1052. Characterisation of the DNA binding properties of ridinilazole, a selective antibiotic currently in phase III trials for the treatment of **Clostridiodes difficile**

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**Session:** P-61 Novel Agents

**Background.** **Clostridiodes difficile** infection (CDI) is recognised by the CDC as an “urgent threat” in the USA, responsible for nearly 13,000 deaths, and carries an economic burden ranging from $5.4 to $6.3 billion per year. In a phase II study, ridinilazole was shown to be effective at treating CDI and decreasing subsequent recurrence compared to vancomycin. However, the precise mechanism of action of ridinilazole has yet to be fully elucidated. In this study, we explored ridinilazole clearly interacts with DNA in **C. difficile** and binds with high affinity to the minor groove of DNA. These interactions are predicted to have consequences on cellular functions within **C. difficile**.

**Methods.** High resolution confocal microscopy was used to track the intracellular localisation of ridinilazole in **C. difficile**. Fluorescence intensity was used to characterise the DNA binding properties of ridinilazole; sequence specificity was demonstrated with AT- or GC-rich DNA patterns, and tight binding was shown using short double-stranded oligonucleotides. Hanging drop vapour diffusion enabled co-crystallisation and subsequent structural determination of DNA-bound ridinilazole.

**Results.** Confoal microscopy revealed clear co-localisation of ridinilazole to the DNA within **C. difficile**. Ridinilazole demonstrated a dose-dependent increase in fluorescence in response to increased concentration of target DNA. Fluorescence binding studies revealed that ridinilazole shows a preference towards AT-rich DNA sequences. Tight binding characteristics were demonstrated by ridinilazole in complex with short double-stranded oligonucleotides, returning dissociation constants (Kd) of 20 – 50 nM. Crystallisation enabled co-structures of ridinilazole bound to the minor groove of double-stranded DNA oligonucleotides to be solved.

**Conclusion.** Ridinilazole demonstrates tight binding with sequence specificity within the minor groove of DNA and co-localises with DNA in **C. difficile**. Further analysis is ongoing to fully understand this novel mechanism of action, the downstream consequences of these interactions and how they contribute to the bacterial activity of DNA.

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1053. The β-Lactam Inhibitor QPX7728 Restores the Activity of β-Lactam Agents against Contemporary Extended-Spectrum β-lactamase (ESBL)-Producing and Carbenapenem-Resistant Enterobacteriales (CRE) Isolates, Including Isolates harbouring Metallo-β-lactamases

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**Session:** P-61 Novel Agents

**Background.** The β-lactam (BL) β-lactamase inhibitor (BLI) combinations approved in the last 10 years are active against most ESBL-producing Enterobacteriales (ENT) and CRE isolates, but have limited activity against metallo-β-lactamase (MBL)-producing ENT. We evaluated the activity of QPX7728 (QPX), a novel BLI with broad-spectrum antibacterial activity. The activity of QPX against COL isolates is depicted as an MBC profile for 200 BLIs.

**Methods.** A total of 1,027 ENT isolates were susceptibility (S) tested by reference broth microdilution against aztreonam (ATM), biapenem (BPM), meropenem (MER), and tebipenem (TEB) combined with QPX at the highest concentration tested (Table). Oral agents, CTB,CDR and TEB were tested with QPX at a fixed 4 mg/L and showed a 32- to 128-fold increase in potency (MIC<sub>90</sub> 0.5–4 mg/L). ATM and TEB were tested with QPX at a fixed 4 and 8 mg/L and displayed MIC<sub>90</sub> values ranging from 0.12-0.5 mg/L. ATM and TEB, tested with QPX, inhibited 90% of ENT isolates at the breakpoint for the BL agent alone. BLI inhibitor combinations PT and CT displayed MIC<sub>90</sub> values of 2 and 4 mg/L with the addition of 8 mg/L. QPX MER with QPX at a fixed 4 mg/L and 8 mg/L inhibited 99.8% and 100% of isolates, respectively.

**Conclusion.** The activity of all BLIs evaluated was restored when combined with QPX and the activity of all combinations was restored when combined with QPX.
resistance mechanisms, including difficult to treat CRE isolates and MBL producers. Further development of QPX with various orally- and IV-available BL agents appears warranted.

### Table

| Antibacterial agent | MIC (μg/mL) of LSVT-1701 |
|---------------------|--------------------------|
| Methicillin-resistant S. aureus | 0.5-16 |
| Methicillin-sensitive S. aureus | 0.12-16 |
| Coagulase-negative staphylococci (CoNS) | 0.12-16 |

### Discussion

Jill Lindley, bracevos biosciences (research grant or support); krasna pharmaceuticals (research grant or support); pfizer (research grant or support); Qixbiopharma (research grant or support); yahye edah, as (research grant or support); olga lomovskaya, PhD. Qexi biopharma (employee) mariana castanheira, PhD. abbvie (formerly allergan) (employee) melinta therapeutics, llc (research grant or support); melinta therapeutics, inc. (research grant or support); cplla therapeutics (research grant or support); cipra usa inc. (research grant or support); gloxsmithkline (research grant or support); melinta therapeutics (research grant or support); pfizer, inc. (research grant or support); qex biopharma (research grant or support); shionogi (research grant or support); salvia biopharma (research grant or support). The use of LSVT-1701 was not associated with any clinical adverse events or drug-related findings. These results support further clinical development of LSVT-1701 for the treatment of S. aureus and CoNS infections. The MIC of LSVT-1701 against methicillin-resistant S. aureus (MIC < 1 μg/mL) was similar to that of other approved agents, but LSVT-1701 demonstrated potent activity against methicillin-resistant S. aureus (MIC < 1 μg/mL) and coagulase-negative staphylococci (CoNS) collected worldwide.

### Methods

LSVT-1701 and comparators were tested against 415 S. aureus (n=315) and CoNS (n=100) clinical isolates expressing various resistance phenotypes. The isolates were collected from medical centers located in the United States (50 medical centers; 174 isolates; 41.9% overall, Europe (37 medical centers; 140 isolates; 33.7% overall), Asia-Pacific region (15 medical centers; 55 isolates; 13.3% overall), and Latin America (12 medical centers; 46 isolates; 11.1% overall). These isolates originated from the year 2019 and the year 2020. The isolates were susceptibility tested by the CLSI broth microdilution method. MIC interpretations were based on CLSI and EUCAST criteria where available.

### Results

LSVT-1701 was highly active against S. aureus and CoNS isolates with MIC<2 mg/mL for all S. aureus, methicillin-susceptible S. aureus (MSSA), methicillin-resistant S. aureus (MRSA), and CoNS (Table). The highest LSVT-1701 MIC values were 4 and 8 mg/mL among S. aureus and CoNS, respectively. LSVT-1701 retained potent activity against S. aureus isolates showing resistance or decreased susceptibility to comparator agents. LSVT-1701 was highly active against clinical isolates with uncommon resistance phenotypes.