Short Communication

Neisseria gonorrhoeae arthritis in a patient with Systemic Lupus Erythematosus: case report and whole genome sequencing

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Abstract: *Neisseria gonorrhoeae* is the most common sexually transmitted bacterium that causes infectious arthritis. In this study, we describe a case report of gonococcal arthritis in a Systemic Lupus Erythematosus patient. We performed whole genome sequencing (WGS) of the etiologic agent involved and molecular analysis using a global collection of *N. gonorrhoeae* strains. Ours is the only sample derived from synovial fluid identified in this collection, the others being from the usual anatomical sites. Antimicrobial susceptibility was determined by disk diffusion and Etest, and WGS was conducted to determine multilocus sequence typing profiles, group isolates based on core genome single nucleotide polymorphisms (SNP), and identify virulence genes and antimicrobial resistance determinants. The *N. gonorrhoeae* samples in the global collection were highly heterogeneous. Our sample displayed resistance to ciprofloxacin (MIC = 2 µg/mL) and tetracycline (zone diameter = 0 mm), among the antimicrobials tested. The isolate had genetic features related to beta-lactam, tetracycline and quinolone resistance. No resistance genes for immune evasion and toxin were identified. Few strains from non-mucosal sites have been sequenced. This makes it difficult to compare cases of disseminated gonococcal

infection with cases of mucosal infection in terms of phylogenetic similarity, mutations in resistance genes and virulence factors.

Keywords: *Neisseria gonorrhoeae*, gonococcal arthritis, whole genome sequencing, resistance, virulence.

1. Introduction

Neisseria gonorrhoeae is the most common sexually transmitted bacterium that causes infectious arthritis (Rice, 2005). Gonococcal arthritis is usually monoarticular and is associated with a positive blood and synovial fluid culture in approximately 50% of patients (García-Arias et al., 2011).

It results from hematogenous dissemination of *N. gonorrhoeae* from the initial mucosal infection (Britigan et al., 1985). Between 0,5-3% of patients with mucosal infection can develop a disseminated gonococcal infection (DGI) (Bardin, 2003).

Previous case reports have shown an association of DGI in patients with Systemic Lupus Erythematosus (SLE). The reported patients are typically young females, with kidney disease and congenital or acquired hypocomplementemia, who may present with all the characteristics of a lupus joint exacerbation (Mitchell et al., 1990).

Here, we described a case report of gonococcal arthritis in a SLE patient. We performed whole genome sequencing (WGS) of the etiologic agent involved, and molecular analysis using a global collection of *N. gonorrhoeae* strains available on Pathogenwatch (https://www.sanger.ac.uk/tool/pathogenwatch/).

2. Case report

A 28-year-old man diagnosed with SLE, being treated with mycophenolate and prednisone 20mg/day, presented reporting symptoms of arthralgia and generalized myalgia for over a month. He also reported urethritis, and had previously self-treated with ciprofloxacin and doxycycline.

The patient developed asymmetric oligoarthritis of large joints, with signs of active synovitis. Joint puncture guided by ultrasound of the left knee was performed, with no diagnostic yield. Aerobic, anaerobic, mycobacterial and fungal cultures were negative, and acid-alcohol-resistant bacillus and PCR tests for *Mycobacterium tuberculosis* DNA were also negative. In addition, culture of urethral secretion, urine and pharynx were negative for *Neisseria*. He was treated on an outpatient basis with cycles of nonsteroidal anti-inflammatory drug for approximately 1 month during investigation and care of the arthritis.

However, the left ankle arthritis got worse, with episodes of night sweating. A new joint puncture was performed on the left ankle, with a positive culture for *N. gonorrhoeae*. This strain produced Beta-lactamase, however, it was susceptible to ceftriaxone. Treatment with ceftriaxone 2g/day was started for 4 weeks. He had a progressive improvement in edema and a decrease in arthralgia.

3. Materials and methods

3.2. Sampling

N. gonorrhoeae (5671) was isolated in the synovial fluid culture and identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF; Bruker Biotyper 3.1,

Bruker Daltonics) at Hospital das Clínicas, University of São Paulo Medical School (USP), Brazil. The specimen was subsequently submitted for WGS at the Institute of Tropical Medicine, USP. Our sample 5671 belonged to Multi Locus Sequence Type (MLST) 1588. Hence, we performed a search on Pathogenwatch for *N. gonorrhoeae* WGS with MLST 1588. A total of 319 isolates were included, 21 in Brazil, 160 in Europe, 35 in Asia, 85 in the United States of America (USA), 16 in Oceania and 2 in Africa, between 2004 to 2018 (Table S1). Ours was the only synovial fluid sample identified in this collection, the others being from mucosal anatomical sites. The most common specimen type was urethral (n = 44), followed by urine (n = 24), endocervical (n = 6), vaginal (n = 3), pharynx (n = 1), rectal (n = 1), penile discharge (n = 1), and urogenital (n = 1), with 237 of unknown source.

3.3. Antimicrobial susceptibility testing

Azithromycin (Gianecini et al., 2020) and tetracycline susceptibility were determined by disk diffusion test, while ciprofloxacin and ceftriaxone MIC values were determined by Etest. *N. gonorrhoeae* isolate ATCC 49226 was used as quality control (CLSI, 2020).

3.4. Genomic analysis

WGS of the *N. gonorrhoeae* isolate was performed using MiSeq Illumina™ (Illumina Inc., San Diego, CA, United States). *N. gonorrhoeae* FA19 (GenBank accession number NZ_CP012026.1) was used as a reference for molecular characterization.

MLST and resistome analysis were performed using MLSTfinder 2.0 (Larsen et al.,2012) and ResFinder 4.0 (Bortolaia et al., 2020). *N. gonorrhoeae* Multi-Antigen Sequence Typing (NG-MAST) and *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR)] were obtained and identified using PubMLST (Jolley et al., 2018). Mutations in resistance genes were manually searched in the genomes using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990).

The Virulence Factor Database (VFDB) tool was used to search for virulence genes (Liu et al., 2019).

3.5. Phylogenetic analysis

A phylogenetic tree was constructed using the REALPHY tool (v. 1.12) using 320 sequenced genomes based on Single Nucleotide Polymorphisms (SNP) with default parameters (Bertels et al., 2014). Briefly, the sequences were mapped using the reference sequence *N. gonorrhoeae* strain FA19 (Accession number NZ_CP012026.1) via Bowtie2. SNPs were calculated using Seaview (Galtier et al., 1996) and multiple sequence alignments were recreated using PhyML with 100 bootstrap replications for the tree construction (Bertels et al., 2014).

4. Results and Discussion

Infection has generally been considered the main cause of morbidity and mortality in patients with SLE (Mitchell et al., 1990). Gonococcal arthritis is mainly observed in sexually active young adults and recent studies have shown a female predominance (Bardin, 2003). Asymptomatic local infection is more frequent in women and may result in delayed antibiotic treatment (Walker and Sweet, 2011). Delayed treatment may facilitate disseminated infection

and partly explain the predominance in young women (Bardin, 2003). However, our case report is of a symptomatic male patient who self-treated inappropriately with antibiotics, the most likely reason for gonococcal dissemination. We characterized an *N. gonorrhoeae* isolate (5671), which has been circulating in São Paulo since 2018, from synovial fluid culture for the first time.

The phylogenetic tree showed that 5671 was not closely related to any isolate in the global collection of *N. gonorrhoeae* strains. However, bootstrap values in this tree structure are generally low. Interestingly, the genomes from Brazil were spread throughout the tree, grouping with samples from other countries, suggesting intercontinental dissemination (Fig. 1A). *N. gonorrhoeae* samples in the global collection were highly heterogeneous; we saw no evidence of clustering by country. This diversity may be more common in sexually transmitted infections than healthcare associated infections, where the presence of within-country clusters is frequent in outbreaks.

The SNP tree had a total 19532 SNPs in 320 samples, and 7338 SNPs in the 80 samples closest to ours, highlighted in blue (Fig. 1B).

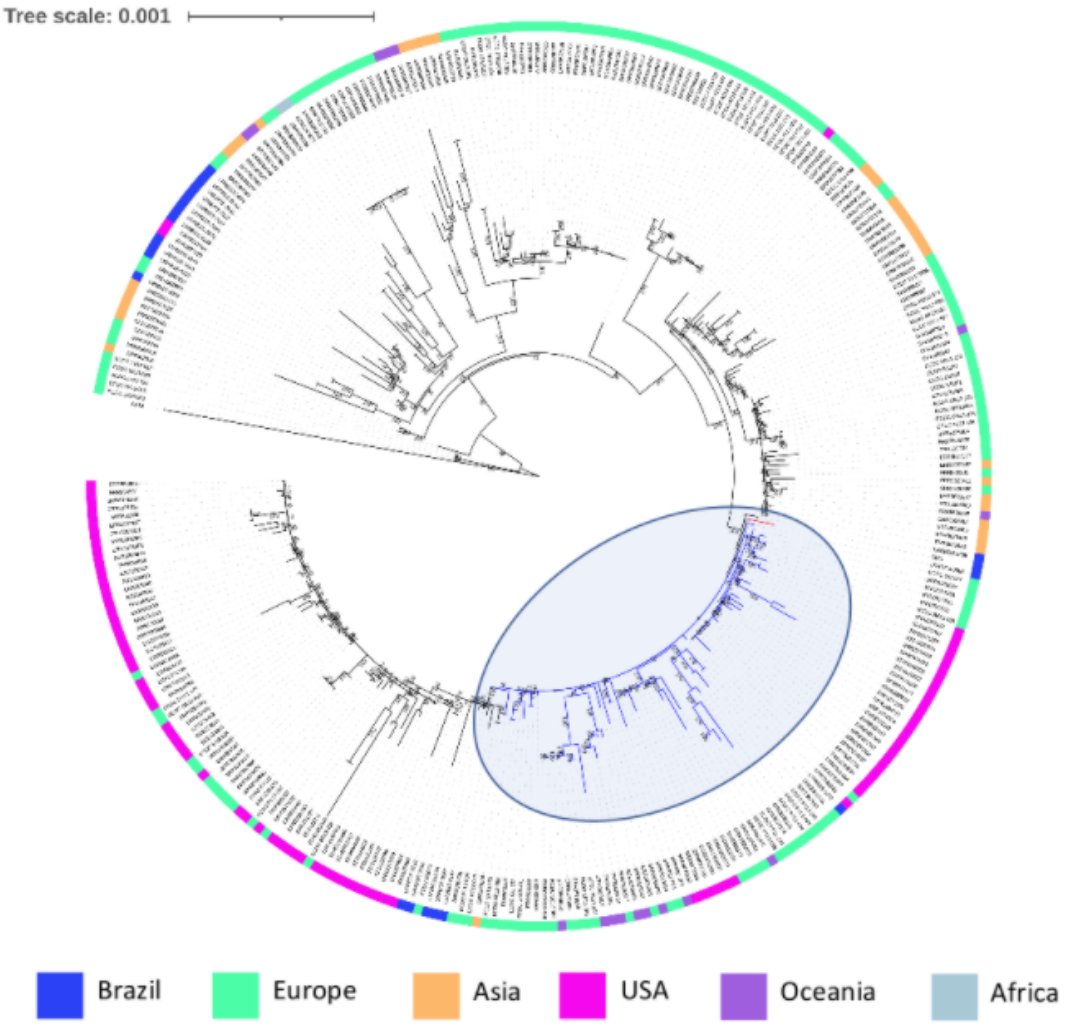

Figure 1A.

Figure 1B.

Analysis of molecular epidemiology was initially performed using the WGS data and three traditional typing schemes, i.e. MLST, NG-MAST and NG-STAR (Table 1). In total, 98 different NG-MAST and 90 NG-STAR STs were found among the 320 isolates. A recent study of genomic diversity among gonococcal strains in Brazil showed that MLST 1588 was the second most common ST (Golparian et al., 2020).

Regarding antibiotic susceptibility testing, our sample displayed resistance only to ciprofloxacin (MIC = 2 µg/mL) and tetracycline (zone diameter = 0 mm), and was susceptible to azithromycin (zone diameter = 55 mm) and ceftriaxone (MIC ≥ 0,016). High levels of *N. gonorrhoeae* resistance to penicillin, tetracycline and ciprofloxacin have been observed in Brazil from 2006 to 2015 (Costa-Lourenço et al., 2018) and the Brazilian Ministry of Health has therefore recommended dual therapy of ceftriaxone with azithromycin to treat gonococcal infections since 2017 (Ministério da Saúde, 2020). However, despite the lack of robust, continuous surveillance programs throughout the country, recent studies have also found emerging resistance to azithromycin and decreasing susceptibility to extended-spectrum cephalosporins (Costa-Lourenço et al., 2018; Bazzo et al., 2018).

Based on the isolate susceptibility profile found, we researched mutations in the resistance genes. The Resfinder tool identified genes related to beta-lactam and tetracycline resistance. The tet(M) gene confers resistance to tetracycline, while the *bla*TEM-104 and *bla*TEM-1B gene confer resistance to beta-lactams. Furthermore, we observed mutation patterns in the *gyr*A and *par*C genes, mechanisms of resistance to quinolones. Two point mutations were found on the *gyr*A gene, leading to two amino acid changes (Ser-91→ Phe

and Asp-95 → Ala). Two point mutations were also found on the *parC* gene, leading to two amino acid changes (Ser-87 → Asn and Val-43 → Ala). Seventy-one virulence genes were identified in our sample, belonging to the following classes: adherence, efflux pump, immune modulator, invasion, iron uptake, protease and stress adaptation (Table 1). However, no virulence genes for immune evasion and toxin were observed.

VFclass	Virulence factors	Related genes
Adherence	Adhesion and penetration protein	app
	LOS sialylation	lst
	LOS synthesis	kdtA/waaA, lgtA, lgtB, lgtC, lgtD, lgtE, lgtF, lgtG, rfaC, rfaF, rfaK
	Phosphoethanolamine modification	lptA
	Type IV pili	pilC, pilD, pilE, pilF, pilG, pilH, pill, pilJ, pilK, pilM, pilN, pilO, pilP, pilQ, pilS, pilT2, pilT, pilU, pilV, pilW, pilX, pilZ
Efflux pump	FarAB	farA, farB
	MtrCDE	mtrC, mtrD, mtrE
Immune modulator	Factor H binding protein	fHbp
	Neisserial surface protein A	nspA
Invasion	Class 5 outer membrane protein	opc
	Opacity protein	opa
	PorA	porA
	PorB	porB
Iron uptake	ABC transporter	fbpA, fbpB, fbpC
	Ferric enterobactin transport protein A / ferric-repressed protein B	fetA/frpB
	Heme uptake	hpuA, hpuB
	Hemoglobin receptor	hmbR
	Lactoferrin-binding protein	lbpA, lbpB
	Ton system	exbB, exbD, tonB
	Transferrin-binding protein	tbpA, tbpB
Protease	IgA protease	iga
Stress adaptation	Catalase	katA
	Manganese transport system	mntA, mntB, mntC
	Methionine sulphoxide reductase	msrA/B(pilB)
	Recombinational repair protein	recN

Table 1.

5. Conclusion

This case highlights the importance of appropriate use of antibiotic therapy for *N. gonorrhoeae* given the high levels of resistance to penicillin, tetracycline and ciprofloxacin observed. Furthermore, inappropriate use can favor development of resistance mechanisms and lead to disseminated gonococcal disease.

Although there are thousands of *N. gonorrhoeae* genomes available, our sample was the only one from a non-mucosal anatomical site among the samples that had the source identified. This hampers comparisons between cases of disseminated gonococcal infection and those of mucosal infection in terms of phylogenetic similarity, mutations in resistance genes and virulence factors.

6. References

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