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1 **Genome characteristics of *Bordetella pertussis* isolates from Tunisia**

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14 **Running title:** *Bordetella pertussis* isolates from Tunisia

15 **Keywords:** *Bordetella pertussis*, Tunisia, genomic epidemiology, phylogeny

16 **Accession numbers**

17 The genomic sequence data generated in this work were submitted to the European
18 Nucleotide Archive and are available from the International Nucleotide Sequence Database
19 Collaboration (NCBI/ENA/DDBJ) databases under project accession number PRJEB27412
20 and run data accession numbers ERS2572942 to ERS2572951.

21 **Abstract**

22 **Purpose:** The recent increase in pertussis cases observed in some countries may have several
23 causes, including the evolution of *Bordetella pertussis* populations towards escape of vaccine-
24 induced immunity. Most genomic studies of *B. pertussis* isolates performed so far are from
25 countries that use acellular vaccines. The objective was to analyze genomic sequences of
26 isolates collected during the 2014 whooping cough epidemic in Tunisia, a country where
27 whole cell vaccines are used. **Methodology:** Ten Tunisian isolates and four vaccine strains
28 were sequenced and compared to 169 isolates from countries where acellular vaccines are
29 used. **Results:** Phylogenetic analysis showed that Tunisian isolates are diverse, demonstrating
30 a multi-strain 2014 epidemic peak, and are intermixed with those circulating in other world
31 regions, showing inter-country transmission. Consistently, Tunisian isolates have antigen
32 variant composition observed in other world regions. No pertactin-deficient strain was
33 observed. **Conclusion:** The Tunisian *B. pertussis* population appears to be largely connected
34 with populations from other countries.

35 **Introduction**

36 Pertussis is a highly contagious infectious disease, caused by the bacterium *Bordetella*
37 *pertussis* and more rarely by *B. parapertussis*. Although the infection is largely controlled by
38 vaccination, a recent increase in pertussis incidence has been observed in several countries
39 [1]. Possible explanations of this increase include sub-optimal vaccine coverage, improved
40 surveillance and diagnosis of the disease, waning of vaccine-induced immunity, and genetic
41 variations in circulating *B. pertussis* isolates that escape vaccine-induced immunity [2–4].

42 In Tunisia, pertussis is still present as a cyclical disease despite high (98%) primo-
43 vaccination coverage [5], and two epidemic peaks were observed in 2009 and 2014. A 9-year
44 surveillance study (2007-2016) among 1,844 infants and children reported 306 (16.6%)
45 *Bordetella* infection that were confirmed by real time PCR [6, 7]. While initial vaccination of
46 Tunisian children is performed at 2, 3 and 6 months of age, a booster dose is received at the
47 age of 18 months. Since April 2011, a pentavalent vaccine (Pentavac, Serum Institute of
48 India), which includes a whole cell pertussis component, is used in Tunisia.

49 *B. pertussis* is an highly monomorphic bacterium [4, 8]. Therefore, molecular typing of
50 *B. pertussis* isolates requires the full resolution of whole genome sequencing (WGS) for
51 accurate phylogenetic comparisons and to follow the spread of sublineages, as other methods
52 largely fail to resolve relationships at strain level [4, 9–11]. Inter-country transmission of
53 *B. pertussis* isolates was demonstrated based on genomic-scale genotyping of a global dataset
54 of isolates collected before 2011 [4] and of isolates collected more recently in countries using
55 acellular vaccines [9, 11, 12]. However, only few data are currently available on the genomic
56 sublineages of *B. pertussis* isolates collected in countries using whole cell vaccines [13, 14].
57 Therefore, whether transmission occurs between these countries and those using acellular
58 vaccines is not enough documented, limiting our understanding of the drivers of *B. pertussis*
59 diversity across countries with distinct vaccination strategies.

60 This study aimed to determine the genomic characteristics of Tunisian *B. pertussis* clinical
61 isolates and to compare them with isolates from other world regions and with strains used to
62 prepare the whooping cough vaccine used in Tunisia.

63

64 **Methods**

65 ***B. pertussis* isolates and genomes.** Tunisian *Bordetella pertussis* clinical isolates were
66 recovered during the epidemic peak of 2014. Their phenotypic characteristics and antigen
67 genotyping of genes *ptxP*, *prn*, *ptxA*, *fim2* and *fim3* were described earlier [7]. Genomic DNA
68 was isolated and purified from subcultured isolates [7] using the MagNA Pure 96 DNA and
69 Viral NA Small Volume Kit (Roche, Germany) according to the manufacturer's instructions.
70 Sequencing libraries were prepared using the Nextera XT DNA Sample Preparation Kit
71 (Illumina, USA). Whole genome sequencing was performed on the Illumina® NextSeq® 500
72 system (Illumina, USA) using a 2×150 paired-end protocol by the Mutualized Platform for
73 Microbiology at Institut Pasteur of Paris. AlienTrimmer v0.4.0, Musket v1.1 and
74 KhmerStream v1.1 software packages were used for paired-end reads clipping, trimming and
75 sequencing errors correction, respectively. SPAdes/3.9.0 was used for genome assembly.
76 Genome sequences were deposited in the European Nucleotide Archive and their accession
77 numbers are available in Table 1.

78

79 **cgMLST and phylogenetic analysis.** Genome sequence data were analyzed using a gene-by-
80 gene approach known as core genome MLST (cgMLST) [11, 15]. cgMLST was performed
81 using the BIGSdb platform of Institut Pasteur (<http://bigsdb.pasteur.fr/bordetella>) as
82 previously described [11]. Briefly, genome assemblies were compared using BLASTN to the
83 reference alleles of 2,038 predefined gene loci. When novel alleles were discovered in the

84 genomes of the Tunisian isolates, they were imported into the reference allele database and an
85 allelic number was assigned. Isolates from other world regions were investigated in a previous
86 study and corresponded to isolates from France, UK and USA [11]. Genomic sequences of
87 vaccine strains from Serum Institute of India J445 (GCA_001831395), J446
88 (GCA_001831415), J447 (GCA_001831435) and J448 (GCA_001831455) were also
89 included. We used IQ-TREE v1.5.4 to infer a maximum likelihood phylogenetic tree based on
90 concatenated alignments of the sequences from each of the 2,038 cgMLST loci.

91

92 **Results and Discussion**

93 Ten isolates collected during the epidemic of 2014 at the Children's Hospital of Tunis, the
94 only pediatric university hospital in Tunisia, were analyzed by genome sequencing. The
95 characteristics of genomes are summarized in **Table 1**. Assemblies characteristics were
96 similar for the ten isolates: about 300 contigs were obtained for each genome, with a mean
97 length of 3,867,263 bp. **Figure 1** presents the genomic sequence-based phylogenetic tree of
98 Tunisian *B. pertussis* isolates and their positioning with respect to isolates from other world
99 regions. Tunisian isolates were all distributed in the *ptxP3* clade but were genetically diverse,
100 with eight of them falling within the *fim3-2* clade and two within the *fim3-1* clade. TN0003
101 and TN0005 were closely related, with only 4 different cgMLST genes among them, whereas
102 they were more distant from other isolates (11 to 14 different loci). Likewise, isolates
103 TN0007, TN0001, TN0006 and TN0011 were closely related to each other within the *fim3-2*
104 branch, displaying only 1 or 2 different loci among themselves. These two groups of
105 genetically very similar Tunisian isolates may correspond to local chains of transmission [11].
106 Nevertheless, the 10 Tunisian isolates were mixed phylogenetically with isolates from other
107 countries, indicating several independent transmissions between Tunisia and other countries.
108 The closest neighbors of the Tunisian strains in this dataset were either from France, UK or

109 the USA, and diverged from them by 3 to 8 loci (**Table 2**). This close genetic relatedness
110 between Tunisian isolates and isolates from other geographic origins is consistent with the
111 high global genetic homogeneity of *B. pertussis* and is likely favored by the increased
112 international exchanges with Tunisia. Hence, despite the monomorphic nature of *B. pertussis*,
113 the genome sequencing approach shows genetic heterogeneity among the Tunisian isolates
114 included in this study, demonstrating that the epidemic peak of 2014 was not due to the spread
115 of a single strain but instead, was caused by the simultaneous infection by various strains as
116 observed previously [9, 11].

117 *B. pertussis* isolates can be characterized by their allelic profile, which recapitulates the allelic
118 variants at virulence and antigen genes loci. As previously observed [7], the allelic profile of
119 all Tunisian isolates is *ptxP3-ptxA1-prn2-fim2-1-fim3-2*. Notably, all Tunisian isolates had
120 alleles *ptxA1* for the subunit 1 of pertussis toxin, and pertactin allele *prn2*, irrespective of the
121 *fim3* clade they belong to, as observed for the majority of current *B. pertussis* isolates
122 collected in countries using acellular *B. pertussis* vaccine [4, 11, 12]. An increase in reported
123 *B. pertussis* isolates not producing pertactin has been observed in many countries using the
124 acellular pertussis vaccine, including the USA [16], Japan [12] and European countries [17].
125 All Tunisian *B. pertussis* isolates were shown to produce pertactin [7], and the genomic
126 sequence confirmed that they all had an intact *prn* gene. The low number of isolates from
127 Tunisia that could be analyzed does not allow to exclude the presence of pertactin non-
128 producing isolates in this country, but the results indicate that they are much less frequent
129 than pertactin-producing ones. This situation therefore contrasts with countries where
130 acellular vaccines are used since nearly two decades such as Japan, USA or some EU
131 countries [17–19]. Therefore, the inter-country transmission of *B. pertussis* sublineages is not
132 intense enough not erase the specificities of *B. pertussis* populations in countries with distinct
133 vaccination strategies.

134 The four vaccine strains (J445 to J448) incorporated in the vaccine used in Tunisia appear to
135 belong to early-branching clades (**Figure 1**), as does the Tohama reference strain previously
136 shown to be distant from most *B. pertussis* isolates that currently cause infection [4, 20].
137 Genetic distances of the four vaccine strains to Tunisian isolates were higher for strain J446
138 (117 to 124 cgMLST gene mismatches) than for strain J445 (28 to 31 mismatches) and for
139 strains J447 and J448 (17 to 24 mismatches). These genetic distances and the phylogenetic
140 position of vaccine strains are consistent with the promoter sequence or antigenic variants of
141 vaccine strains (J446: *ptxP2/ptxA4/prn7/fim2-2/fim3-1*; J445: *ptxP1/ptxA2/prn1/fim2-1/fim3-*
142 *1*; J447 and J448: *ptxP1/ptxA1/prn1/fim2-1/fim3-1*), which do not match entirely with the
143 profiles of most circulating isolates (*ptxP3/ptxA1/prn2/fim2-1/fim3-2*). Vaccine efficacy might
144 be improved by the use of vaccine strains that have a better phylogenetic and antigenic match
145 with the circulating isolates.

146 In conclusion, although based on a limited collection of isolates, this study provides a
147 snapshot of the genomic make-up of Tunisian isolates. Despite the use of whole cell vaccines
148 in Tunisia, *B. pertussis* isolates from this country are phylogenetically and antigenically
149 closely related to those circulating in countries using acellular vaccines, whereas they are less
150 related to vaccine strains. However, our sample of *B. pertussis* isolates did not reveal the
151 presence of pertactin-deficient strain in Tunisia. The relative contributions of long-range
152 transmission and local selection by vaccine-induced immunity in shaping *B. pertussis*
153 populations is an important subject for future research. Surveillance of pertussis infections in
154 Tunisia and other countries using whole-cell vaccines should be reinforced to inform control
155 measures and vaccination policy.

156 **Author statements**

157 **Author contributions:** Conceptualization: IBF, AK, HS and SB; Experiments: IBF, VB;
158 Data analysis: IBF, VB; Original draft preparation: IBF, VB, HS, SB; Review and editing:
159 SB; Supervision: HS, SB; Funding: SB.

160 **Conflict of Interest:** The authors declare that there are no conflicts of interest.

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232

Table 1: Characteristics of Tunisian *Bordetella pertussis* genomes used in this study

Isolate Name	Month of collection	Age of patient	Number of contigs	Genome size (nt)	N50 length (nt)	Genotype	Accession number (PRJEB27412)
TN0001	April 2014	2 months	300	3,872,771	20,131	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572942
TN0002	April 2014	3 weeks	293	3,864,234	20,328	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572943
TN0003	April 2014	6 weeks	298	3,863,302	20,108	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-1</i>	ERS2572944
TN0004	April 2014	3 weeks	301	3,878,136	19,862	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572945
TN0005	April 2014	1 month	290	3,868,375	20,544	<i>ptxP3 ptxA1 prn3 fim2-1 fim3-1</i>	ERS2572946
TN0006	May 2014	6 weeks	283	3,868,089	20,787	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572947
TN0007	May 2014	2 months	288	3,865,925	20,544	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572948
TN0008	May 2014	2 months	298	3,862,980	19,926	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572949
TN0009	May 2014	2 months	293	3,865,015	20,346	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572950
TN0011	May 2014	3 months	289	3,863,801	20,541	<i>ptxP3 ptxA1 prn2 fim2-1 fim3-2</i>	ERS2572951

Table 2: Genetically closest isolate(s) for each Tunisian *Bordetella pertussis* isolate

Isolate Name	Closest isolate(s) by cgMLST distance	No. of cgMLST mismatches
TN001	FR3903; FR3916	2
TN002	FR3903; FR3916	3
TN003	FR5438; FR5439	5
TN004	FR5791; P2M	3
TN005	I538; I539; I656	2
TN006	FR5859; FR5860; FR5942; FR6031; H379; H788; H918	2
TN007	FR3903; FR3916	2
TN008	FR3903; FR3916; FR5943; FR5793	2
TN009	FR5942; FR6031; H379; H788; H918; FR5859; FR5860	2
TN011	FR3903; FR3916	2

239 **Figure 1:** Maximum-likelihood phylogenetic tree of Tunisian *Bordetella pertussis* isolates
240 and isolates from other geographical origins based on the concatenated alignments of 2,038
241 cgMLST gene sequences. The tree was rooted with strain J446. Black branches correspond to
242 the *fim3-1* clade and green branches to the *fim3-2* clade. The external circle indicates the
243 geographic origin of isolates (long red bars, Tunisia; long blue bars: vaccine strains of Serum
244 Institute of India; light green, France; light blue, United Kingdom; light pink, USA). The
245 scale bar indicates the number of nucleotide substitutions per site.