

**Supplementary Table S1.** Strain typing of 49 amoxicillin resistant *Escherichia coli* isolates from grey-headed flying fox pups using a combination of phylotyping, genome fingerprints (ERIC and CGG) and class 1 integron PCR data.

Isolate ID	PG PCR	ERIC PCR type	CGG PCR type	Integron Integrase PCR	Integron gene cassette PCRs	WGS
FF1568B1	A	A-1	A-1	Negative	-	Yes
FF1563	A/C	A/C-1	A/C-1	Negative	-	Yes
FF1575	A/C	A/C-1	A/C-1	Negative	-	No
FF1576A2	A/C	A/C-1	A/C-1	Negative	-	No
FF1565	A/C	A/C-2	A/C-2	Negative	-	Yes
FF1566	A/C	A/C-2	A/C-2	Negative	-	No
FF1600	A/C	A/C-2	A/C-2	Negative	-	No
FF1568A	A/C	A/C-3	A/C-3	Negative	-	Yes
FF1584B	A/C	A/C-3	A/C-3	Negative	-	No
FF1570A	A/C	A/C-4	A/C-4	Positive	HS458/JL-D2 positive (1 band, ~2kbp)	Yes
FF1571	A/C	A/C-4	A/C-4	Positive	HS458/JL-D2 positive (1 band, ~2kbp)	No
FF1576A1-2	A/C	A/C-4	A/C-4	Positive	HS458/JL-D2 positive (1 band, ~2kbp)	No
FF1585B	A/C	A/C-4	A/C-4	Positive	HS458/JL-D2 positive (1 band, ~2kbp)	No
FF1589	A/C	A/C-4	A/C-4	Positive	HS458/JL-D2 positive (1 band, ~2kbp)	No
FF1570B1	A/C	A/C-5	A/C-5	Negative	-	Yes
FF1397	B1	B1-1	B1-1	Negative	-	No
FF1410	B1	B1-1	B1-1	Negative	-	No
FF1412	B1	B1-1	B1-1	Negative	-	No
FF1418	B1	B1-1	B1-1	Negative	-	No
FF1419	B1	B1-1	B1-1	Negative	-	No
FF1532	B1	B1-1	B1-1	Negative	-	Yes
FF1533	B1	B1-1	B1-1	Negative	-	No
FF1534	B1	B1-1	B1-1	Negative	-	No
FF1535	B1	B1-1	B1-1	Negative	-	No
FF1536	B1	B1-1	B1-1	Negative	-	No
FF1562	B1	B1-2	B1-2	Negative	-	Yes
FF1572	B1	B1-3	B1-3	Negative	-	Yes
FF1576A1-1	B1	B1-3	B1-3	Negative	-	No
FF1598	B1	B1-4	B1-4	Negative	-	Yes
FF1582	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	Yes
FF1583	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1584A	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1586	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1587	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1588	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1590	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1591	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1593	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1594	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1595	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1596	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1597	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1599	B1	B1-5	B1-5	Positive	HS458/JL-D2 positive (3 bands, >800bp)	No
FF1567	D/E	D/E-1	D/E-1	Negative	-	Yes
FF1569	D/E	D/E-1	D/E-1	Negative	-	No
FF1573	D/E	D/E-1	D/E-1	Negative	-	No
FF1574	D/E	D/E-1	D/E-1	Negative	-	No
FF1579	D/E	D/E-1	D/E-1	Negative	-	No
FF1581	D/E	D/E-1	D/E-1	Negative	-	No

**Supplementary Table S2.** Individual BioSample accession numbers under NCBI Sequence Read Archive (SRA) BioProject ID PRJNA814458, EnteroBase Barcodes and GenBank accession numbers for class 1 integrons from 12 antimicrobial resistant *Escherichia coli* isolated from grey-headed flying fox pups in care.

Isolate ID	ST	Serotype	BioSample No.	EnteroBase Barcode	Integron GenBank accession No.
FF1563	10	ONT:H32	SAMN26553891	ESC_UA2847AA	-
FF1570A	48	O4:H26	SAMN26553892	ESC_UA2853AA	ON087280
FF1570B1	48	B18:H11	SAMN26553893	ESC_UA2854AA	-
FF1582	58	O8:H25	SAMN26553894	ESC_UA2849AA	ON087281
FF1598	58	ONT:H37	SAMN26553895	ESC_UA2856AA	-
FF1568A	361	O9:H30	SAMN26553896	ESC_UA2851AA	-
FF1562	641	O70:H10	SAMN26553897	ESC_UA2846AA	-
FF1565	710	B9:H30	SAMN26553898	ESC_UA2848AA	-
FF1567	963	ONT:H18	SAMN26553899	ESC_UA2850AA	-
FF1568B1	1421	O9:H4	SAMN26553900	ESC_UA2852AA	-
FF1572	1727	ONT:H14	SAMN26553901	ESC_UA2855AA	-
FF1532	2144	O166:H49	SAMN26553902	ESC_UA2845AA	-

**Supplementary Table S3.** Antibiotic discs used for EUCAST susceptibility testing.

Antibiotic and disc content (µg)	OXOID disc	Antibiotic category and generation	Breakpoint
Ampicillin (10 µg)	AMP10	β-lactam/Penicillins	EUCAST
Amoxicillin/Clavulanic Acid (30 µg)	AMC30	β-lactam/Penicillins + β-lactamase inhibitors	EUCAST
Cephalexin (30 µg)	CL30	β-lactam/Cephalosporins/1st Generation (Non-extended spectrum cephalosporin)	EUCAST
Cefotaxime (5 µg)	CTX5	β-lactam/Cephalosporins/3rd Generation (Extended-spectrum cephalosporin)	EUCAST
Imipenem (10 µg)	IPM10	β-lactam/Carbapenems	EUCAST
Nalidixic acid (30 µg)	NA30	Quinolones-Fluoroquinolones/ 1st Generation Quinolone	CLSI
Trimethoprim (5 µg)	W5	Trimethoprim (Folate pathway inhibitors)	EUCAST
Trimethoprim/Sulfamethoxazole (30 µg)	SXT30	Trimethoprim + Sulfonamide (Folate pathway inhibitors)	EUCAST
Amikacin (30 µg)	AK30	Aminoglycosides	EUCAST
Gentamycin (10 µg)	CN10	Aminoglycosides	EUCAST
Streptomycin (25 µg)	S25	Aminoglycosides	NA*
Spectinomycin (25 µg)	SH25	Aminoglycosides	NA*
Chloramphenicol (30 µg)	C30	Phenicol	EUCAST
Nitrofurantoin (200 µg)	F200	Nitrofurantoin	NA*
Tetracycline (30 µg)	TE30	Tetracyclines	CLSI

\* NA = Not Available: Breakpoint criteria unavailable from EUCAST or CLSI tables.

**Supplementary Table S4.** URL links to interactive versions of GrapeTree cgMLST phylogeny trees for GHFF pup and closely related *E. coli* isolates in EnteroBase.

ST and serotype	URL link
ST 10 ONT:H32	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST10_ONT_H32_n=39_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST10_ONT_H32_n=39_ms_tree.json</a>
ST48 O4:H26	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST48_O4_H26_n=122_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST48_O4_H26_n=122_ms_tree.json</a>
ST48 B18:H11	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST48_B18_H11_n=166_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST48_B18_H11_n=166_ms_tree.json</a>
ST58 O8:H25	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST58_O8_H25_n=439_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST58_O8_H25_n=439_ms_tree.json</a>
ST58 ONT:H37	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST58_ONT_H37_n=130_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST58_ONT_H37_n=130_ms_tree.json</a>
ST361 O9:H30	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST361_O9_H30_n=323_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST361_O9_H30_n=323_ms_tree.json</a>
ST641 O70:H10	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST641_O70_H10_n=40_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST641_O70_H10_n=40_ms_tree.json</a>
ST710 B9:H30	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST710_B9_H30_n=27_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST710_B9_H30_n=27_ms_tree.json</a>
ST963 ONT:H18	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST963_ONT_H18_n=184_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST963_ONT_H18_n=184_ms_tree.json</a>
ST1421 O9:H4	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST1421_O9_H4_n=76_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST1421_O9_H4_n=76_ms_tree.json</a>
ST1727 ONT:H14	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST1727_ALL_MINUS_H49_n=153_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST1727_ALL_MINUS_H49_n=153_ms_tree.json</a>
ST2144 O166:H49	<a href="https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST2144_O166_H49_n=80_ms_tree.json">https://achtman-lab.github.io/GrapeTree/MSTree_holder.html?tree=https://github.com/Fiona-McDougall/GHFF-Phylogenetics/blob/master/ST2144_O166_H49_n=80_ms_tree.json</a>

