AMMI Canada Medical Student Research Proposal

Project: *Corynebacterium diphtheriae* infections in an impoverished, inner-city patient population: epidemiology and mechanisms of antibiotic resistance

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Background

Outbreaks and severe disease associated with non-toxigenic Corynebacterium diphtheriae are increasingly described worldwide, including Canada.

Globally, routine vaccination with diphtheria toxoid has greatly reduced cases of diphtheria due to infection by toxigenic *Corynebacterium diphtheriae* (1). Despite this, there have been numerous outbreaks of diphtheria reported globally since 2000 (2). Furthermore, the vaccine does not protect against non-toxigenic *C. diphtheriae*, which has been increasingly recognized as an emergent pathogen, especially among urban, impoverished populations in the developed world (3). Reports of non-toxigenic *C. diphtheriae* causing significant disease in the form of cutaneous diphtheria, as well as invasive infections such as bacteremia and endocarditis, are occurring with greater frequency (4-10).

Interpretive breakpoints for susceptibility testing have been updated, but clinical implications of isolates previously characterized as susceptible now reported intermediate are uncertain.

Penicillin and erythromycin are considered first-line antibiotics used to treat diphtheria (11). In recent years, a limited number of *C. diphtheriae* cases from Canada, the US, and the UK have exhibited resistance to penicillin and other conventional antimicrobials (10, 12, 13). The Clinical & Laboratory Standards Institute (CLSI) recently lowered the penicillin susceptible breakpoint from ≤1 mg/L to ≤0.12 mg/L. This has resulted in a reclassification of many *C. diphtheriae* isolates from being classified as penicillin susceptible to penicillin intermediate (14). However, clinical evidence of penicillin resistant *C. diphtheriae* is still limited to isolated case reports; moreover, it is unclear whether these cases reflect overall trends in the prevalence of resistant *C. diphtheriae.* Treatment options for penicillin-intermediate *C. diphtheriae* are limited by gastrointestinal side effects, risk of *C. difficile* infection, and broad spectrum antibiotic exposure for erythromycin, clindamycin and vancomycin, respectively. Therefore, there is a need for larger-scale susceptibility data to help establish the underlying epidemiology of resistant *C. diphtheriae* strains, and the potential implications of resistance on the interpretation of *C. diphtheriae* susceptibility testing in the clinical microbiology laboratory.

There is an opportunity to better understand the epidemiology of non-toxigenic C. diphtheriae resistance in a Canadian centre where this emerging pathogen is prevalent.

Our institution (St. Paul's Hospital, Vancouver BC) serves an impoverished, inner-city community in which cutaneous *C. diphtheriae* infection is not uncommon (3, 4, 15). Unlike many other microbiology laboratories in Canada, this has allowed for the accumulation of a significant number of clinical *C. diphtheriae* isolates over recent years. We aim to use this repository of strains to study the prevalence and evolving patterns of antibiotic susceptibility among *C. diphtheriae* isolates over the span of several years, as well as characterize potential mechanisms of antibiotic resistance.

Objectives

1. Evaluate the prevalence and patterns of antibiotic susceptibility among *C. diphtheriae* isolates collected over several years at an inner-city, tertiary care hospital.
2. Identify mechanisms of resistance through whole-genome sequencing (WGS).
3. Compare phenotypic interpretations of resistance (14, 16) with genotypic resistance analysis.
4. Determine clonality of *C. diphtheriae* isolates through both multilocus sequence typing (MLST) and WGS subtyping.
5. Determine toxigenic status of *C. diphtheriae* isolates by WGS and compare results with genotypic and phenotypic toxin testing performed by the national reference laboratory.

Methods

C. diphtheriae isolates

Approximately 100 *C. diphtheriae* isolates recovered from patients presenting to our hospital over the past 5 years will be included in this study. Isolates were recovered primarily from wound and blood cultures, and confirmed as *C. diphtheriae* as previously outlined (4). All isolates in this study were non-toxigenic as determined by the reference laboratory.

Antimicrobial susceptibility testing

Susceptibility testing will be carried out on *C. diphtheriae* isolates utilizing both Etest and agar dilution. Testing for penicillin, clindamycin, erythromycin and vancomycin will be completed based on CLSI M-45 guidelines (14). Susceptibility of isolates will be interpreted utilizing both the 2010 (16) and 2015 (14) breakpoints.

Genomic analysis

Whole-genome sequencing will be performed on all study isolates with next-generation sequencing (MiSeq, Illumina, San Diego, CA). Library preparation will be done using the KAPA Library HyperPlus Kit (Roche Sequencing, Pleasanton, CA).

Sequencing data will be analyzed for:

- MLST typing
- Identification of resistance markers for penicillin, macrolides and clindamycin
- Presence of diphtheria toxin gene

Clinical Significance

C. diphtheriae continues to cause significant invasive and cutaneous disease, for which penicillin and erythromycin remain the mainstays of therapy. Despite isolated reports, the prevalence of antibiotic-resistant *C. diphtheriae* remains unknown. To our knowledge, this study will be the first to utilize a large sample of *C. diphtheriae* isolates to estimate the prevalence of antibiotic resistance, as well as monitor developing trends in resistance over time. These results may have implications for interpretation and reporting of *C. diphtheriae* susceptibility testing within clinical microbiology laboratories and, more broadly, the choice of first-line antibiotic (penicillin vs. erythromycin) for clinicians treating *C. diphtheriae* infections.

References

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