Background. Klebsiella pneumoniae carbapenemase (KPC) and Verona inte
P
, Merck: Investigator, Research support.

Methods. We tested isolates for responses to CZA alone (1 and 4x MIC; avibac
amixed at 4 µg/mL), and in combination with colistin (COL; 2 µg/mL), fosfomycin
(FOS; 100 µg/mL + 25 µg/mL G6P), gentamicin (GEN; 2 µg/mL), MER (8 µg/mL),
and tigecycline (TGC; 2 µg/mL) by time-kill using a starting inoculum of 1 x 10^6 CFU/mL.
Log kills were calculated as log cFU/mL decrease from time 0; 24 hours was the
primary endpoint.

Results. Thirty KPC-Kp isolates were studied (22 KPC-2 and 8 KPC-3); all iso-
lates were CZA-susceptible (MIC range: 0.125–4 µg/mL). Fifty-three percent harbored
omk56 mutations (eight each with IS55 and 134–135 DG insertions). Mean log kills by
CZA at 1x and 4x MIC were 2.00 and 2.35, respectively; CZA was bactericidal (≥2-log
kill) at 24 hours against 33% and 50%, respectively. CZA mean log kills at 4x MIC were
greater than KPC-3 (3.81) vs. KPC-2 (1.82) isolates (P = 0.003), but did not vary by
porin genotype (P = 0.44). GEN was the most active single agent and was bactericidal
against 70% of isolates. Mean log kill in combination with CZA was greater (2-log
kill in combination with COL, FOS, GEN, and TGC) and was 83%, 60%, 40%, 87%, and 7%, respectively. The corresponding rates of bactericidal activity of CZA and
GEN were 90%, 69%, 50%, 100%, and 30%, respectively. Antagonism (>1-log kill by most active single
agent) was rare (6%; 2% with COL, 3% with GEN, and 0% with TGC). CZA and MER were
mean log kills by CZA + MER were greater among iso-
lates with wild-type (6.58 ± vs. mutant (5.48) omk56 (P = 0.0006), and isolates harbor-
ing KPC-3 (7.02) vs. KPC-2 (5.63; P = 0.0004). CZA + COL responses were attenuated among isolates with COL MICs ≥2 (log kills 2.88 ± vs. 7.94; P = 0.0009), but not affected
by omk56 genotype (P = 0.53). Among isolates with COL MICs <2; log kills were
greater for CZA + COL (7.94) than CZA + MER (6.44; P = 0.0001).

Conclusion. A two-drug combination of CZA + MEM results in high rates of
synergy and bactericidal activity against genetically diverse KPC-Kp. Mean log kills by
CZA were less among isolates with mutations in omk56. CZA + COL was highly active
against isolates omk56 mutations, but contingent on COL susceptibility.

Disclosures. M. H. Nguyen, Merck: Grant Investigator, Research grant. Astellas: Grant Investigator, Research grant. R. K. Shields, Allergan: Allergan Investigator, Research grant. Pfizer: Consultant and Scientific Advisor, Speaker honorarium. Shionogi: Scientific Advisor, Consulting fee. Roche: Grant Investigator, Research grant. Venatora: Grant Investigator, Research grant. Medicines Company: Grant Investigator and Scientific Advisor, Consulting fee and Research grant. Accelerate Diagnostics: Scientific Advisor, Consulting fee.

707. Clarifying the Role of CfrB in Polymyxin-resistant Klebsiella pneumoniae Clinical Isolates Utilizing a Novel CRISPR-Cas9 System

Thomas McConville, MD; Marla Giddins, BA; Nenad Macesic, MBBS and Anne-Carroll Uehleman, MD, PhD; Columbia University Medical Center, New York, New York

Session: 67. Resistance Mechanisms: Gram-Negative Thursday, October 4, 2018: 12:30 PM

Background. Polymyxin resistance (PR) threatens the mainstay of therapy for
carbapenem-resistant Enterobacteriaceae (CRE) infections. While mrgR disruption accounts for most cases of PR, mssbA mutations in cfrB have been proposed as an alternative
pathway for PR through PmrA/B/C upregulation of the pmrHFKJKLM operon. It remains
unknown if CfrB acts as a positive or negative regulator on its downstream targets.

Methods. We assembled a CRISPR-Cas9 system for gene knockouts (KO) in CRE
K. pneumoniae (CRKP) using zeocin as a selectable marker. We chose a polymyxin
susceptible (PS) and a PR isolate with a missense mutation in cfrB (L87V) (NR5377
and NR5083, respectively) for KO. Isolates were transformed with a cfrB KO pla-
smid, grown with zeocin selection, induced with arabinose, and plated on low-
salt LB-zeocin/ara/kan. KO strains were confirmed via PCR and Sanger sequencing. Polymyxin
susceptibility was performed with broth-microdilution. Gene expression was deter-
mined by qRT-PCR of cfrB target genes and a non-targeting control.

Results. Colistin MIC following cfrB KO of NR5377 (PS) remained unchanged. In
contrast, cfrB KO of NR5083 (PR), decreased polymyxin MIC (MIC ≥128 to 1.0 µg/mL;
qRT-PCR of NR5083 did not show increased expression of pmrA/C, pmrHFKJKLM
operon. NR5083 cfrB showed a small decrease in phoP expression, compared with NR5083,
but similar expression of phoQ, pmrA/C and pmrK (Table 1).

Conclusion. Polymyxin MIC decreased >12-fold after cfrB KO in a PR isolate, but
colistin MIC remained unchanged after KO in a PS isolate. CfrB mutations in PR isolates
may contribute to function with or without acting as a positive regulator on its downstream
targets. Contrary to previous literature, no upregulation of pmrA/C and pmrHFKJKLM
was detected. Differences in cfrB mutations or clonal background may explain this find-
ing. CRISPR-Cas9 may serve as a reliable system for genetic manipulation of CRKP.
Further data on the impact of individual cfrB missense mutations are needed.