

1 **TITLE**

2 Sentinel Case of *Candida auris* in the Western United States Following Prolonged
3 Occult Colonization in a Returned Traveler from India

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27

28 **RUNNING TITLE**

29 Emergence of *Candida auris* in Western USA

30 **FOOTNOTE PAGE**

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46 **ABSTRACT**

47 *Candida auris* is an emerging multidrug-resistant yeast with high mortality. We
48 report the sentinel *C. auris* case on the United States West Coast in a patient who
49 relocated from India. We identified close phylogenetic relatedness to the South Asia
50 clade and *ERG11* Y132F and *FKS1* S639Y mutations potentially explaining antifungal
51 resistance.

52

53

54 **Introduction**

55 *Candida auris* is an emerging fungal pathogen with high minimum inhibitory
56 concentrations (MIC) for many antifungals. Since identification in 2009, it is increasingly
57 recognized as an important cause of invasive disease and nosocomial outbreaks, with
58 high associated in-hospital mortality of 40-72%(1). Genomic evaluation of strains from
59 multiple geographic regions suggests simultaneous emergence of distinct geographic
60 clades on three continents, as opposed to dissemination from a single source (1). This
61 observation suggests environmental factors such as increased antifungal use may have
62 contributed to *C. auris* emergence(1).

63 In addition to high fluconazole MICs, *C. auris* isolates also frequently have high
64 MICs for other antifungals including amphotericin and less frequently echinocandins(1).
65 Despite the alarming frequency of elevated antifungal MICs in *C. auris*, the underlying
66 mechanisms and alleles associated with this resistance have not been fully
67 characterized. In *C. auris* as well as in other *Candida* species, mutations in *ERG11*
68 (ergosterol synthetase), *FKS1* (1,3 beta-D-glucan synthetase) and *FUR1* (uracil
69 phosphoribosyltransferase) have been associated with resistance to fluconazole,
70 echinocandins and flucytosine, respectively(1, 2). Previous studies suggest that
71 mutations in these genes can arise in the setting of systemic antifungal therapy(3).

72 Despite first appearing in the eastern United States in 2013, *C. auris* had not
73 been detected on the US West Coast(2). Here we report the identification of *C. auris* in
74 this region, which was unusual in that it did not establish endemicity, and use whole
75 genome sequencing (WGS) to identify strain origin and evaluate genetic mechanisms of
76 antifungal resistance.

77

78 **Methods**

79 *Case description*

80 An elderly man with metastatic rectal cancer relocated from India to California.
81 He had received chemotherapy and radiation while in India and had also undergone
82 intraabdominal surgeries complicated by sepsis. In the year following his move, he
83 required multiple admissions to the University of California, San Francisco Medical
84 Center (UCSF) for management of his malignancy and for secondary infections with
85 carbapenem-resistant *Enterobacteriaceae* (CRE), for which he was placed in contact
86 isolation. During his initial multi-month admission, two cultures from his urostomy grew
87 10,000 colony forming units of a non-*Candida albicans* yeast that was not further
88 speciated due to unclear clinical significance. In the course of his care, he was treated
89 with echinocandins with prophylactic intent. Several months after initial admission, he
90 was transitioned to palliative care. Three days prior to death, a nephrostomy culture
91 returned positive for yeast, which was ultimately speciated as *Candida auris*.

92

93 *Clinical microbiology and antifungal susceptibility testing*

94 Urine collected from the patient's nephrostomy tube into a sterile container
95 underwent quantitative culture for bacteria and yeast using standard culture methods.
96 Species identification was made using MALDI-TOF mass spectrometry (Brucker
97 Diagnostics), which returned a score value of 2.14, and was additionally confirmed by
98 the California Department of Public Health. Antifungal susceptibility testing was
99 performed using Sensititre YeastOne MIC plates (Trek Diagnostic Systems, Inc.), which

100 has >95% agreement with the Clinical Laboratory Standards Institute reference
101 method.(4)

102

103 *Whole genome sequencing*

104 DNA was extracted from the cultured *C. auris* isolate using the Zymo ZR
105 Bacterial/Fungal DNA kit. Library preparation was completed with the New England
106 Biolabs NEBNext Ultra II DNA library prep kit and WGS was performed using an
107 Illumina NextSeq. The same DNA also underwent library prep using the Oxford
108 Nanopore Rapid Low Input by PCR Barcoding Kit and WGS on a MinION instrument.

109

110 *Genome assembly, phylogenetic analyses and antifungal resistance gene analysis*

111 Raw Illumina sequencing reads were quality filtered using PriceSeqFilter(5) and
112 then parsed with Nanopore reads for hybrid *de novo* assembly using DBG2OLC (6).
113 Reference-based whole genome phylogenetic analysis constructed from core genome
114 single nucleotide polymorphisms (SNPs) was carried out with the NASP pipeline(7)
115 using Pakistan strain B8441 as the reference genome and incorporating genomes from
116 Lockhart et al.(1) as well as *C. auris* isolate 16B15b containing the *FKS1* S639P
117 mutation identified by Rhodes et al (3). RAxML-ng (8) was used to build maximum
118 likelihood phylogenetic trees as detailed in Supplemental Methods. To identify genetic
119 mutations associated with fluconazole or echinocandin resistance, Illumina sequences
120 were aligned against *ERG11* (Genbank KY410388.1) and *FKS1* (Genbank
121 XM_018312471.1) using BowTie2(9). Mutations were confirmed by *ERG11* and *FKS1*

122 PCR followed by Sanger Sequencing (Table S1) following previously described
123 methods.(10)

124

125 **Results**

126 *Assembly and Phylogenetic Characteristics*

127 *De novo* hybrid assembly of Illumina and Oxford Nanopore reads produced a
128 total of 33 contigs spanning 12 Megabases (Mb), characterized by 44.9% GC content,
129 consistent with prior estimates(1, 3). Whole genome phylogenetic analysis based on a
130 core genome of 208,384 SNPs placed this isolate within the South Asia clade (Figure
131 A). On average, 56 SNPs separated this isolate from others from the South Asia clade
132 (Figure B).

133

134 *Phenotypic and Genotypic Assessment of Antifungal Resistance*

135 The California isolate demonstrated low MICs to amphotericin (1 µg/mL),
136 flucytosine (0.5 µg/mL), and voriconazole (0.032 µg/mL). The isolate had an elevated
137 fluconazole MIC of 32 µg/mL. Assessment of this isolate's *ERG11* (encoding ergosterol
138 synthetase) allele revealed the well-characterized Y132F substitution in the azole
139 resistance hotspot region (1, 3). Unlike most *C. auris* strains, this California isolate also
140 exhibited a high caspofungin MIC of 8 µg/mL. Interrogation of *FKS1* (encoding (1,3)-β-
141 D-glucan synthetase) revealed a S639Y mutation in the echinocandin resistance
142 hotspot 1 region (Table S1) (3, 10).

143

144 **Discussion**

145 *C. auris* emerges on the West Coast of the United States

146 Here we report the first case of *C. auris* on the US West Coast, a region that had
147 no previous reports of the pathogen despite emergence in New York in 2013. The
148 patient's history of healthcare exposure in India combined with the clustering of his *C.*
149 *auris* isolate with the South Asia clade by WGS phylogenetic analysis suggests that he
150 acquired *C. auris* abroad prior to hospitalization in California. This finding supports
151 current guidance from the US Centers for Disease Control and Prevention to speciate
152 all *Candida* in high risk patients including those from regions of high *C. auris*
153 prevalence, to allow for early implementation of infection control measures(1, 11).
154 Following identification of *C. auris*, enhanced infection control measures were
155 implemented at UCSF including surface disinfection, a unit-level point prevalence
156 survey and prospective surveillance. No additional cases of *C. auris* at our medical
157 center have been identified in over a year. This case represents an unusual interruption
158 in spread and prolonged healthcare environmental contamination that has been
159 characteristic of detection of healthcare-associated *C. auris*. Early implementation of
160 contact precautions for CRE may have contributed to curbing transmission of *C. auris* in
161 this case.

162 This isolate had a high fluconazole MIC with an observed *ERG11* Y132F
163 mutation (1, 3). The California *C. auris* isolate also demonstrated a high echinocandin
164 MIC, which is observed in less than 10% of *C. auris* strains (1). It is possible that this
165 patient's prophylactic treatment with echinocandins could have selected for resistance
166 as observed in this isolate. This *C. auris* isolate also had a *FKS1* hotspot-1 region
167 mutation, which has been associated with echinocandin resistance in multiple other

168 *Candida* species (3, 10). The identified *FKS1* S639 substitutions of nonpolar residues
169 (Y,F,P) has also been identified in other *C. auris* strains with high echinocandin MIC
170 values, suggesting a key role for this amino acid in echinocandin resistance (3, 10).

171 Further study is needed to estimate the prevalence and duration of colonization
172 by this emerging pathogen. Future work using WGS is needed to clarify the origins of *C.*
173 *auris*, transmission patterns, and mechanisms of resistance to prevent and manage this
174 emerging fungal pathogen of global significance.

175

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184 **DATA AVAILABILITY**

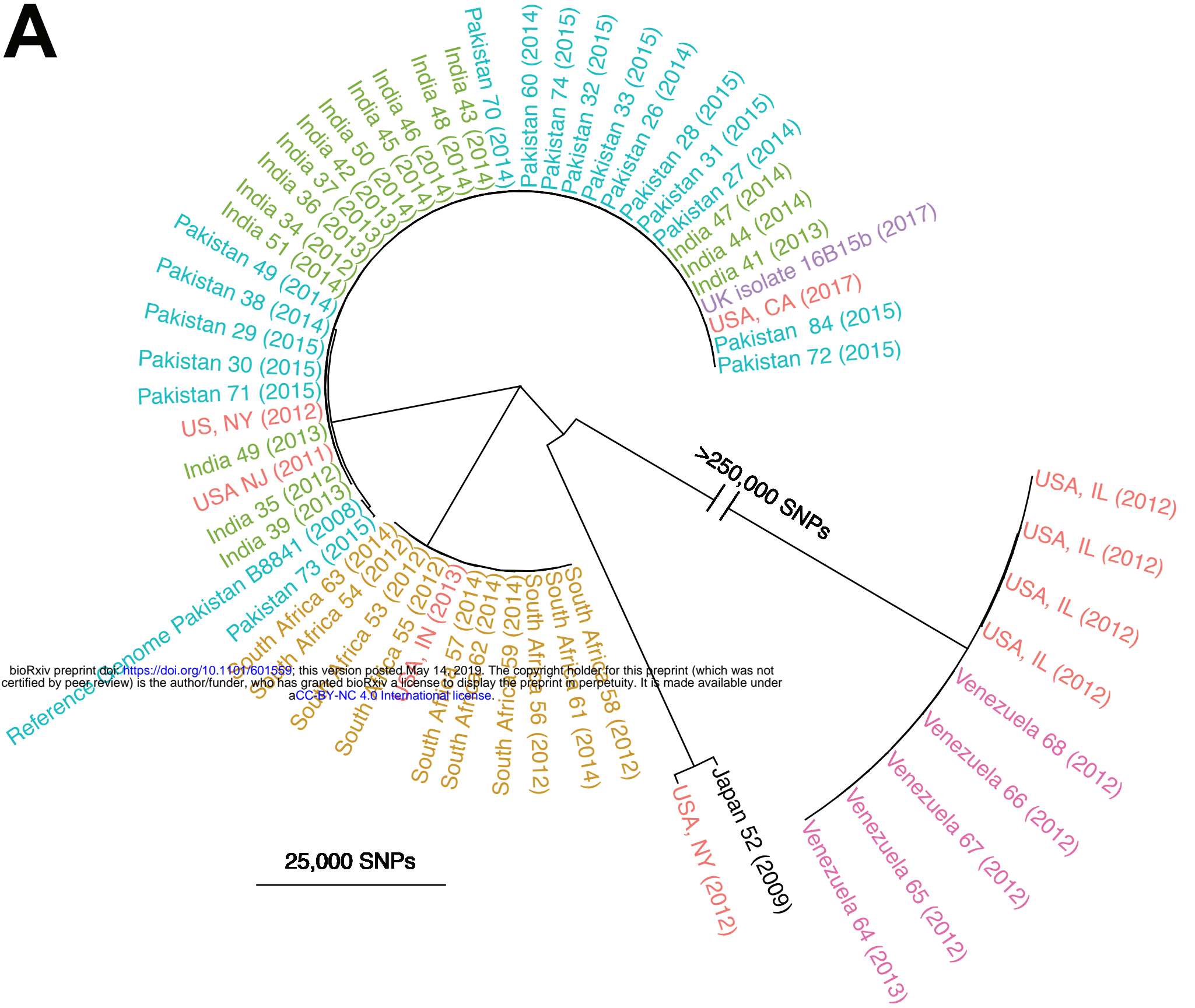
185 Raw sequences are available via Bioproject ID PRJNA480539.

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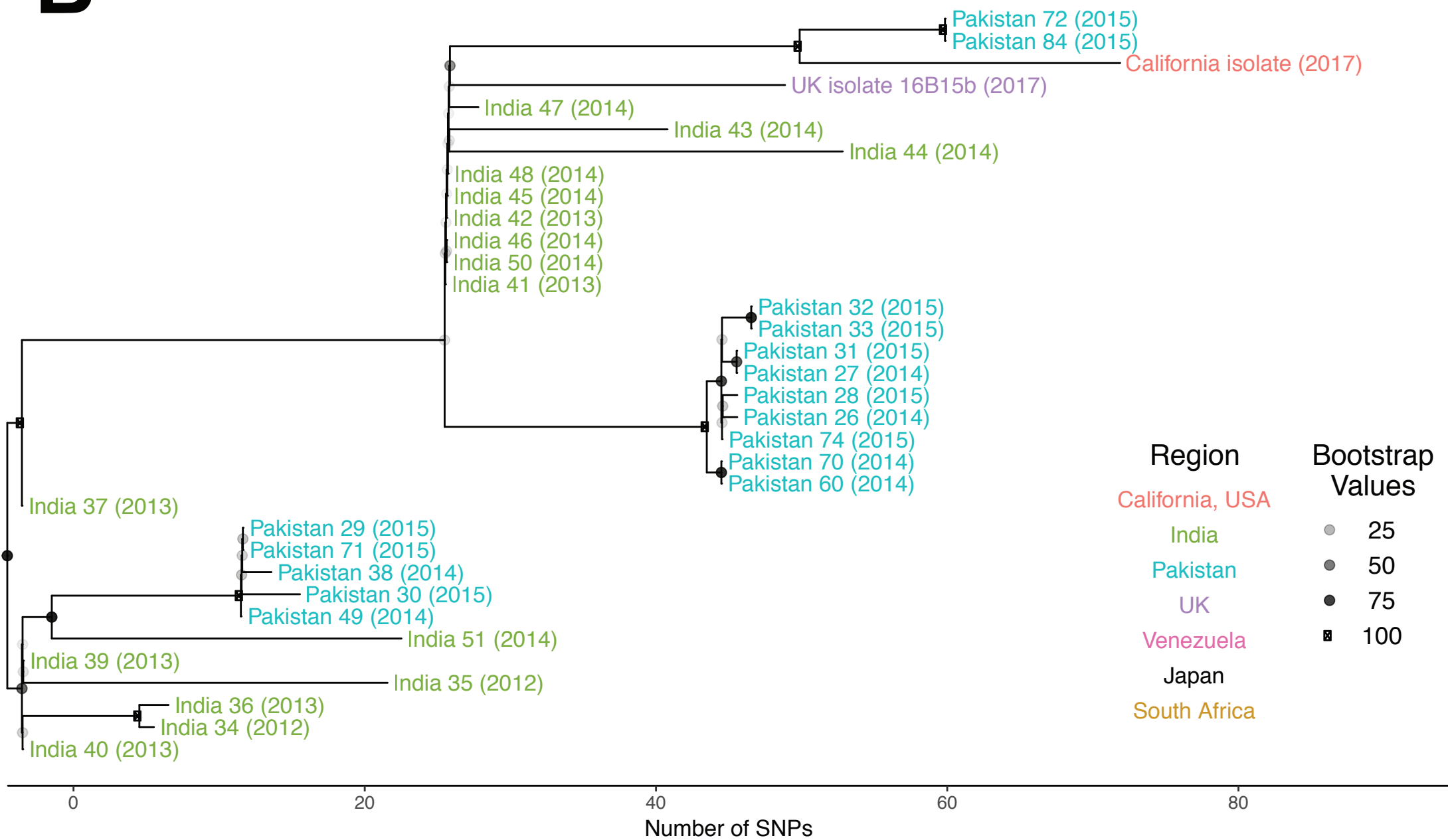
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234

A



B



235 **FIGURE LEGEND**

236 A) Phylogenetic assessment based on core genome SNPs demonstrated the four
237 known geographic clades(1) and placed the California isolate within the South Asia
238 clade. B) Detailed phylogenetic tree describing the South Asia clade including the
239 California isolate and UK outbreak isolate16B15b (3), which both harbored the *FKS1*
240 S639P mutation.