Mechanisms of antibiotic resistance in *Haemophilus parainfluenzae*

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*Haemophilus parainfluenzae* is part of the HACEK group of fastidious bacteria commonly implicated in endocarditis and bacteremia. Previously considered as a normal respiratory, oral and sometimes genitourinary commensal, it has been recognised as a pathogen that can cause life-threatening infections in both immunocompromised and healthy individuals. It has also been reported as a bacterium that can harbor transferable antibiotic resistance genes. This paper presents a literature review on the molecular mechanisms of resistance of *H. parainfluenzae* to commonly prescribed antibiotics and discusses areas for further research.

**Keywords:** β-lactams, chloramphenicol, fluoroquinolones, HACEK, *Haemophilus parainfluenzae*, macrolides, resistance mechanisms, tetracycline

Introduction

*Haemophilus parainfluenzae*, a Gram-negative coccobacillus, is a pleomorphic, non-motile bacterium. It belongs to the family Pasteurellaceae and is most closely related to *Actinobacillus* spp. and *Aggregatibacter segnis*. Although considered a normal part of the respiratory, oral and genitourinary flora, it has pathogenic potential. *H. parainfluenzae* forms part of the HACEK Haemophilus parainfluenzae, Aggregatibacter actinomycetemcomitans, Aggregatibacter aphrophilus, Aggregatibacter paraphrophilus, Cardiobacterium spp., *Eikenella corrodens* and *Kingella* spp.) group of bacteria, which are implicated in bacteremia and infective endocarditis.

A literature search revealed a paucity of research and information on antibiotic resistance mechanisms in this pathogen. The aim of this paper is, therefore, to provide a comprehensive review on molecular resistance mechanisms of *H. parainfluenzae* to commonly prescribed antibiotics.

Infections caused by *H. parainfluenzae*

*H. parainfluenzae* has been implicated in a number of infections. In addition to endocarditis and bacteremia, other pathologies associated with *H. parainfluenzae* include cellulitis, myositis, biliary tract infections, meningitis, septic arthritis, neonatal sepsis, peritonitis, acute exacerbation of chronic obstructive pulmonary disease (AECOPD), bronchitis, sinusitis, otitis media, prostatic/native joint infections, brain abscess, soft tissue infection, chorioamnionitis in women, hepatic infections, ophthalmic infections, and urinary tract infections.

Molecular mechanisms of resistance of *H. parainfluenzae* to antibiotics

The molecular mechanisms of resistance employed by *H. parainfluenzae* against antibiotics are diverse but similar to mechanisms employed by other Gram-negative bacteria. Investigation of these mechanisms involved polymerase chain reaction (PCR) amplification, sequence and alignment of genes to detect amino acid substitutions and expression of certain target proteins. The molecular mechanisms of antibiotic resistance reported in *H. parainfluenzae* are summarised in Table 1. The resistance mechanisms against each group of antibiotic are discussed below.

### β-lactam antibiotics

Generally, resistance to β-lactam antibiotics is mediated through the production of β-lactam hydrolyzing enzymes (β-lactamases), changes in outer membrane permeability (modifications in porins), penicillin binding proteins (PBPs) with poor affinity for the antibiotic and the expression of efflux pumps that actively eject the antibiotics from the cell.

The first report of β-lactam resistance in *H. parainfluenzae* was in 1976 by Groves et al. Since then, resistance has been observed globally at varying rates. The bacterium is notorious for β-lactamase production, and its β-lactamase producing ability is typically mediated by transferable plasmids.

**H. parainfluenzae** is also considered as the origin and reservoir for the dissemination of β-lactam-carrying plasmids to other bacterial species. The β-lactamases detected include the TEM-1 and TEM-15 types, which are usually associated with plasmids. It was reported that the plasmid that carried the TEM-1 β-lactamase was identical to one isolated from *African-type* penicillinase-producing *Neisseria gonorrhoeae* strain. Inhibitor resistant β-lactamases TEM-34 and TEM-182, which were both carried by TnA transposon of the Tn2 type, were also detected in *H. parainfluenzae* in 2011.

β-lactam resistance in *H. parainfluenzae* not mediated by β-lactamases has also been reported. Some of these resistant bacteria utilise mutations in the PBPs to circumvent the lethal effects of the antibiotic. Molecular analysis revealed these mutations include amino acid substitutions in the PBPs: Lys276Asn, Ala307Asn, Val329Ile, Ser385Thr, Val511Ala, Asn526Lys, Asn526Ser, Ala343Val and Asn526His, Ala530Ser, Thr574Ala. Efflux-mediated mechanisms of resistance and under expression of porins in β-lactam resistant strains of *H. parainfluenzae* were not evident from the literature search.
Resistance to these agents include: alternative metabolic pathways; impermeability of the cell wall; production of a resistant chromosomal enzyme; and, the production of a plasmid mediated inhibitor-resistant enzyme.

Plasmid and transposon-borne sulfonamide (sul) and trimethoprim (dfr) resistance genes have been reported, as have mutations in chromosomal gene folP for dihydropteroate synthase, leading to a reduced affinity for the inhibiting sulfonamide. These mutations for dihydropteroate synthase, leading to a reduced affinity for the inhibiting sulfonamide. These mutations are responsible for resistance to sulfonamides.

Resistance of H. parainfluenzae to aminoglycosides has been reported, but to the best of our knowledge, the underlying molecular mechanisms remain unreported. There is, therefore, the need to study the molecular mechanisms of resistance.

Macrolides, azalides, ketolides, lincosamides and streptogramins

Resistance to the macrolides, azalides, ketolides, lincosamides and streptogramins is mediated by three main mechanisms: active efflux encoded by the msr (A), msr (B), msr (D), and mef (A) genes; drug inactivation through esterases, phosphotransferases (encoded by mph genes); and, most importantly, target alteration by methylation (usually mediated by methylases encoded by mph genes) or the mutations in the domain V of rRNA and L4 and L22 that inhibit the antibiotic binding to its ribosomal target.

Resistance of H. parainfluenzae to macrolides is attributed to the presence of the efflux mediated resistance mechanism mef (A) and msr (D), and to the Ala69Ser substitution in the L4 protein, a mutation formerly associated with H. influenzae. The ErmB gene which codes for methylases has also been implicated in macrolide, azalide and ketolide resistance by H. parainfluenzae.

Substitutions in the L22 ribosomal protein and in the 23S rRNA have not yet been reported in macrolide resistant H. parainfluenzae. However, the involvement of other resistance mechanisms of the bacteria to macrolides is still to be determined.

Tetracyclines

Resistance to tetracyclines is mediated through the following mechanisms: (1) enzymatic breakdown of tetracyclines; (2) mutations in rRNA; (3) efflux mediated mechanisms; (4) production of ribosomal protection proteins; and, (5) undetermined means that work by mechanisms completely different from well-documented determinants. The most important is the production of ribosomal protection proteins, which include Tet (T), Tet (S), Tet (Q), Tet (B), Tet (W), Tet (O), Tet (M) and OtrA. Tet (O) and Tet (M) are the most extensively studied and were originally described in Campylobacter jejuni and Streptococcus spp.

In H. parainfluenzae, production of the ribosomal protective protein Tet (M), Tet (B), Tet (C), Tet (D) has been reported to cause resistance to tetracyclines, while enzymatic breakdown, mutations in rRNA and efflux pump mediated tetracycline resistance remain under-investigated.

Quinolones

Molecular detection of mechanisms carried out on fluoroquinolone resistant H. parainfluenzae over the past few years have revealed that mutations in genes that led to amino acid substitutions in Quinolone Resistance Determining Region (QRDR) of gyrA, gyrB, parC and parE are responsible for resistance to fluoroquinolones. Some of the substitutions detected included Ser84Phe in gyrA, Ser84Phe, Ser84Leu, Ser84Thr and Met198Leu in parC, and Asp420Asn and Ala451Ser in parE. Of the studies that investigated the involvement of plasmid mediated resistance genes (qnrA, qnrB, qnrS, qnrC, qnrD, qnrVC, qepA and aac(6’)-ib-cr genes), only one detected aac(6’)-ib-cr in four isolates of H. parainfluenzae.

Chloramphenicol

Resistance of H. parainfluenzae to chloramphenicol was reported in 1976, with the molecular mechanism underlying this resistance being the production of CatS acetyltansferase enzyme. This enzyme is coded by the cat gene and is located on the chromosome of H. parainfluenzae as opposed to being plasmid-borne as observed in H. ducreyi and H. influenzae. The CatS of H. parainfluenzae share some similarity with type II CatS from Enterobacteriaceae.

Aminoglycosides

Molecular mechanisms underlying resistance to this class of antibiotics include active efflux, reduced outer membrane permeability, mutations in the target molecule and inactivation by enzymes, the latter being the most common. This mechanism is mediated by three groups of enzymes: phosphotransferases, acetyltransferases and nucleotidyltransferases.

Resistance of H. parainfluenzae to aminoglycosides has been reported, but to the best of our knowledge, the underlying molecular mechanisms remain unreported. There is, therefore, the need to study the molecular mechanisms of resistance.

Folic acid metabolism inhibitors

Resistance to these agents include: alternative metabolic pathways; impermeability of the cell wall; production of a resistant chromosomal enzyme; overproduction of a susceptible chromosomal enzyme; and, the production of a plasmid mediated inhibitor-resistant enzyme.

Plasmid and transposon-borne sulfonamide (sul) and trimethoprim (dfr) resistance genes have been reported, as have mutations in chromosomal gene folP for dihydropteroate synthase, leading to a reduced affinity for the inhibiting sulfonamide. These mutations include Leu186Phe; Asp238Asn and Phe246Tyr amino acid substitutions. Although high level resistance of H. parainfluenzae to anti-folate agents has been reported, the molecular mechanisms underlying the observed resistance (to the best of our knowledge) was not reported.
Conclusion
This literature review revealed that the molecular mechanisms of resistance of *H. parainfluenzae* to a number of commonly prescribed antibiotics have not been fully investigated, although it has been shown to harbor transferable resistance determinants that can spread to more clinically significant bacteria. There is, therefore, an ongoing need for antimicrobial susceptibility surveillance and elucidation of genotypic mechanisms of resistance.

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