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Neisseria species and their complicated relationships with human health

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Abstract. *Neisseria* spp. are a transient low abundance member of the human microbiome. This species contains the very well described pathogens, *Neisseria gonorrhoeae* and *N. meningitidis*. Recent advances in molecular typing have revealed that this genus is more diverse than previously thought and that commensal species may have important roles in inhibiting the growth the pathogens. This short review summates these new findings and examines the evidence that the relatively under-reported *Neisseria* commensal species maybe beneficial to human health.

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In 1879 Albert Ludwig Neisser observed diplococci found within neutrophils present in urethral exudates of men and women suffering from gonorrhoea and gonorrhoeal conjunctivitis. This organism was later named *Neisseria gonorrhoeae* and marks the first ever description of a member of the genus *Neisseria*[1]. The genus *Neisseria* belongs to the family *Neisseriaceae* within the phylum β-*Proteobacteria*[2]. Other genera of the family *Neisseriaceae* of medical importance include *Kingella* and *Eikinella*[2].

The *Neisseria* genus is larger and more diverse than first thought

The *Neisseria* genus contains diverse species inhabiting mammals, reptiles and environmental sites[3]. Members of the genus are Gram-negative, generally diplococci. Some *Neisseria* species such as *N. weaveri*, *N. elongata* and *N. bacilliformis* do not conform to the general diplococcus morphology, instead existing as chains of bacilli or filaments[4]. Other classical characteristics of the genus *Neisseria* include lack of motility, absence of flagella, aerobic fermentation of sugars and oxidase production. *Neisseria* speciation is continuously being revised and so far there are 10 established species associated with humans (Table 1) with a further seven recently identified from a nasopharyngeal carriage study in an African population[5]. The current robust phylogeny of this species has been developed by applying multi-locus sequence typing (MLST)[6,7]. The MLST scheme uses the single nucleotide polymorphisms in each gene to create a unique sequence type (ST) for every isolate. STs can be grouped into larger clusters based on their similarity to one another. The schemes use different numbers of genes with the basic approach using seven housekeeping genes, ribosomal MLST (rMLST) using 53 ribosomal genes[8] and a core genome MLST (cgMLST) using 246 conserved loci[9]. This has resulted in the condensation of older isolates classified as *N. subflava* biovar *subflava*, *perflava*, *flava* and *flavescens* into a single species, *N. subflava*[9]. Isolates previously termed *N. sicca* are now variants of *N. mucosa*[9] and those previously termed *N. mucosa* var *heidelbergenisis* are now called *N. oralis*[10]. Genomic approaches have been more robust than matrix-assisted laser desorption ionisation-time of flight mass spectroscopy (MALDI-ToF) at discriminating these species due to their close relatedness[11]. In the case of laboratory diagnostic identification, whole genome sequencing is the best approach to identify an unknown *Neisseria* sp.

Neisseria spp. that act as pathogens in the human host

Neisseria spp. have multiple modes of interfacing with the human host. *N. gonorrhoeae* is considered to be a true pathogen[12] as it elicits an inflammatory response upon urethral infection of the human male and causes a delayed inflammatory response, pelvic inflammatory disease, in women. Interestingly, although classified as a pathogen it can asymptomatically colonise the oral mucosa and anorectal sites that self-resolve over 4–12 months[13]. *N. meningitidis*, the causative agent of invasive meningococcal disease (IMD), is considered an opportunistic pathogen. Whereas *N. gonorrhoeae* is highly clonal[7], *N. meningitidis* has diversified into at least 11 clonal complexes that are highly associated with the risk of IMD[14]. A much wider array of genetic lineages are colonisers of the human host but act as commensals as they are infrequently associated with IMD. These two groups are broadly distinguished by the possession of a capsule polysaccharide synthesis (*cps*) operon. Among many virulence factors[15], the possession of a capsule by *N. meningitidis* is

Table 1. Summary of characteristics of human commensal *Neisseria* species.

Neisseria spp.	Micro/macroscopic morphology	Host	Biotic relationship	Site/niche	Reference
N. meningitidis	Gram-negative diplococcus	Human	Commensal and/or pathogen	Nasopharynx (commensal/pathogen)	19
				Urethra	
N. gonorrhoeae	Gram-negative diplococcus	Human	Pathogen	Mucous membranes of nasopharynx, genital mucosa, urethra, conjunctiva, rectum	13
N. bacilliformis	Gram-negative bacilli or filamentous rods	Human (may not be human exclusive)	Commensal	Mucous membranes of oral cavity	8,9
N. lactamica	Gram-negative diplococcus	Human	Commensal	Nasopharynx	9,39
	Yellow pigment production, some strains haemolytic on horse blood agar				
N. mucosa	Gram-negative diplococcus	Human	Commensal	Nasopharynx, dental plaque and buccal mucosa	9
	Most strains non-pigmented, some produce grey to yellow pigment (formerly known as *N. sicca*)				
N. cinerea	Gram-negative diplococcus	Human	Commensal	Respiratory tract: nasopharynx, sputum	9,40
	Some strains produce yellow pigment in colonies			Urogenital tract: vagina, cervix, urethra and urine	
				Other sites: eyes, ears, blood	
N. elongata	Gram-negative filamentous rods	Human	Commensal	Nasopharynx, blood	9,24
N. oralis	Gram-negative diplococcus, (may be present in chains, formerly known as *N. mucosa* var heidelbergensis)	Human	Commensal	Nasopharynx, blood	10
				Gingival plaque	
N. polysaccharea	Gram-negative diplococcus	Human	Commensal	Nasopharynx	9,41
N. subflava	Gram-negative diplococcus	Human	Commensal	Gingival crevice/upper respiratory tract	9
	Yellow colonies				
	Spontaneous agglutination in saline (formerly known as *N. subflava biovar subflava*, *N. perflava*, *N. flava*, *N. flavescens*)				

a key factor enabling survival of IMD-causing bacteria within the blood stream to cause bacteraemia and meningitis. This feature is the basis of genogrouping isolates by quantitative real-time PCR in meningococcal carriage studies. Isolates that are non-disease-causing and disease-causing isolates are stratified by the presence of a capsule null locus (*cnl*) and capsule transporter A (*ctrA*), respectively[16]. Meningococcal carriage studies have shown that the prevalence of nasopharyngeal carriage of the meningococcus ranges from 10–30% dependent upon a variety of community and behavioural factors[14]. However, since the incidence of IMD is much lower than this, other factors are involved in the risk of progressing to IMD after colonisation. This fulcrum rests on the virulence of the isolate and the underlying health of the host[17,18]. Until recently, *N. meningitidis* was not associated with urogenital disease and was considered to be a transient asymptomatic coloniser of the urogenital compartment. This concept was dramatically revised with the report in 2017 of an outbreak of urogenital urethritis attributed to meningococci closely related to an IMD outbreak clade[19]. A retrospective review of published case reports of meningococcal disease has uncovered consistent reporting of sporadic cases of horizontal mother to child transmission in pregnancy resulting in rare cases of sepsis, anorectal infection and conjunctivitis[20].

Neisseria spp. that are low abundance, transient commensals of the human host

In comparison to the two pathogenic species, the remaining eight species are atypical infectious disease agents[3, 21]. Collectively they

are sporadically associated with a wide variety of conditions usually in immunocompromised patients[21]. Since they are not widely known as infectious disease agents, it is also possible that the reports of their involvement in these disease manifestations is under-reported. Nevertheless, genomic comparisons of these commensal species with the pathogenic *N. meningitidis* shows that they lack multiple virulence determinants[22] supporting the conclusion that they are naturally commensal and act as opportunistic pathogens in a disregulated host immune environment. Prevalence studies have typically examined pharyngeal carriage and have shown that all of these species are transient low abundance (<2% abundance) members of the human microbiome. *N. lactamica* has the highest prevalence of all species and with the highest incidence in children under the age of 4 (14%) before declining in young adults[23]. *N. polysaccharea* also showed a similar distribution as *N. lactamica* but at a much lower incidence of 2%. In this study *N. bergeri* and *N. subflava* had very low prevalence and showed no age-related variation in incidence. Co-colonisation studies have not been performed recently, but an older study from the 1980s that used culture as the means of detection, found multiple *Neisseria* spp. occurred in 57% of people while 41% of carriage was with *N. subflava* alone[24]. The high prevalence of *N. subflava* appears to be due to its role as a contributor to periodontal disease. Although multiple *Neisseria* spp. are present in both healthy teeth and dental caries samples, an increase in the abundance of *N. subflava* is a key signal as the microbial community changes in composition to become acid-secreting, resulting in tooth enamel erosion[25].

The role of *Neisseria* spp. in the human microbiome

Human microbiome studies have begun to unravel some relationships of the *Neisseria* spp. within their relevant mucosal microbiome communities. Unfortunately, *Neisseria* spp. are typically reported at the genus level as variation in the 16S rRNA alone is insufficient to speciate them. Nevertheless, some generalities can be gained from the current literature. Numerous studies have shown that *Neisseria* spp. are absent from normal flora in the vulvovaginal mucosal surfaces of women[26]. This suggests that the isolation of any *Neisseria* spp. from this compartment should be investigated as a potential pathogen related to an infection particularly urethritis[3,21]. Commensal *Neisseria* spp. are transient, low abundance residents of the rhinopharynx and oropharynx[27] that are not associated with any known disease-state[28].

There are hints that there are complex interference patterns at both intra- and inter-species levels that influence colonisation by *Neisseria* spp. Many of these interactions have been examined through the lens of preventing or interfering with colonisation by the pathogens. Exposure to *N. gonorrhoeae* does not necessarily result in human infection. In surveys of human disease, the risk of contracting gonorrhoea has been linked to a syndrome termed bacterial vaginosis, in which the microbiome has a reduced abundance of *Lactobacillus* sp.[29]. Although co-culture of the two species confirms *Lactobacillus* sp. will inhibit *N. gonorrhoeae* growth, probiotic treatment of mice with *Lactobacillus* shows no efficacy in mouse models of gonorrhoea infection[30]. *Streptococcus pneumoniae* has been shown to inhibit *N. meningitidis* using two mechanisms: the secretion of hydrogen peroxide[31] and a neuraminidase[32]. Inter-species antagonism is also a feature of the commensal *Neisseria* spp. against both *N. gonorrhoeae* and *N. meningitidis*. *N. cinerea* and *N. lactamica* impair early colonisation steps and reduce meningococcal invasion into host cells[33,34] while *N. mucosa* secretes a small molecule secondary metabolite that inhibits *N. gonorrhoeae*[35]. However, all commensal *Neisseria* spp. could kill *N. gonorrhoeae* through a DNA-dependent mechanism[36]. This mechanism is dependent on the expression of type IV pili, which enable the uptake of DNA into the bacterial cell. The DNA from the commensal bacteria have a different methylation pattern and this appears to poison the gonococcal and meningococcal bacteria[33]. Direct synergism between *Neisseria* spp. and other species has not been extensively reported. However, a recent innovative model of meningococcal colonisation conducted by Audry *et al*.[37] showed that meningococcal colonisation of the human oropharyngeal site may not elicit an immediate inflammatory response as the bacteria can be trapped in the mucus layer, preventing invasion of the mucosal epithelium. This state of homeostasis can be perturbed by co-colonisation with other bacteria, and in this model, *Streptococcus mitis* but not *Moraxella catarrhalis* triggered the escape of the meningococcus from the mucus layer and invasion into the host cells. *S. mitis* potentiated growth of the meningococcus by degrading the mucins.

Future directions

In summary, the taxonomy of the genus *Neisseria* is continually being redefined by modern molecular typing tools and the recent observation that the diversity of this group remains largely unexplored. This genus contains species that are either pathogenic or commensal with humans, whereas *N. meningitidis* contains clonal complexes that are pathogenic or commensal. Since its discovery 142 years ago, the interest in this genus has been driven by the medical interest in devising preventative measures against gonorrhoea and meningitis. Other members of this genus, such as *N. lactamica* have been investigated as a probiotic intervention strategy against IMD[34], while the recent observation that commensal

Neisseria spp. may kill *N. gonorrhoeae* via a DNA-dependent mechanism has been recently patented (International Patent Application No. PCT/US2015/048114). Future work is likely to focus on whether commensal *Neisseria* spp. have a benefit to human health and are necessary for development of a healthy immune system.

Conflicts of interest

The author declares no conflicts of interest.

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Biography

Associate Professor Charlene Kahler is a teaching/research academic specialising in bacterial pathogenesis. She obtained her BSc (honours in Microbiology) from the University of Queensland. She completed her PhD in the field of microbiology with Dr John Pemberton at the University of Queensland. She travelled to the USA to undertake post-doctoral training with Dr David Stephens at Emory University (Atlanta, Georgia). In this position, she studied the pathogenesis of *Neisseria meningitidis* and described the biosynthesis pathway of lipooligosaccharide. She returned to Australia to work with Professor John Davies at Monash University where she studied regulatory pathways in *N. gonorrhoeae*. She moved to the University of Western Australia to establish her own laboratory studying both pathogens. She is currently Head of Discipline for Microbiology and Immunology and the Deputy Director of the Marshall Centre for Infectious Diseases Research and Training at University of Western Australia. Her greatest accomplishment is assisting her students through their PhDs and seeing them fulfil their dreams in microbiology. She is thankful to the members of the Centre who contributed to this issue: Dr Tim Inglis, Dr Allison Imrie, Professor Jeff Keelan and Professor Barry Marshall.