Figures and figure supplements

Global diversity and antimicrobial resistance of typhoid fever pathogens: Insights from a meta-analysis of 13,000 *Salmonella* Typhi genomes

Megan E Carey *et al*.

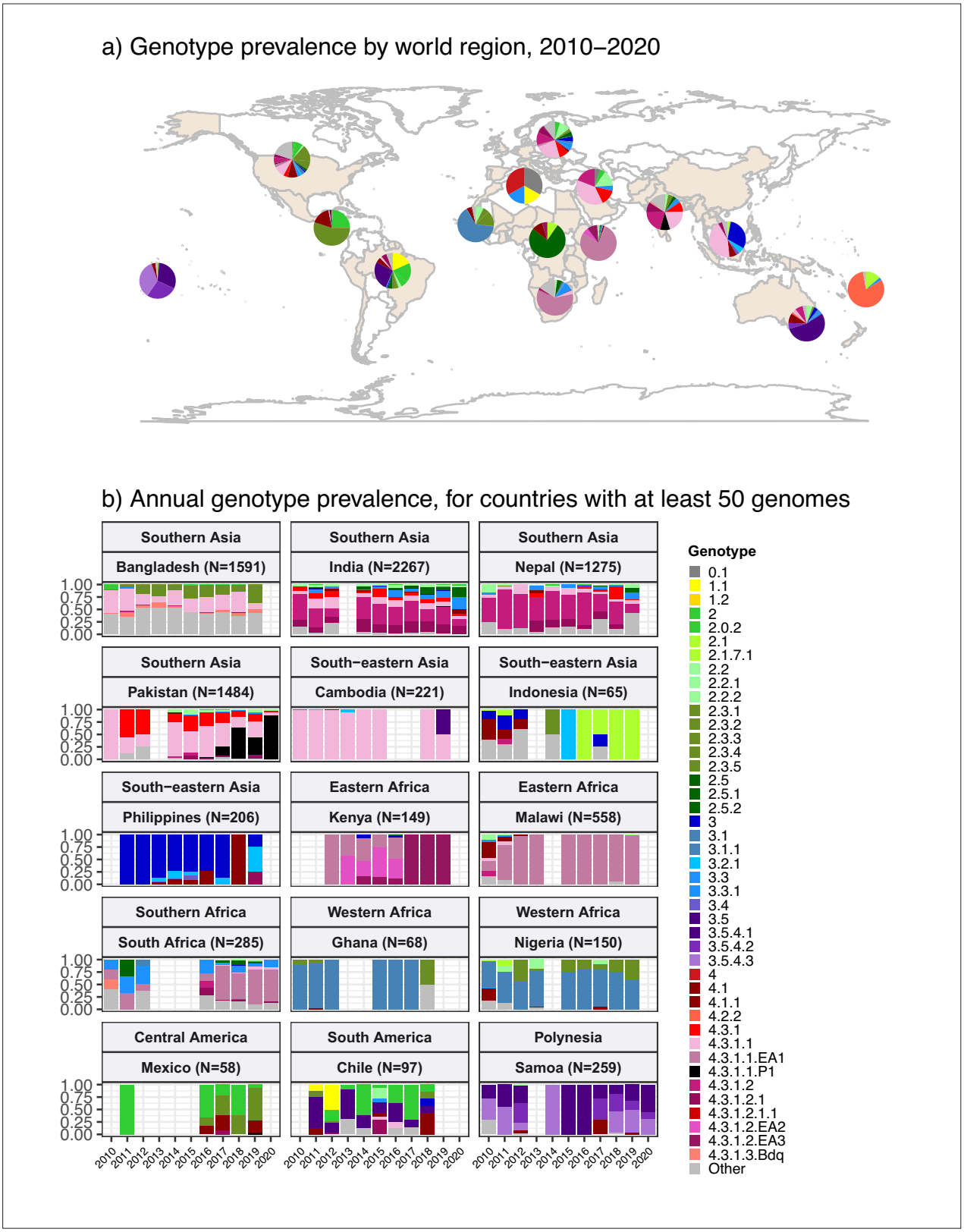

Figure 1. Global genotype prevalence estimates. Based on assumed acute cases isolated from untargeted sampling frames from 2010 onwards, with known country of origin (total N=9478 genomes). (**a**) Genotype prevalence by world region, 2010–2020. Countries contributing data are shaded in beige, and are grouped by regions as defined by the UN statistics division. (**b**) Annual genotype prevalence for countries with ≥50 genomes where typhoid is endemic. In both plots, colours indicate prevalence of Typhi genotypes, as per inset legend. Genotypes not exceeding 20% frequency in at least one

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country are aggregated as 'other'. Full data on regional and national genotype prevalences, including raw counts, proportions, and 95% confidence intervals, are given in ***Supplementary files 5 and 6***, respectively.

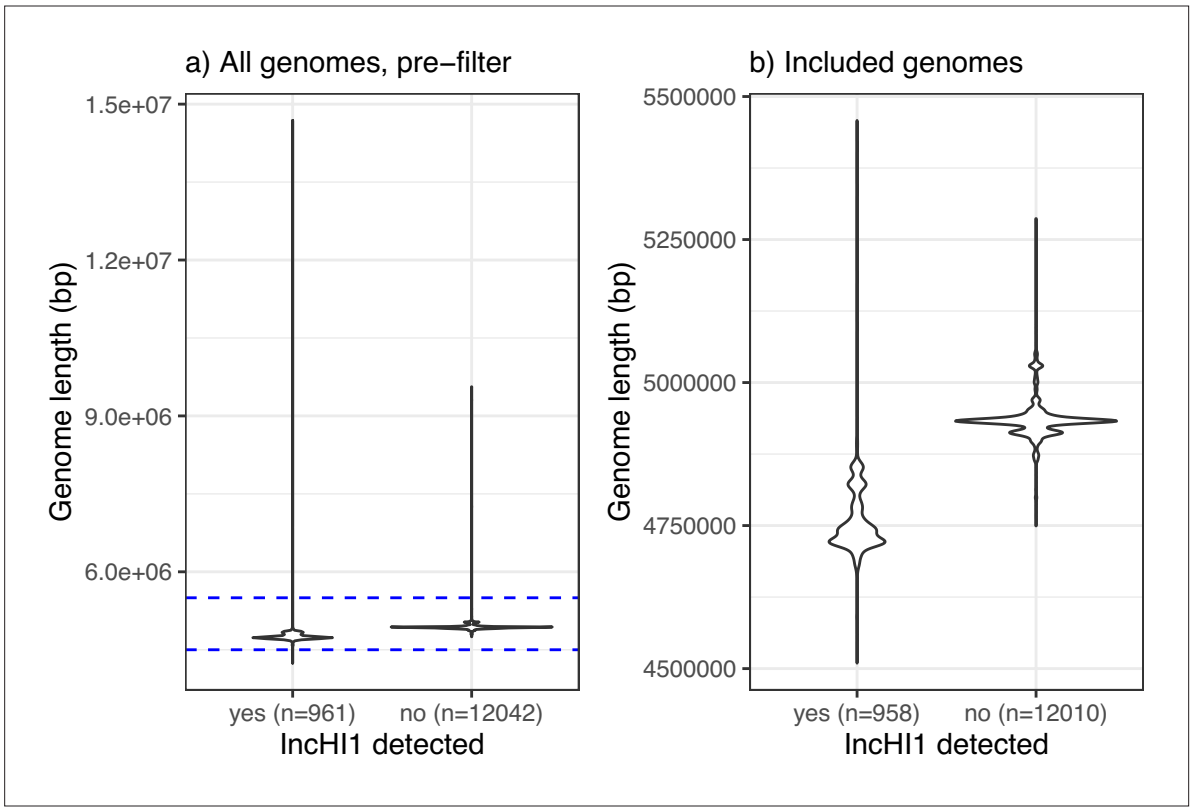

Figure 1—figure supplement 1. Genome size pre- and post-filtering, stratified by detection of an IncHI1 plasmid replicon marker. (**a**) All assemblies examined (n=13,000). (**b**) Assemblies of genomes included in the analysis (n=12,965), inclusion criterion being size between 4.5 and 5.5 Mbp.

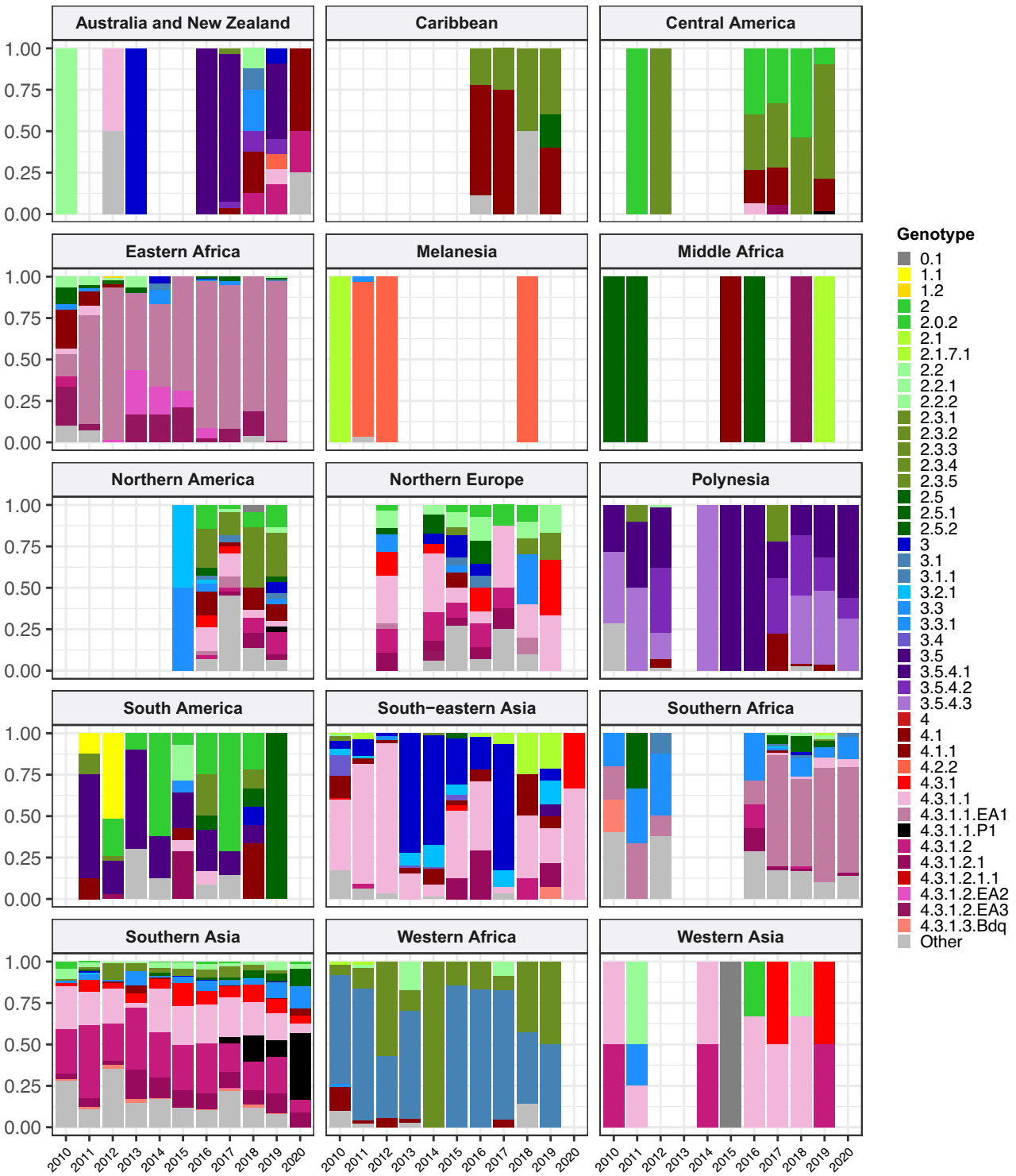

Figure 1—figure supplement 2. Annual breakdown of genotypes per world region, 2010–2020, for regions with ≥20 representative genomes. Bars show genotype prevalence rates observed per annum, coloured as per inset legend. Genotypes present at ≥20% frequency in any country are indicated separately, rare genotypes are aggregated as 'other'. Full data, including raw counts, proportions, and 95% confidence intervals, are available in ***Supplementary file 5***.

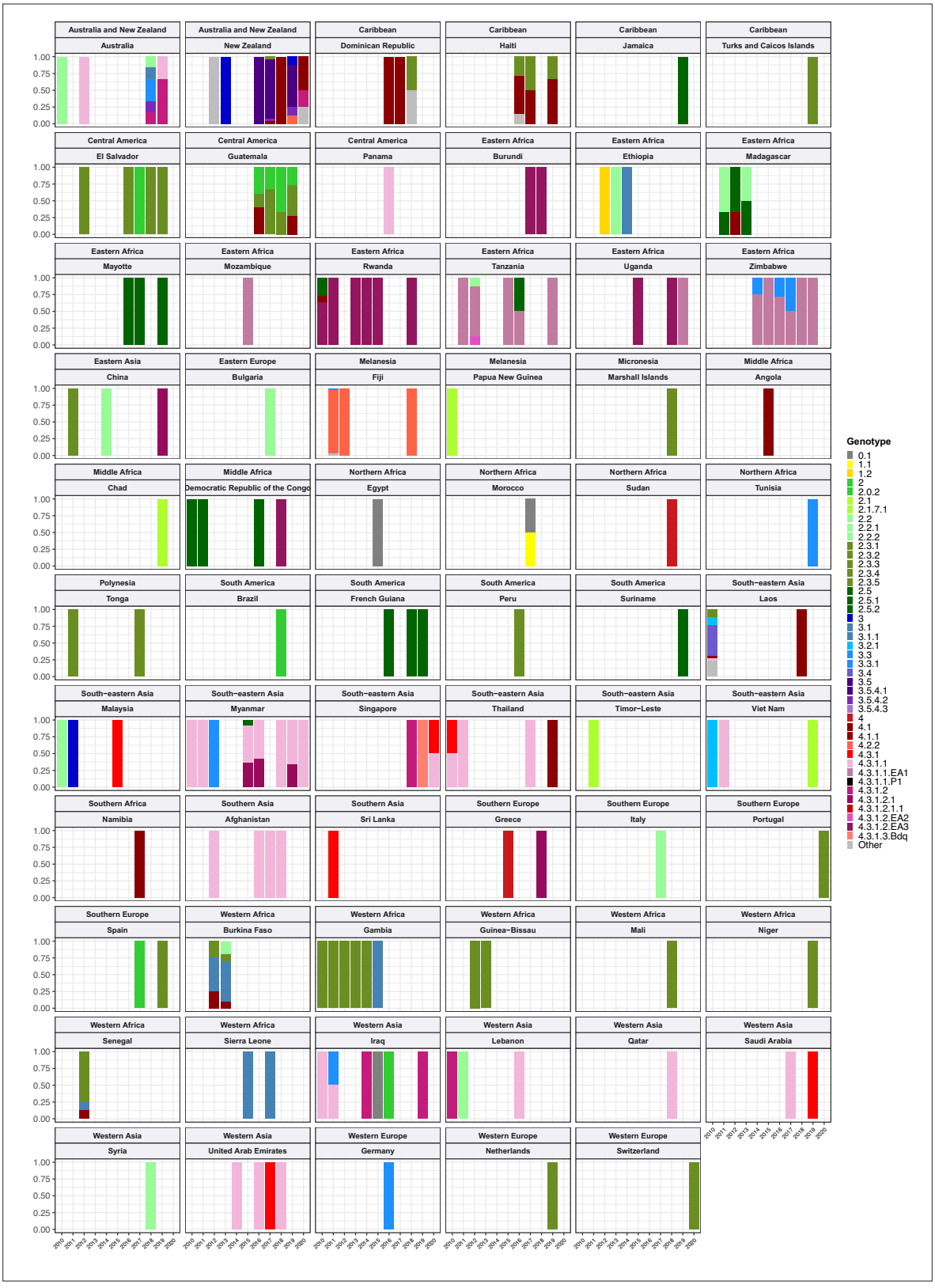

Figure 1—figure supplement 3. Annual breakdown of genotypes per country, for countries with <50 representative genomes between 2010 and 2020. (Note plots for countries with ≥50 genomes are shown in ***Figure 1b***, full data including raw counts, proportions, and 95% confidence intervals, are in ***Supplementary file 6***). Bars show genotype prevalence rates observed per annum, coloured as per inset legend. Genotypes present at ≥20% frequency in any country are indicated separately, rare genotypes are aggregated as 'other'.

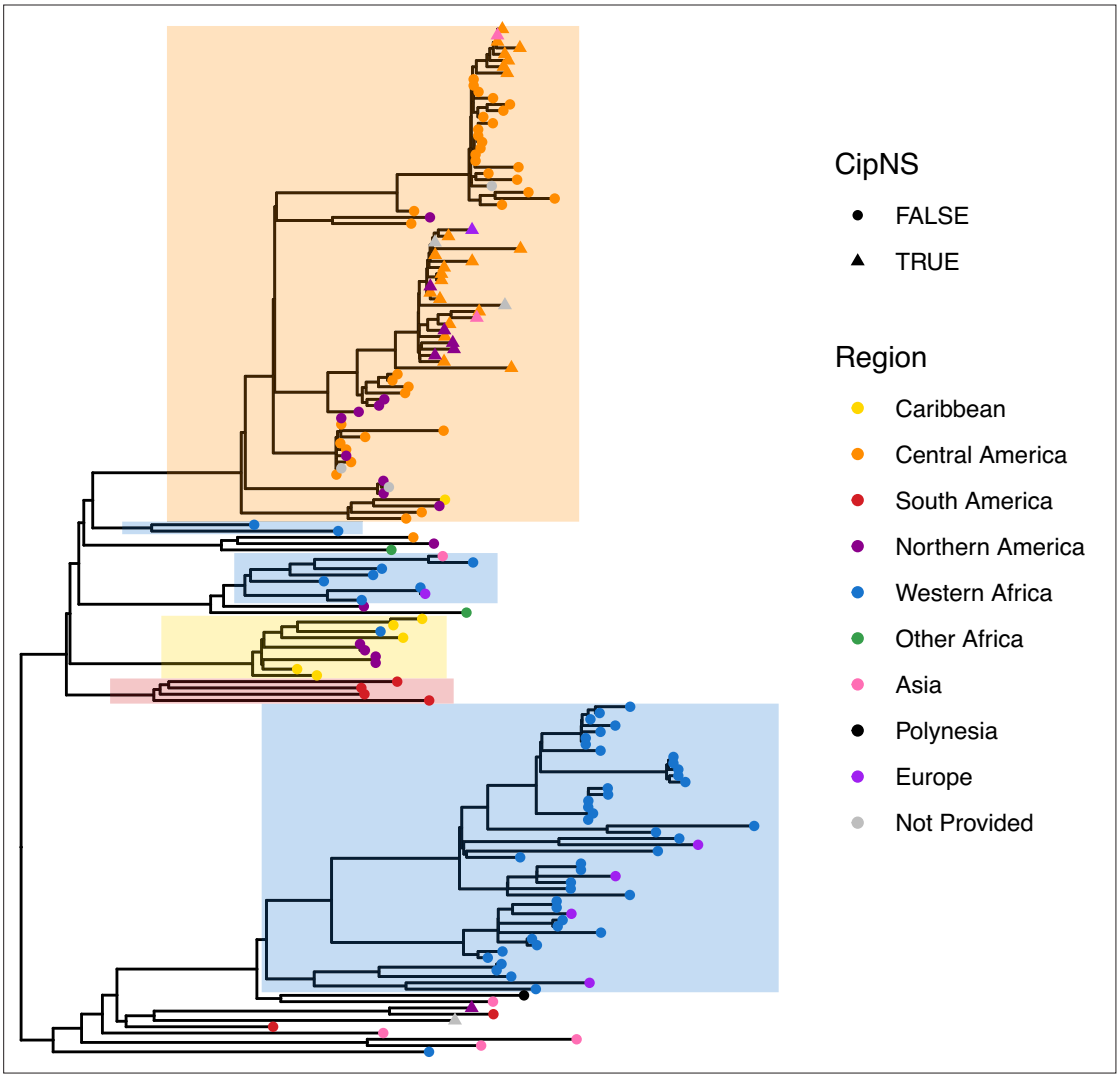

Figure 1—figure supplement 4. Phylogenetic tree showing relationships amongst genotype 2.3.2 genomes. The tree is a core-genome distance-based neighbour-joining tree generated from assemblies using Pathogenwatch, including n=164 genotype 2.3.2 genomes, outgroup rooted using a diverse set of genomes from ***Ingle et al., 2021*** (n=115 genomes from 16 genotypes). Tips are coloured by world region, according to inset legend; triangles indicate genomes harbouring QRDR mutations resulting in predicted non-susceptibility to ciprofloxacin (CipNS). Clades representing putative local clonal expansions are shaded.

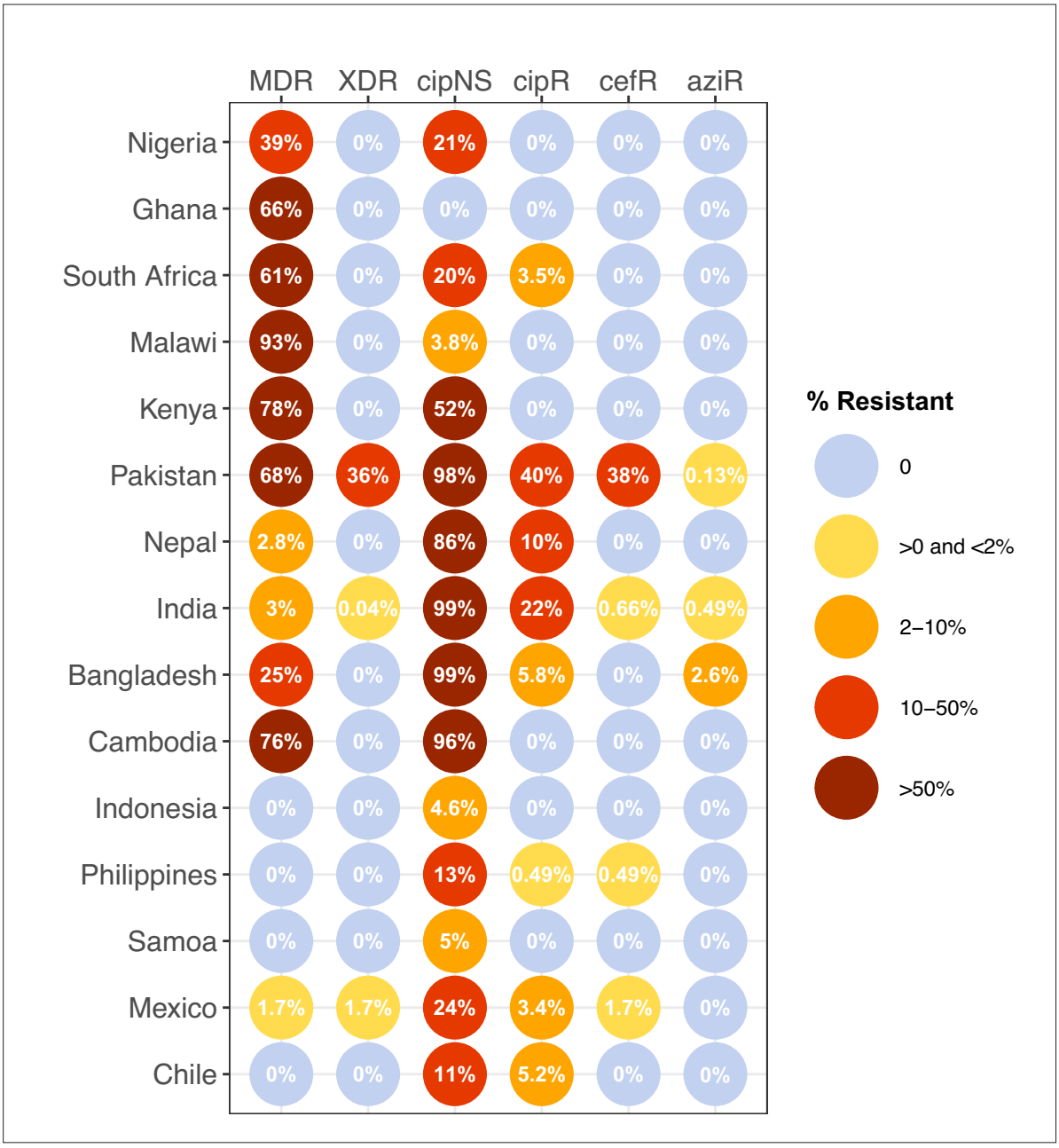

Figure 2. Prevalence of key antimicrobial resistance (AMR) genotype profiles by country. For all countries with ≥50 representative genomes (untargeted, assumed acute cases) from 2010 to 2020, where typhoid is endemic. Percentage resistance values are printed for each country/drug combination, and are coloured by categorical ranges to reflect escalating levels of concern for empirical antimicrobial use: (i) 0: no resistance detected; (ii) >0 and ≤2%: resistance present but rare; (iii) 2–10%: emerging resistance; (iv) 10–50%: resistance common; (v) >50%: established resistance. Annual rates underlying these summary rates are shown in ***Figure 3*** and ***Supplementary file 8***. Full data including counts and confidence intervals are included in ***Supplementary file 8***. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant; AziR, azithromycin resistant. Countries are grouped by geographical region.

Figure 2—figure supplement 1. Prevalence of key antimicrobial resistance (AMR) genotype profiles by world region, for non-targeted samples, 2010–2020. Percentage resistance values are printed for each region/drug combination, and are coloured by categorical ranges to reflect escalating levels of concern for empirical antimicrobial use: (i) 0: no resistance detected; (ii) >0 and ≤2%: resistance present but rare; (iii) 2–10%: emerging resistance; (iv) 10–50%: resistance common; (v) >50%: established resistance. Full data including counts and confidence intervals are in ***Supplementary file 7***. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant; AziR, azithromycin resistant.

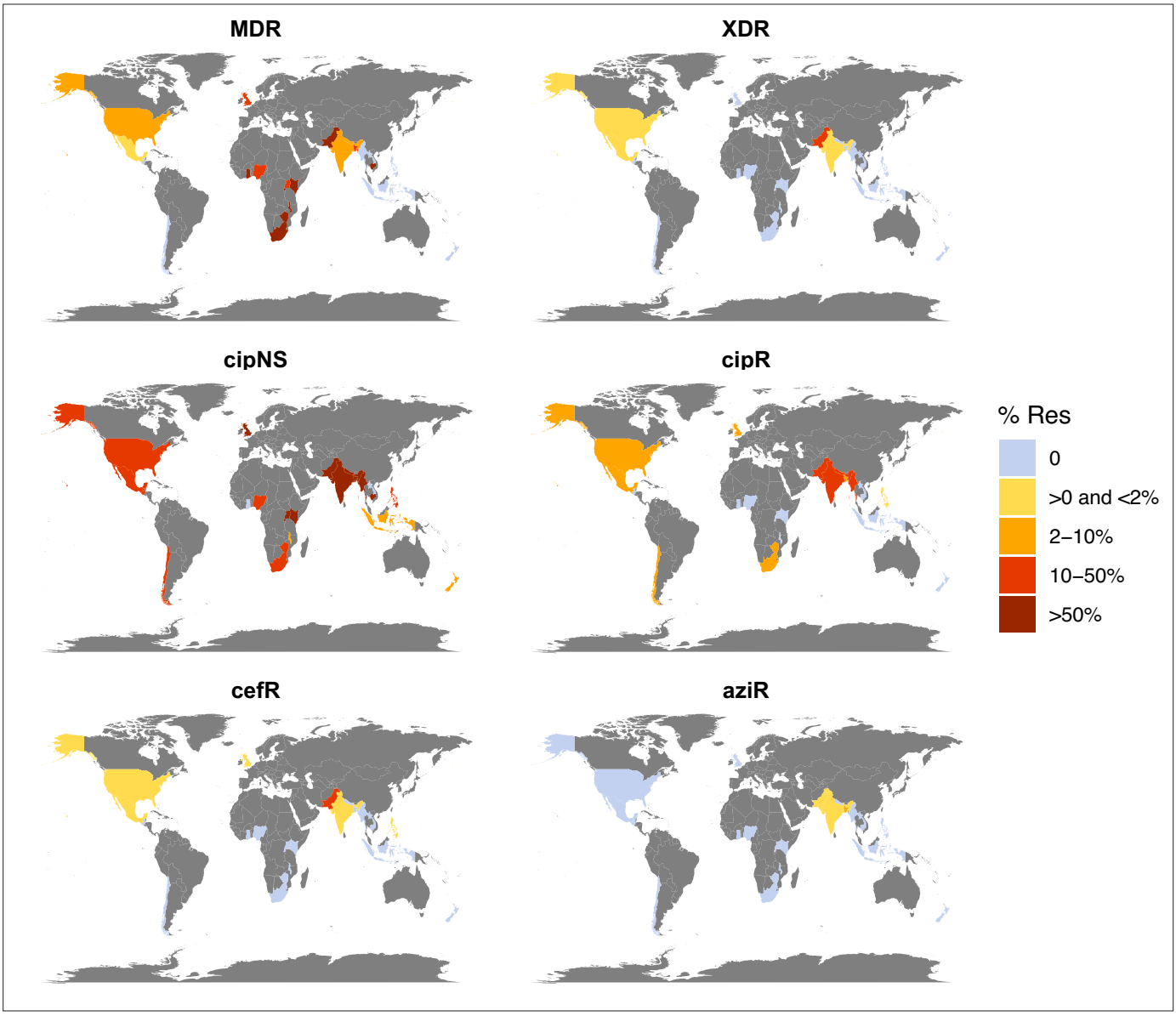

Figure 2—figure supplement 2. Antimicrobial resistance (AMR) prevalence for non-targeted samples, 2010–2020. Data are shown only for countries with N≥20 isolates (others are coloured grey). Countries are coloured by the prevalence of resistance per country, as per the inset legend. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant; AziR, azithromycin resistant.

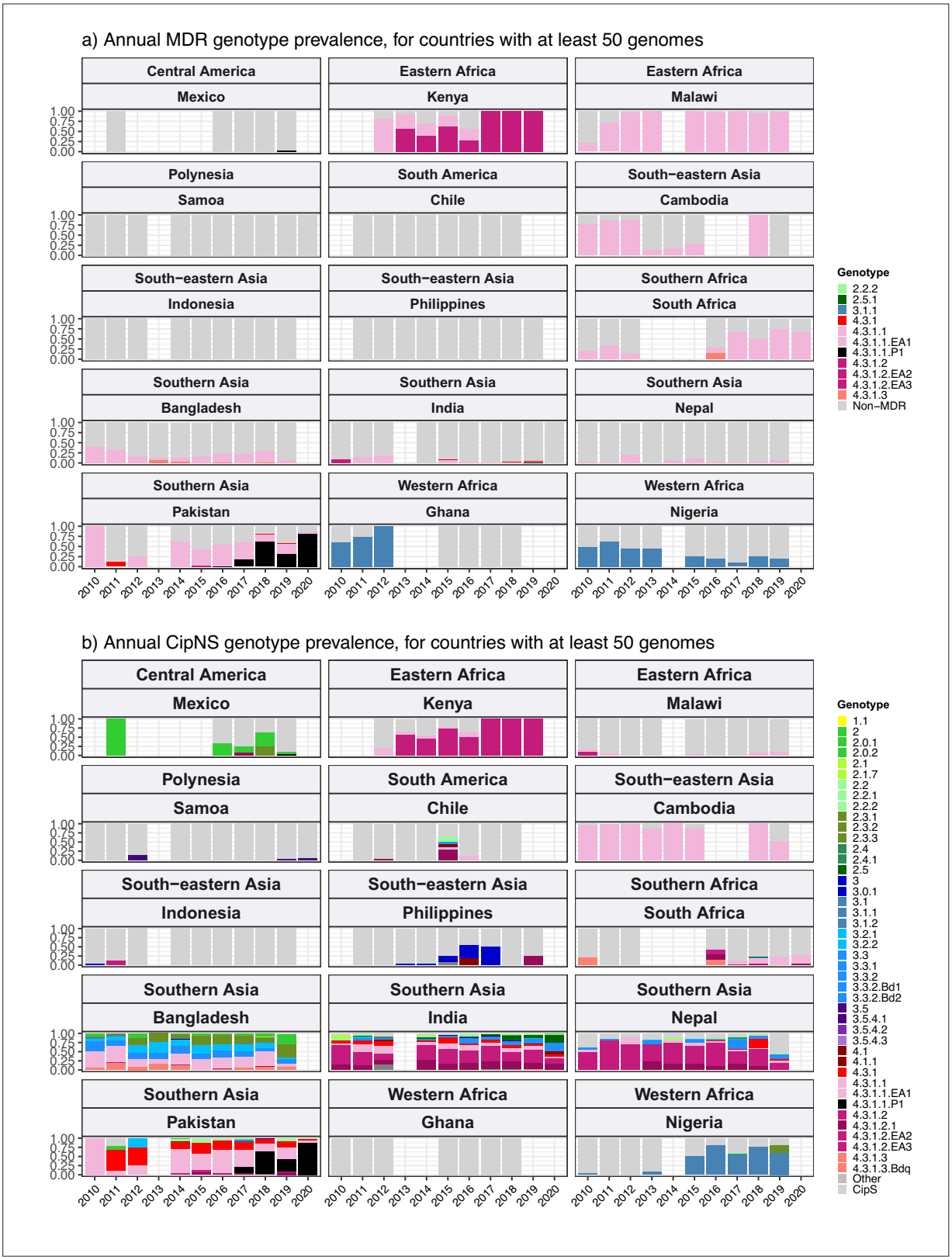

Figure 2—figure supplement 3. Annual genotype prevalence amongst multidrug-resistant (MDR) and ciprofloxacin non-susceptible (CipNS) genomes. For countries with ≥50 representative genomes between 2010 and 2020 and endemic typhoid. Genotypes for (a) MDR and (b) CipNS genomes are coloured according to the inset legends; sensitive genomes of all genotypes are aggregated and coloured grey.

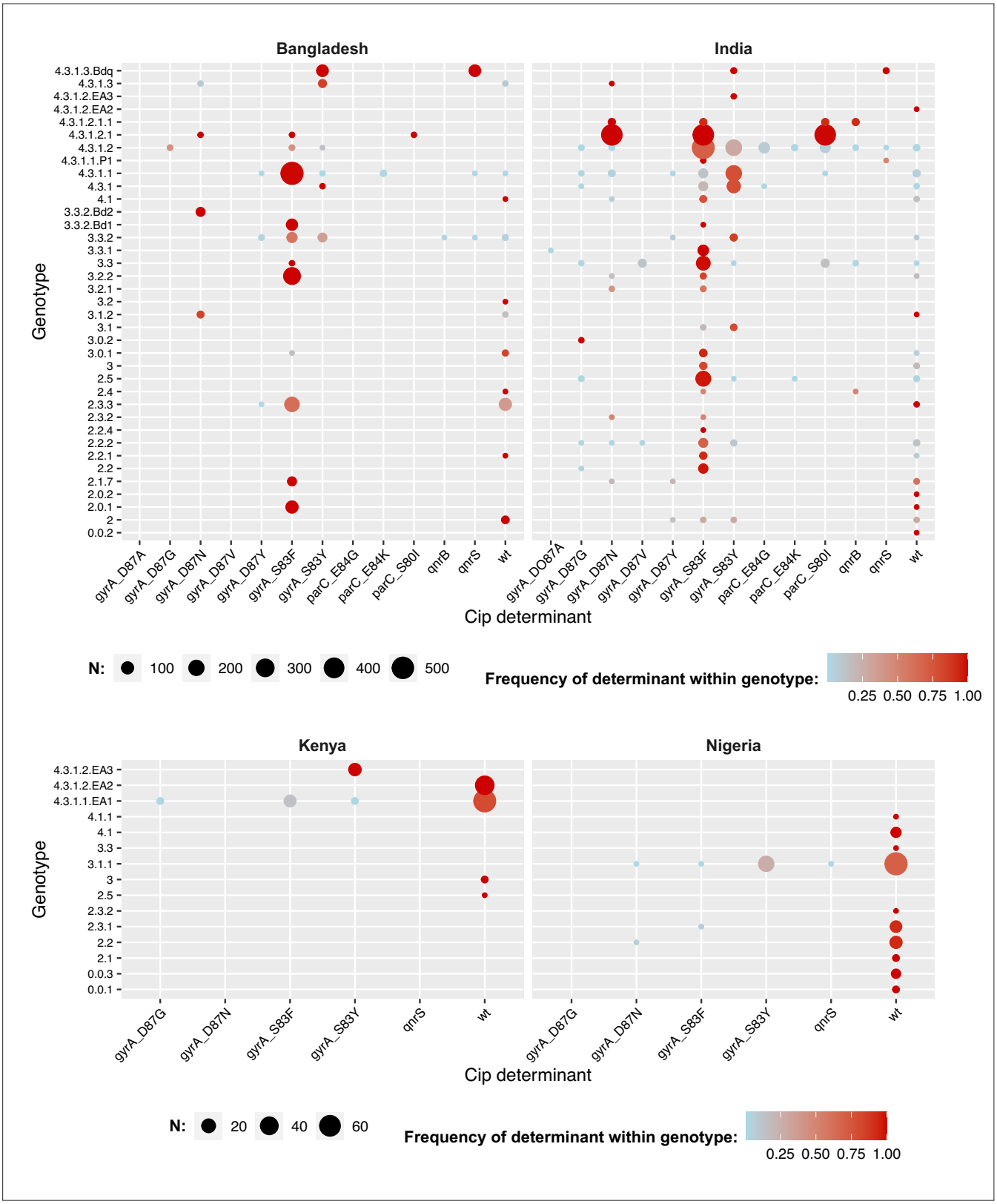

Figure 2—figure supplement 4. Distribution of fluoroquinolone resistance determinants by genotype. For selected countries discussed in text. Node size indicates total number of isolates for a given combination of genotype (row) and determinant (column); nodes are coloured to indicate the frequency of the determinant within that genotype. Wt = wildtype; that is, no quinolone resistance determining mutations was detected in *gyrA* or *parC* and no plasmid-borne quinolone resistance (*qnr*) genes were detected.

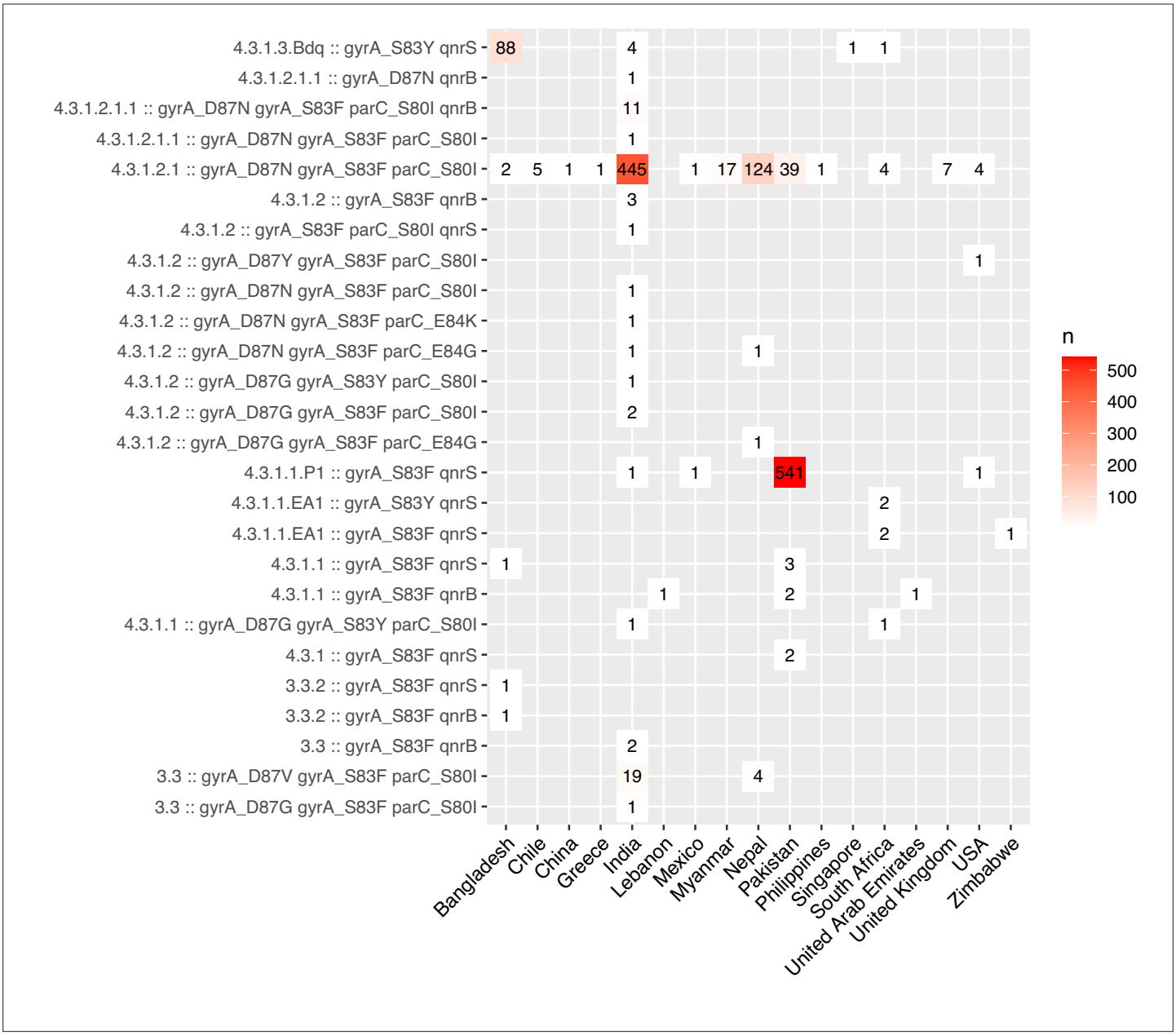

Figure 2—figure supplement 5. Ciprofloxacin-resistant genotypes identified. Rows show all n=24 unique combinations of Typhi genotype, quinolone-resistance determining region (QRDR) mutations (in *gyrA*, *gyrB*, *parC*, see **Methods**) and acquired plasmid-mediated quinolone resistance (PMQR) genes (qnrB, qnrD, qnrS) identified in genomes that are predicted to result in ciprofloxacin resistance (presence of ≥1 QRDR mutation+≥1 PMQR gene, or presence of ≥3 QRDR mutations).

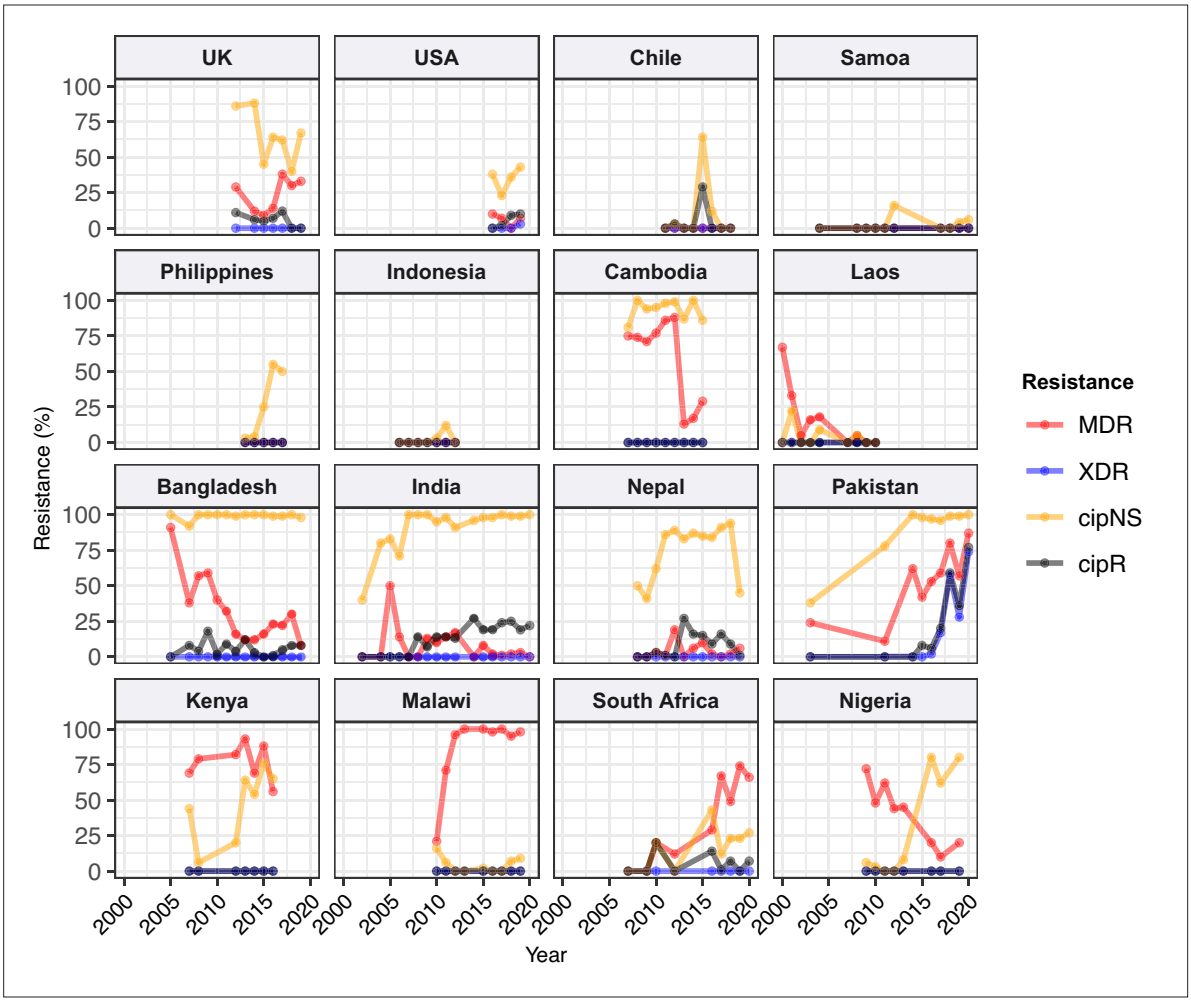

Figure 3. Annual prevalence of key antimicrobial resistance (AMR) profiles. For countries with ≥3 years with ≥10 representative genomes (untargeted, assumed acute cases) from 2000 to 2020. Data are shown only for country/year combinations with N≥5 isolates. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant.

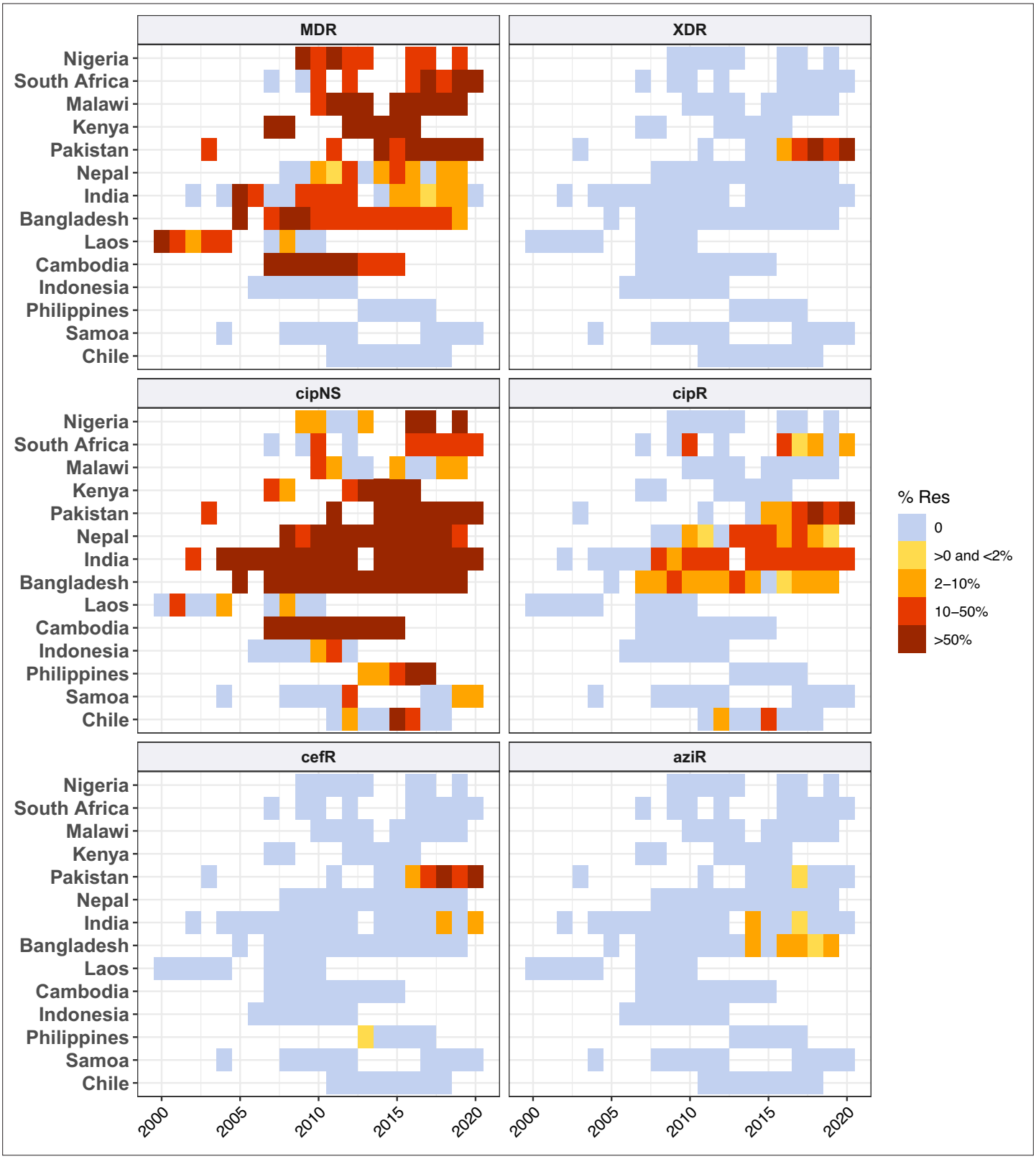

Figure 3—figure supplement 1. Annual prevalence of key antimicrobial resistance (AMR) profiles. For countries with ≥3 years with ≥10 representative genomes (untargeted, assumed acute cases) from 2000 to 2020 and endemic typhoid. Data are shown only for country/year combinations with N≥5 isolates. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant; AziR, azithromycin resistant.

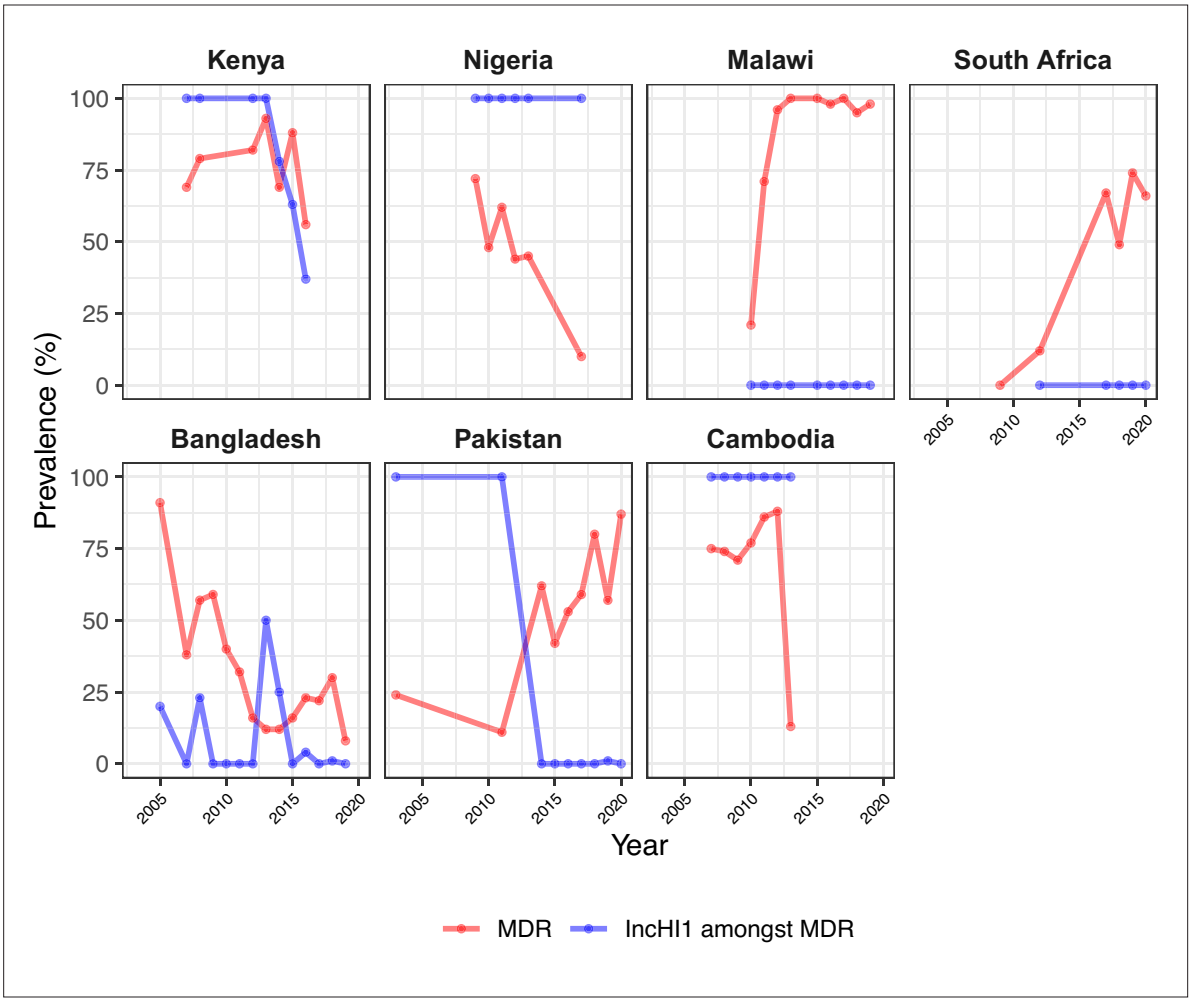

Figure 3—figure supplement 2. Trends in annual frequency of multidrug-resistant (MDR) genomes and proportion of MDR explained by IncHI1 plasmids. For countries with endemic typhoid and ≥5% MDR prevalence between 2000 and 2020.

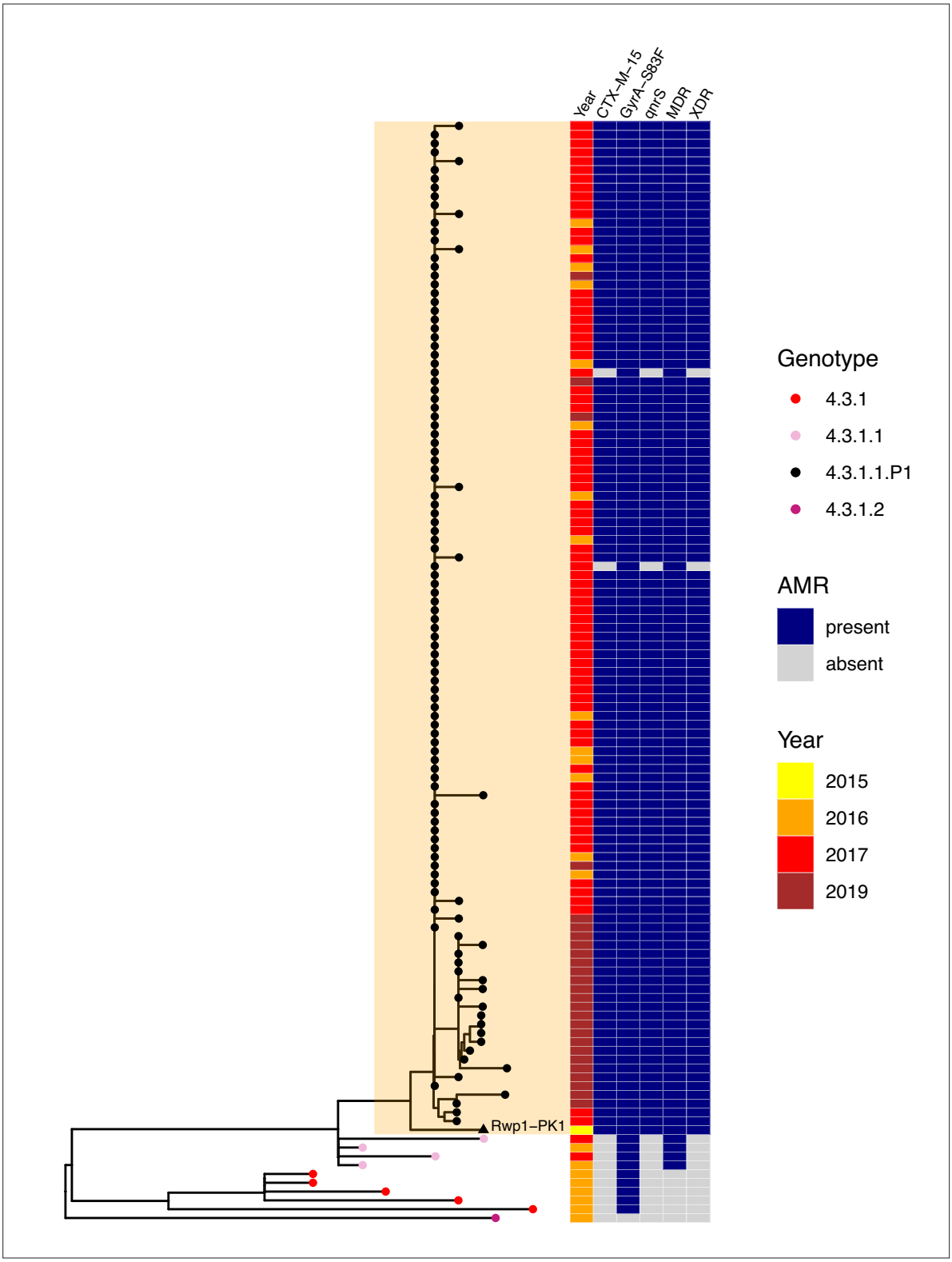

Figure 4. Phylogenetic tree showing position of 2015 Rwalpindi isolate, Rwp1-PK1, in context with other genomes from Pakistan. Core-genome distance-based neighbour-joining tree generated in Pathogenwatch, using all genomes from ***Klemm et al., 2018*** (the first genomic characterisation of the extensively drug-resistant [XDR] outbreak clade, including outbreak strains and local context strains from Sindh Province in 2016–2017) and ***Rasheed et al., 2020*** (genomic report of XDR outbreak strains from Lahore in 2019). Tree tips are coloured by genotype, according to inset legend; the 2015 strain Rwp1-PK1 is labelled in the tree and indicated with a triangle. Year of isolation and presence of antimicrobial resistance (AMR) determinants are indicated in the heatmap, according to inset legend.

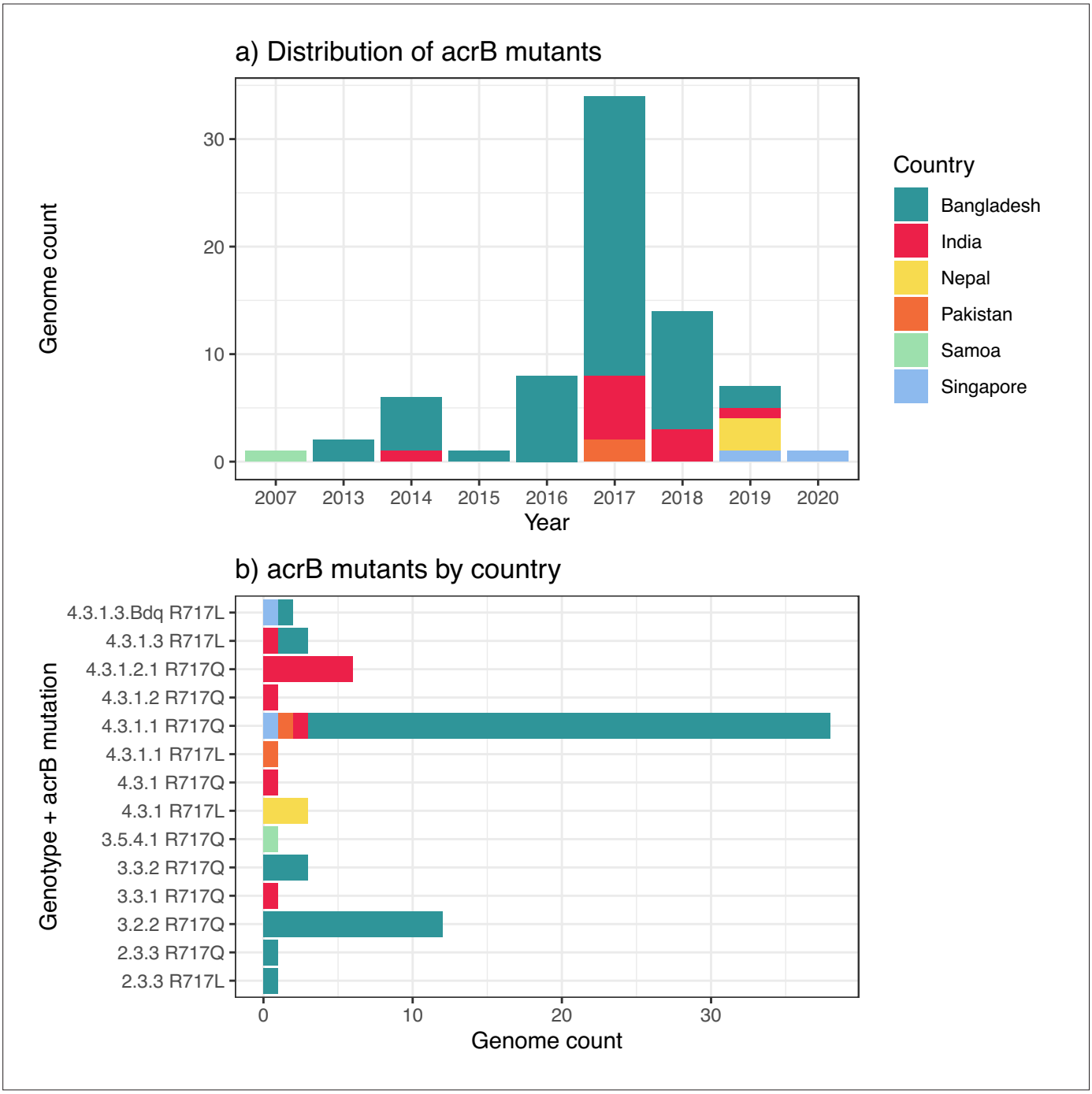

Figure 5. Distribution of azithromycin resistance-associated *acrB* mutations detected in Typhi genomes. (**a**) Temporal distribution of *acrB* mutants. (**b**) Distribution of *acrB* mutants by genotype and mutation. The first *acrB* mutant appeared in Samoa in 2007. Other mutants have appeared independently across a range of genetic backgrounds, largely in South Asian countries, but remain at low prevalence levels overall (see ***Figure 2***). Country of origin is coloured as per inset label.

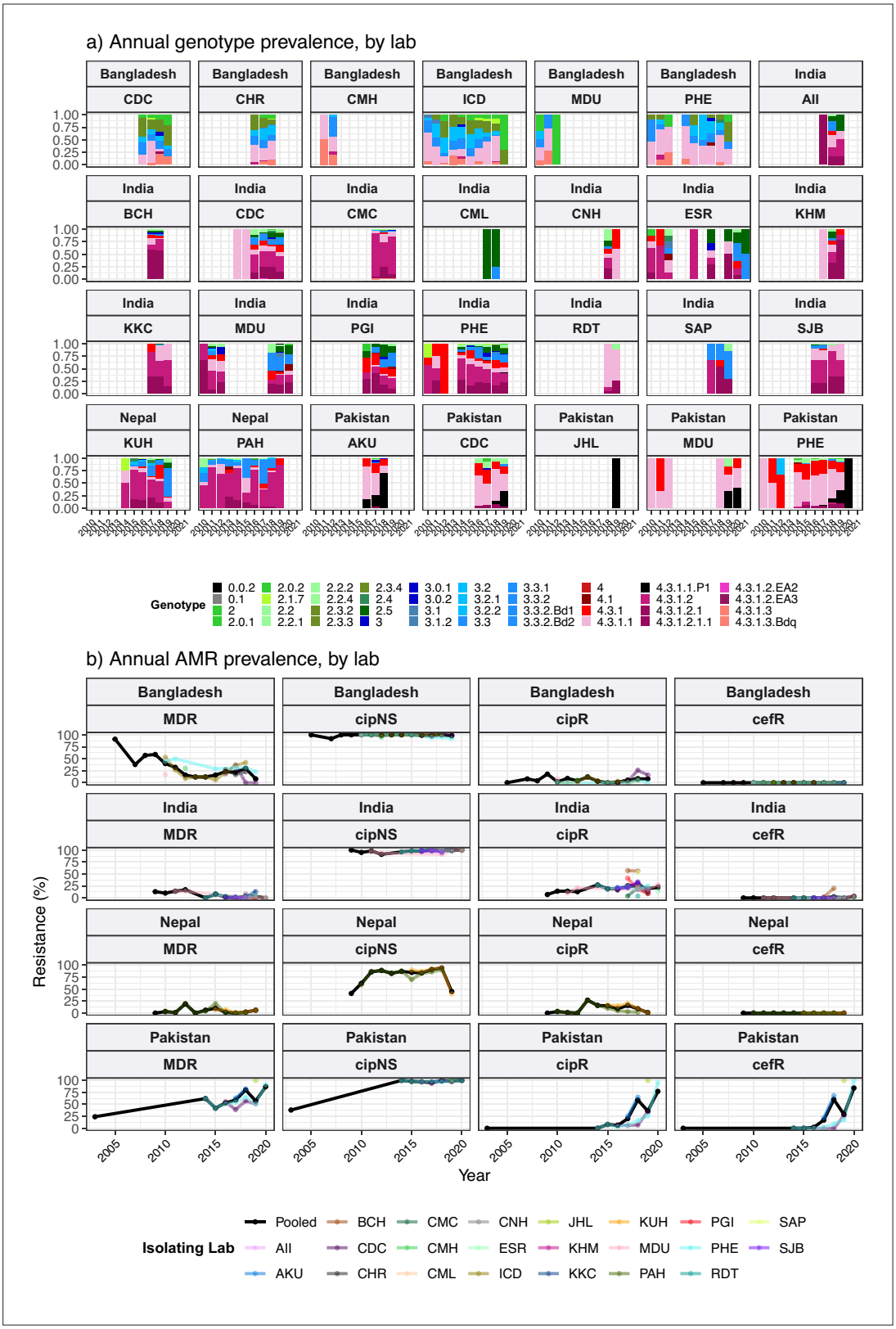

Figure 6. Annual genotype and antimicrobial resistance (AMR) frequencies by isolating lab, for South Asian countries with multiple data sources. Labs shown are those with ≥20 isolates; and years shown for each lab are those with N≥5 isolates from that year. (a) Bars are coloured to indicate annual genotype prevalence, as per inset legend. (b) Lines indicate annual frequencies of key AMR profiles, coloured by isolating laboratory as per inset legend. MDR, multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant. See ***Supplementary file 9*** for three-letter laboratory code master list.

Figure 6—figure supplement 1. Genotype prevalence estimated from different data sources, for South Asian countries. For source laboratories with N≥20 isolates. Lines show 95% confidence interval for each proportion (prevalence) estimate; solid circles highlight the pooled point estimate for national prevalence in each country. Lines are coloured by country as per the inset legend. See ***Supplementary file 9*** for three-letter laboratory code master list.

Figure 6—figure supplement 2. Antimicrobial resistance (AMR) prevalence estimated from different sources, for South Asian countries. For source laboratories with N≥20 isolates from which to estimate prevalence. Lines show 95% confidence interval for each proportion (prevalence) estimate; solid circles highlight the pooled point estimate for national prevalence in each country. Lines are coloured by country as per the inset legend. See ***Supplementary file 9*** for three-letter laboratory code master list.

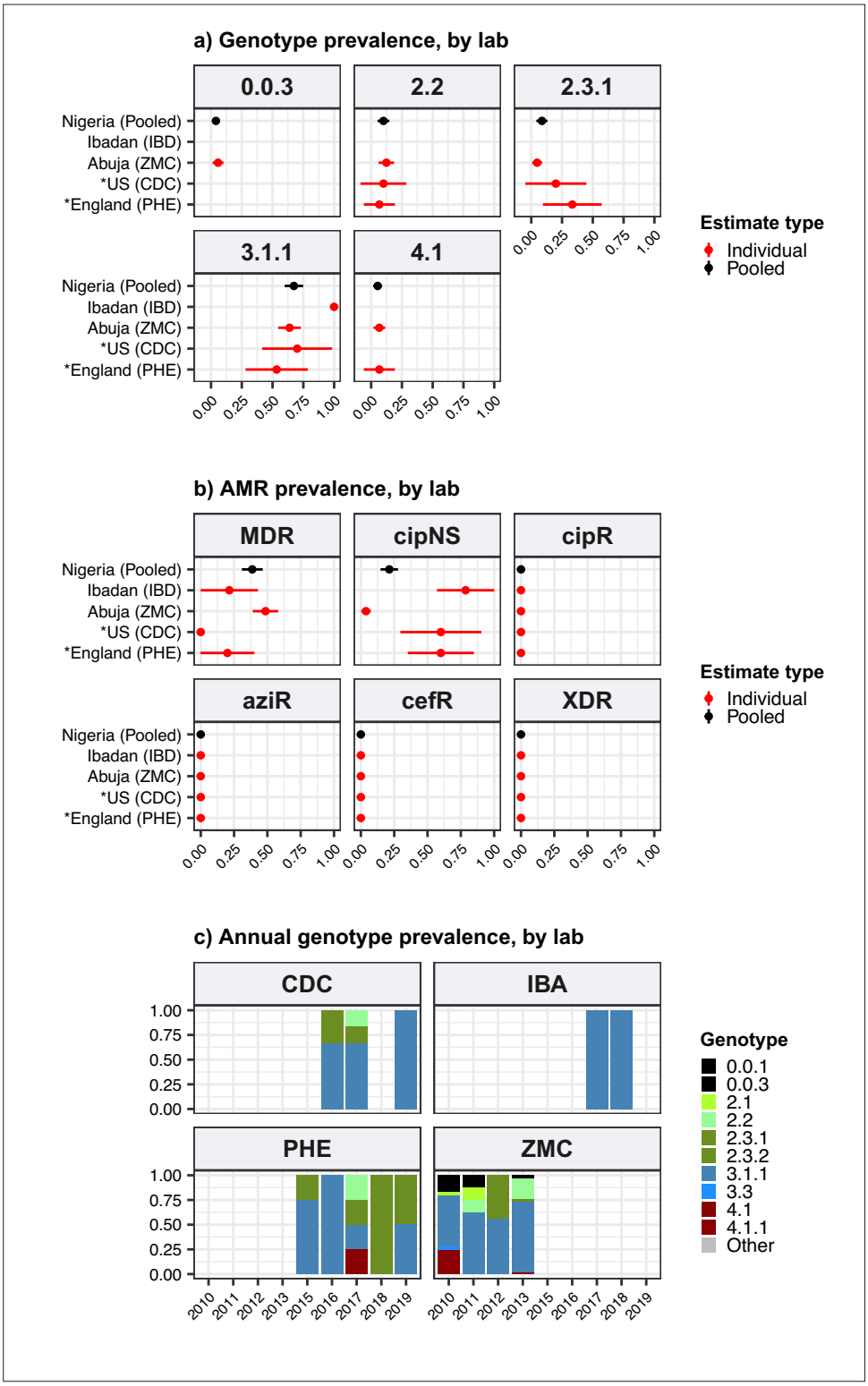

Figure 7. Genotype and antimicrobial resistance (AMR) prevalence rates estimated for Nigeria from different data sources. Data are shown only for source labs with N≥10 isolates from which to estimate prevalence. (**a**) Genotype prevalence and (**b**) AMR prevalence, using all available isolates per lab, 2010–2020. Lines show 95% confidence interval for each proportion (prevalence) estimate. Red indicates estimates based on data from individual labs, black indicates pooled estimates (i.e. from all labs), as per inset legend. (**c**) Annual genotype frequencies. Bars are coloured by genotype as per inset legend. Lab abbreviations are shown in y-axis labels for panels (**a–b**). MDR,

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multidrug resistant; XDR, extensively drug resistant; CipNS, ciprofloxacin non-susceptible; CipR, ciprofloxacin resistant; CefR, ceftriaxone resistant; AziR, azithromycin resistant. See ***Supplementary file 9*** for three-letter laboratory code master list.