Whole Genome Sequencing and Penicillin Susceptibility Testing for *Corynebacterium diphtheriae:* Are the 2015 CLSI M-45 Breakpoints Relevant?

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Introduction

- *C. diphtheriae* is a re-emerging pathogen in urban disadvantaged populations, and has the potential to cause significant cutaneous and invasive disease[1]
- First line therapies for *C. diphtheriae* infections include penicillin and erythromycin
- Amidst limited reports of penicillin resistance, the CLSI breakpoint for penicillin susceptibility was lowered in 2015 from ≤1 mg/L to ≤0.12 mg/L[2]
- The net effect is a higher proportion of previously susceptible isolates now labelled penicillin-intermediate
- It is not known whether this re-classification truly reflects an increasing prevalence of penicillin resistance at the phenotypic and genotypic level

Methods

Collection of *C. diphtheriae* isolates

- Unique isolates collected and identified as *C. diphtheriae* between March 2015 and June 2018 at St. Paul's Hospital, Vancouver, BC were included

Antimicrobial susceptibility testing (AST)

- Performed using Etest (bioMérieux, Marcy-l'Étoile, France) for penicillin (PEN), erythromycin (ERY), clindamycin (CLI), and vancomycin (VAN)
- Interpreted using the breakpoints from CLSI M-45 2nd (2010) and 3rd (2015) editions

Whole Genome Sequencing (WGS) analysis

- Performed with next-generation sequencing (MiSeq, Illumina, San Diego, CA)
- Sequences analyzed for identification of MLST and antimicrobial resistance markers using ARIBA[3], encompassing 5 public databases (ARG-ANNOT, CARD, MEGARes, ResFinder and SRST2-ARG-ANNOT)

Results

- 56 non-toxigenic *C. diphtheriae* isolates were identified over the study period recovered from multiple anatomic sites: wound (54), blood (1), and throat (1)
- AST results available for 39/56 isolates; WGS results available for 48/56 isolates

Figure 1: Distribution of MICs from penicillin susceptibility testing on *C. diphtheriae* isolates

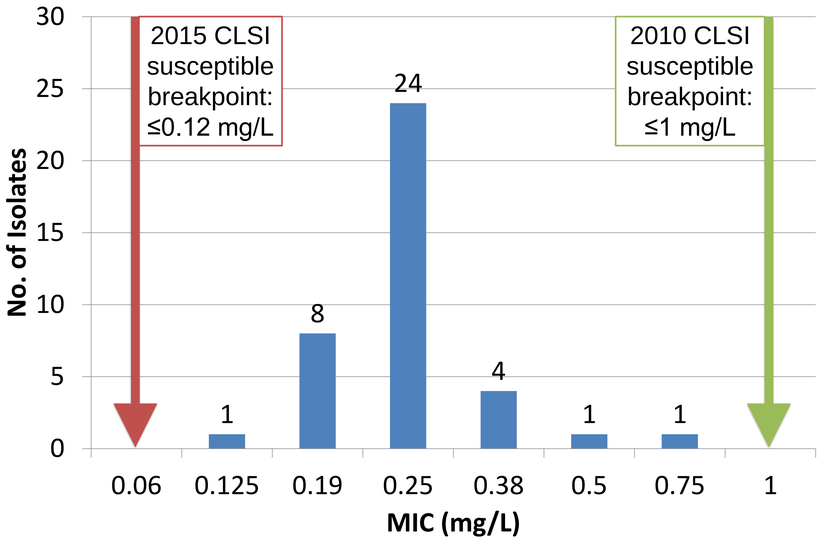
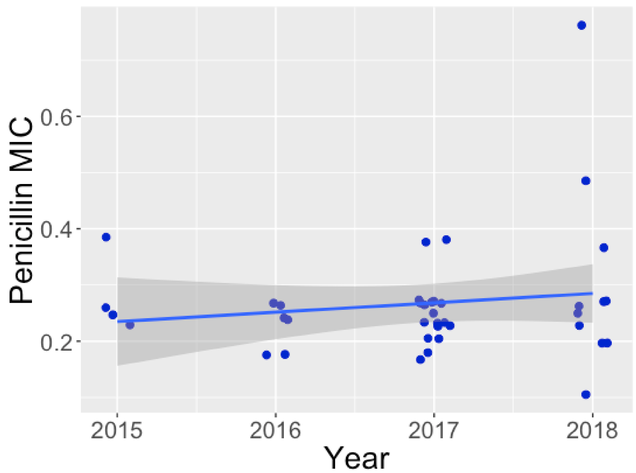


Figure 2: Trend in penicillin susceptibility for *C. diphtheriae* isolates collected over 4 years from 2015-2018



- No change in distribution of PEN MICs over 4-year study period (Fig. 2)
- No mutations associated with beta-lactam resistance identified through WGS for samples with sufficient sequencing depth (0/37)
- Resistance genes preliminarily identified in 42/48 isolates (87.5%), associated with resistance to sulfonamides (41/48, *sul1*), tetracyclines (1/48, *tet*), and macrolides (1/48, *ermX*)
- MLST analysis: ST-76 (45/48), ST-05 (1/48), ST-32 (1/48), and one novel ST

Discussion

- All *C. diphtheriae* isolates collected at our institution during the study period were reported as penicillin non-susceptible following adoption of 2015 CLSI breakpoints
- However, no genetic resistance to penicillin was identified in our *C. diphtheriae* isolates using WGS
- Resistance to erythromycin was more prevalent than penicillin resistance, in keeping with the results of another Canadian study of 195 *C. diphtheriae* isolates[4]
- Misclassification of PEN susceptibility may lead to selection of less effective and/or less well-tolerated antimicrobials (e.g. ERY, CLI, VAN)
- Further study is required to understand the generalizability of these findings to other *Corynebacterium* species, as well as other patient populations, where epidemiology of *C. diphtheriae* may be different

Acknowledgements

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References

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