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REVIEW ARTICLE

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 cocco-bacillus, is a pleomorphic, non-motile bacterium. It belongs to the family Pasteurellaceae and is most closely related to *Actinobacillus spp.* and *Aggregatibacter segnis*.[1] 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.[2]

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,[3] other pathologies associated with *H. parainfluenzae* include cellulitis, myositis,[4] biliary tract infections,[5] meningitis,[6] septic arthritis,[7,8] neonatal sepsis,[9] peritonitis,[10] acute exacerbation of chronic obstructive pulmonary disease (AECOPD),[11] bronchitis, sinusitis, otitis media, prosthetic/native joint infections, brain abscess, soft tissue infection, chorioamnionitis in women, hepatic infections,[2] ophthalmic infections,[12] and urinary tract infections.[13]

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.[22] The first report of β-lactam resistance in *H. parainfluenzae* was in 1976 by Groves *et al.*[23] 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.[17,24–26]

H. parainfluenzae is also considered as the origin and reservoir for the dissemination of β-lactamase-carrying plasmids to other bacterial species.[27] The β-lactamases detected include the TEM-1 and TEM-15 types, which are usually associated with plasmids.[14,16,28,29] 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.[26,30] 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.[16]

β-lactam resistance in *H. parainfluenzae* not mediated by β-lactamases has also been reported.[16,31] 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 PBP3; Lys276Asn, Ala307Asn, Val329Ile, Ser385Thr, Ile442F, Val511Ala, Asn526Lys, Asn526Ser, Ala343Val and Asn526His, Ala530Ser, Thr574Ala.[14–17] 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.

Table 1: Molecular resistance mechanisms of *H. parainfluenzae*

Antibacterial agent	Molecular mechanisms of resistance	Reference
Beta lactams	PBP3: Lys276Asn, Ala307Asn, Val329Ile, Ser-385Thr, Ile442Phe, Val511A-la, Asn526Lys, Asn526Ser, Ala343Val, Asn526His, Ala530Ser, Thr574Ala, Val-562Ile, Val488Ile, Glu398Asp, Ile414Val,	(14–17)
	TEM 15, TEM-182, TEM-34, TEM-1	(14, 16)
Macrolides	*Mef* (A), *Msr* (D), *Erm B*; L4: Ala69Ser	(15, 18)
Quinolones	*GyrA*: Ser84Phe, Asp88Tyr, Ser84Leu;	(19, 20)
	ParC (Ser84Phe, Ser84Leu, Ser84Tyr, Ser138Thr and Met198Leu)	(15, 17, 18, 21)
	ParE (Asp420Asn and Ala451Ser)	
	Aac-(6′)-Ib-cr	
Tetracycline	*Tet* (M)	(15)
Trimethoprim-Sulfameth-oxazole	None reported	
Rifampin	None reported	
Chloramphenicol	CatS	(15)
Aminoglycosides	None reported	

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 *erm* genes) or the mutations in the domain V of rRNA and L4 and L22 that inhibit the antibiotic binding to its ribosomal target.[32]

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*.[18] Substitutions in the L22 ribosomal protein and in the 23S rRNA have not yet been reported in macrolide resistant *H. parainfluenzae*.[15] 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.*[33]

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,[15,29] 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 and Asp88Tyr in *gyrA*, Ser84Phe, Ser84Leu, Ser84Tyr, Ser138Thr 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),[15,17,19,20,34] only one detected *aac(6′)-Ib-cr* in four isolates of *H. parainfluenzae*.[18]

Chloramphenicol

Resistance of *H. parainfluenzae* to chloramphenicol was reported in 1976,[35] with the molecular mechanism underlying this resistance being the production of *CatS* acetyltransferase enzyme.[15] 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.[36]

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.[37]

Resistance of *H. parainfluenzae* to aminoglycosides has been reported,[38] 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; Asn245Lys and Phe246Tyr amino acid substitutions.[39] Although high level resistance of *H. parainfluenzae* to anti-folate agents has been reported,[34] 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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References

1. Pollard A, St Michael F, Connor L, et al. Structural characterization of *Haemophilus parainfluenzae* lipooligosaccharide and elucidation of its role in adherence using an outer core mutant. Can J Microbiol. 2008 Nov;54(11):906–17. PubMed PMID: 18997847. https://doi.org/10.1139/W08-082
2. Janda WM. Update on the HACEK group of fastidious gram-negative bacilli Part I. Clin Microbiol Newsl. 2013;35(11):87–92. https://doi.org/10.1016/j.clinmicnews.2013.05.001
3. Yew HS, Chambers ST, Roberts SA, et al. Association between HACEK bacteraemia and endocarditis. J Med Microbiol. 2014 Jun;63 (Pt 6):892–5. PubMed PMID: 24681996.
4. Owensby JE, Elliott S, Tu K, et al. Cellulitis and myositis caused by agrobacterium radiobacter and *haemophilus parainfluenzae* after influenza virus vaccination. South Med J. 1997 Jul;90(7):752–4. PubMed PMID: 9225903. https://doi.org/10.1097/00007611-199707000-00022
5. Frankard J, Rodriguez-Villalobos H, Struelens MJ, et al. *Haemophilus parainfluenzae*: an underdiagnosed pathogen of biliary tract infections? Eur J Clin Microbiol Infect Dis. 2004 Jan;23(1):46–8. PubMed PMID: 14669072. https://doi.org/10.1007/s10096-003-1050-z
6. Cardines R, Giufre M, Ciofi degli Atti ML, et al. *Haemophilus parainfluenzae* meningitis in an adult associated with acute otitis media. New Microbiol. 2009 Apr;32(2):213–5. PubMed PMID: 19579703.
7. Carey TW, Jackson K, Roure R, et al. Acromioclavicular septic arthritis: a case report of a novel pathogen. Am J Orthop. 2010 Mar;39(3): 134–6. PubMed PMID: 20463985.
8. Mora A, Marimón I, Mesquida J, et al. *Haemophilus parainfluenzae* septic arthritis: report of a case and review of the literature. Enferm Infecc Microbiol Clin. 2011 Jun–Jul;29(6):472–3. PubMed PMID: 21507531. Epub 2011/04/22. eng. https://doi.org/10.1016/j.eimc.2011.02.015
9. Govind B, Veeraraghavan B, Anandan S, et al. *Haemophilus parainfluenzae*: report of an unusual cause of neonatal sepsis and a literature review. J Infect Dev Ctries. 2012 Oct;6(10):748–50. PubMed PMID: 23103898.
10. Gardenier JC, Sawyer RG, Sifri CD, et al. Peritonitis caused by *haemophilus parainfluenzae*, *leifsonia aquatica*, and *gordonia* spp. in a patient undergoing continuous ambulatory peritoneal dialysis. Surg Infect. 2012 Dec;13(6):409–12. PubMed PMID: 23268614. Epub 2012/12/28. eng. https://doi.org/10.1089/sur.2011.009
11. Ye F, He LX, Cai BQ, et al. Spectrum and antimicrobial resistance of common pathogenic bacteria isolated from patients with acute exacerbation of chronic obstructive pulmonary disease in mainland of China. Chin Med J (Engl). 2013 Jun;126(12):2207–14. PubMed PMID: 23786927.
12. Khauv P, Turner P, Soeng C, et al. Ophthalmic infections in children presenting to Angkor Hospital for Children, Siem Reap, Cambodia. BMC Res Notes. 2014;7:784. PubMed PMID: 25369774. Pubmed Central PMCID: 4228269. https://doi.org/10.1186/1756-0500-7-784
13. Chow SK, Clarridge JE, 3rd. Necessity of 16S rRNA gene sequencing for identifying *Haemophilus parainfluenzae*-like strains associated with opportunistic urinary tract infections. J Med Microbiol. 2014 Jun;63(Pt_6):805–11. PubMed PMID: 24648471. Epub 2014/03/22. eng. https://doi.org/10.1099/jmm.0.071803-0
14. Tristram SG, Pitout MJ, Forward K, et al. Characterization of extended-spectrum beta-lactamase-producing isolates of *Haemophilus parainfluenzae*. J Antimicrob Chemother. 2008 Mar;61(3):509–14. PubMed PMID: 18245789. Epub 2008/02/05. eng. https://doi.org/10.1093/jac/dkm523
15. Tinguely R, Seiffert SN, Furrer H, et al. Emergence of extensively drug-resistant *haemophilus parainfluenzae* in Switzerland. Antimicrob Agents Chemother. 2013 Jun;57(6):2867–9. PubMed PMID: 23545526. Pubmed Central PMCID: 3716184. https://doi.org/10.1128/AAC.00221-13
16. Garcia-Cobos S, Arroyo M, Campos J, et al. Novel mechanisms of resistance to beta-lactam antibiotics in *Haemophilus parainfluenzae*: beta-lactamase-negative ampicillin resistance and inhibitor-resistant TEM beta-lactamases. J Antimicrob Chemother. 2013 May;68(5):1054–9. PubMed PMID: 23335113. https://doi.org/10.1093/jac/dks525
17. Giufrè M, Daprai L, Garlaschi M, et al. Genital carriage of the genus Haemophilus in pregnancy: species distribution and antibiotic susceptibility. J Med Microbiol. 2015;64(7):724–30. https://doi.org/10.1099/jmm.0.000083
18. Abotsi RE, Govinden U, Moodley K, et al. Fluoroquinolone, Macrolide, and Ketolide Resistance in *Haemophilus parainfluenzae* from South Africa. Microb Drug Resist. 2017 Jan 19. PubMed PMID: 28103180. Epub 2017/01/20. eng.
19. Elliott E, Oosthuizen D, Johnson MM, et al. Fluoroquinolone resistance in *Haemophilus influenzae*. J Antimicrob Chemother. 2003;52(4):734–5. https://doi.org/10.1093/jac/dkg420
20. Law DK, Shuel M, Bekal S, et al. Genetic detection of quinolone resistance in *Haemophilus parainfluenzae*: Mutations in the quinolone resistance-determining regions of gyrA and parC. Can J Infect Dis Med Microbiol. 2010 Spring;21(1):e20–2. PubMed PMID: 21358875. Pubmed Central PMCID: 2852290.
21. Rodriguez-Martinez JM, Lopez-Hernandez I, Pascual A. Molecular characterization of high-level fluoroquinolone resistance in a clinical isolate of *Haemophilus parainfluenzae*. J Antimicrob Chemother. 2011 Mar;66(3):673–5. PubMed PMID: 21127039. Epub 2010/12/04. eng. https://doi.org/10.1093/jac/dkq473
22. Wilke MS, Lovering AL, Strynadka NCJ. β-Lactam antibiotic resistance: a current structural perspective. Curr Opin Microbiol. 2005 10;8(5):525–33. https://doi.org/10.1016/j.mib.2005.08.016
23. Groves DJ, Adeniyi-Jones C. Ampicillin-resistant *Hemophilus* sp. Can Medical Assoc J. 1976;114(5):407.
24. Scheifele DW, Fussell SJ, Roberts MC. Characterization of ampicillin-resistant *Haemophilus parainfluenzae*. Antimicrob Agents Chemother. 1982 May;21(5):734–9. PubMed PMID: 6980626. Pubmed Central PMCID: 182003. https://doi.org/10.1128/AAC.21.5.734
25. Brunton J, Meier M, Erhman N, et al. Origin of small beta-lactamase-specifying plasmids in *Haemophilus species* and *Neisseria gonorrhoeae*. J Bacteriol. 1986 Oct;168(1):374–9. PubMed PMID: 3020002. Pubmed Central PMCID: 213461. https://doi.org/10.1128/jb.168.1.374-379.1986

26. Martel AY, Gosselin P, Ouellette M, et al. Isolation and molecular characterization of beta-lactamase-producing *Haemophilus parainfluenzae* from the genital tract. Antimicrob Agents Chemother. 1987 Jun;31(6):966–8. PubMed PMID: 3497610. Pubmed Central PMCID: 284226. https://doi.org/10.1128/AAC.31.6.966
27. Facinelli B, Montanari MP, Varaldo PE. *Haemophilus parainfluenzae* causing sexually transmitted urethritis. Sexually Transmitted Diseases. 1991 Jul–Sep;18(3):166–9. PubMed PMID: 1948515. Epub 1991/07/01. eng. https://doi.org/10.1097/00007435-199107000-00008
28. Marchese A, Ardito F, Fadda G, et al. The Sentinel Project: an update on the prevalence of antimicrobial resistance in community-acquired respiratory *Streptococcus pneumoniae* and *Haemophilus* spp. in Italy. Int J Antimicrob Agents. 2005 Jul;26(1): 8–12. PubMed PMID: 15967638. https://doi.org/10.1016/j.ijantimicag.2005.02.017
29. Dimopoulou ID, Kartali SI, Harding RM, et al. Diversity of antibiotic resistance integrative and conjugative elements among haemophili. J Med Microbiol. 2007 Jun;56(6):838–46. PubMed PMID: 17510272. https://doi.org/10.1099/jmm.0.47125-0
30. Brunton J, Clare D, Meier MA. Molecular epidemiology of antibiotic resistance plasmids of haemophilus species and neisseria gonorrhoeae. Rev Infect Dis. 1986 Sep–Oct;8(5):713–24. PubMed PMID: 3024290. https://doi.org/10.1093/clinids/8.5.713
31. Smith PW, Chambers WA, Walker CA. Ampicillin resistant *Haemophilus parainfluenzae* endocarditis. Am J Med Sci. 1979 Sep–Oct;278(2):173–6. PubMed PMID: 517570. Epub 1979/09/01. eng. https://doi.org/10.1097/00000441-197909000-00011
32. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis. 2002;34(4):482–92. https://doi.org/10.1086/324626
33. Taylor DE, Chau A. Tetracycline resistance mediated by ribosomal protection. Antimicrob Agents Chemother. 1996;40(1):1–5.
34. Rodriguez-Martinez J-M, Lopez-Hernandez I, Pascual A.. Molecular characterization of high-level fluoroquinolone resistance in a clinical isolate of *Haemophilus parainfluenzae*. J Antimicrob Chemother. 2011;66(3):673–5. https://doi.org/10.1093/jac/dkq473
35. Kosakai N, Oguri T. Distribution and changes of antibiotic susceptibility of genus Haemophilus (author's transl). Jpn J Antibiot. 1976 Feb;29(2):159–66. PubMed PMID: 1046091.
36. Roberts MC, Actis LA, Crosa JH. Molecular characterization of chloramphenicol-resistant *Haemophilus parainfluenzae* and *Haemophilus ducreyi*. Antimicrob Agents Chemother. 1985 Aug;28(2):176–80. PubMed PMID: 3879658. Pubmed Central PMCID: 180214. https://doi.org/10.1128/AAC.28.2.176
37. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis. 2007;45(1):88–94. https://doi.org/10.1086/518605
38. Liu JD, Lu HX, Hu L. The isolation results and the antimicrobial agents susceptibility analysis of Haemophilus from the sputum of the elderly suffering from respiratory tract disease. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi. 1999 Dec;10(2):71–5. PubMed PMID: 10681708.
39. Sköld O. Resistance to trimethoprim and sulfonamides. Vet Res. 2001;32(3/4):261–73. https://doi.org/10.1051/vetres:2001123

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