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Application of "Precision Medicine" Through the Molecular Characterization of Extensively Drug Resistant *Klebsiella pneumoniae* in a Multivisceral Transplant Candidate

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ABSTRACT

Background: Treatment of carbapenemase-producing organisms is clinically challenging. We elucidated the mechanisms of carbapenem resistance in order to design an effective antibiotic regimen for a patient with XDR *K. pneumoniae*.

Methods: A *K. pneumoniae* isolate was recovered as a cause of complicated urinary tract infection in a patient undergoing evaluation for multi-visceral transplantation. The patient, originally from Turkey, required multiple bowel resections for the treatment of a desmoid tumor. Antimicrobial susceptibility (AST) testing was performed using the Vitek-automated system and Etest. The CarbaNP assay was used to detect carbapenemases, followed by amplification of bla genes with PCR and sequencing. Multilocus sequence typing (MLST) and plasmid replicon typing were performed. Antibiotic combinations were tested using disk diffusion and Etest.

Results: AST demonstrated resistance to all b-lactams, fluoroquinolones and aminoglycosides: colistin MIC = 8 µg/mL; tigecycline MIC = 2µg/mL; and fosfomycin MIC = 12µg/mL. CarbaNP detected a carbapenemase. PCR amplification and DNA sequencing revealed presence of blaNDM-1, blaOXA-48, and blaCTX-M. MLST determined that the isolate belonged to sequence type (ST) 14. Three different plasmids were identified containing blaNDM-1, blaOXA-48/blaCTX-M, and blaCTX-M. Combination of ceftazidime-avibactam (TAZ-AVI) plus aztreonam, both by disk diffusion and Etest suggested synergy. The patient initially received empirical treatment with oral fosfomycin and IV meropenem/ertapenem with eradication of the XDR K. pneumoniae in the urine. Subsequently, TAZ AVI/ aztreonam were used as part of peri-operative antibiotic prophylaxis effectively preventing post-surgical infections with XDR K. pneumoniae, despite persistent rectal colonization with this organism.

Conclusion: *K. pneumoniae* harboring blaNDM-1 and blaOXA-48 is being increasingly recognized in the US and globally. Molecular characterization of the genetic background and mechanisms of resistance in this isolate, precision medicine, guided the design of an effective antibiotic regimen (TAZ-AVI/aztreonam) for prevention of infections with XDR K. pneumoniae.

CASE PRESENTATION

- A 35-year-old woman from Edremit, Turkey, was admitted to a hospital in Miami, Florida, USA, to undergo evaluation for kidney and intestinal transplants.
- The patient suffered from Gardner syndrome with familial polyposis and invasive desmoid tumors that required multiple bowel resections, resulting in small bowel obstruction, intestinal fistulas and bilateral ureteral obstruction with nephropathy, for which she had placement of bilateral nephrostomy catheters.
- The patient did not have a history of recent infections with MDR organisms before referral to our center.
- The patient presented with dysuria and right flank pain. Physical exam revealed a temperature of 36.6 °C, normal pulse and blood pressure, and purulent discharge from the exit site of a nephrostomy catheter. Urine culture grew *K. pneumoniae*.
- Antimicrobial susceptibility (AST) testing was performed using the Vitek 2 automated system and Etest and interpreted according to breakpoints established by Clinical Laboratories Standard Institute (CLSI). Results are displayed in Table 1.

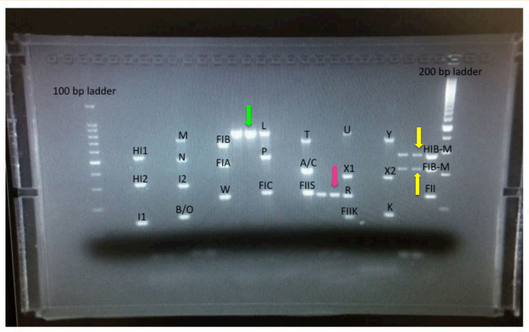
Table 1. Susceptibility of *Klebsiella pneumonia* isolate to various antimicrobials

Antibiotic	MIC (ug/mL)	Method	Interpretation
Ampicillin-sulbactam	≥64	Vitek 2	Resistant
Aztreonam	≥32	Vitek 2	Resistant
Cefazolin	≥64	Vitek 2	Resistant
Cefepime	≥64	Vitek 2	Resistant
Cefoxitin	≥64	Vitek 2	Resistant
Ceftazidime	≥64	Vitek 2	Resistant
Ceftazidime-avibactam	>256	E-test	Resistant
Ceftriaxone	≥64	Vitek 2	Resistant
Ertapenem	8	E-test	Resistant
Meropenem	≥16	Vitek 2	Resistant
Piperacillin-Tazobactam	≥128	Vitek 2	Resistant
Levofloxacin	≥8	Vitek 2	Resistant
Amikacin	≥64	Vitek 2	Resistant
Gentamicin	≥16	Vitek 2	Resistant
Tobramycin	≥16	Vitek 2	Resistant
Colistin[a]	8	Broth macrodilution	Resistant
Fosfomycin	12	E-test	Susceptible
Nitrofurantoin	256	Vitek 2	Resistant
Tetracycline	8	Vitek 2	Resistant
Tigecycline[b]	1	E-test	Susceptible
Trimethroprim-Sulfamethoxazole	≥32	Vitek 2	Resistant

MOLECULAR CHARACTERIZATION

- Rapid Carb Screen assay suggested presence of a carbapenemase.
- Due to the patient's origin in Turkey, where OXA-48-producing *K. pneumoniae* is endemic, further genetic characterization of the mechanism of carbapenem resistance was pursued.
- Colonies of a lactose fermenter obtained from the McConkey plate where the original urine sample had been plated were inoculated into blood culture bottles and incubated overnight, and subsequently analyzed with the Verigene Gram-Negative Blood Culture Test.
- Verigene detected bla_{NDM}, bla_{OXA}, and $bla_{CTX-M,}$.
- The presence of bla_{NDM-1}, bla_{OXA-48}, and $bla_{CTX-M-15}$ was subsequently confirmed by PCR amplification and DNA sequencing. Multi-locus Sequence Typing determined that the isolate belonged to sequence type 14.
- Results of plasmid replicon typing are shown in Figure 1.

Figure 1. Plasmid identification using PCR Based Replicon Typing Kit (Diatheva, Fano PU, Italy). Each replicon control is labeled; arrows indicate the amplicons obtained for the *Klebsiella pneumonia isolate.*



The bla_{NDM-1} harboring plasmid contained both the HIB-M and FIB-M replicons, bla_{OXA-48}/$bla_{CTX-M,}$ plasmid contained the L replicon and bla_{CTX-M} plasmid contained the R replicon

THERAPEUTIC MANAGEMENT

- The patient was initially treated with a combination of oral fosfomycin and double carbapenem therapy (intravenous meropenem and ertapenem) for 14 days. Nephrostomy catheters were exchanged. *K. pneumoniae* was successfully eradicated from the urine and signs of infection resolved.
- Since the patient was listed for kidney and isolated intestine transplant, a peri-operative antibiotic prophylaxis regimen was designed.
- The activity of the combination of ceftazidime-avibactam (TAZ-AVI) plus aztreonam (AZT) was predicted, considering the ability of avibactam to inactivate OXA-48 and CTX-M, and the stability of aztreonam against NDM-1.
- This concept was tested both by Etest and disk diffusion with *in-vitro* studies that suggested synergistic activity (Figure 2).

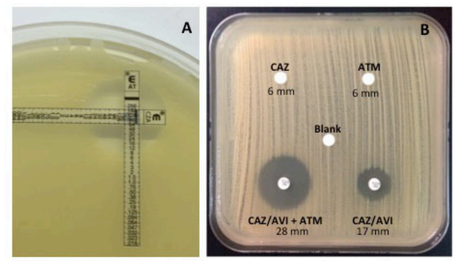


Figure 2. Synergy testing between ceftazidime-avibactam and aztreonam performed by Etest (A) and disk diffusion (B).

PERI-OPERATIVE PROPHYLAXIS

- The patient underwent kidney and isolated intestinal transplant, and TAZ-AVI and AZT were used as part of peri-operative surgical prophylaxis. Other antibiotics used at the time included daptomycin (due to co-colonization with vancomycin-resistant enterococci), metronidazole, and fluconazole.
- Antimicrobial coverage was extended to 4 weeks because of repeated surgeries to address bleeding and anastomotic leak.
- Post-surgical infections did not occur while receiving this five-drug regimen or after discontinuation of prophylactic antibiotics.
- Four months after transplantation the patient remained well despite evidence of rectal colonization with XDR *K. pneumoniae* in surveillance cultures.

CONCLUSION

- Epidemiological and molecular background should be considered when treating patients with infections due to carbapenemase producing CRE.
- Rapid diagnostic technologies that can uncover bacterial mechanisms of resistance can guide individualized therapy, and in this case led to the successful use of ceftazidime-avibactam and aztreonam for prevention of surgical site infections after kidney and intestine transplantation.

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