The Broad Street Pump

Aug 2022

Issue 57

A Centre for Infectious Diseases and Microbiology – Public Health (CIDM-PH) and Sydney Institute for Infectious Diseases (Sydney ID) publication

Genomic variation in *Bordetella* spp. following induction of erythromycin resistance

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Published text can be found:

https://academic.oup.com/jac/advance-article/doi/10.1093/jac/dkac272/6667673

The *Bordetella* genus is comprised of several species and includes the mammalian pathogens *B. pertussis, B. parapertussis* and *B. bronchiseptica*. The human pathogens, *B. pertussis* and *B. parapertussis,* are the causative agents of pertussis, a highly infectious respiratory disease associated with prolonged coughing episodes.[1] *B. pertussis* is the primary cause of pertussis, however, it is estimated that *B. parapertussis* is responsible for approximately 1% of pertussis cases globally[2]. In recent years, the emergence of a closely related species *B. holmesii*, has impacted *B. pertussis* surveillance, as both species contain the PCR target used to diagnose *B. pertussis* infections. In Australia, *B. holmesii* has a prevalence of between 0 - 16.8% and this reflects its prevalence in other developed countries.[3, 4]

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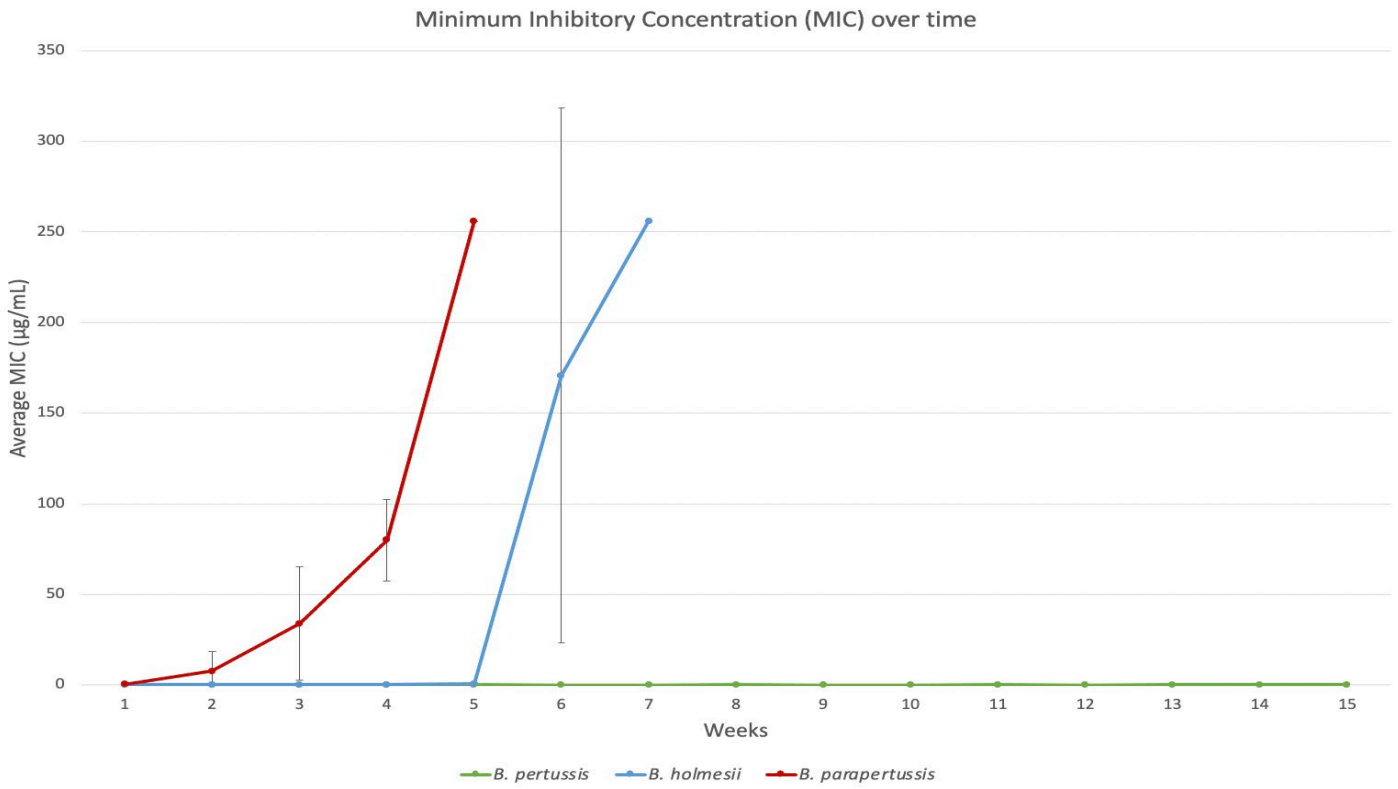

Figure 1: Average erythromycin MIC of *Bordetella* spp. across all 15 weeks. *B. parapertussis* became resistant in 2 weeks (average 4.5 weeks), while *B, holmesii* took on average 6 weeks. However, erythromycin MIC of *B. pertussis* remained persistently low. Error bars represent the standard deviation of MIC at the time point.

The currently recommended treatment for pertussis infections and post-exposure prophylaxis are macrolide antibiotics. However, macrolide-resistant strains of *B. pertussis* have been reported for some years in the U.S.A,[5] France,[6] China,[7-9] Iran[10] and Vietnam.[11] The resistance is due to a A2037G mutation in the 23S rRNA gene of *B. pertussis* in comparison to *B. pertussis* Tohama I.[5, 9] The increased prevalence of these strains in recent years has raised concerns for their global expansion.[12] Given pertussis can also be caused by other *Bordetella* species, namely, *B. parapertussis* and *B. holmesii*, the ability to recognise and monitor macrolide resistance in clinical strains of all *Bordetella* spp. becomes crucial.

We examined the comparative ability of several strains of *B. pertussis* and other significant *Bordetella* spp. (*B. parapertussis* and *B. holmesii*) to develop induced phenotypic resistance following exposure to erythromycin *in vitro*. Further strains were sequenced, and the genomes interrogated for any potential variation that may indicate erythromycin resistance.

Induction of resistance was performed on four *B. pertussis*, three *B. parapertussis* and three *B. holmesii* strains, where strains were grown on media containing erythromycin. Passaged isolates were sequenced every month (i.e., 4 weeks/8 passages) to monitor any intermediate genomic variation that may have contributed to phenotypic increase in MIC (Figure 1).

Despite some elevation of MIC to erythromycin, the genomes of the *B. pertussis* and *B. parapertussis* isolates contained no mutations in the 23S rRNA gene sequence reported in in macrolide-resistant *B. pertussis*[5]. However, such mutations in the 23S rRNA gene were detected in all resistant *B. holmesii* with each having a distinct mutation in positions G2031A (strain CIDM-BH2), A2032G (CIDM-BH3), and C2585T (CIDM-BH1). Long read sequencing allowed the differentiation of the three 23S rRNA gene copies, which cannot be resolved with short read sequencing. Mapping the short read CIDM-BH3 resistant (CIDM-BH3R) reads to the closed CIDM-BH3 genome confirmed that all three copies of the 23S rRNA carried the A to G mutation in position 2032. No mutations were observed in the 23S rRNA gene for both CIDM-BPP2 and CIDM-BPP2 resistant strain (CIDM-BPP2R).

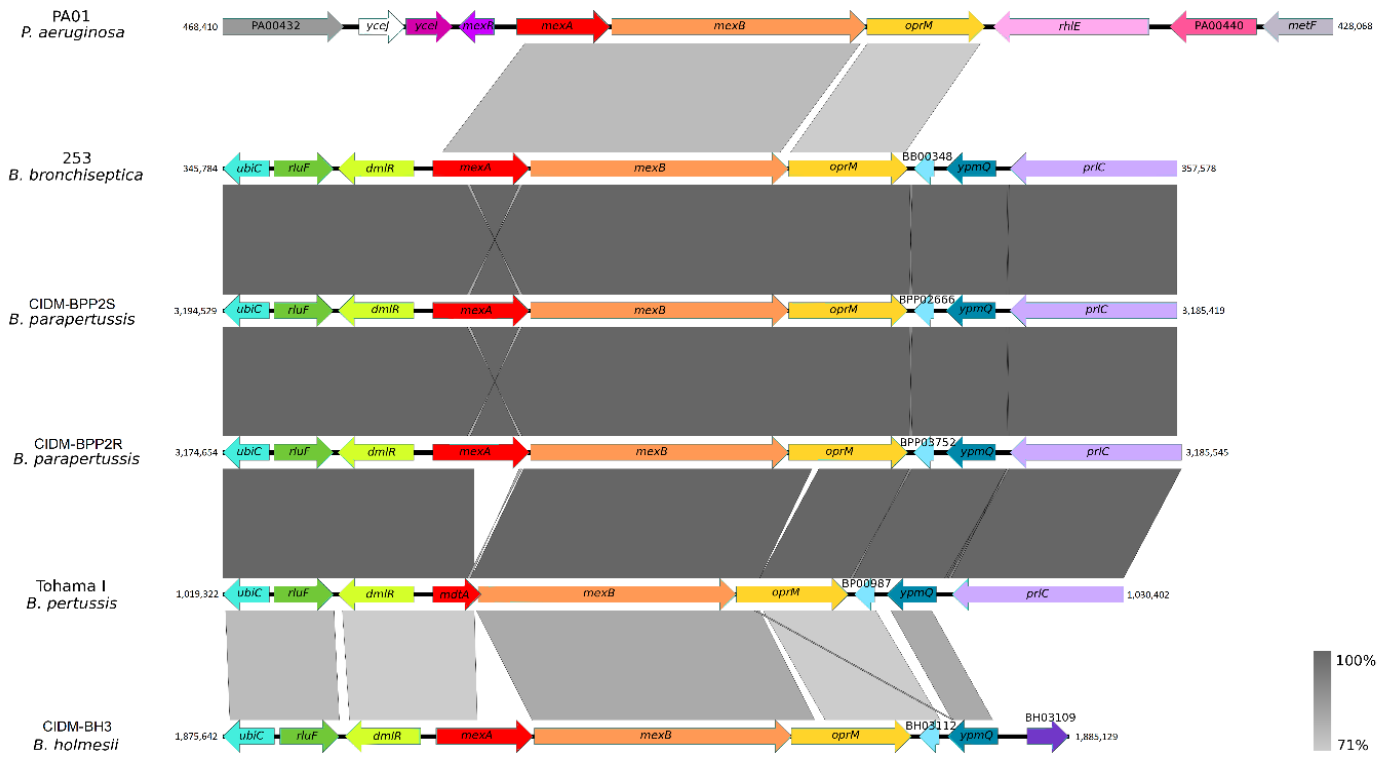

Figure 2: Comparison (BLASTn) of *mexAB-oprM* operon ± 3 flanking genes of *Pseudomonas aeruginosa* PA01 (NC_002516.2), *Bordetella bronchiseptica* 253 (NC_019382.1), *B. parapertussis* CIDM-BPP2 and CIDM-BPP2R, *B. pertussis* Tohama I (NC_002929.2), and *B. holmesii* CIDM-BH3. The primary pathogens from the *Bordetella* spp. carry a >71% ortholog by BLASTn of the *mexAB-oprM* operon, however, there is a gene deletion present in the *mexA* and *oprM* genes in *B. pertussis*. Nucleotide sequence similarity is scaled according to the scale bar.

We demonstrated that repeated exposure to erythromycin induced *in vitro* resistance in *B. parapertussis* and *B. holmesii* but not in *B. pertussis* for the duration of our study. While exposure decreased susceptibility to erythromycin in *B. pertussis*, the MICs did not reach levels defined as *in vitro* resistance. The predicted mechanisms of resistance varied between species, with *B. holmesii* containing a 23S rRNA gene mutation and *B. parapertussis* having no obvious mutations relating to macrolide resistance in this gene. The *B. holmesii* strains each acquired unique 23S rRNA mutations in different nucleotide positions, all of which have conferred resistance against macrolides in previous reports.[5] As induced erythromycin resistant *B. parapertussis* did not possess mutations in the 23S rRNA gene, other possible resistant mechanisms such as the presence of *erm, mef, mex* or *ere* were investigated.

While the 23S rRNA mutation was the most likely explanation of induced resistance in *B. holmesii,* isolates of *B. parapertussis* did not contain mutations in the same region. We found that none of the *erm, mef* or *ere* genes were present in the *Bordetella* species, however, an ortholog of the *mexAB-oprM* operon with >71% homology was detected in the genomes of all mammalian *Bordetella* spp. (*B. pertussis*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica*) (Figure 2). The *mexAB-oprM* operon is present in *Pseudomonas aeruginosa* and encodes for an efflux pump which confers macrolide resistance. The *mexAB-oprM* operon in *B. parapertussis* had high sequence similarity (99.7%) to *B. bronchiseptica*, and the function of *mexAB-oprM* is predicted to be the same in both species. *B. bronchiseptica*, the common ancestor of *B. parapertussis* and *B. pertussis,*[13, 14] is inherently macrolide resistant with an MIC between 4-32 mg/L,[15, 16] and is also known to rapidly develop macrolide resistance upon antibiotic pressure.[17]

In conclusion, our findings have indicated that *B. holmesii* and *B. parapertussis* can readily develop phenotypic resistance to erythromycin under antibiotic pressure while *B. pertussis* did not in the conditions described here. The predicted mechanisms of resistance varied between species, with *B. holmesii* containing a recognised 23S rRNA gene mutation and *B. parapertussis* having no obvious mutations relating to macrolide resistance. The presence of the *mexAB-oprM* ortholog (*acrAB-cusC* operon) has the potential to confer macrolide resistance in *B. parapertussis*. Genomic data and isolates with induced resistance can serve as reference points for development of diagnostic assays and surveillance of macrolide resistance in *Bordetella* recovered from patients with clinical pertussis.

These findings have significant implications for the development of antibiotic guidelines on treatment and prophylaxis of pertussis caused by these pathogens as infection with *B. parapertussis* or *B. holmesii* can be misdiagnosed as *B. pertussis*. The understanding of mechanisms of macrolide resistance and ability to detect resistance in a timely fashion can improve patient outcomes and reduce the spread of the disease. The ability of *B. parapertussis* and *B. holmesii* to rapidly acquire macrolide resistance highlights the need for better surveillance and antibiotic stewardship in the management and control of pertussis cases and outbreaks.

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Staff Profile

Jocelyne Basseal

Dr Jocelyne Basseal has recently joined the Sydney Infectious Diseases Institute (Sydney ID) core executive team leading the strategic development of the Institute and fostering national and global partnerships. Prior to this role, from April 2020, Jocelyne was an infection prevention and control consultant for the World Health Organization's COVID-19 response supporting member states in the Western Pacific Region. During this time, she worked closely with low-middle income countries responding to their IPC challenges, developing regional specific guidance and resources, quality improvement tools, and a communication toolkit for long-term care facilities. She has vast experience working with global stakeholders, has been invited to present at international conferences and is continually engaged with research collaborators across Australia.

By way of background, Jocelyne is a graduate from the University of Sydney with a PhD in Medical Microbiology, has supervised post-graduate students, delivered lectures, organised conferences, published in journals and textbooks. After leaving academia, Jocelyne spent 8 years as the Managing Editor, Research and Policy Manager for the Australasian Society for Ultrasound in Medicine. With strong governance knowledge, skills in policy development and communication, Jocelyne was instrumental in advocating for best practices in infection control for medical imaging. During this time, she was a consultant for corporate organisations as an IPC advisor, delivered educational workshops, supported associations with their peer-reviewed journals and developed a research grants scheme for a philanthropic organisation. Currently, Jocelyne is a member of the Standards Australia HE-023 committee on reusable medical devices and is facilitating guideline development for Radiology Across Borders. As President for the Australasian Medical Writers Association, Jocelyne is passionate about promoting excellence in health and medical communications.

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