the Expanded Antimicrobial Susceptibility Testing (ExAST) program (Figure 1). The program provides an in-house prepared broth microdilution (BMD) panel of 3 drug combinations and ATM using the Tecan D300e Digital Dispenser. The drug combinations include TAZ-AVI, ATM-AVI, and aztreonam-ceftazidime-avibactam (ATM-TAZ-AVI).
- Enterobacterales isolates that test aztreonam non-susceptible and possess *bla*_{NDM}, *bla*_{VIM}, or *bla*_{IMP} carbapenemase genes meet the inclusion criteria for testing. Public health laboratories, treatment facilities, physicians, and providers can request testing. Submitted isolates are MBL-producing Enterobacterales demonstrating resistance to ATM.
- WC performed ExAST on 344 clinical isolates from 2019-2024. There was a notable increase in isolate submissions in 2023 (Figure 2). This increase may be attributed to an increase in resistant isolates due to misuse of antibiotics and lack of infection control in facilities during the COVID-19 pandemic and increased awareness of the program.



Figure 1. Antimicrobial Resistance Laboratory Network Testing | ARLN | CDC

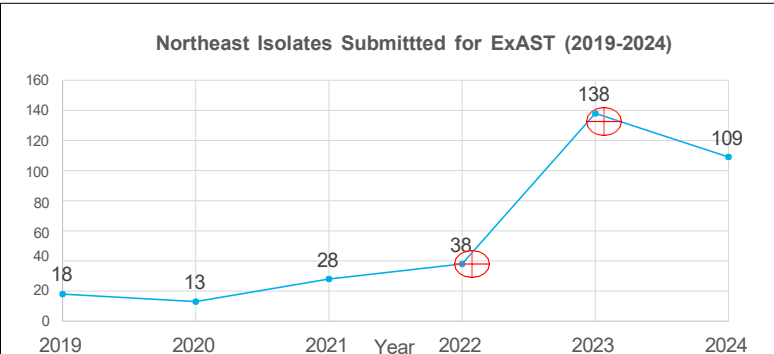


Figure 2.

Methods and Workflow

- Upon submission, isolates are confirmed via MALDI-TOF MS for organism identification, laboratory developed real-time PCR for the detection of *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{VIM}, and *bla*_{IMP}, antimicrobial susceptibility testing (AST) using the Sensititre™ Negative GN2F panel followed by testing with the in-house prepared ExAST BMD plate. Whole-genome sequencing (WGS) is performed for a subset of isolates submitted.
- The ExAST BMD plates are prepared on the Tecan D300e Digital Dispenser using antibiotic stock solutions and cation adjusted mueller hinton broth (CAMHB) provided by the Centers for Disease Control (CDC) (Figure 3).
- Clinical isolates are inoculated into prepared BMD plates using the Sensititre AIM™ Automated Inoculation Delivery System (AIM). The inoculated plate is incubated at 36°C for 16-20 hours and read using a magnifying mirror (Figure 3). The MICs are read for TAZ-AVI, ATM, ATM-AVI, and ATM-TAZ-AVI and interpreted according to current CLSI guidelines. As breakpoints have not been established for ATM-AVI the MIC is reported without interpretation.

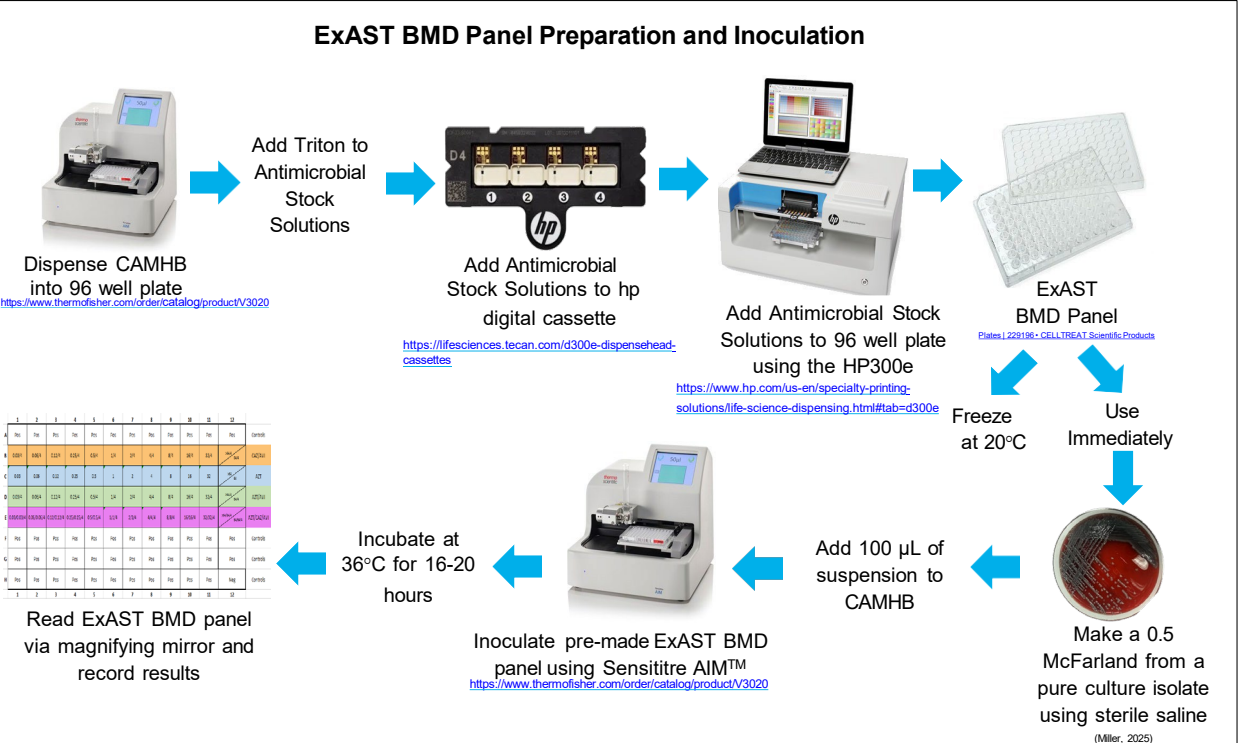


Figure 3.

ExAST Results

Organism	ExAST Performed	Real-time PCR Positive for Genes that Express MBLs				
		NDM	NDM/ OXA-48	NDM/ KPC	IMP	IMP/ KPC
<i>Citrobacter amalonaticus</i>	1	1				
<i>Citrobacter freundii</i> complex	2	1		1		
<i>Enterobacter cloacae</i> complex	23	20		2		1
<i>Enterobacter hormaechei</i>	15	12		3		
<i>Escherichia coli</i>	100	94	6			
<i>Klebsiella aerogenes</i>	1	1				
<i>Klebsiella oxytoca</i>	4	2		2		
<i>Klebsiella pneumoniae</i>	185	150	31	2	1	1
<i>Klebsiella variicola</i>	1	1				
<i>Morganella morganii</i>	1			1		
<i>Pluralibacter gergoviae</i>	1	1				
<i>Proteus mirabilis</i>	4	4				
<i>Providencia rettgeri</i>	3	3				
<i>Raoultella ornithinolytica</i>	1	1				
<i>Serratia marcescens</i>	2	2				
Total	344	293	37	11	1	2

Table 1.

- WC performed ExAST on 344 clinical isolates from 2019-2024: *Escherichia coli* (100), *Klebsiella pneumoniae* (185), *Enterobacter cloacae* complex (23), *Enterobacter hormaechei* (15), and 21 additional Enterobacterales. The majority (95%) of isolates tested represented *Enterobacter* species, *Escherichia coli* and *Klebsiella* species (Big 3; indicated with pink shading).
- The majority (99%) of isolates contained the MBL NDM either alone (293) or in combination with an additional carbapenemase (NDM/OXA-48 (37) and NDM/KPC (11)). The remaining three contained IMP (1) and IMP/KPC (2).

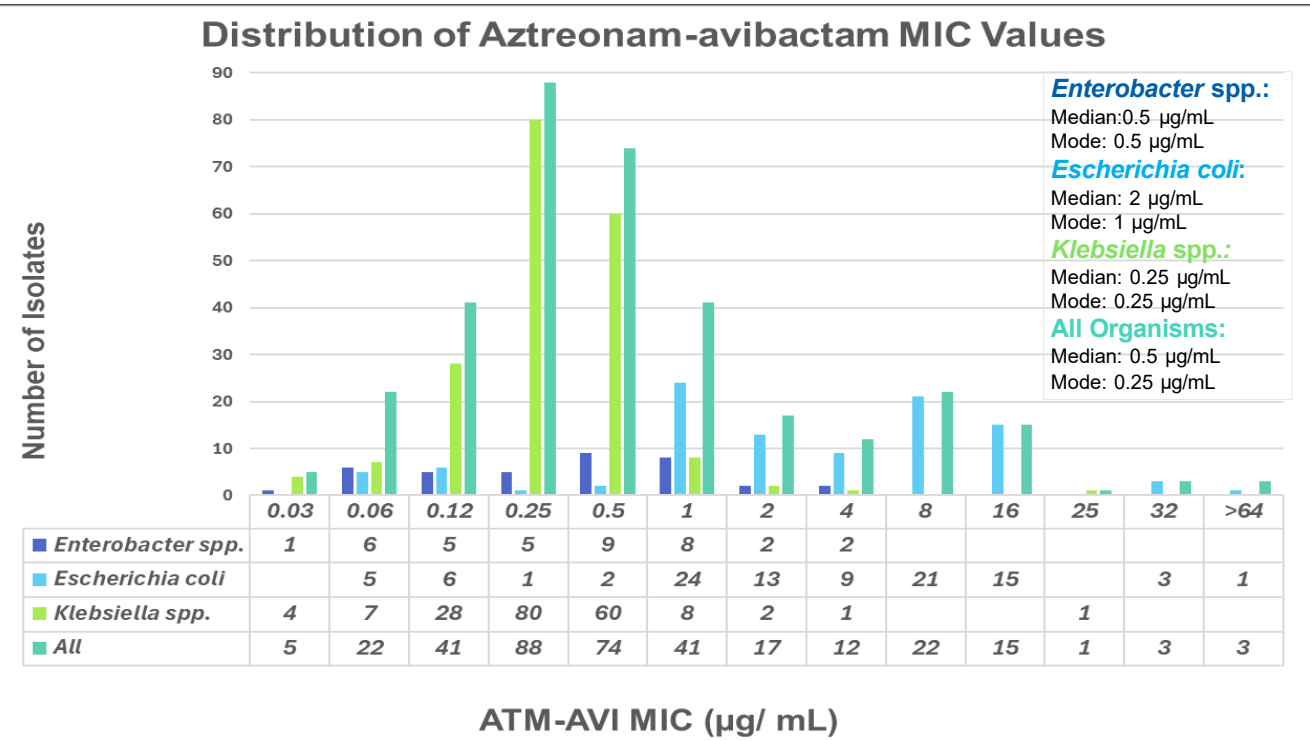


Figure 4.

- The ATM-AVI MIC panel range was 0.03/4 ug/mL to ≥64/4 µg/mL. The figure displays the distribution ATM-AVI MIC values for the “Big 3” organisms compared to all ExAST isolates tested.
- The median and mode were calculated for the “Big 3” organisms individually and all isolates together. The modal ATM-AVI MIC value for all organisms was ≤1.0 µg/mL.
- It can be noted that 49% of *Escherichia coli* isolates demonstrated ATM-AVI MIC values ≥4 µg/mL.

Whole-genome Sequencing (WGS)

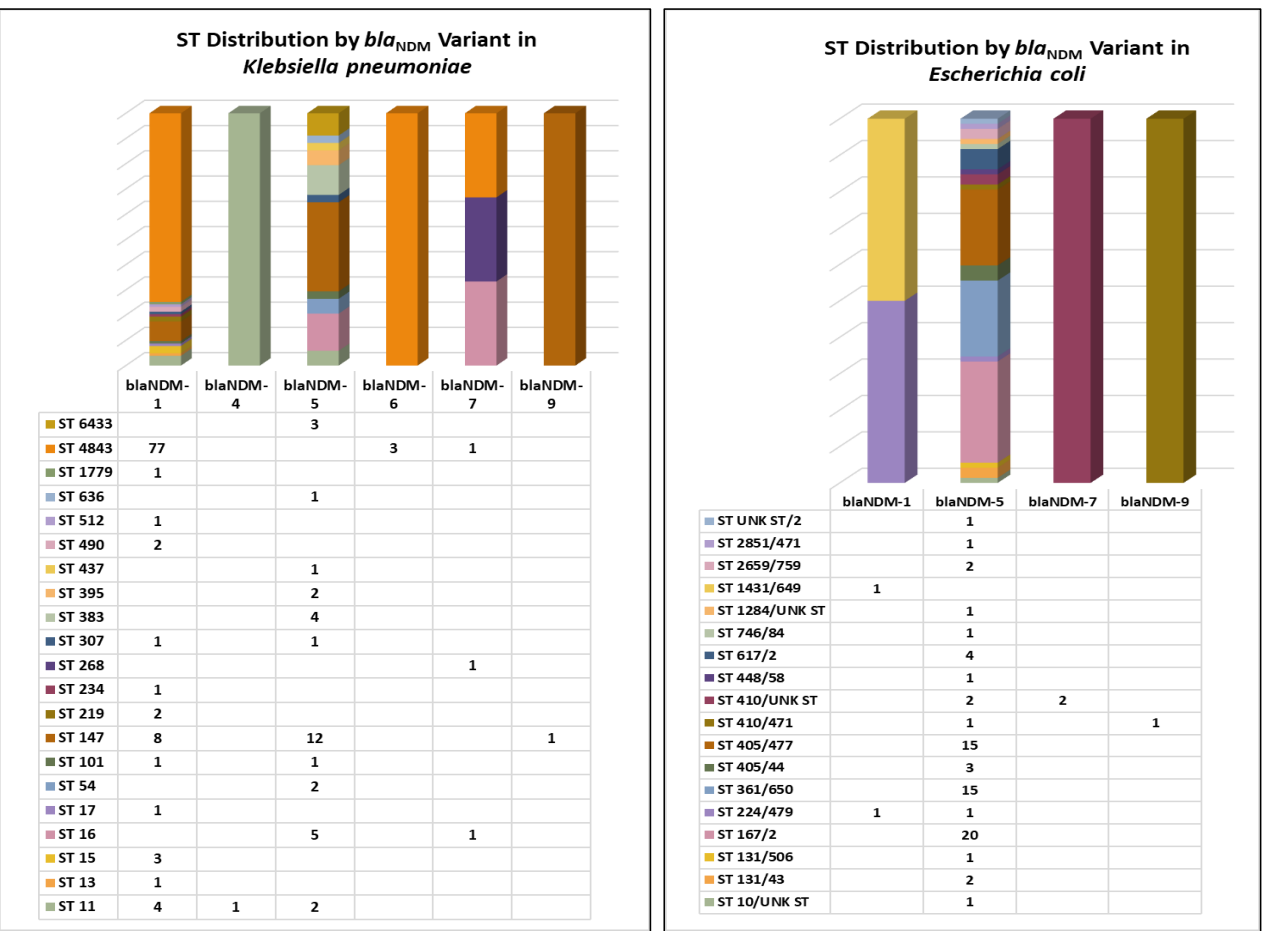


Figure 5.

- WGS provided additional characterization of the carbapenemase gene variant and organism multi-locus sequence type (ST) on a subset (288) of these highly resistant strains.
- bla*_{NDM-1} (150/288) was the most frequently identified (52%) followed by *bla*_{NDM-5} (119/288) at 42%.
- K. pneumoniae*, *bla*_{NDM-1}, ST 4843, and *E. coli*, *bla*_{NDM-5}, ST 167/2 were the strains most frequently submitted for testing.

Discussion

- Aztreonam targets bacterial cell wall synthesis and retains activity against MBL-producing isolates, though its effectiveness is limited due to co-production of ATM-hydrolyzing β-lactamases.
- TAZ-AVI is often used for treating resistant infections but is ineffective against MBL-producers; hence, ATM-AVI is under continued study, as AVI may shield ATM from degradation.
- WC's ExAST program offers specialized testing not broadly available and plays a key role in identifying additional treatment options for difficult-to-treat Enterobacterales isolates carrying a MBL.
- The lab prepares and performs ExAST BMD panel testing for ATM, TAZ-AVI, ATM-AVI, and ATM-TAZ-AVI on submitted isolates. The majority of isolates tested harbor the NDM-type MBL, either alone or alongside another carbapenemase.
- The data demonstrates that most isolates have an ATM-AVI MIC range of ≤0.03/4 to 2/4 µg/mL, while those with higher MICs (4/4 to >64 µg/mL) are primarily from *E. coli*.
- Further characterization by WGS identified *bla*_{NDM-1} and *bla*_{NDM-5} in the majority of isolates tested.
- Between 2019 and 2024, WC conducted ExAST testing on 344 clinical isolates, with test volume increasing significantly as awareness has grown among healthcare providers.
- Overall, ATM-AVI has shown promise as an effective therapy for infections caused by carbapenem-resistant Enterobacterales carrying MBLs.

Acknowledgements

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- We thank the Wadsworth Center Advanced Genomic Technologies Cluster for conducting whole genome sequencing.