Dynamics of antimicrobial resistance in intestinal *Escherichia coli* from children in community settings in South Asia and sub-Saharan Africa

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Supplementary Note

2.
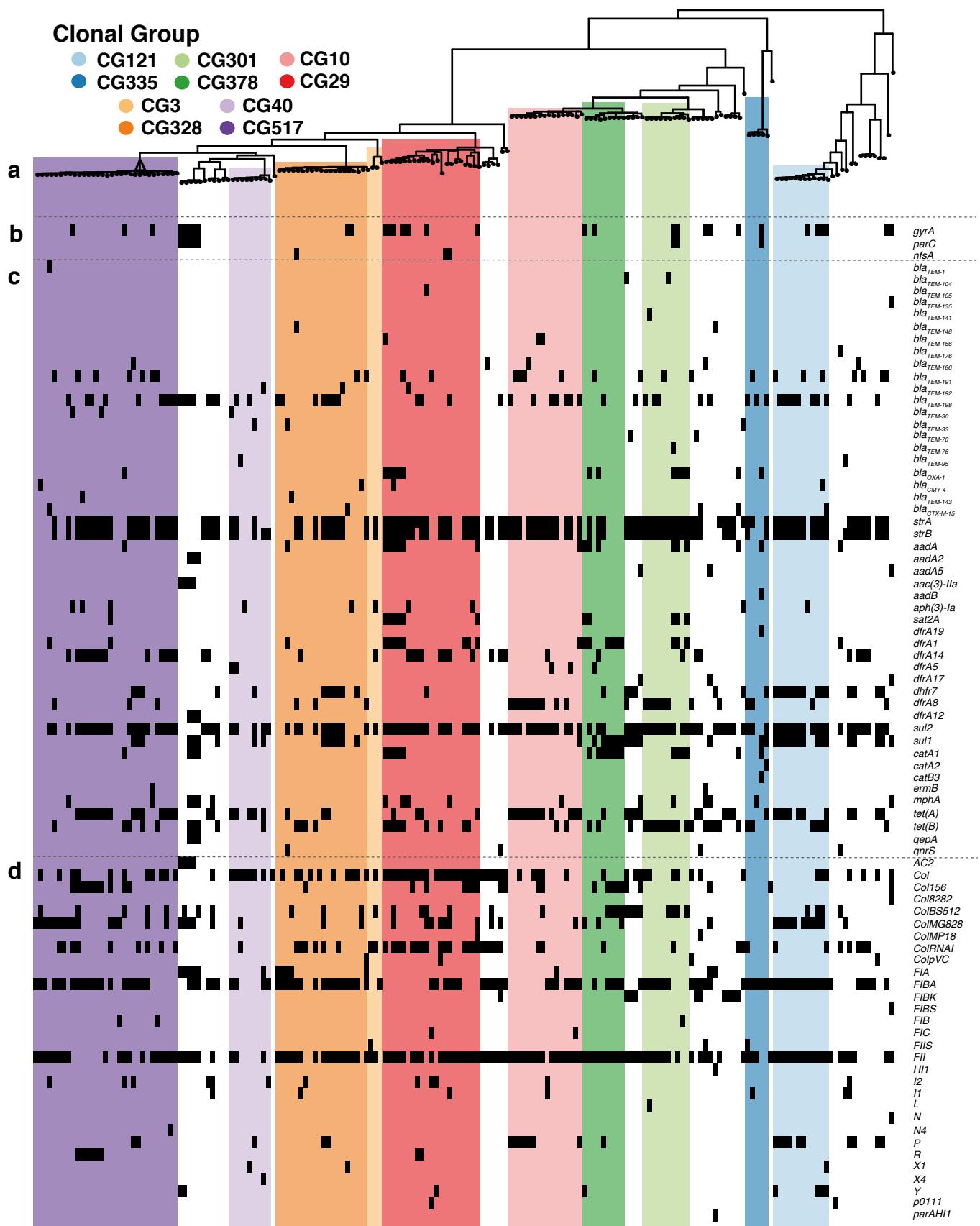
Supplementary Figure 1. Presence of common acquired antimicrobial resistance (AMR) genes and plasmid replicon genes in the GEMS aEPEC population. (a) The population structure of 185 EPEC isolates is shown at the top of the figure (sub-setted from core chromosomal phylogeny, see Methods). The ten common aEPEC clonal groups (CG) are depicted by different colours, as per the inset colour key. The presence of AMR genetic determinants are plotted as a binary heatmap with black indicating the presence within each bacterial genome of: (b) point mutations associated with AMR, (c) genes associated with AMR acquired by horizontal gene transfer, and (d) plasmid replicon genes.
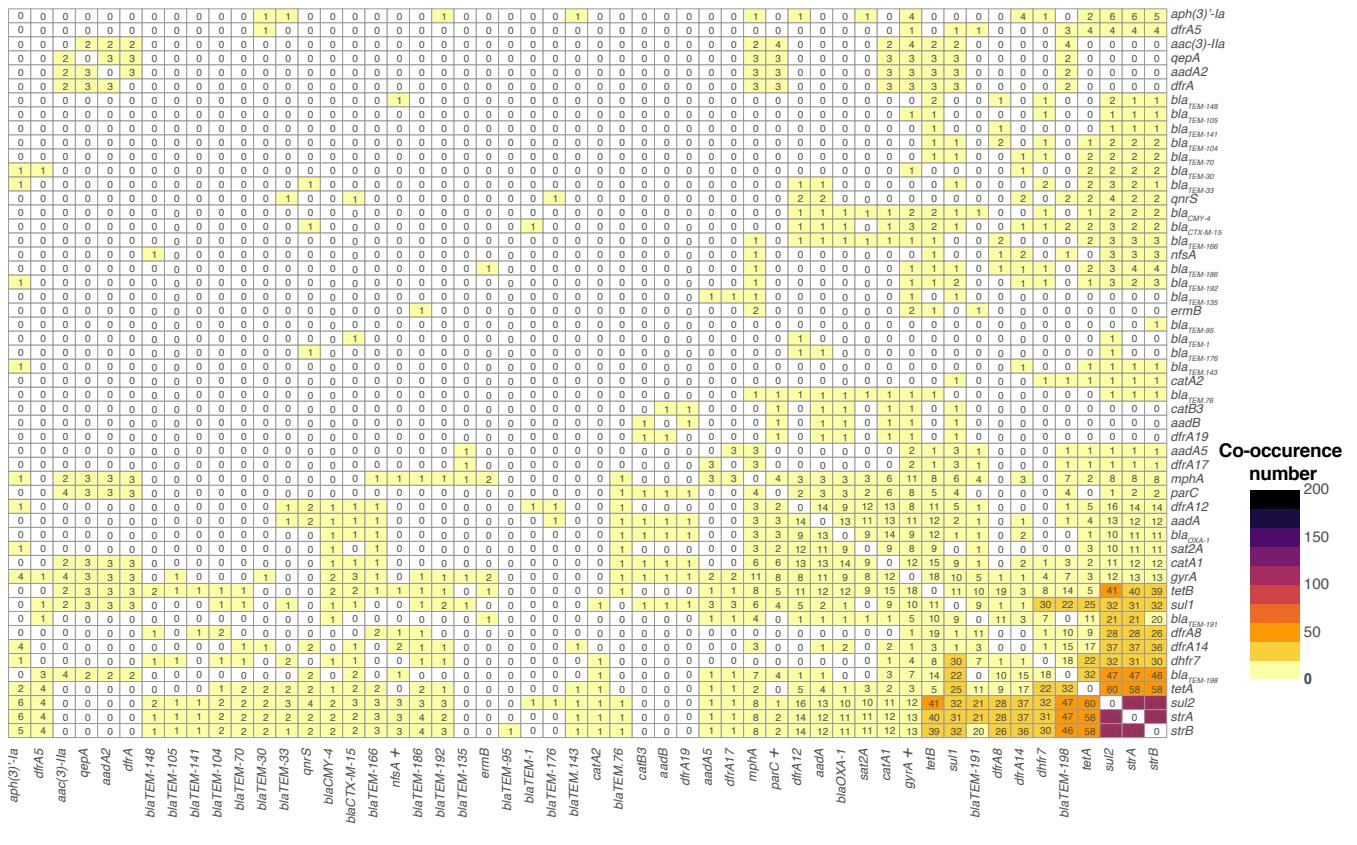
Supplementary Figure 2. Co-occurrence matrix of acquired antimicrobial resistance genes.
Number of aEPEC genomes containing different combinations of resistance-encoding genes. These data
were used to develop the co-occurrence networks in Figure 3.
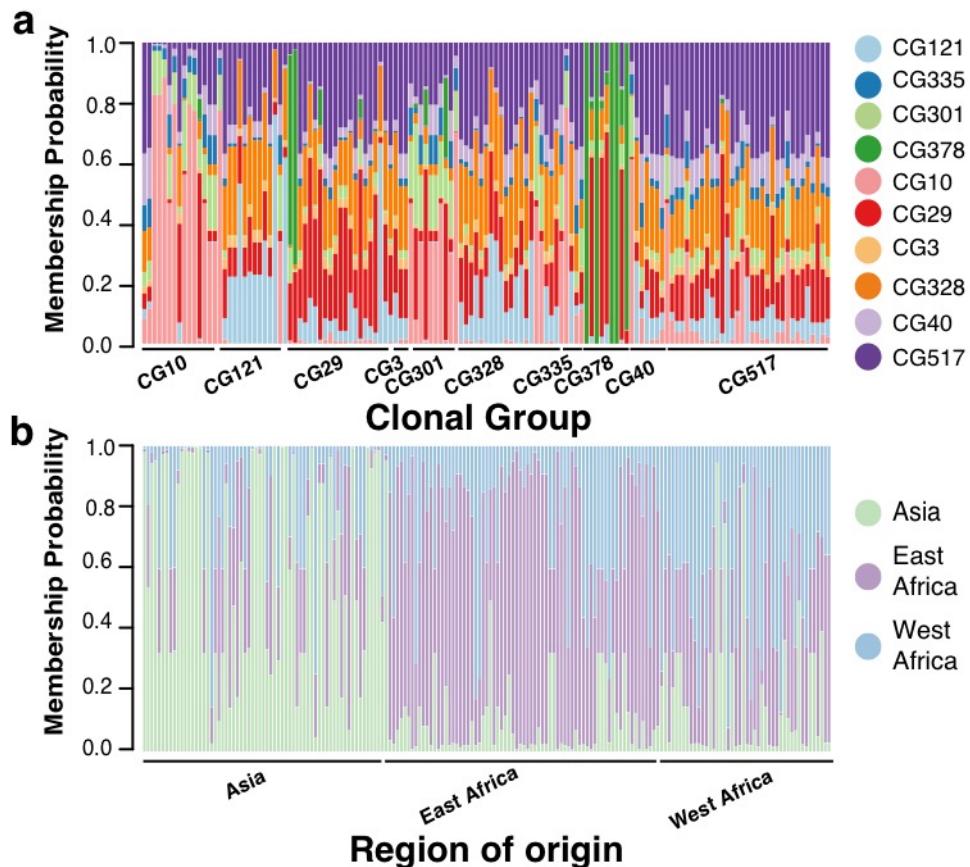
Supplementary Figure 3. Discriminant analysis of principal components (DAPC) group membership probabilities, indicating discrimination of isolates into groups on the basis of the AMR gene content matrix. Groupings subjected to DAPC analysis were (a) clonal groups (CG) (n = 137), and (b) region of origin (n = 185). Each vertical line represents a single isolate, and the colours indicate the probability of assignment to each of the possible groups based on the isolate's AMR gene profile.

Supplementary Tables

The following tables are included with this file:

Supplementary Table 2: MIC breakpoint values used to determine phenotypic resistance

Supplementary Table 5: Predictive power of AMR-associated genotypes for AMR phenotypes for 185 aEPEC

Supplementary Table 6: Frequency of antimicrobial resistance by drug class, stratified by confirmed diseases status (n= 182)

The tables listed below are in separate xlsx files available for download:

Supplementary Table 1. Details of 185 isolates of atypical enteropathogenic *E. coli* analysed in this study.

Supplementary Table 3. Antimicrobial susceptibility profiles of all isolates, inferred from VITEK and agar plate dilution methods.

Supplementary Table 4. Presence/absence of point mutations, acquired antimicrobial resistance (AMR) genes and plasmid replicon genes in all isolates.

Supplementary Table 7. MIC data from VITEK2 for 185 isolates of atypical enteropathogenic *E. coli*
(aEPEC).

Supplementary Table 2: MIC breakpoint values used to determine phenotypic resistance

Antimicrobial	Method	MIC breakpoint	Susceptible ≤ (µg/mL)	Intermediate (µg/mL)	Resistant > (µg/mL)
Beta-Lactam					
Ampicillin	VITEK2	EUCAST	8	NA*	8
Ceftazidime	VITEK2	EUCAST	1	4	4
Ceftriaxone	VITEK2	EUCAST	1	2	2
Cefepime	VITEK2	EUCAST	1	4	4
Meropenem	VITEK2	EUCAST	2	8	8
Aminoglycoside					
Streptomycin ^	Agar-dilution (16 µg/mL)	Neither	8	NA	≥16
Gentamicin	VITEK2	EUCAST	2	4	4
Tobramycin	VITEK2	EUCAST	2	4	4
Amikacin	VITEK2	EUCAST	8	16	16
Folate Pathway Inhibitors					
Trimethoprim	VITEK2	EUCAST	2	4	4
Trimethoprim/ sulphamethoxazole ^^	VITEK2	EUCAST	2	4	4
Nitrofurantoin					
Nitrofurantoin	VITEK2	EUCAST	64	NA	64
Chloramphenicol					
Chloramphenicol	Agar-dilution (16 µg/mL)	EUCAST	8	NA	8
Macrolides					
Azithromycin^	Agar-dilution (16 µg/mL)	Neither	8	NA	≥16
Tetracyclines					
Tetracycline #	Agar-dilution (8 µg/mL)	CLSI	4	8	16
Fluoroquinolones					
Ciprofloxacin	VITEK2	EUCAST	0.5	1	1
Norfloxacin	VITEK2	EUCAST	0.5	1	1

* NA, not applicable

^ The breakpoint for *E. coli* is uncertain and is not provided by EUCAST or CLSI. A resistance breakpoint of MIC 16 µg/mL was used in keeping with that used for *Salmonella enterica* .

^^ Ratio of 1:19 used for trimethoprim:sulphamethoxazole

CLSI cut-off used as no MIC breakpoint given under EUCAST. Intermediate and resistant phenotyp

Supplementary Table 5: Predictive power of AMR-associated genotypes for AMR phenotypes for 185 aEPEC

Antimicrobial	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Beta-lactam				
Ampicillin	0.93 (0.86 - 0.97)	0.80 (0.68 - 0.89)	0.90 (0.83 - 0.94)	0.85 (0.73 - 0.93)
Cefepime	1 (0.29 - 1)	0.98 (0.94 - 0.99)	0.43 (0.1 - 0.82)	1 (0.98 - 1)
Ceftazidime	1 (0.54 - 1)	0.97 (0.94 - 0.99)	0.55 (0.23 - 0.83)	1 (0.98 - 1)
Ceftriaxone	1 (0.54 - 1)	0.97 (0.94 - 0.99)	0.55 (0.23 - 0.83)	1 (0.98 - 1)
Meropenem	NA	1 (0.98 - 1)	NA	1 (0.98 - 1)
Aminoglycoside				
Streptomycin	0.95 (0.88 - 0.99)	0.53 (0.43 - 0.63)	0.61 (0.52 - 0.69)	0.93 (0.84 - 0.98)
Gentamicin	1 (0.48 - 1)	1 (0.98 - 1)	1 (0.48 - 1)	1 (0.98 - 1)
Tobramycin	1 (0.48 - 1)	1 (0.98 - 1)	1 (0.48 - 1)	1 (0.98 - 1)
Amikacin	NA	1 (0.98 - 1)	NA	1 (0.98 - 1)
Folate Pathway Inhibitor				
Trimethoprim	0.97 (0.92 - 0.99)	0.82 (0.7 - 0.91)	0.92 (0.85 - 0.96)	0.93 (0.82 - 0.98)
Trimethoprim/Sulphonamide	0.97 (0.92 - 0.99)	0.84 (0.73 - 0.92)	0.92 (0.86 - 0.96)	0.93 (0.83 - 0.98)
Nitrofurantoin				
Nitrofurantoin	NA	0.98 (0.95 - 1)	0 (0 - 0.71)	1 (0.98 - 1)
Chloramphenicol				
Chloraphenicol	0.9 (0.7 - 0.99)	0.98 (0.94 - 0.99)	0.83 (0.61 - 0.95)	0.99 (0.96 - 1)
Macrolides				
Azithromycin	0.85 (0.55 - 0.98)	0.97 (0.93 - 0.99)	0.65 (0.38 - 0.86)	0.99 (0.96 - 1)
Tetracyclines				
Tetracycline	0.96 (0.9 - 0.99)	0.9 (0.81 - 0.96)	0.93 (0.86 - 0.97)	0.95 (0.87 - 0.99)
Fluoroquinolone				
Ciprofloxacin	1 (0.63 - 1)	0.98 (0.94 - 0.99)	0.67 (0.35 - 0.9)	1 (0.98 - 1)
Norfloxacin	0.97 (0.83 - 1)	0.97 (0.93 - 0.99)	0.86 (0.7 - 0.95)	0.99 (0.96 - 1)

Point estimates for Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) with 95% confidence intervals

NA, not applicable

8.

Supplementary Table 6: Frequency of antimicrobial resistance by drug class, stratified by confirmed diseases status (n=182)

Drug Class	Phenotypic resistance				Resistance predicted from genotype			
	% Cases (n=94)	% Controls (n=88)	P-value (Fisher's exact test)	Odds Ratio [95% CI]	% Cases (n=94)	% Controls (n=88)	P value (Fisher's exact test)	Odds Ratio [95% CI]
Ampicillin	71.3	60.2	0.1	0.6 [0.3-1.2]	73.4	62.5	0.2	0.6 [0.3-1.2]
3rd-4th gen. cephalosporins	3.2	2.3	1	0.7 [0.1-6.3]	6.4	4.5	0.7	0.7 [0.1-3.1]
Meropenem	0.0	0.0	-	-	0.0	0.0	-	-
Aminoglycoside	46.8	45.5	0.9	0.9 [0.5-1.8]	72.3	76.1	0.6	1.2 [0.6-2.5]
Amikacin	0.0	0.0	-	-	0.0	0.0	-	-
Trimethoprim	68.1	64.8	0.6	0.9 [0.4-1.7]	72.3	68.2	0.6	0.8 [0.4-1.6]
Trimethoprim/ Sulphonamide	67.0	63.6	0.6	0.9 [0.4-1.7]	72.3	71.6	1	1 [0.5-1.9]
Nitrofurantoin	-	-	-	-	1.1	2.3	0.6	2.2 [0.1-128.8]
Chloramphenicol	16.0	6.8	0.1	0.4 [0.1-1.1]	17.0	8.0	0.1	0.4 [0.1-1.2]
Macrolide	8.5	5.7	0.6	0.6 [0.2-2.4]	8.5	10.2	0.8	1.2 [0.4-3.8]
Tetracyclines	52.1	60.2	0.3	1.4 [0.7-2.6]	55.3	61.4	0.5	1.3 [0.7-2.4]
Fluoroquinolones	19.1	13.6	0.4	0.7 [0.3-1.6]	19.1	18.2	1	0.9 [0.4-2.1]