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RESEARCH

Genomic characterization of invasive meningococcal X isolates from Brazil, 1992–2022

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Abstract

Introduction Invasive meningococcal disease (IMD) is a major health problem. Given the post-COVID-19 pandemic scenario with the loosening of the non-pharmacological measures to control the virus transmission and considering the observed global reduction of meningococcal vaccination coverage, an increase in IMD cases can be expected.

Methodology Using whole-genome sequencing, we characterized six *Neisseria meningitidis* serogroup X (MenX) isolates recovered from IMD cases in Brazil in the last 30 years.

Results The predominance (66.6%, 4/6) of ST2888 presenting fHbp 160, NHBA 129, NadA 21, and PorA 19,15 was found on isolates. Two novel STs, 15458 and 15477, were described.

Conclusion This study describes the circulation of MenX lineage ST2888 in Brazil, previously reported only in Europe. Continuous universal surveillance is crucial to implement prompt public health measures aiming to prevent and control non-vaccine preventable serogroup X IMD cases.

Keywords Meningococcal X disease · Invasive meningococcal disease · *Neisseria meningitidis* serogroup X · MenX

Abbreviations

CC	Clonal complex
cgMLST	Core-genome MLST
IAL	Adolfo Lutz Institute
IMD	Invasive meningococcal disease
fHbp	Factor-binding protein
MATS	Meningococcal Antigen Typing System

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MenDeVAR	Meningococcal deduced vaccine antigen reactivity
MenX	*Neisseria meningitidis* serogroup X
MLST	Multilocus sequence typing
NadA	Neisserial adhesin A
NHBA	Neisserial heparin binding antigen
NmCV-5	Pentavalent meningococcal conjugate vaccine
ST	Sequence type
WGS	Whole-genome sequencing

Introduction

Invasive meningococcal disease (IMD) remains a serious public health issue impacting morbidity and mortality worldwide. Globally, most IMD cases are caused by serogroups A, B, C, W, Y, and more rarely X (Chang, Tzeng and Stephens, 2012).

Until recently, *Neisseria meningitidis* serogroup X (MenX) was considered to have low virulence, frequently found in immunocompromised individuals (Fijen et al., 1989; Vicente, Esnal and Pérez-Trallero, 2012), and usually displays a high level of effective carriage (Kristiansen et al., 2013). Although serogroup X isolates have been responsible for scarce IMD cases outside Africa, with most of the cases reported from Europe (Hansman, 1983; Grahlow, Ocklitz and Mochmann, 1986; Fazio et al., 2010; Pan et al., 2014; Wang et al., 2015; Stefanelli et al., 2017), outbreaks and huge epidemics are reported from Burkina Faso, Ghana, Kenya, Niger, and Togo from 1990 until recently (Gagneux et al., 2002; Boisier et al., 2007; Mutonga et al., 2009; Delrieu et al., 2011).

In Brazil, the first reported serogroup X IMD occurred in 1992, and its occurrence remains very rare. To date, nine MenX IMD cases have been officially notified in the last 15 years, of which the latest two cases occurred during the COVID-19 pandemic in 2021 and 2022 (Fukasawa et al., 2022)

The emergence of serogroup X is especially concerning since there is no vaccine available against this serogroup. Currently, the licensed meningococcal conjugate vaccines protect only against serogroups A, C, W, and Y. A pentavalent meningococcal conjugate vaccine against the A, C, W, Y, and X serogroups (NmCV-5) is in an advanced stage of development (Tapia et al., 2021), but is not yet available for large-scale vaccination.

Since 2013, two protein-based vaccines against serogroup B have been licensed in Europe and the Americas (MenB-FHbp and 4CMenB). The MenB-FHbp vaccine is composed of two recombinant lipidated factor H-binding protein (fHbp) from subfamilies A (A05) and B (B01). The 4CMenB vaccine is composed of multiple recombinant antigens: a non-lipidated fHbp peptide 1 from subfamily B/variant 1, neisserial adhesin A (NadA) peptide 8 variant NadA-2/3, neisserial heparin binding antigen (NHBA) peptide 2, and PorA P1.7-2,4 from the New Zealand epidemic strain (Serruto et al., 2012; Zlotnick et al., 2015).

Since these vaccinal antigens may also be present and expressed in non-serogroup B isolates, these available protein-based vaccines may be a potential option against other serogroups including X (Hong et al., 2013; Biolchi et al., 2020; Leo et al., 2020).

In front of two recent unexpected serogroup X IMD cases in Brazil (Fukasawa et al., 2022) and considering that the genomic characteristics of Brazilian MenX isolates are unknown, this study was conducted in six available serogroup X IMD isolates of the Brazilian National Reference Laboratory for meningitis. We described the genetic lineages, vaccine antigen types, virulence factor types, and allele types of antimicrobial-associated resistance genes based on whole-genome sequencing (WGS) and evaluated the phylogenetic structure of Brazilian isolates in the context of global serogroup X population structure.

Material and methods

Neisseria meningitidis isolates

Since 1982, laboratories throughout the country have sent isolates of invasive *N. meningitidis* to our National Reference Laboratory for Meningitis at Adolfo Lutz Institute, São Paulo, herein named IAL, for species confirmation and further characterization. From 1992 to May 2022, we received seven invasive MenX isolates from the whole country. In this study, we have characterized based on whole-genome sequencing six available serogroup X IMD isolates belonging to the Brazilian collection.

Whole-genome sequencing and analysis

Genomic DNA extraction and purification were performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genome library preparation was performed using the Ion Xpress Plus Fragment Kit (Thermo Fisher Scientific, Waltham, MA, USA), and genome libraries corresponding to 400 base pairs were obtained using E-Gel SizeSelect Agarose Gel, 2% (Thermo Fisher Scientific, Waltham, MA, USA). Genome sequencing was performed using the Ion Torrent S5 sequencer (Thermo Fisher Scientific, Waltham, MA, USA), and the reads were assembled *de novo* using SPAdes v.5.6.0.1. The assembled contigs were functionally annotated by the NCBI Prokaryotic Genome Annotation Pipeline. Sequence assignment was performed using the gene-by-gene approach with the PubMLST *Neisseria* database (https://pubmlst.org/neisseria/).

Vaccinal antigen analysis

Peptide sequences of fHbp, NadA, and NHBA were extracted from PubMLST and aligned with our sequences using ClustalO in Geneious Prime 2022.0.2 Software. Next, SplitsTree v.5.0 was employed to create phylogenetic networks. Genome reference sequence(s) were included for each antigen as follows: peptide 1 (B24/1.1; accession number NZ_AEQZ00000000.1), peptide 45 (A05/3.45; accession number AY330361.1), and peptide 55 (B01/1.55; accession number AY330406.01) for fHbp; peptide 8 (NadA-2/3; accession number GQ302859.1) for NadA; and peptide 2 for NHBA; accession number AF226445.1).

Phylogenetic analysis

The sequenced genomes of this study ($N=6$) were compared with all the MenX genome entries available at PubMLST (https://pubmlst.org/) ($N=129$) by using the BIGSdb Genome Comparator tool (Bratcher et al., 2014). After the generation of a distance matrix based on the number of variable alleles based on cgMLST, a phylogenetic network was generated in the software SplitsTree v.5.0.

Results

MLST, fHbp, NHBA, NadA, PorA, FetA, and lpt3 typing

Six invasive *N. meningitidis* X isolates were identified in the Brazilian National Reference Laboratory for meningitis in the last 30 years. The IMD cases occurred in patients with ages ranging from 3 to 11 years old, and only one IMD case was reported in 2013 from an elderly patient. All IMD cases showed a clinical presentation of meningitis. All of them came from only two (of the five) administrative regions in Brazil. Three IMD cases occurred in the same administrative region (South) in the same year (2016), but no obvious epidemiological link was recorded for these cases. None of them reported travel to countries endemic to MenX (Table 1).

WGS analysis revealed a prevalence of lineage ST2888 among the recent invasive MenX isolates ($n=4/6$; 67.0%). The oldest isolates (N.971-92 and N.764-01) presented novel STs (15458 and 15477, respectively). All isolates were assigned without clonal complex (Table 2). The lineage ST2888 presented a unique antigenic profile. ST2888 isolates were predicted to encode the fHbp peptide 160 (subfamily A/variant 3), NHBA peptide 129, NadA peptide 21, PorA 19,15, and FetA 5-5. On the other hand, the oldest isolates, except for NHBA peptide 789, presented a diversity of antigens. Regarding the gene involved in phosphoethanolamine addition to lipooligosaccharide, the ST2888 lacked the *lpt3* that encodes the phosphoethanolamine transferase (Table 2).

Genetic variability and distribution of antimicrobial resistance-associated genes

All isolates were found to present the *gyrA* gene without mutations, and only the oldest isolate (N.971-92) contained H552N target mutation in the *rpoB* gene, conferring resistance to rifampin. In addition, 3/4 (75.0%) of ST2888 isolates carried the mutated *penA* allele 19 that harbor reduced susceptibility-associated mutations (amino acid substitutions: F504L, A510V, I515V, G541N, and I566V), per observed phenotype (Table 2).

Vaccinal antigen analysis

Pairwise analysis indicated similarities of 81.8% and 82.1% between the NHBA peptides 129 and 789, respectively, and the vaccinal peptide 2. For the NadA peptide, a similarity of 54.6% was found between the detected peptide 21 and the vaccinal antigen 8. Regarding the fHbp peptides found in this study, in comparison with the vaccinal antigens 1, 55, and 45, similarities range from 56.3% to 95.6%. Phylogenetic networks created from the fHbp, NadA, and NHBA peptide sequences are presented in Fig. 1.

Table 1 Epidemiological data for the six studied invasive *Neisseria meningitidis* serogroup X isolates from Brazil

Isolate identification	Year of isolation	Source	Patient age	Clinical presentation	City	State	Administrative region
N.971-92	1992	CSF*	11 y	Meningitis	Sorocaba	São Paulo	Southeast
N.764-01	2001	CSF	9 y	Meningitis	Contagem	Minas Gerais	Southeast
N.68-13	2013	CSF	67 y	Meningitis	São João Batista	Santa Catarina	South
N.61-16	2016	CSF	3 y	Meningitis	Santa Cruz do Sul	Rio Grande do Sul	South
N.72-16	2016	CSF	10 y	Meningitis	Passos de Torres	Santa Catarina	South
N.133-16	2016	CSF	4 y	Meningitis	Porto Alegre	Rio Grande do Sul	South

*Cerebrospinal fluid

Table 2 Genomic characteristics of invasive *Neisseria meningitidis* serogroup X isolates from Brazil

Isolate	Sequence type	Clonal complex	PorA	FetA	fHbp Subfamily/ variant; peptide	NadA Variant; allele; peptide	NHBA Allele; peptide	*penA*	*gyrA*	*rpoB*
N.971-92	**15458**	na[¶]	21,16	F4-38	B/1; 1150	–	**1872;** 789	1	2	267 (mutation H552N)
N.764-01	**15477**	na	19,15-1	F3-67	B/1; 14	–	774; 789	1	2	38
N.68-13	2888	na	19,15	F5-5	A/3; 160	Nad-4/5; 109; 21	234; 129	3	4	7
N.61-16	2888	na	19,15	F5-5	A/3; 160	Nad-4/5; 109; 21	234; 129	19	4	7
N.72-16	2888	na	19,15	F5-5	A/3; 160	Nad-4/5; 109; 21	234; 129	19	4	7
N.133-16	2888	na	19,15	F5-5	A/3; 160	Nad-4/5; 109; 21	234; 129	19	4	7

[¶]na: not assigned

In bold, new features described in this study

Vaccine coverage prediction

The theoretical coverage of MenX isolates by 4CMenB and MenB-FHbp vaccines was predicted using MenDeVAR (meningococcal deduced vaccine antigen reactivity) index following the criteria reported on https://pubmlst.org/organisms/neisseria-spp/mendevar as "green," exact matches to the sequence variants; "amber," cross-reactive in experimental studies; "red," not cross-reactive in experimental studies; and "grey," insufficient data (Rodrigues et al., 2021). By the MenDeVAR index, only one isolate (N.764/01) was classified as being covered by both vaccines due to the cross-reactivity of fHbp peptide 14. The ST2888 carried completely unknown peptides, and since no experimental data is available, the MenDeVAR index was unable to classify it.

Phylogenetic analysis

The core-genome MLST (cgMLST) analysis with the gene-by-gene approach was conducted with 135 MenX sequences, 129 available on PubMLST (https://pubmlst.org/), and the six generated in this study, presented in Fig. 2. The generated phylogenetic network showed the close relation of ST181 isolates, mainly from sub-Saharan Africa and less frequently from Europe, while a star-like distribution is observed with the other ST, including those identified in our isolates, ST2888 ($N=4$) and the newly described ST15458 and ST15477 ($N=1$, each). Intriguingly, most of the isolates recovered in this study (4/6) were ST2888, the only ST presenting a potential dispersion along with ST24 and ST750 (Fig. 2).

Further, a second phylogenetic network was constructed with the four ST2888 identified in this study and the available complete genome sequences available on PubMLST ($N=4$). Despite the long distance of the European isolates

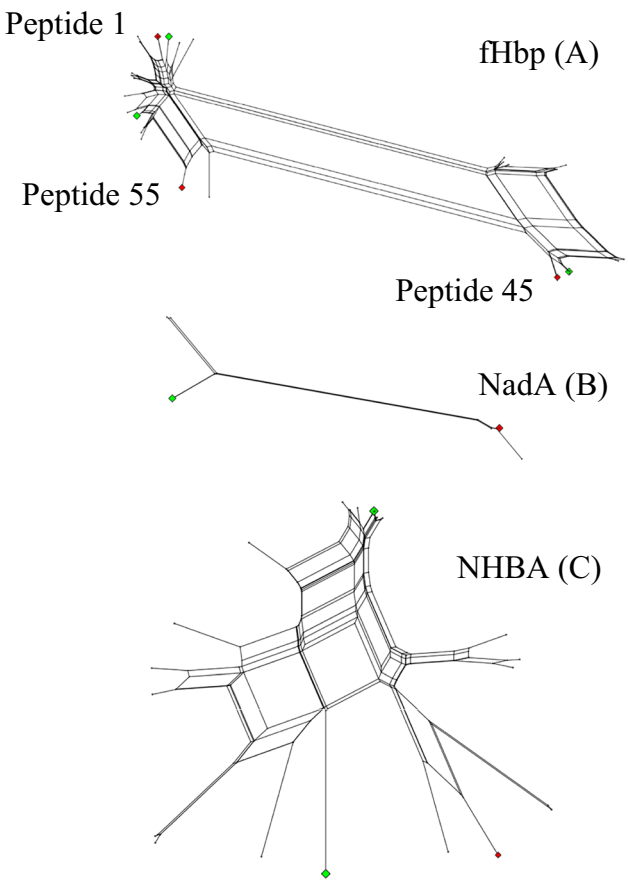

Fig. 1 Phylogenetic networks created on SplitsTree v.5.0 based on the fHbp (**A**), NadA (**B**), and NHBA (**C**) peptide sequences. The reference peptide sequences (in red) fHbp 45 and 55 correspond to antigens in MenB-fHbp, and fHbp 1, NadA3-8, and NHBA 2 correspond to antigens in 4CMenB vaccines. The reference peptides are indicated by red diamonds in the figures, and the peptides found in this study are in green

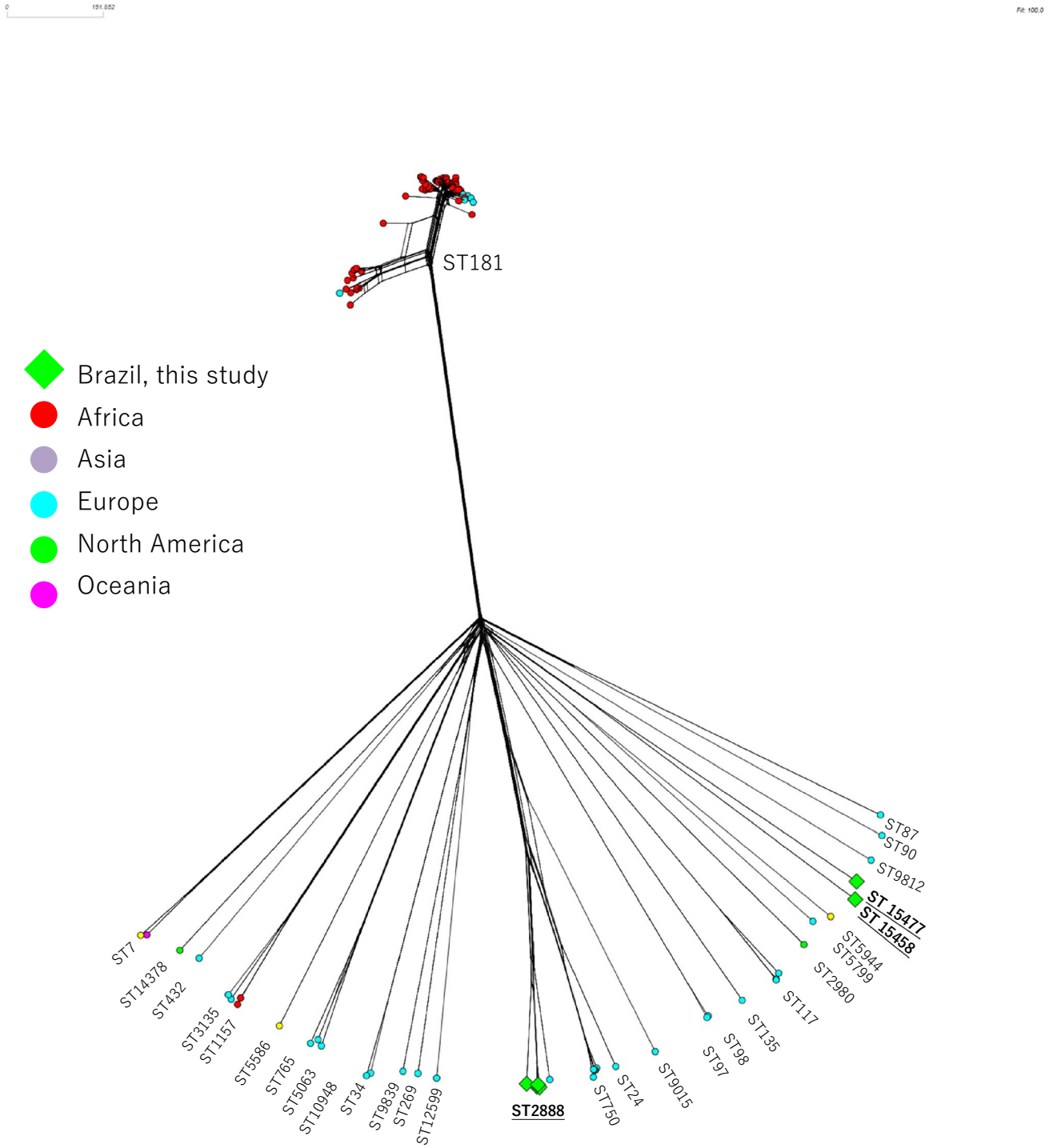

Fig. 2 Phylogenetic network created on SplitsTree v.5.0 based on cgMLST analysis of MenX available in PubMLST ($n=129$) and sequenced in this study ($n=6$, green diamonds). Each circle represents one sequence, and the color indicates its geographic location

collected between 2002 and 2015 (which presented at least 325 different loci among each other), three Brazilian MenX isolates from 2016 were grouped into a derived branch with reduced diversity (70–80 different loci), as shown in Fig. 3.

Discussion

This study is the largest genomic characterization of MenX isolates collected outside Africa causing IMD in patients without travel reported to countries of the meningitis

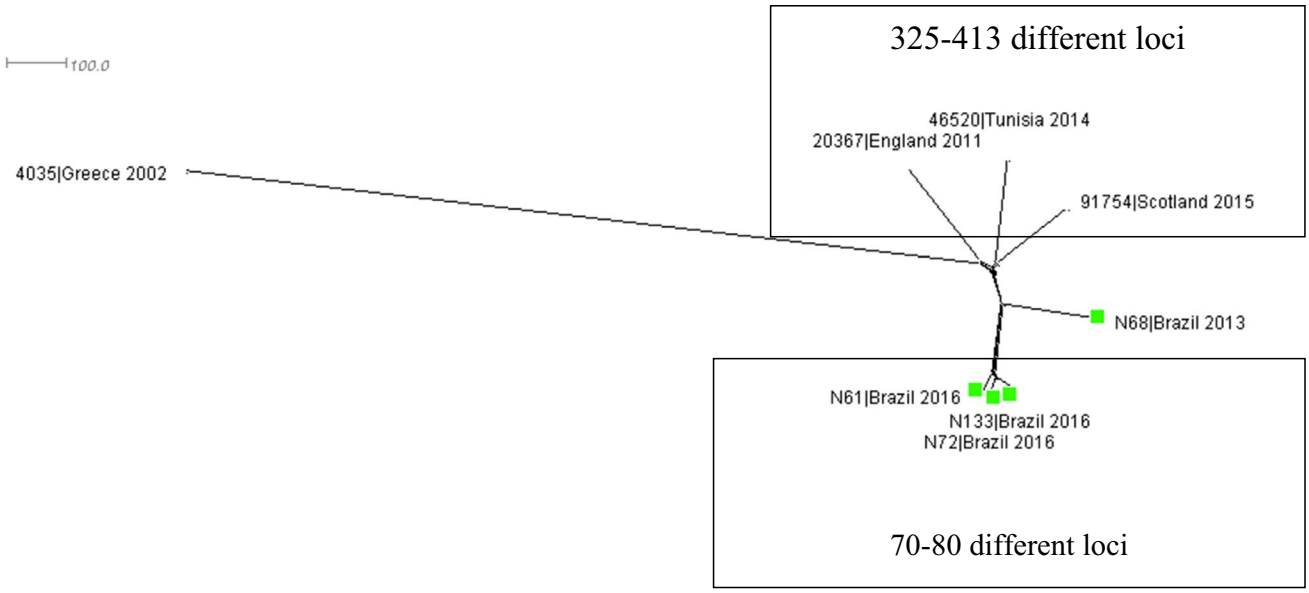

Fig. 3 Phylogenetic network created on SplitsTree v.5.0 based on cgMLST analysis of *N. meningitidis* ST2888 available in PubMLST ($n=4$) and sequenced in this study ($n=4$, green diamonds). The entry number, country, and year of isolation for each isolate are indicated

belt of Africa. Although there is a low number ($N=6$) of studied Brazilian MenX IMD isolates, its frequency is per serogroup X IMD epidemiology reported in Europe and North America (Fazio et al., 2010; Wang et al., 2015; Agnememel et al., 2016; Stefanelli et al., 2017) and identified the internal circulation of MenX ST2888 lineage in Brazil since 2013.

WGS has been used to investigate the population structure diversity between African and European MenX isolates and to identify potential virulence factors (Agnememel et al., 2016). The genomic study in sub-Saharan Africa displayed that the population structure of MenX belongs to a single main lineage, ST181 (cc181), contrasting with the diversified MenX population found in Europe (Agnememel et al., 2016). Recently, four MenX isolates belonging to ST181 lineage were detected outside Africa. All cases occurred in migrants living in refugee camps or reception centers in Italy (Stefanelli et al., 2017). Humanitarian crises and conflicts are resulting in an increase in the deprived population with difficulty accessing health services. Migrants, refugees, and asylum seekers are reported to have an increased risk condition for infectious disease transmission, including IMD (Dinleyici and Borrow, 2020). Despite being a global movement, the increase in the number of international migrants was not associated with any of the recent MenX IMD cases reported in our study.

The low genomic diversity characteristic of circulating MenX lineages in sub-Saharan Africa was also observed in our collection. The prevalent ST2888 lineage characterized in this study was recently associated with notified MenX IMD case in 2022 and was proven to circulate in Brazil since 2013. The MenX ST2888 had been only reported in Italy in 2009, in a patient that had not traveled abroad (Fazio et al., 2010). With the size of Brazil, some well-marked regional variations in meningococcal epidemiology have been previously observed (Aparecido Nunes et al., 2021). We have reported the prevalence of serogroup W ST11/cc11 throughout the Brazilian territory predominantly in the Southern region (De Lemos et al., 2022). In this study, we also have reinforced the observation that the Southern region was highlighted as a hotspot of MenX ST2888 since the last four isolates with no apparent association among them came from this region. Recently, one MenX ST2888 isolate was notified in the Brazilian Southeast region, warning of a possible expansion across the country.

Several studies have hypothesized genomic markers to explain the emergence and spread of a new clone in a naïve population (Mustapha et al., 2015; Brynildsrud et al., 2018). Genomic analysis and animal models have suggested *lpt3* gene allele 45 as a possible mechanism involved in enhanced transmissibility and virulence of MenX ST181 isolates (Agnememel et al., 2016). Unlike previously reported data, our data show that none of the studied isolates of ST2888 presented the potential expression of a functional phosphoethanolamine transferase. It is well documented in meningococcal epidemiology that certain hypervirulent meningococcal lineages are known to transmit globally (Caugant, 1998). Thus, further studies are needed to better understand the emergence of ST2888 and how it can soon impact transmission.

Since no licensed vaccine is available against serogroup X, an attempt to investigate the possibility of the use of the

4CMenB vaccine against serogroup X isolates was demonstrated in a previous study (Hong et al., 2013). Data predicted by Meningococcal Antigen Typing System (MATS) and correlated with serum bactericidal assays suggested that the 4CMenB vaccine could cover the studied MenX isolates from Africa by at least fHbp antigen unlike the studied MenX isolates from Europe (Hong et al., 2013). Since there is lack of cross-reactivity data from the new peptides carried by MenX isolates belonging to ST2888, with the available protein-based vaccines, the hypothetical potential protection in our MenX collection could not be observed by 4CMenB and MenB-FHbp vaccines using the MenDeVAR index (Rodrigues et al., 2021).

Despite the deep genomic characterization of the largest collection of MenX from non-African countries, this study has potential limitations. First of all is lacking data on the clinical status of patients since serogroup X is related to immunocompromised conditions (Fijen et al., 1989; Vicente, Esnal and Pérez-Trallero, 2012). The quality of epidemiological surveillance and meningococcal diagnosis varies throughout the country. Although there has been an improvement with the incorporation of real-time PCR in our surveillance system, approximately one-third of IMD cases are still confirmed by clinical criteria, and only around 50% of Brazilian IMD cases had serogroup information (http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/cnv/meninbr.def).

The present data reinforce that continuous universal and real-time surveillance is important to monitor the emergence and persistence of meningococcal hypervirulent lineages, including the non-vaccine preventable serogroup X to effective public health prevention strategies.

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Authors' contributions All authors attest they meet the ICMJE criteria for authorship.

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Data availability Sequencing reads and genome assemblies from this study have been deposited in GenBank under the following accessions: N.972-92 (JAAARH010000092); N.764-01 (JABDST010000000); N.68-13 (JAAARI010000000); N.61-16 (JAAARJ010000000); N.72-16 (JAAFZQ00000000); N.133-16 (JAAFZR00000000)

Declarations

Conflict of interest The authors declare no competing interests.

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