MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Novel Bacterial Species in the Human Nasal Microbiome

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Abstract

Human respiratory problems have been linked to *Candidatus Ornithobacterium hominis* (*O. hominis*), which was discovered in nasopharyngeal swabs from all over the world. The largest family in the Bacteroidetes phylum is Flavobacteriaceae, which includes *O. hominis*, a recently discovered member. This family has 90 genera and hundreds of species, including important human infections like *Elizabethkingia meningoseptica and Capnocytophaga canimorsus*. This review aims to obtain a more profound understanding of the genomic landscape of *O. hominis*, with an emphasis on identifying the associated virulence, antimicrobial genes, and distinct defense mechanisms, In this review, we summarized the sum of the previous works done on the *O. hominis* genome to determine the antimicrobial resistance genes and virulence factors that are present in the core genome. We discussed the initial description of the culture conditions used to isolate this bacterium. A deeper understanding of the therapeutic significance of this species is expected to become easier with the availability of an easily reproducible culture method. We also discussed the phylogenetic relationship of *O. hominis* and *O. rhinotracheal*, which shows close genus relationships indicated by the two genomes' approximately 40% amino acid similarity over 50% of their length.

Keywords: *Ornithobacterium*, nasal microbiome, antimicrobial, resistance genes, virulence factors.

Introduction:

The Flavobacteriaceae family, which comprises the recently identified member OH, is the largest in the Bacteroidetes phylum. There are hundreds of species and 90 genera in this family, including important human infections like *Capnocytophaga canimorsus* and *Elizabethkingia meningoseptica*. Human respiratory diseases have been linked to *O. hominis*, which was discovered in nasopharyngeal swabs from all over the world. It's still unclear exactly what part *O. hominis* plays in the emergence of disease. The biological roles of OH and the molecular pathways by which it interacts with the host microbiota are still unknown (Marsh et al., 2020)

Although the role of *O. hominis* in ear and respiratory diseases remains uncertain, its initial discovery was in infants from refugee camps in Thailand and Australia with elevated incidences of respiratory diseases (Salter et al., 2017). Notably, children lacking a history of otitis media (OM)

either did not harbor *O. hominis* in their nasal microbiome or possessed it at a low relative abundance. OH, represents the sole known human-associated species within the genus *Ornithobacterium* (Coleman et al., 2021). Its presence in children with OM suggests a potential role as a novel otopathogen within this population. Moreover, network correlations revealed associations between *Ornithobacterium*, *Helcococcus*, and *Dichelobacter*, indicating their potential influence on clinical outcomes. Recent investigations propose the existence of new bacterial species within genera lacking known human representatives, such as *Dichelobacter* and *Gracilibacteria*, or containing only one species, such as by *Dolosigranulum*, within the nasal microbial community (Coleman et al. 2021). Previous research on the nasopharyngeal microbiota of children in the Maela refugee camp in Thailand used 16S rRNA gene sequencing to identify unclassified taxon (Salter et al. 2017). The gene shared 93 percent nucleotide identity with that of the avian respiratory pathogen *Ornithobacterium rhinotracheale*, and it was >99 percent identical to other unclassified sequences identified in nasopharyngeal samples from the Gambia (Kwambana-Adams et al., 2017), Kenya (Feazel et al., 2015), and Australia (Marsh et al., 2016). Being a component of the respiratory system, the nasopharynx is home to a special microbial community that develops during infancy and evolves throughout life. The nasopharyngeal microbiome is significant because it contains both pathogenic and non-pathogenic types of bacteria that might result in illnesses like otitis media or pneumonia (Salter et al., 2019). Children under the age of two and occasionally their mothers were found to be colonized by *O. hominis* in Maela, a refugee camp in Thailand (Salter et al. 2017). Therefore, it is essential to study the pathogenic potential and clinical significance of *O. hominis*. Most respiratory bacteria that can result in middle ear and respiratory infections reside in the nasopharynx.

The prevalence and persistence of *O. hominis*, in the nasopharynx of a pediatric population at high risk of respiratory infection were discovered by a polymerase chain reaction (PCR)-based study, which has increased interest in this bacterium **(Salter *et al.*, 2019)**. The closest known relative of *O. hominis* is *Ornithobacterium rhinotracheale;* a bird respiratory pathogen. These findings compel investigation into the clinical relevance and pathogenic potential of *O. hominis*. Even though metagenomic data can be used to deduce genomes, OH isolates are required to fully understand the bacterium's role in human respiratory diseases **(Lawrence *et al.*, 2019)**.

The ability of co-residing commensal nasopharyngeal bacteria to alter the behavior of pathogenic species is becoming more widely acknowledged, which can directly influence research on probiotic techniques for preventing pathogen colonization of the nasopharynx. For the development of such cutting-edge treatments, it is essential to comprehend the interactions between commensal and pathogenic species that co-occur in the nasopharynx (Kang and Kang, 2021). This review aimed to shed light on the genomic landscape of *O. hominis* and its potential role in human respiratory infections.

Culture of Ornithobacterium hominis

Following techniques suitable for *O. rhinotracheale* or other nasopharyngeal bacteria, initial attempts have been undertaken to culture the bacteria from nasopharyngeal swabs anticipated to contain a significant amount of this species. *O. hominis* has not yet been successfully cultured from any archived sample (Salter et al. 2019). Previous study shows that culture was performed using bio-banked nasopharyngeal swabs that were collected from four Australian children (age 1–2 years) immediately right before bronchoscopy for investigation for chronic suppurative lung disease (Lawrence et al., 2019). Using Tryptic Soy Agar with 5% Sheep Blood (TSA), Horse Blood Columbia agar (HBA), Chocolate agar, and Brain Heart Infusion agar (BHI), ten microliters of the STGGB swab media were inoculated. The plates were incubated for up to five days at 35 °C aerobically, microaerophilically (Campygen, Oxoid), and anaerobically (Anaerogen, Oxoid). The sole study reported that the primary isolation of the *O. hominis* was challenging due to extensive overgrowth by other species. (Lawrence et al. 2019).

Genetic similarities between O. rhinotracheale and O. hominis

Based on 16S rRNA gene sequences, *O. hominis*"s place within the Flavobacteriaceae family. A limited portion of the genome can be used to compute the ANI between *O. rhinotracheale* UMN-88 (Zehr et al., 2014) and *O. hominis*. Based on 75% of predicted proteins, the two-way AAI between *O. hominis* and UMN-88 is almost 62%. A different metric, POCP (Qin et al., 2014), can be used to determine how similar two genomes are to one another at the genus level. For this measure, a gene needs to share more than 40% of its amino acid similarity over >50% of its length in order to be deemed conserved for this measure; two members of the same genus are anticipated to share at least half of their proteins. Between UMN-88 and *O. hominis*, the POCP is almost 58%. Since *O. hominis'* has 50.7% of the UMN-88 proteins

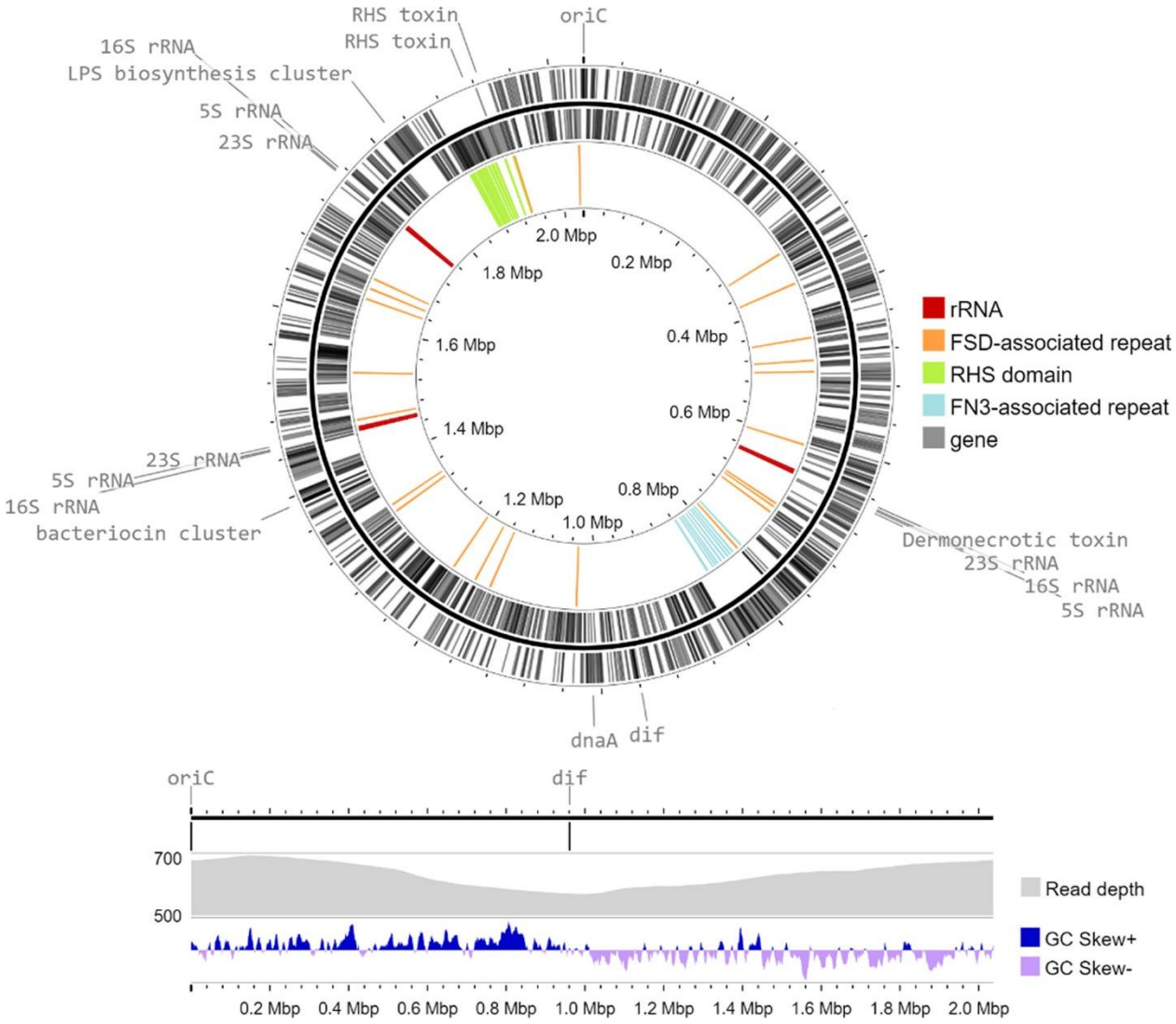

Figure 1. Circular chromosome of O. hominis MSHR-COH1, displaying genes in gray and highlighting repeat locations on the inner ring that could interfere with short-read assemblies. Key landmarks around the genome are labeled, including a putative dermonecrotic toxin gene, a bacteriocin cluster, a lipopolysaccharide (LPS) biosynthesis cluster, and rRNA gene loci. (Bottom) Proposed origin of replication and different sites, aligned with GC skew and mapped read depth (Salter et al., 2023)

conserved, despite the fact that these numbers are based on draft genomes, it seems likely that these are closely related members of the same genus. The ANI of the two genomes, OH-22767 and OH-22803, is 98.78%, which is higher than the 96% criteria for strains belonging to the same species (Salter et al., 2019).

Genome annotation

O. hominis MSHR-COH1 has a single 2,036,909 bp circular chromosome with a 35.72% GC content, 1,899 genes (1,830 of which are coding sequences), and 3 rRNA operons that make up its genome as shown in Fig 1. The rRNA operons, 450-bp noncoding regions linked to putative Fibrobacter succinogenes domain genes, >500-bp rearrangement hotspot (RHS) domain sequences, and >1-kb sequences within putative type III fibronectin domain genes are among the sites that may disrupt short-read assemblies (Salter et al., 2023) . Remarkably, the DNA gene is located close to the hypothesised genome terminus instead of the replication origin. The increased mapped read depth close to the replication origin, the positive GC skew on the leading strand, and the probable dif sequence—which resembles that of Escherichia coli—all support the hypothesised origin (Salter et al., 2023).

Core Genome

The *O. hominis* core genome had many genes that encoded resistance to antibiotics. The core genome included several virulence factors as well. The toxin-encoding genes toxA and parE1, together with genes linked to gliding motility, were found to be among the intriguing genes in the core genome (Kumar et al., 2009). The gliding motility lipoproteins (GldJ, GldD, and GldN) and the gliding motility-associated ABC transporter substrate-binding protein (GldA) have been demonstrated to be important for virulence in related Flavobacterium species. The genome had genes that encoded different proteins. Three of these proteins—TraB, TraD, and TraA—are thought to be conjugative transposons. This implies that there may be a function for mobile genetic components in the genome (Salter et al., 2019).

Accessory genome

Approximately half of the MAGN1 *O. hominis* genome consists of genes encoding hypothetical proteins. The genome also includes evidence of mobile elements, transfer and mobilization genes, phages, and various lipopolysaccharide (LPS) synthesis clusters. A prophage region, appearing as short segments less than 13 kb, was identified. The draft genome also contained the cell wall-associated hydrolase family ID PGF_08065842, as well as genes encoding penicillin-binding proteins linked to multidrug resistance. Additionally, it included genes encoding unique proteins, such as ParA and ParB-related ThiF family proteins, which were identified as conjugative transposons. The genome also had one pathogenic protein family that matched Flavobacterium johnsoniae UW101 with accession ID CP000685. This protein exhibited 88% identity with the Bacteroides MobC/BfmC-like conjugative transposon protein (protein ID ABQ06040). Moreover, there were significant similarities between VapD (COG3309), a virulence-associated protein, and residues 1-91 in chain A of the immunoglobulin G-binding protein G. Specifically, protein D (PDB 3UI3_A) showed 51.69% identity (e-value 1e-24). The membrane protein insertion efficiency factor YidD was encoded by one of the genes uniquely present in the MAGN1 draft genome. The MAGN2 draft genome included the RelK toxin-encoding gene, which is predicted to play a vital role in antibiotic persistence. Additionally, virulence-associated genes encoding protein domains were annotated as LbR_YadA-like domains (cd12820), a group that includes virulence factors with collagen-binding domains. The SpvB domain (PF03534) of the Salmonella virulence plasmid 65 kDa B protein and the BrkB virulence factor (PF03631) were also identified (Salter et al., 2019).

Conclusion

The *O. hominis* draft genome contains antimicrobial resistance-encoding genes and virulence factors, including toxA and gliding motility genes. Extracted genomes contain virulence factors like VapD, gliding motility lipoproteins, and unique proteins like class C-like beta-lactamases and type 2 metallo-beta-lactamases. The *O. hominis* genome sequence offers a valuable resource for future research into the possible involvement of *O. hominis* in respiratory disorders, as well as paving the way for additional in-depth studies and a greater understanding of the genetic structure of this bacterium. However further research and testing are required to determine the genetic underpinnings of this bacterium's pathogenicity. Future research should look at the role of many *O. hominis* genes that encode hypothetical proteins. Furthermore, more investigation is required to ascertain the phenotypic details surrounding *O. hominis'* antibiotic resistance.

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