Antimicrobial resistance: biology and evolution

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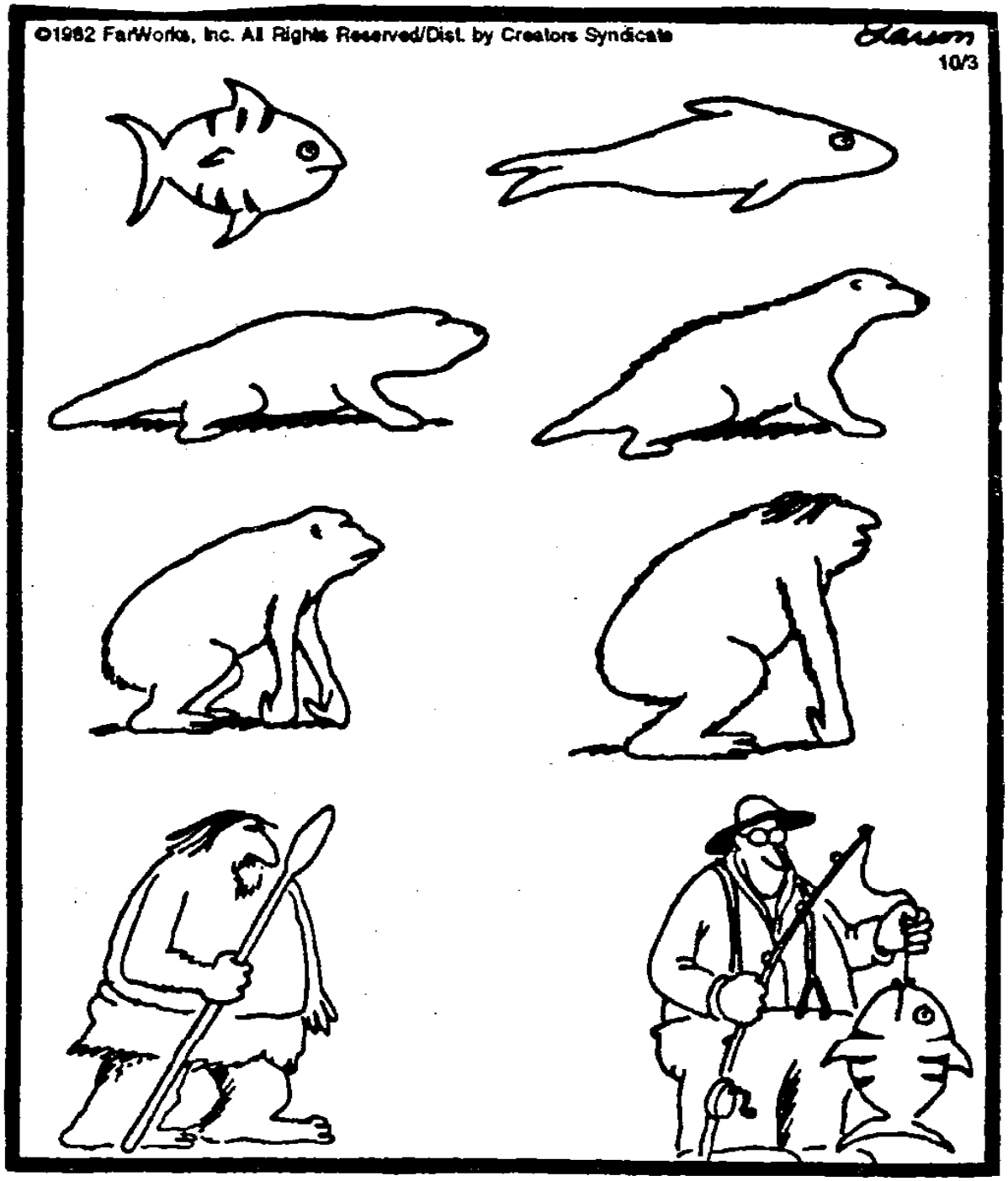
THE FAR SIDE

by GARY LARSON



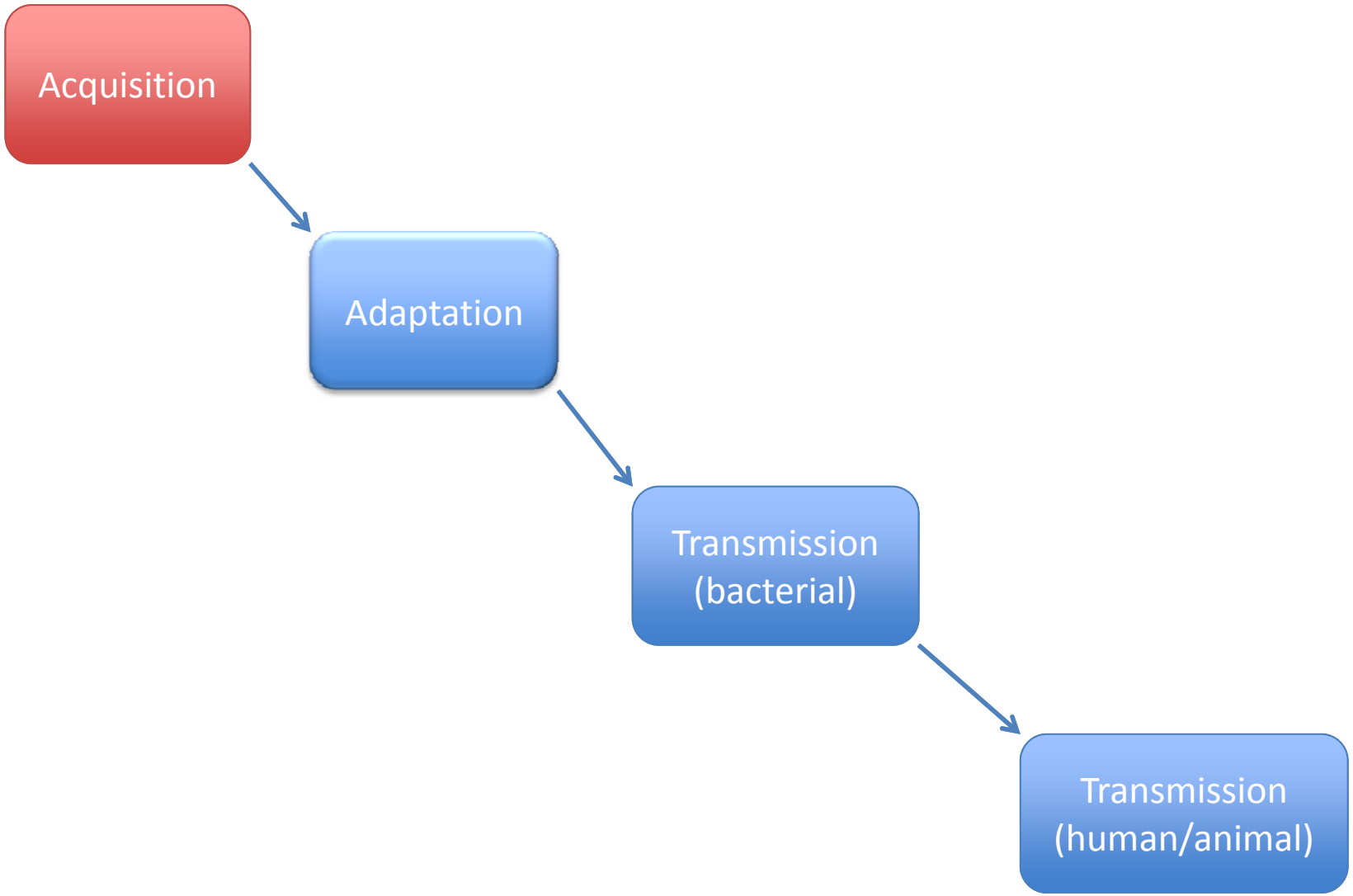
Evolution

Overview

- Introduction, definitions and scope
- Acquisition of resistance
- Adaptation to resistance
- Transmission among bacteria
- Transmission in the community
- Conclusions

Anti-microbial resistance: definition

Natural resistance	Organisms lack the target of the antimicrobial or the antimicrobial is unable to penetrate cellular structures
Acquired resistance	Previously susceptible organism that has acquired new mechanisms to overcome the effect of the anti-microbial



Emergence of anti-microbial resistance overview

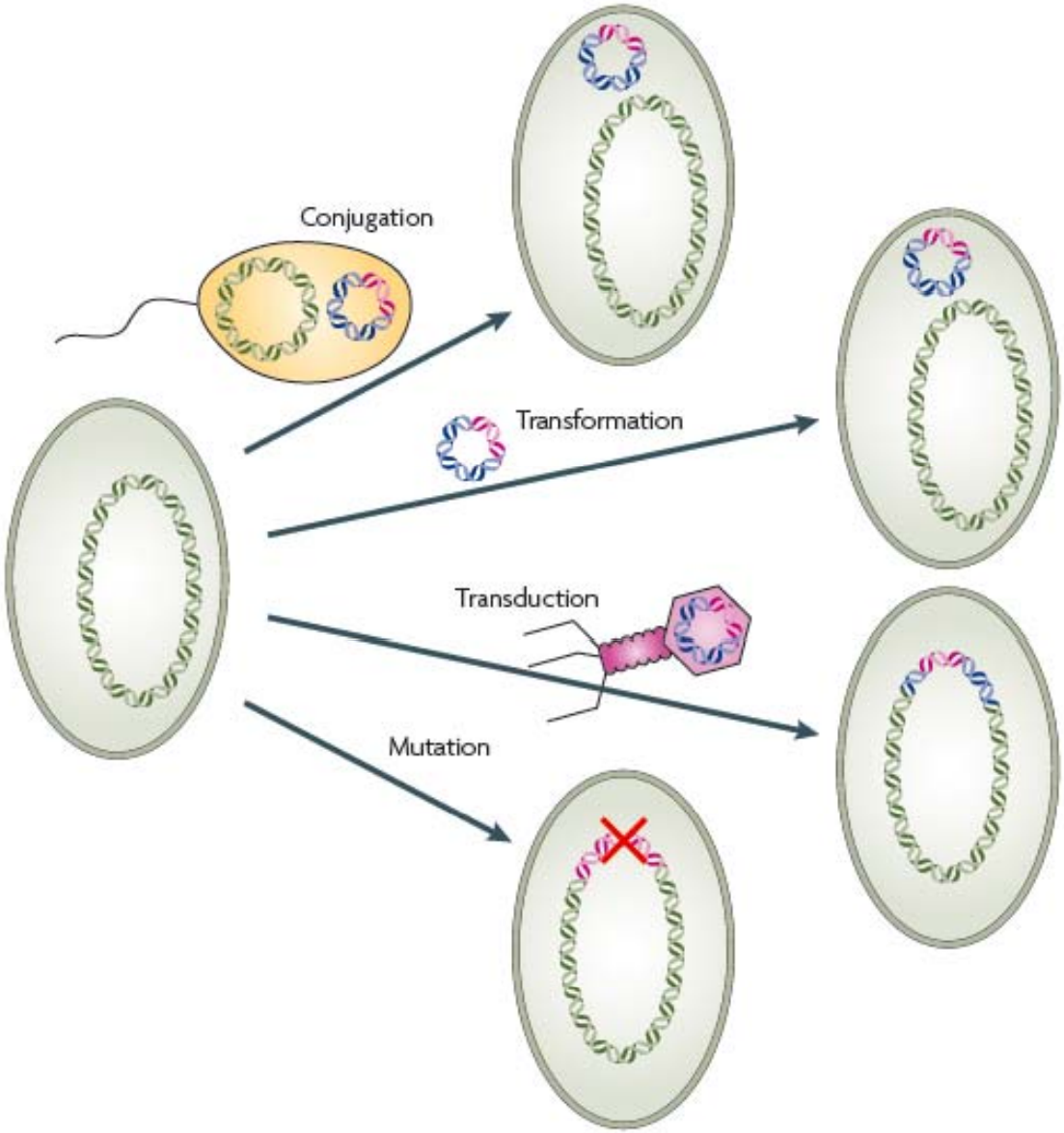


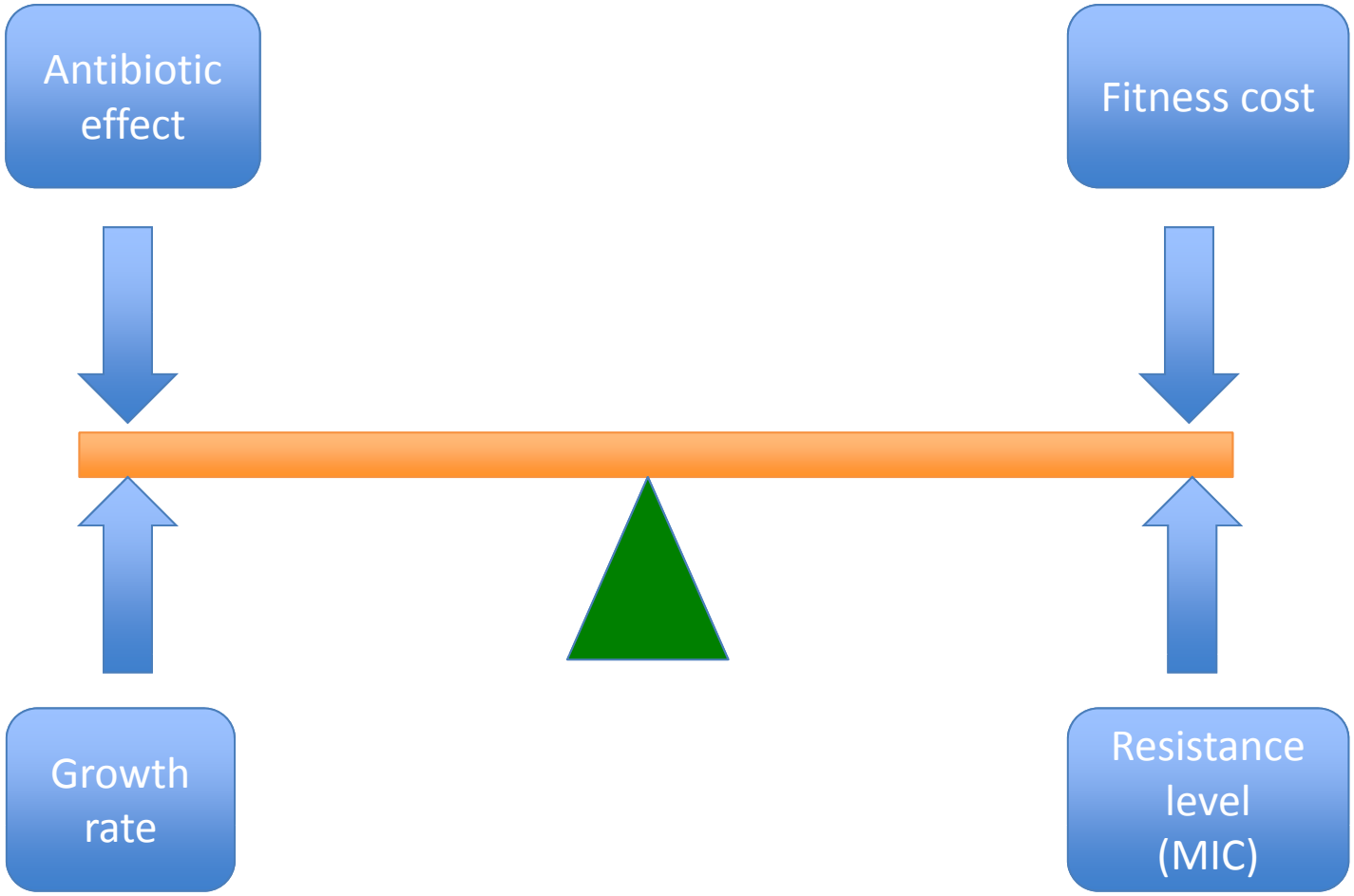
Figure 1 | **Mechanisms of resistance acquisition.** DNA from the biosphere containing an antibiotic resistance gene (pink) can be transferred by horizontal gene transfer into a recipient by several paths: cell-to-cell conjugation; transformation by naked DNA (on plasmids or as linear DNA) that is released by dead cells; or phage-mediated transduction. Resistance can also arise by *de novo* mutation (indicated by a red cross).

Nat Rev Microbiol 2010, 8

Acquisition of anti-microbial resistance

Examples of acquisition mechanisms	
Chromosomal mutation	gene level change
Gene inactivation/deletion	gene level change
Gene mosaicism	gene level change
Plasmid	gene acquisition
Integron	gene acquisition
Phage	gene acquisition

Emergence of resistance: a dynamic balance



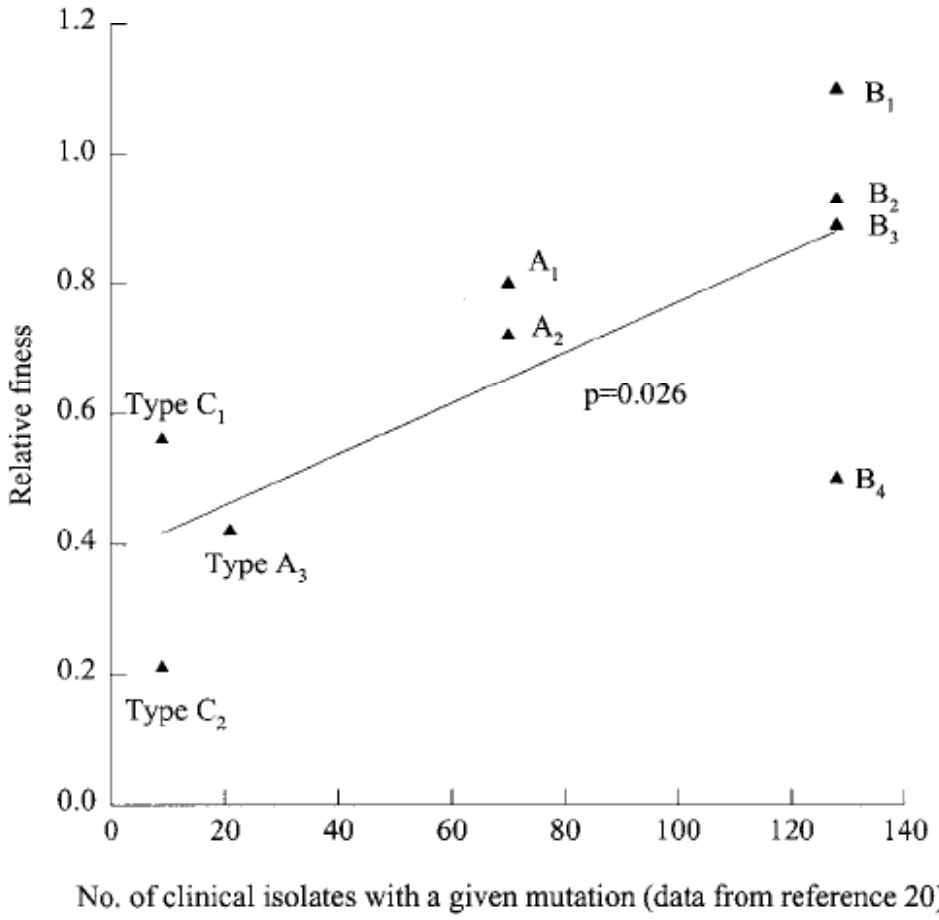


FIG. 2. Relationship between relative fitness and the clinical isolation rate for mutants A, B, and C.

For *M. tuberculosis*/rifampicin resistance clinical frequency is related to the primary fitness cost

TABLE 4. Growth rates of resistant mutants and susceptible *M. tuberculosis* in U937 macrophages

Strain	Mutation	MIC (μg/ml)	Doubling time (h)	No. of divisions	Relative fitness
Harlingen	None (wild type)	0.25	46.0	3.6	1.00
	Ser531→Trp (TCG→TGG)	>32	166.0	1.0	0.28 ± 0.06
	His526→Tyr (CAC→TAC)	>32	72.9	2.4	0.63 ± 0.02
	Ser522→Leu (TCG→TTG)	>16	91.3	1.9	0.50 ± 0.16
H37Ra	None (wild type)	0.25	111.2	1.5	0.41 ± 0.12
H37Rv	None (wild type)	0.25	50.6	3.3	0.91 ± 0.06

[a] Data shown represent the average of two to three independent replicate experiments for each of two independent isolates of the same mutant type. Standard errors for relative fitness are indicated. The doubling time was estimated from a plot of ln(visible cells) = *f*(time), where the slope is ln2/doubling time (hours). The relative fitness is the ratio of doubling time (wild type)/doubling time (mutant).

TABLE 1. Relative fitness of induced H37Rv rifampin-resistant mutant alleles

SSCP pattern	No. of replicates	Mean no. of generations of H37Rv		Location of mutation	Relative fitness
		Sensitive	Resistant		
A_1	4	8.65	6.95	His_{526}→Tyr	0.80
A_2	4	5.90	2.80	His_{526}→Tyr	0.78
A_3	3	6.73	4.62	His_{526}→Asp	0.42
B_1	5	7.88	8.30	Ser_{531}→Leu	1.05
B_2	3	7.73	7.43	Ser_{531}→Leu	0.93
B_3	3	6.83	6.29	Ser_{531}→Leu	0.89
B_4	3	9.60	4.83	Ser_{531}→Leu	0.50
C_1	2	5.68	10.04	His_{526}→Arg	0.56
C_2	5	6.40	1.35	His_{526}→Arg	0.21

What is defined as a “fit” mutant depends on context

Mariam et al., *AAC* 2004; **48:**1289-1294

Table 1. Distribution and percentage of each mutation type detected in strains sampled from the chemostat from days 1 to 37 at pH 7.0 and days 1 to 6 at pH 6.2

	Time from start of steady-state						
	day 1, pH 7.0 (n=104)	day 6, pH 7.0 (n=101)	day 16, pH 7.0 (n=105)	day 23, pH 7.0 (n=102)	day 37, pH 7.0 (n=102)	day 1, pH 6.2 (n=105)	day 6, pH 6.2 (n=104)
Proportion mutants	5.5×10^{-8}	8.9×10^{-8}	3.6×10^{-8}	4.8×10^{-8}	6.2×10^{-8}	7.6×10^{-8}	1.0×10^{-7}
S531L	40	44	86	96	89	50	59
S531W	6	14	5	1	1	10	7
H526Y	14	19	1	1	9	12	1
H526D	18	14	6	2	1	18	10
H526R	11	0	1	0	0	5	2
S522L	4	1	0	0	0	3	2
D516V	2	0	0	0	0	2	1
H526P[a]	0	0	0	0	0	0	1
H526R[a,b]	0	0	0	0	0	0	1
H526A[a]	0	0	0	0	0	0	1
K527Q[a]	0	0	0	0	0	0	1
No RRDR mutation	4	8	1	0	0	0	7
Double mutation[c]	0	0	0	0	0	0	7

[a]Rare mutations not described previously.
[b]This mutation involved a change at codon 526 from CAC to CGA. The common H526R mutation is CAC to CGC.
[c]Double mutations were H526A/K527Q, H526P/K527Q, H526R/K527Q, S531L/K527Q (3) and S531W/K527Q.

Context changes selection and "fitness"

Jenkins et al., *JAC* 2009; 63: 1118-1120

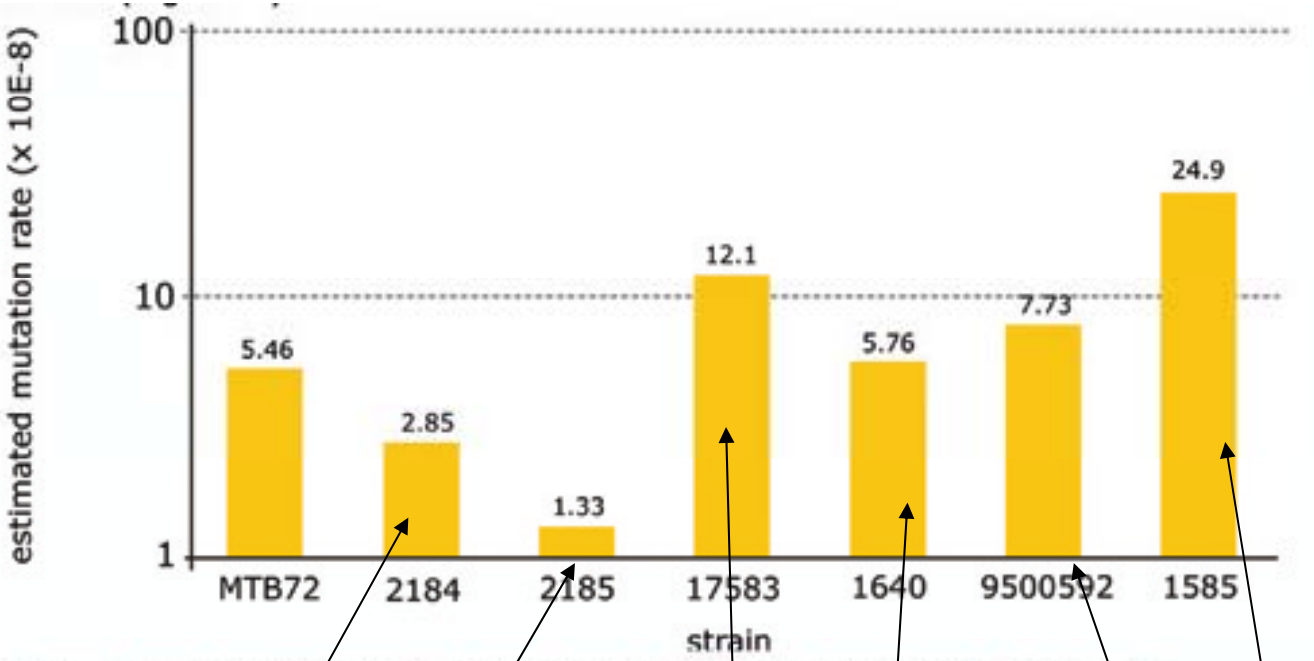


Figure 4. Estimated mutation rates of strains used in this study as determined by the p0-method

Clinical isolates

Ancestral Beijing

Modern Beijing

Bergval. personal communication

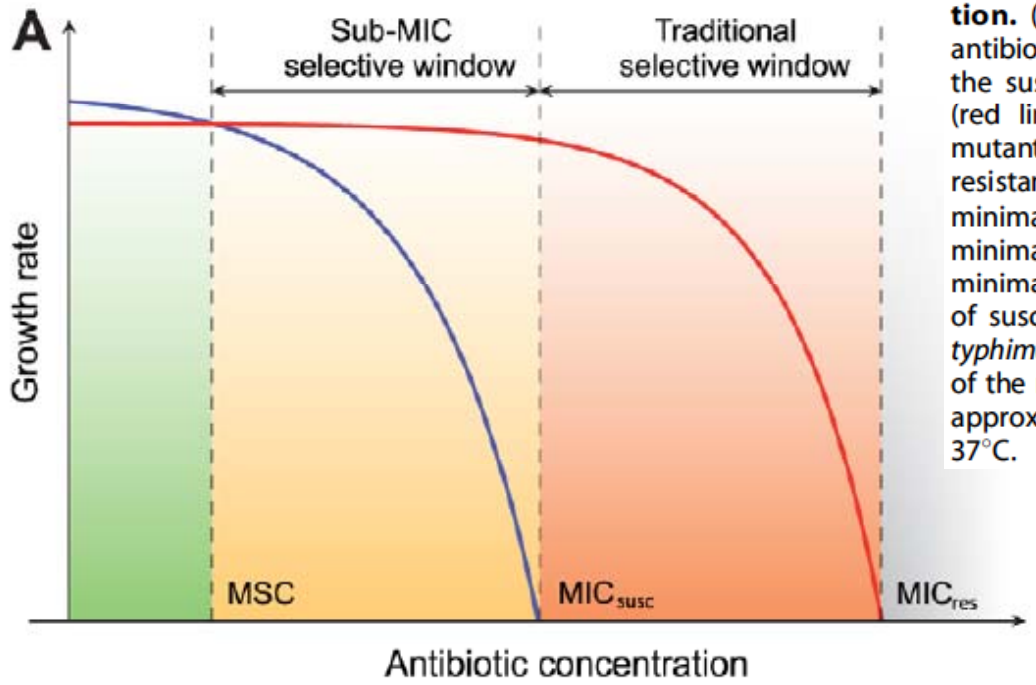
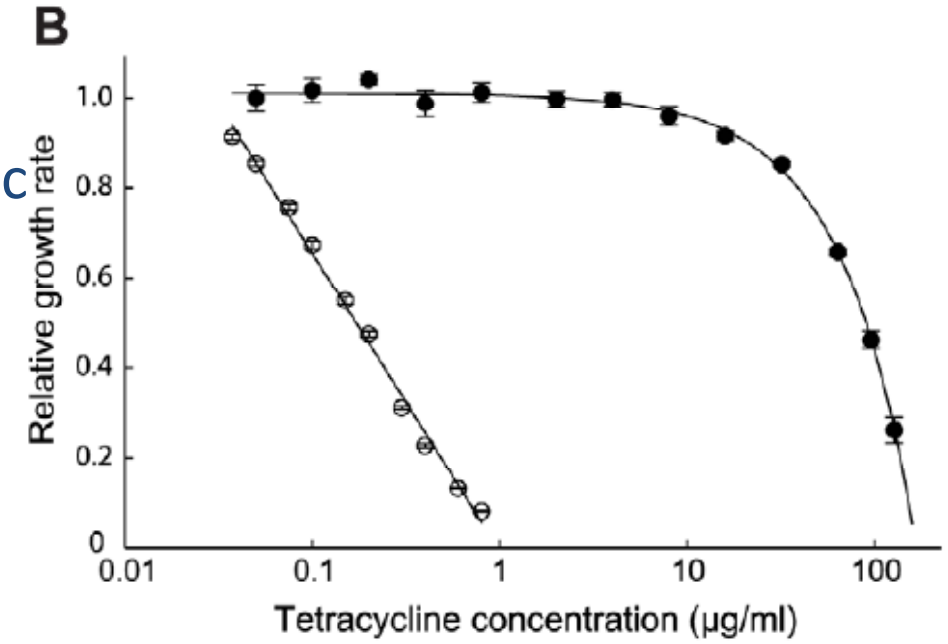


Figure 1. Growth rates as a function of antibiotic concentration. (**A**) Schematic representation of growth rates as a function of antibiotic concentration. Green indicates a concentration interval where the susceptible strain (blue line) will outcompete the resistant strain (red line). Orange (sub-MIC selective window) and red (traditional mutant selective window) indicate concentration intervals where the resistant strain will outcompete the susceptible strain. MIC_{susc} = minimal inhibitory concentration of the susceptible strain, MIC_{res} = minimal inhibitory concentration of the resistant strain and MSC = minimal selective concentration. (**B**). Relative exponential growth rates of susceptible (open circles) and resistant (closed circles) strains of *S. typhimurium* as a function of tetracycline concentration. Standard errors of the mean are indicated. A relative growth rate of 1.0 corresponds to approximately 1.8 hr^{-1}. Cells were grown in Mueller Hinton medium at 37°C.

Mutation at sub-therapeutic concentrations



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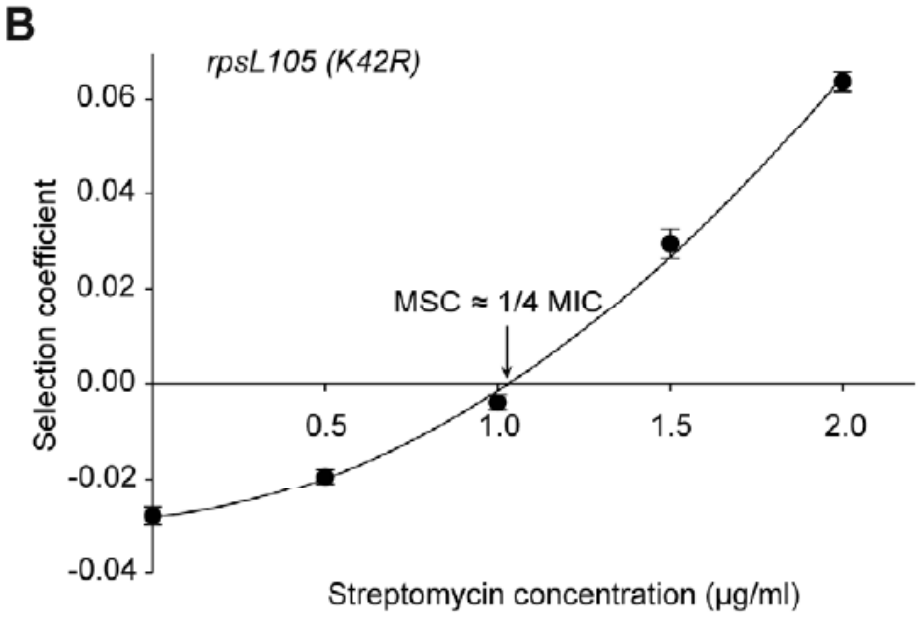

Interplay of selection and fitness varies by different antibiotic

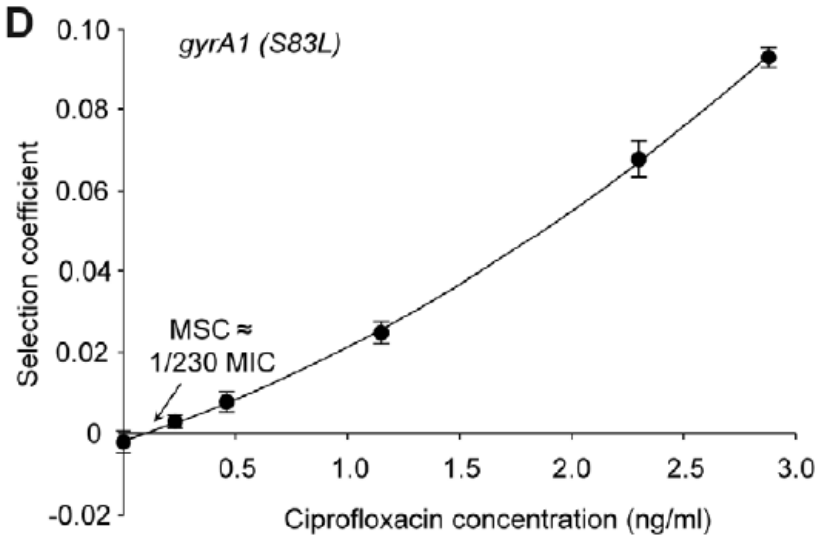

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Table 1. Summary of the mean mutation rates for *M. fortuitum* (MF01332) grown in differing concentrations of ciprofloxacin and selected on various antibiotics by the median mutation method

Selective agent	Median mutation rate in the drug-free broth (mutations/cell division)	$\frac{1}{2}$ MIC median mutation rate (mutations/cell division)	$\frac{1}{4}$ MIC mean mutation rate (mutations/cell division)	$\frac{1}{8}$ MIC mean mutation rate (mutations/cell division)
Ciprofloxacin	5.1×10^{-9}	2.6×10^{-7}	2.2×10^{-8}	1.6×10^{-8}
Levofloxacin	3.8×10^{-9}	2.0×10^{-7}	1.4×10^{-8}	9.6×10^{-9}
Moxifloxacin	4.2×10^{-9}	3.6×10^{-7}	1.5×10^{-8}	1.3×10^{-8}
Erythromycin	1.3×10^{-8}	4.9×10^{-7}	3.3×10^{-7}	3.6×10^{-8}
Rifampicin	2.6×10^{-9}	3.4×10^{-7}	5.3×10^{-8}	7.0×10^{-9}
Gentamicin	7.8×10^{-9}	3.5×10^{-7}	2.3×10^{-7}	3.3×10^{-8}

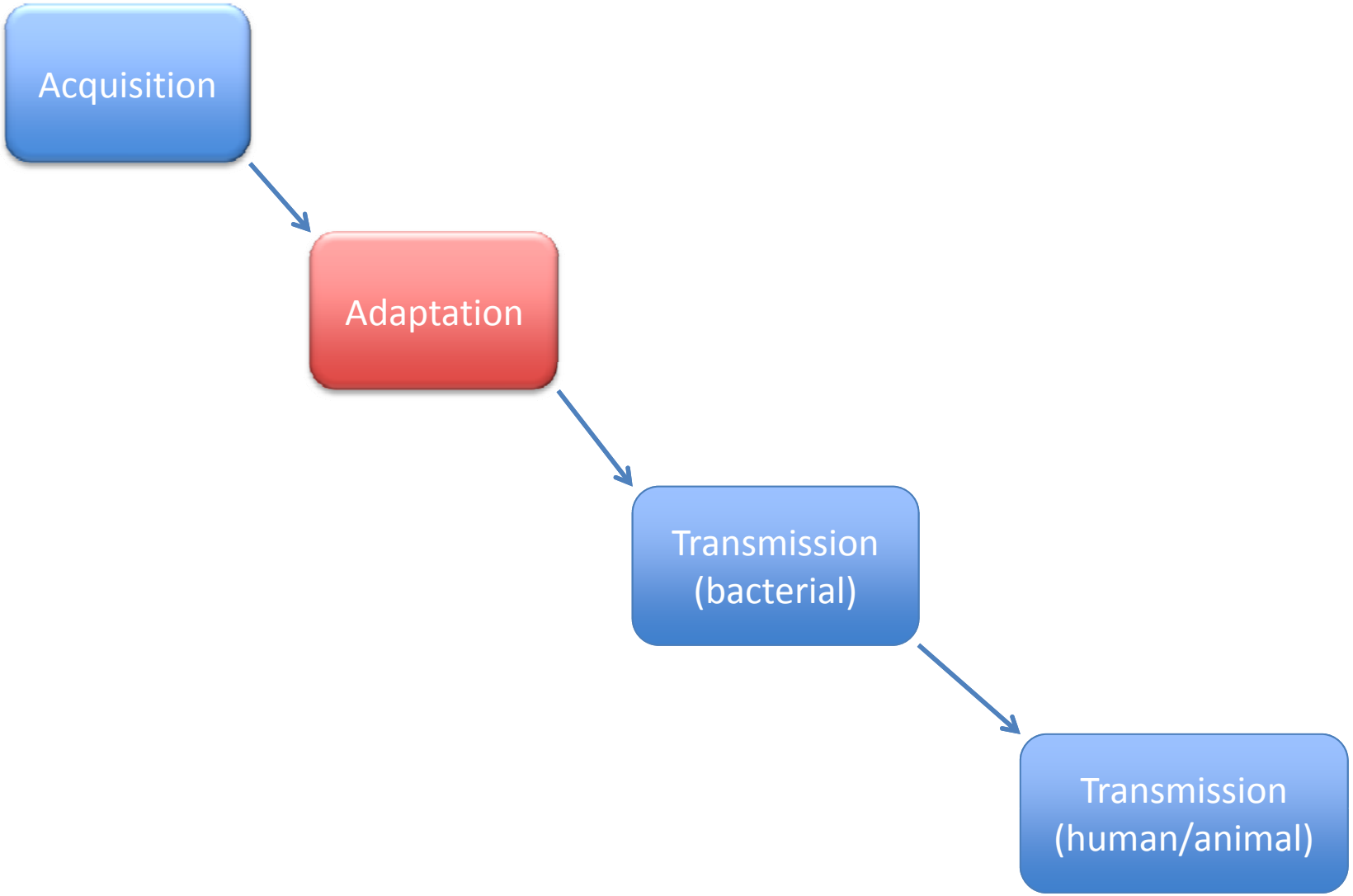
Table 2. Ratio (mean and standard error of mean for five median mutation estimates) between the mutation rates for *M. fortuitum* (MF01332) grown with and without ciprofloxacin in the broth for six antibiotics

	Concentration of ciprofloxacin in test broth			
Selective agent	$\frac{1}{2}$ MIC (SEM)	$\frac{1}{4}$ MIC (SEM)	$\frac{1}{8}$ MIC (SEM)	*P* (ANOVA)
Ciprofloxacin	88.8 (36.6)	5.0 (1.2)	3.8 (0.6)	0.02
Levofloxacin	94.9 (35.7)	5.2 (1.3)	3.1 (0.5)	0.01
Moxifloxacin	121.1 (32.9)	5.6 (1.1)	3.9 (0.8)	0.0006
Rifampicin	81.7 (36.9)	21.2 (11.6)	4.2 (1.8)	0.003
Erythromycin	72.1 (29.4)	21.8 (10.8)	9.6 (4.9)	0.04
Gentamicin	102.5 (41.6)	29.7 (15.3)	6.8 (3.3)	0.007
	Growth in $\frac{1}{2}$ MIC rifampicin			
Rifampicin	1.8	(mean of two median mutation experiments)		

Gillespie et al., *JAC* 2005; 56:344-8

An obvious evolutionary progression

The selective pressures may change



Emergence of anti-microbial resistance overview

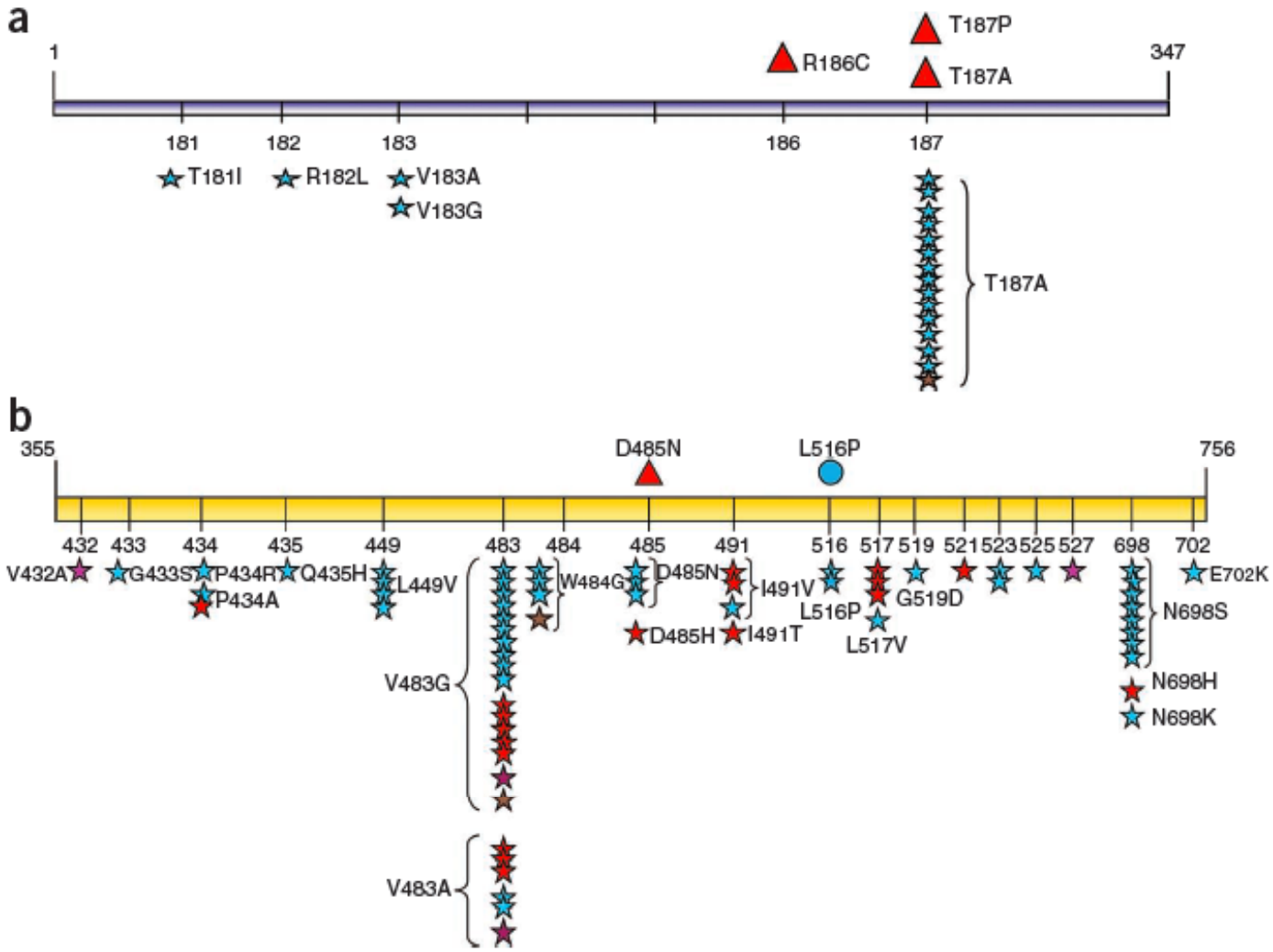


Figure 1 Putative compensatory mutations in *rpoA* and *rpoC* of *M. tuberculosis*. (**a**,**b**) Mutations identified after genome sequencing of experimentally evolved strains (circle) or paired clinical isolates (triangles) are indicated above the gene diagrams of *rpoA* (**a**) and *rpoC* (**b**). Mutations identified by screening a global and a high-burden collection of MDR strains are indicated by stars below the gene diagrams. Colors indicate the respective strain lineage (blue, lineage 2; red, lineage 4; brown, lineage 5; pink, lineage 1). Some of these mutations occurred in multiple lineages or affect the same codon position.

Table 1. Compensatory mutations selected in *rpoA*, *rpoB* and *rpoC*.

Mutations in *rpoA*, *B* or *C*					Selection[d]	
Original	Compensatory	MIC[a]	Dt (min) ± SD[b]	*N*[c]	LB	LB + RIF
Wild-type	Wild-type	12	19.55 ± 0.01	–	–	–
rpoB R529C	Wild-type	3000	54.28 ± 1.08	–	–	–
rpoB R529C	*rpoA* R191C	3000	26.74 ± 0.07	2	–	+ (2)
rpoB R529C	*rpoA* R191S	3000	29.73 ± 0.73	1	–	+
rpoB R529C	*rpoA* Q194P	3000	30.50 ± 0.32	1	+	–
rpoB R529C	*rpoB* D516G	>3000	22.24 ± 0.28	6	+ (3)	+ (3)
rpoB R529C	*rpoB* P560L	3000	26.41 ± 0.34	1	+	–
rpoB R529C	*rpoB* P564S	1500	34.18 ± 0.21	1	+	–
rpoB R529C	*rpoB* E565A	1500	23.96 ± 0.30	1	–	+
rpoB R529C	*rpoB* R637C	3000	31.96 ± 0.42	1	–	+
rpoB R529C	*rpoB* H673Y	3000	29.22 ± 0.36	1	+	–
rpoB R529C	*rpoC* P64L	3000	27.86 ± 0.68	1	+	–
rpoB R529C	*rpoC* L770P	3000	26.79 ± 0.09	1	–	+
rpoB R529C	*rpoC* R1075P	3000	26.17 ± 1.02	1	–	+
rpoB R529C	*rpoC* R1075H	3000	28.30 ± 0.30	1	–	+
rpoB R529C	*rpoC* G1136A	3000	27.96 ± 1.04	1	+	–
rpoB R529C	*rpoC* R1140H	3000	29.83 ± 0.93	1	+	–
rpoB R529C	*rpoC* V1198E	3000	26.13 ± 0.83	1	+	–

a. MIC RIF in μg ml^{-1}.
b. Dt is doubling time (generation time) in LB at 37°C, ± standard deviation (SD) based on three independent measurements for each strain.
c. *N* is the number of independent isolates (from independent lineages) of each mutation.
d. Growth medium in which growth-compensatory mutants were selected. LB or LB with rifampicin at 100 μg ml^{-1}. Numbers in brackets are number of independent isolates of each mutation.

Table 2. Phenotypes of *rpoA* and *rpoC* compensatory mutations.

Mutation	RIF MIC[a]	Dt (min) ± SD[b]
Wild-type	12	19.55 ± 0.01
rpoA R191C	16	20.72 ± 1.65
rpoA R191S	**32**	19.96 ± 0.22
rpoA Q194P	**24**	20.00 ± 0.61
rpoC P64L	**24**	21.39 ± 0.31
rpoC L770P	**>32**	27.32 ± 0.60
rpoC R1075P	**24**	22.78 ± 1.53
rpoC R1075H	**24**	21.92 ± 0.71
rpoC G1136A	12	21.23 ± 0.40
rpoC R1140H	**32**	24.67 ± 0.52
rpoC V1198E	12	19.89 ± 0.67

a. MIC RIF in μg ml^{-1} based on at least three independent measurements for each strain. Values in **bold** are statistically significantly higher than the MIC of the wild-type (≥ 2 MIC steps increase relative to the wild-type MIC).
b. Dt is doubling time (generation time) in LB with shaking aeration at 37°C, ± standard deviation (S.D.), based on three independent measurements for each strain.

Table 1. Minimal Inhibitory Concentrations (MIC) of quinolones against the strains of the two isogenic systems derived from *E. coli* CFT073.

E. coli strains	MIC[a] (μg/ml)					
	NAL	NOR	OFX	CIP	AMK	TOB
E. coli CFT073	2	0.064	0.094	0.012	1.5	0.75
E. coli CFT073(pBR322)	2	0.064	0.094	0.012	1.5	0.5
E. coli CFT073(pBRΔ*tetA*)	2	0.064	0.094	0.012	1.5	0.5
E. coli CFT073(pBRAM1)	8	1	0.75	0.125	1	0.5
E. coli CFT073(pBRAM2)	8	1	0.75	0.125	1.5	0.5
E. coli CFT073-Sm^R	2	0.064	0.094	0.012	1.5	0.75
E. coli CFT073-Sm^R(pHe96)	6	3	0.75	0.75	48	32
E. coli CFT073-Sm^R(pHe96) "R42"	6	3	0.75	0.75	48	32

[a]Minimal inhibitory concentrations measured by E-test for quinolones and aminoglycosides. NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; CIP, ciprofloxacin; AMK, amikacin; TOB, tobramycin.

doi:10.1371/journal.pone.0024552.t001

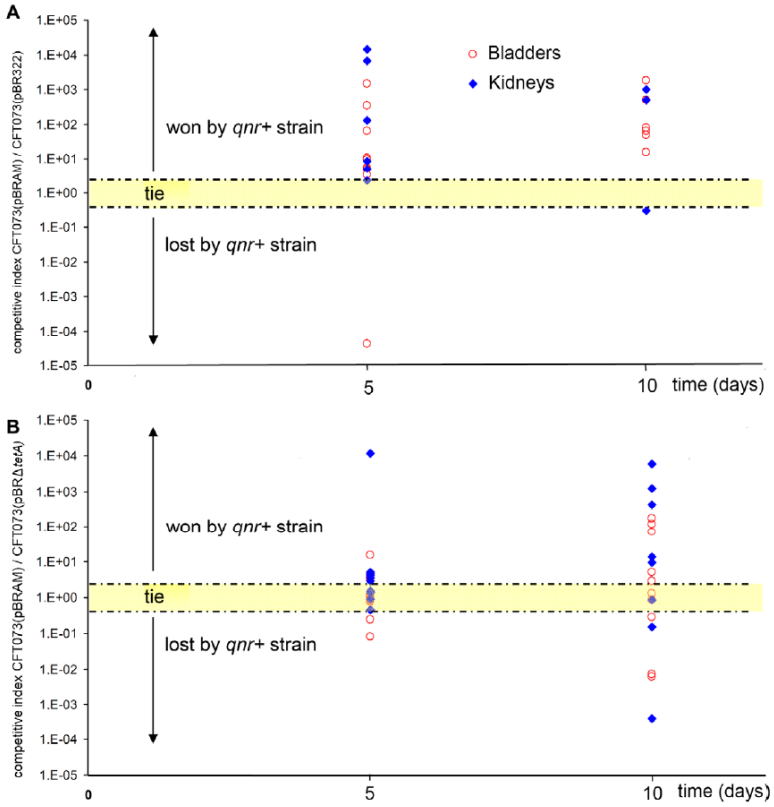


Figure 2. Enhanced fitness observed in competitive infections for *E. coli* CFT073 after *qnrA3* acquisition onto pBR322. Each symbol represents the bacterial ratio (number of CFU for the *qnr*-positive strain/number of CFU for the *qnr*-negative isogenic strain) measured in organs (blue diamond = kidneys, red circle = bladder) collected five and ten days after inoculation of a 1:1 mix of the two strains. When the ratio was equal to 1+/−0.2, it was considered as tie. Part A: competitions experiments opposing *E. coli* CFT073(pBR322) (*qnr*−, *tetA*+) and *E. coli* CFT073(pBRAM2) (*qnrA3*+, *tetA*−). Fifteen mice were inoculated, 15 bladders and 10 pairs of kidneys were efficiently infected. Competition was won 22 times by *E. coli* CFT073(pBRAM2) (*qnrA3*+, *tetA*−), was lost 2 times, and one was tie ($p<0.0001$). Part B: competitions opposing *E. coli* CFT073(pBRΔ*tetA*) (*qnr*−, *tet*−) and *E. coli* CFT073(pBRAM2) (*qnrA3*+, *tet*−). Twenty-three mice were inoculated, 20 bladders and 17 pairs of kidneys were efficiently infected. Competition was won 24 times by *E. coli* CFT073(pBRAM2) (*qnrA3*+, *tetA*−), was lost 9 times, and 6 was tie ($p<0.0001$).
doi:10.1371/journal.pone.0024552.g002

pBR322 background

pHe96 background

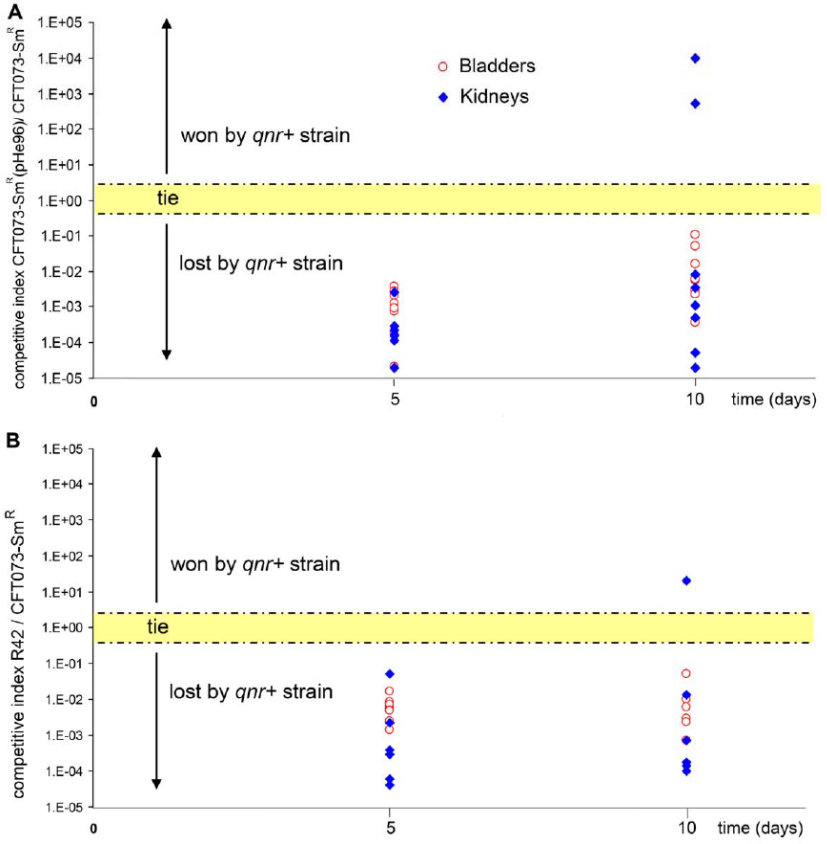


Figure 4. Reduced fitness observed after pHe96 acquisition in competitive infections in absence of antimicrobial exposure. Each symbol represents the ratio (number of CFU for the *qnr*-positive strain/number of CFU for the *qnr*-negative isogenic strain) in organs (blue diamond = kidneys, red circle = bladder), collected five and ten days after inoculation of a 1:1 mix of the two strains. Part A: competition experiments opposing *E. coli* CFT073-Sm^R (*qnr*−) and *E. coli* CFT073-Sm^R(pHe96) (*qnrA3*+). Twenty mice were inoculated, 19 bladders and 16 pairs of kidneys were efficiently infected. Competition was lost 33 times by *E. coli* CFT073-Sm^R(pHe96) (*qnrA3*+), and won only 2 times ($p<0.0001$). Part B: competition experiments opposing *E. coli* CFT073-Sm^R (*qnr*−) and *E. coli* CFT073-Sm^R(pHe96) variant "R42" (*qnrA3*+). The R42 variant was selected from kidneys that were infected by *E. coli* CFT073-Sm^R(pHe96). Twenty mice were inoculated, 18 bladders and 16 pairs of kidneys were efficiently infected. Competition was lost 33 times by the *qnrA3*-positive strain with only one won ($p<0.0001$).
doi:10.1371/journal.pone.0024552.g004

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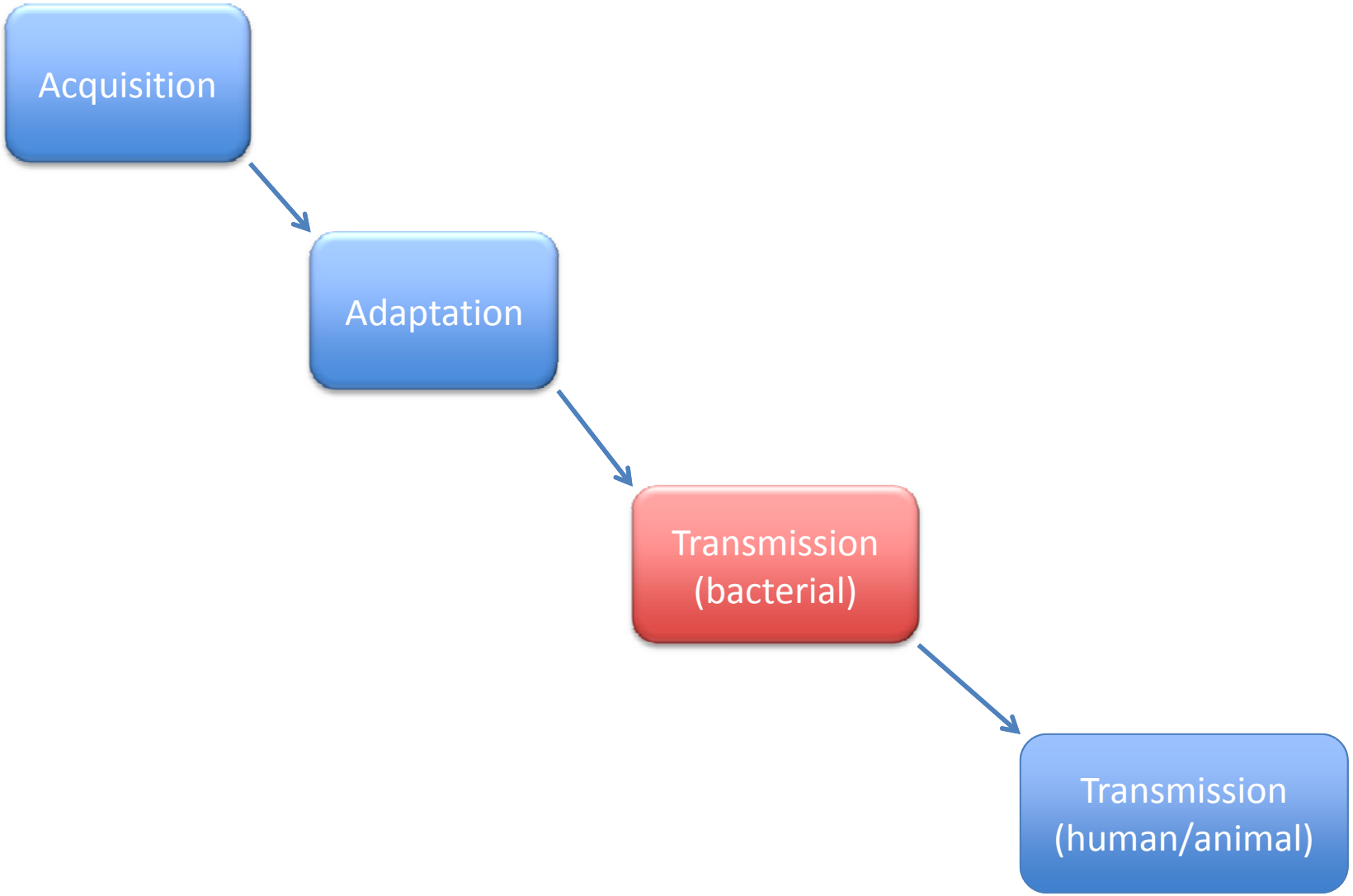
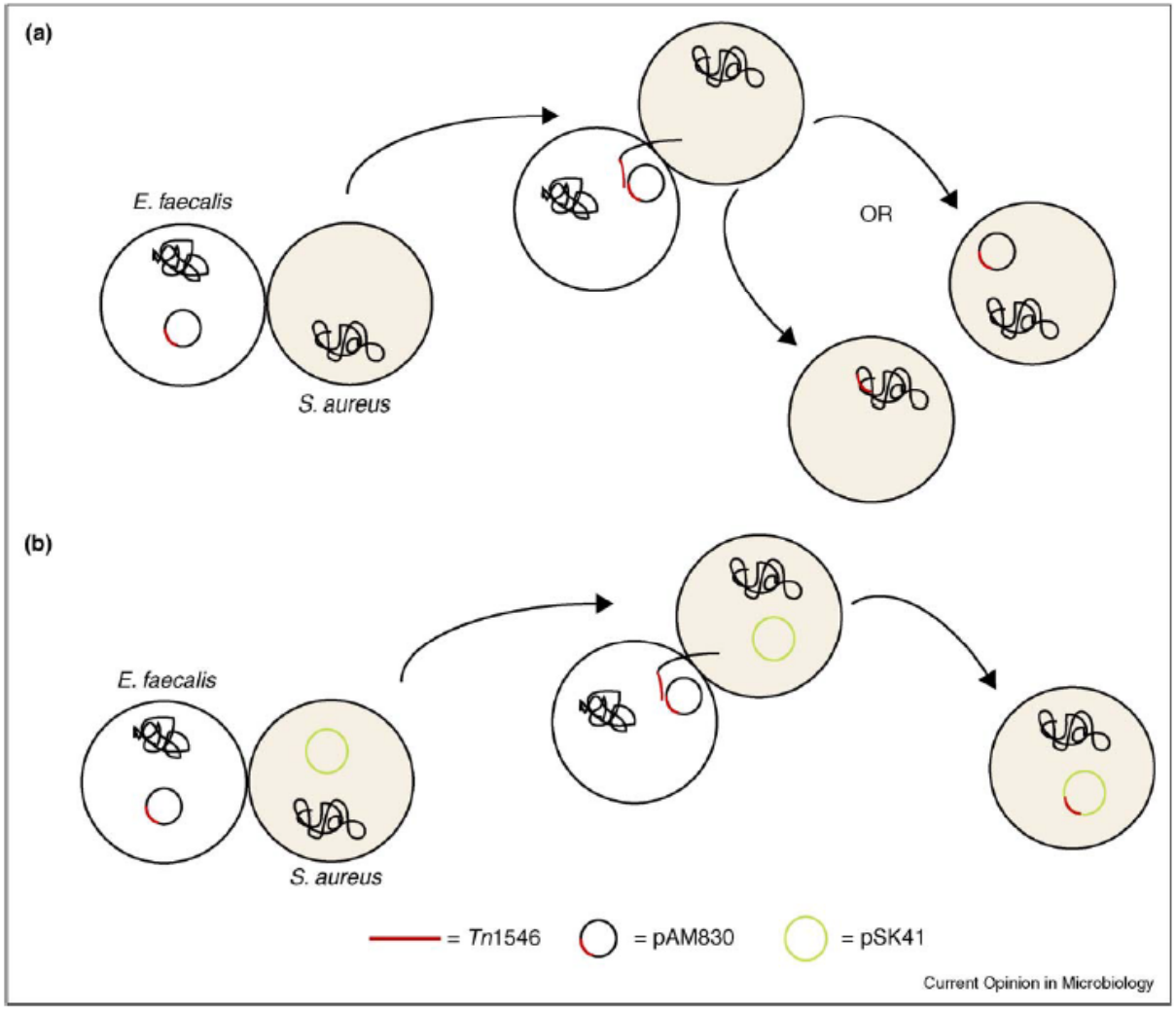


Emergence of anti-microbial resistance overview

Figure 2



Possible scenarios which allowed for dissemination of vancomycin resistance to MRSA from VRE. Co-colonization of patients with VRE and MRSA allowed for the transfer of pAM830 which carried the transposon (Tn*1546*) encoding genes associated with high level glycopeptide resistance. Several outcomes appeared to have occurred based upon the analysis of the resultant VRSA strains. It would appear that: (a) only Tn*1546* may have been transferred during the conjugation process instigated by pAM830 and recombined on the chromosome of the *S. aureus* recipient or the complete plasmid was transferred to the *S. aureus* recipient and was stably maintained; (b) the plasmid carrying Tn*1546* was not completely transferred to the recipient cell or incompatibility issues were encountered between pAM830 and existing plasmids in the MRSA strain and the transposon was found in the recipient strain on a native plasmid.

Current Opinion in Microbiology 2010, **13**:632–639

Transfer of antibiotic resistance between species and by a variety of mobile genetic elements

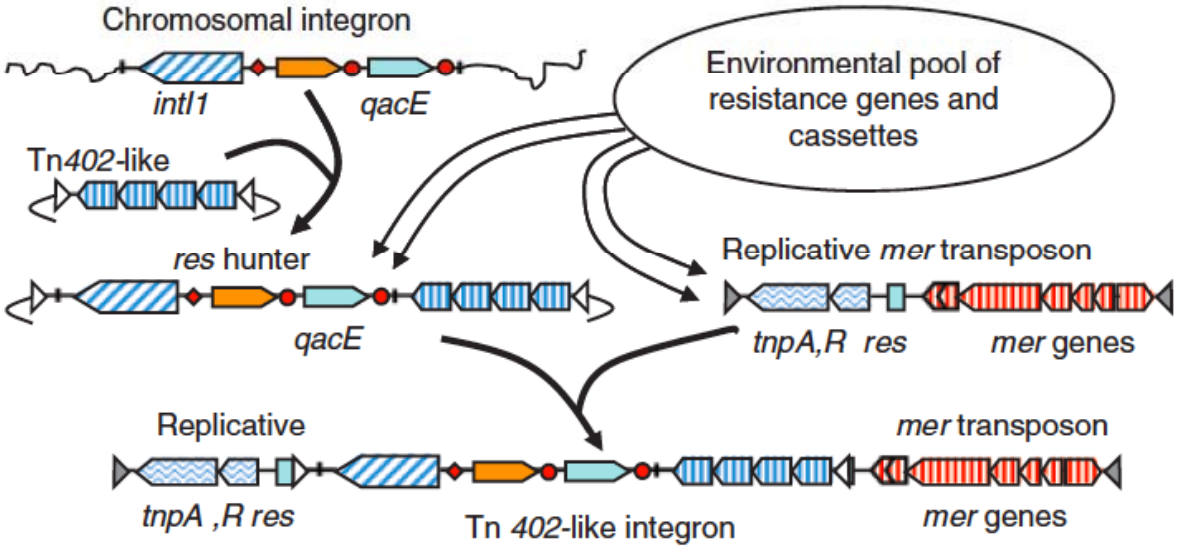
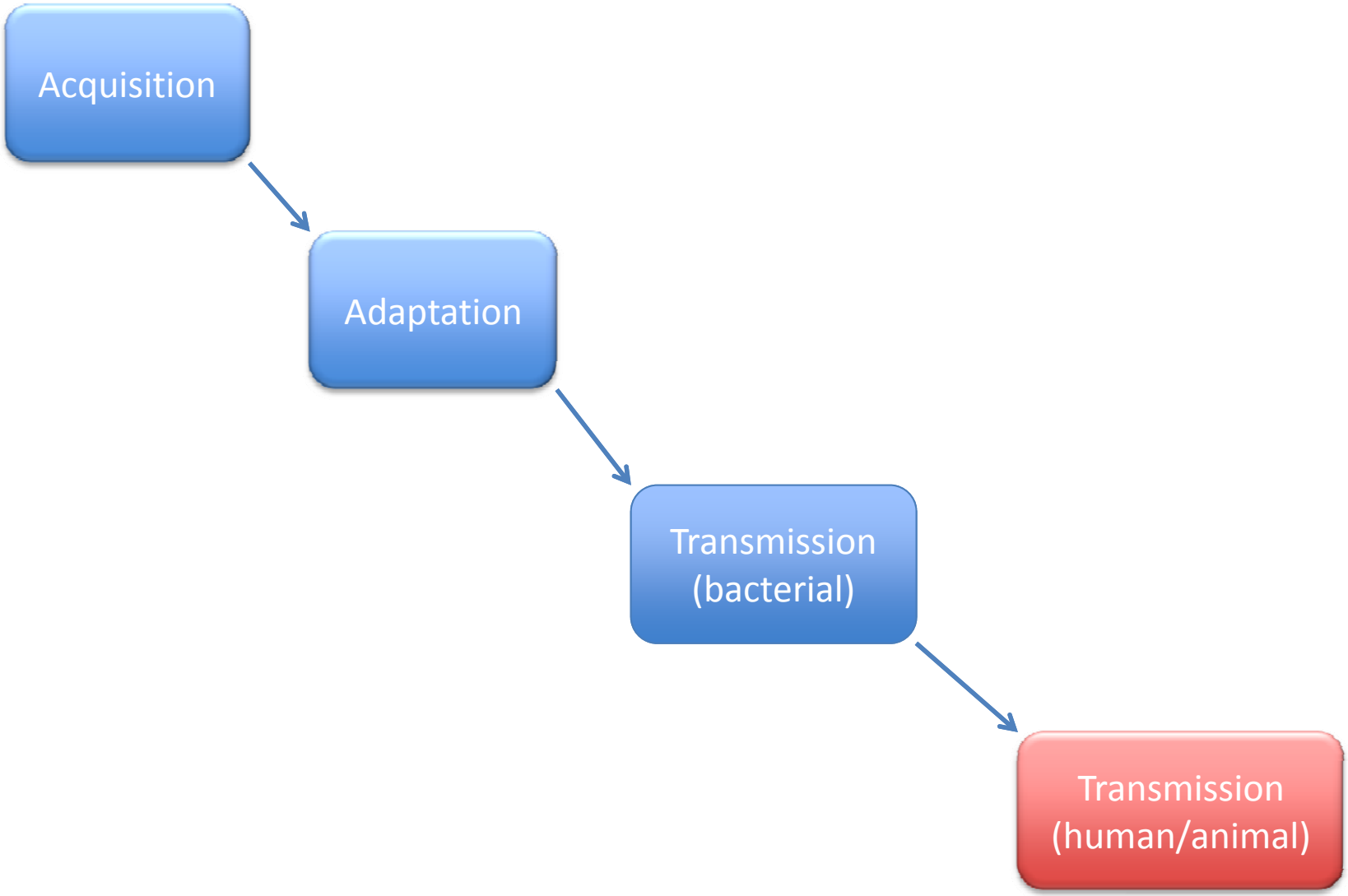


Fig. 5. Coselection and recruitment of transposons and class 1 integrons into pathogens. The schematic represents a model describing the order of events leading to complex and highly mobile multiresistance regions in contemporary Gram-negative pathogens. The use of disinfectants led to the linking of *qac* genes to class 1 integrons before the antibiotic era. This structure then linked to a Tn*402*-like transposition module and became mobile. At about the same time, with the onset of extreme selection pressure via the use of antibiotics, antibiotic resistance genes began to be recruited into this structure and its descendants. In parallel, the presence of mercury in the environment – both natural and human induced – led to the enrichment for mercury-resistant transposons. These also began to independently recruit resistance genes from the onset of the antibiotic era. At the same time and subsequently, the *res* targeting mechanism associated with Tn*402*-like class 1 integrons made the linking of a broad range of transposition modules to site-specific recombination functions inevitable.



Emergence of anti-microbial resistance overview

Sequence Type	PMEN designation	*pbp1a*	*pbp2b*	*pbp2x*	*erm/mef*
90	Spain^{6B}-2	1	2	2	-
156	Spain^{9V}-3	1	1	1	-
173	Poland^{23F}-16	4	13	1	-
185	S. Africa^{6B}-8	5	7	6	-
268	Hungary^{19A}-6	3	5	4	*erm*
270	Finland^{6B}-12	1	9	1	*erm*
273	Greece^{6B}-22	12	5	12	*erm*
338	Columbia^{23F}-26	12	6	17	-
384	Maryland^{6B}-17	10	14	1	*mef*

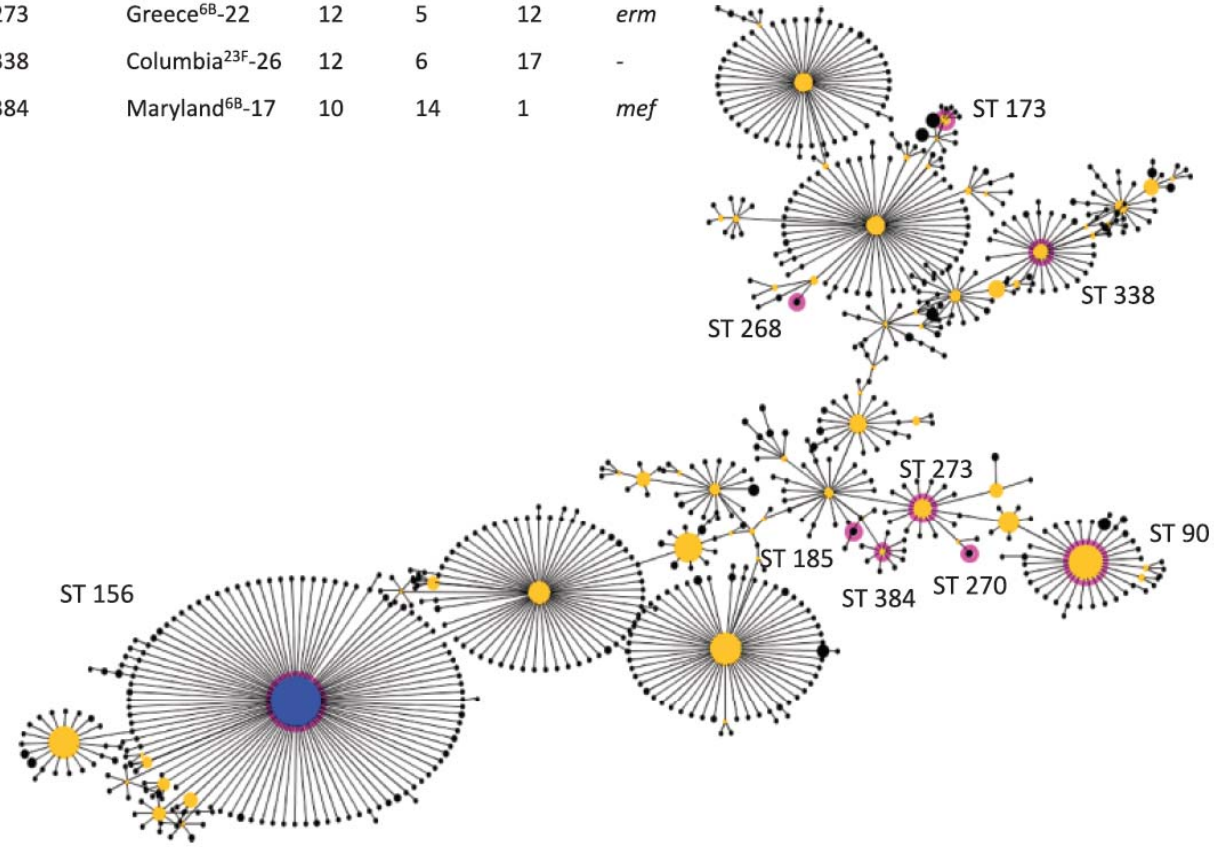


Fig. 3. The largest CC in the *Streptococcus pneumoniae* MLST database (CC156), accessed on 6 August 2010 and visualized using eBURST. Resistant PMEN clones are highlighted in dark pink, and their ST is indicated. A cluster of 6B clones is evident at right. The pbp profiles and erm/mef data are drawn from the PMEN website at http://www.sph.emory.edu/PMEN/.

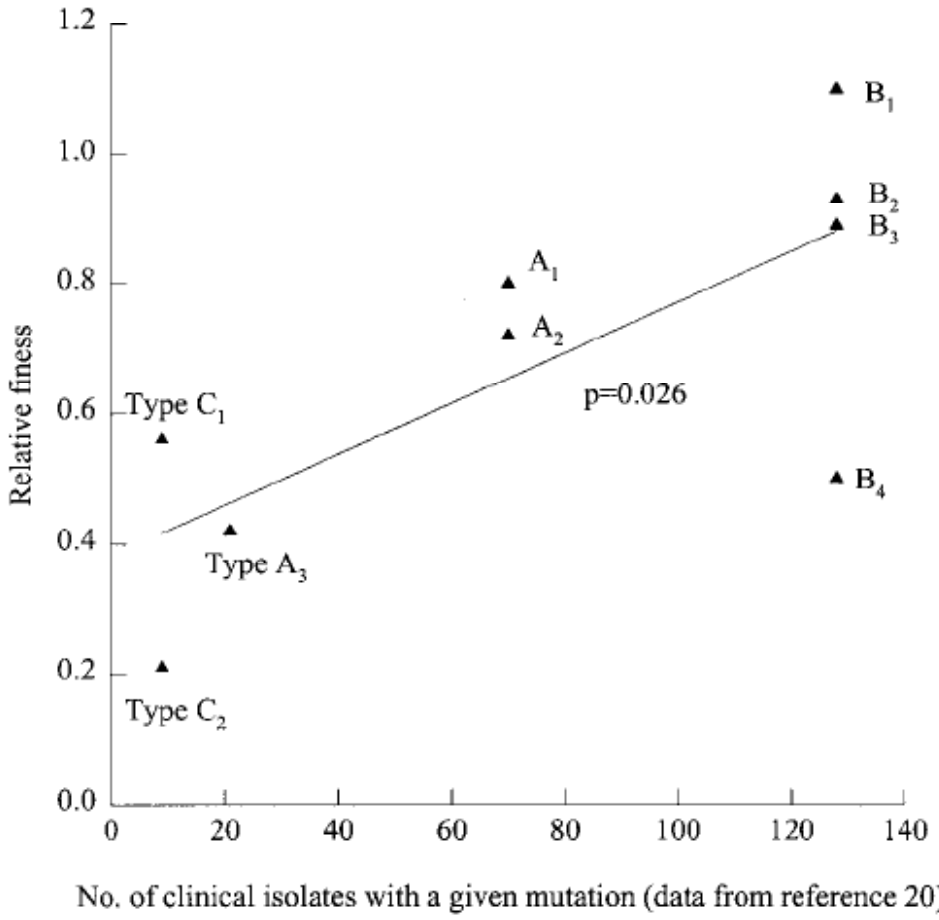


FIG. 2. Relationship between relative fitness and the clinical isolation rate for mutants A, B, and C.

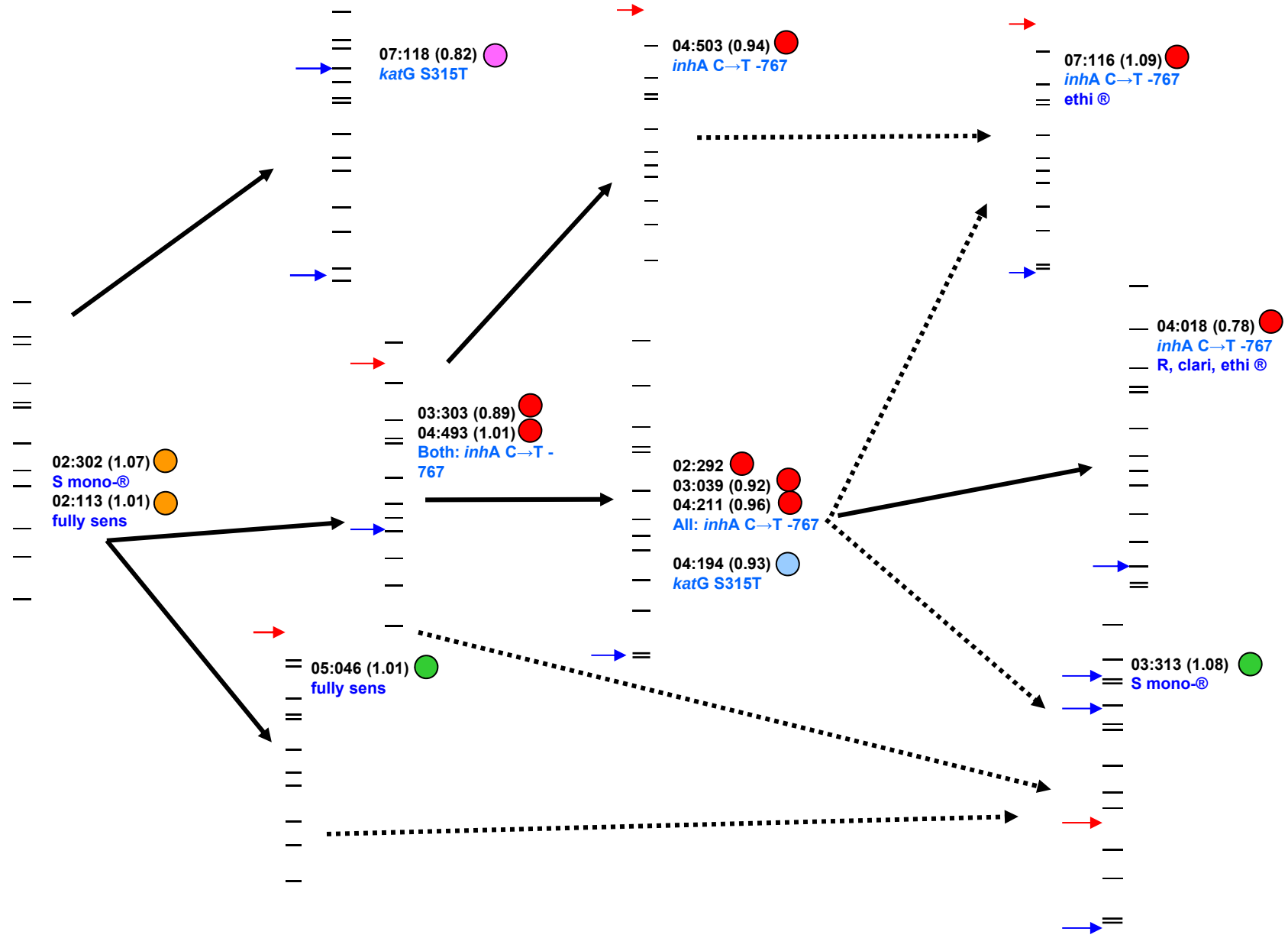
For *M. tuberculosis*/rifampicin resistance clinical frequency is related to the primary fitness cost

Table 1. Antibiotic resistance profile determined by MGIT DST grouped according to patient-reported receipt of antibiotics in the previous 14 days

	Patient category according to antibiotics received in the previous 14 days			TOTAL
Resistance profile	A (No antibiotics)	B (Received non-fluoroquinolone antibiotics)	C (Received unknown antibiotics)	
No resistance	99	9	15	123
M only	15	8	0	23
H only	18	3	1	22
M + H only	6	1	1	8
R + H only (MDR-TB)	3	1	2	6
R+H+M	4	2	1	7
TOTAL	145	24	20	189

M – moxifloxacin, H – isoniazid, R – rifampicin

Lineage No.	Number of isolates	% (number) resistant	Resistance profile(s)	Relative Rate of Transmission (RRT)
15	16	87.5% (14)	10 x INH mono-resistance 1 x INH and eth resistance 1 x INH, RIF, clari & eth resistance 2 x streptomycin mono-resistance	0.14
19	15	6.7% (1) U = 3	INH mono-resistance	11
41	21	9.5% (2) U = 2	INH mono-resistance RIF & INH resistance	8.50
43	10	0% (0)	N/A	N/A
54	13	0.8% (1) U = 1	INH mono-resistance	11
61	13	0.8% (1) U = 1	INH mono-resistance	12



Resistance, fitness and a tuberculosis outbreak

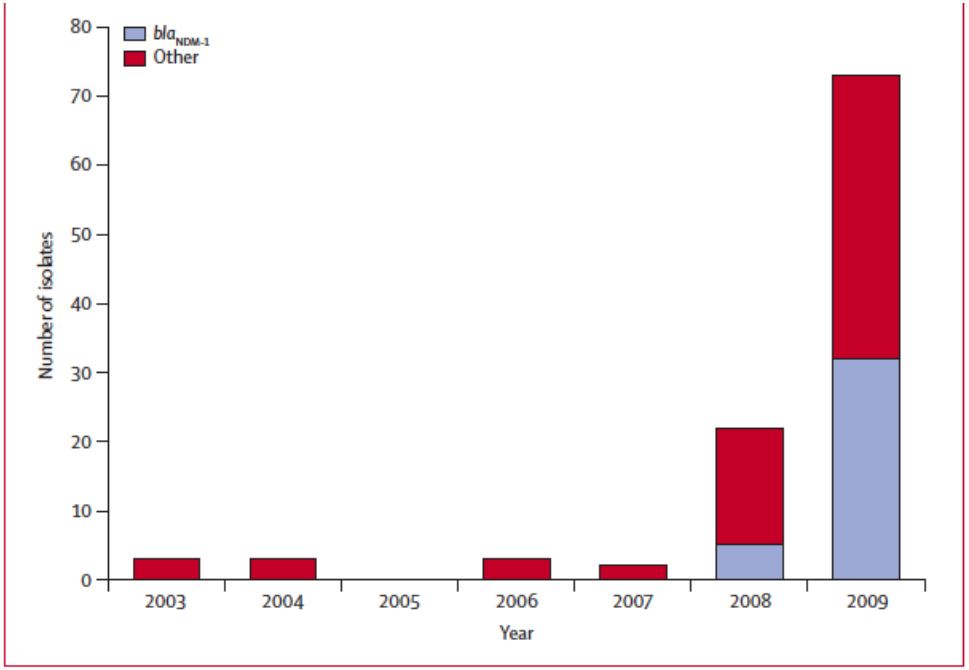


***Figure 1*: Numbers of carbapenemase-producing Enterobacteriaceae referred from UK laboratories to the UK Health Protection Agency's national reference laboratory from 2003 to 2009**
The predominant gene is bla_{NDM-1}, which was first identified in 2008. The other group includes diverse producers of KPC, OXA-48, IMP, and VIM enzymes.

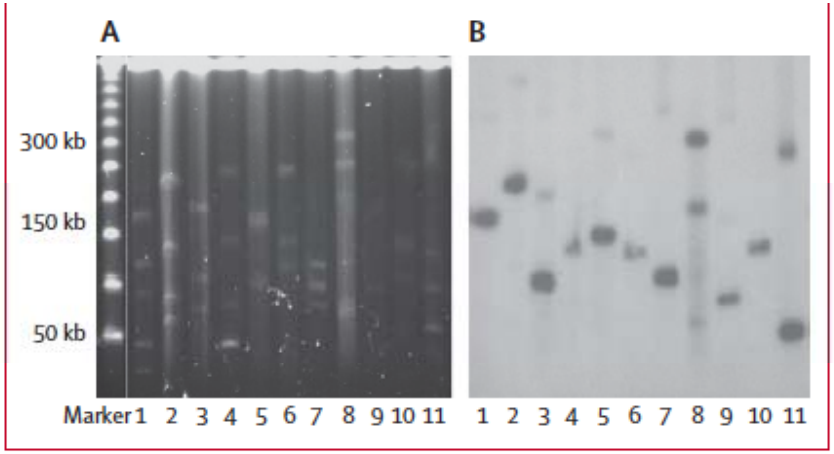


***Figure 4*: Hybridisation results of UK isolates with bla_{NDM-1}**
Pulsed-field gel of S1-treated plasmid DNA of UK isolates M15–M27 stained with ethidium bromide (A). Molecular weight marker is Lambda concatamer 50–1000 kb. The chromosome of each isolate is the bright band at the top of each lane and bright bands below are plasmids of various sizes. Autoradiogram of gel A probed with a bla_{NDM-1} showing individual or multiple plasmids in each strain carrying bla_{NDM-1} (B).

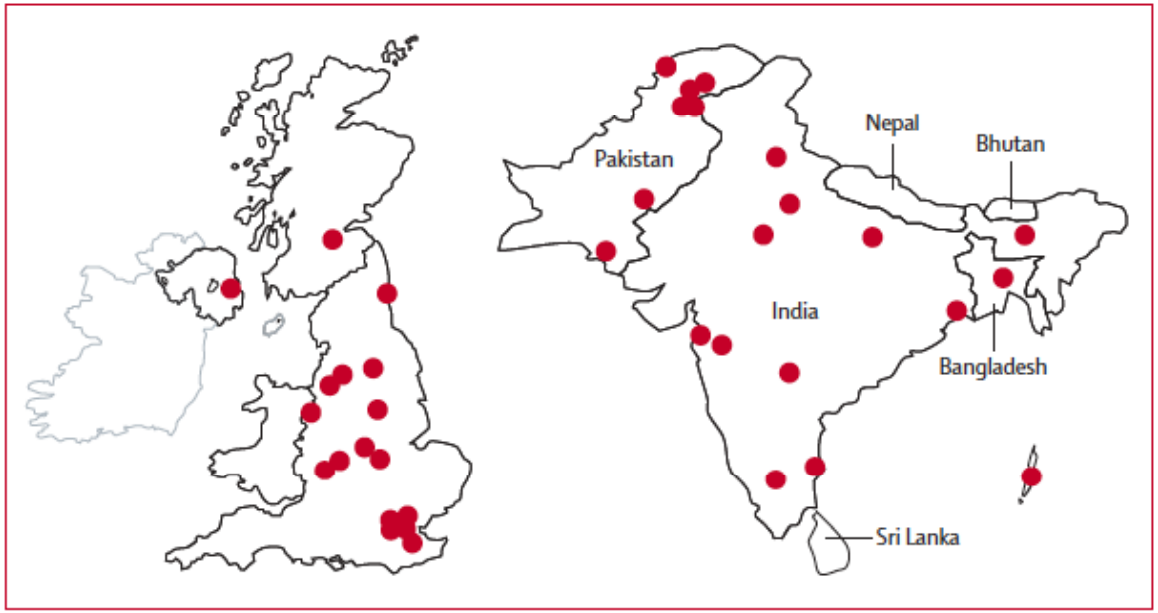


***Figure 5*: Distribution of NDM-1-producing Enterobacteriaceae strains in Bangladesh, Indian, Pakistan, and the UK**

Lancet Infect Dis 2010; 10: 597–602

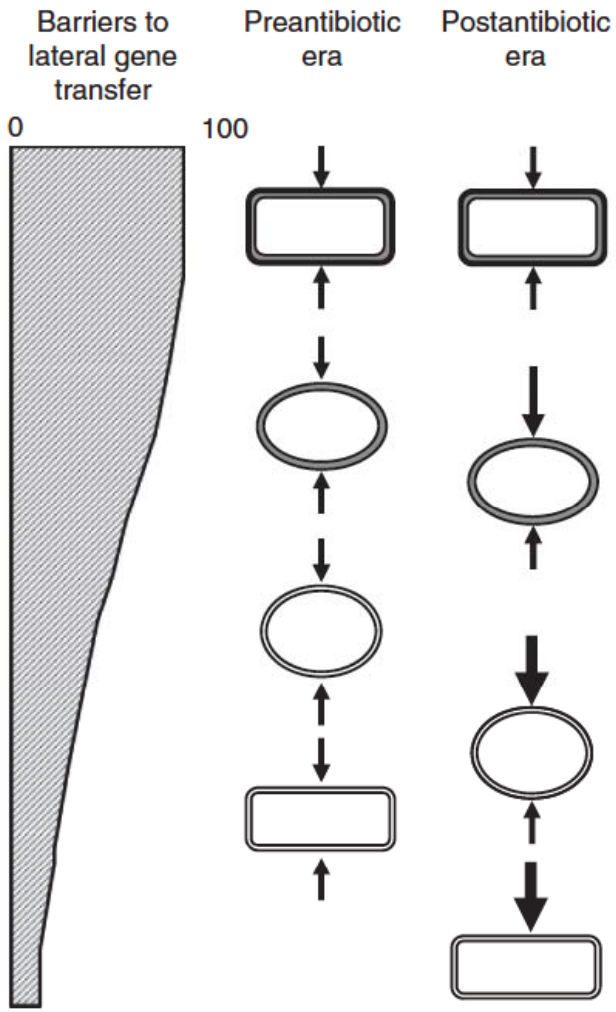


Fig. 4. Barriers to lateral gene transfer. For any species, the barriers to lateral gene transfer are set by two opposing and balanced selective forces: the ability to resist infection by bacteriophage and/or barriers to transposons (upward arrows) and the advantage conferred by the ability to acquire new phenotypes (downward arrows). The widespread dissemination of antibiotics may have altered this equilibrium, selecting for increased lateral transfer capability.

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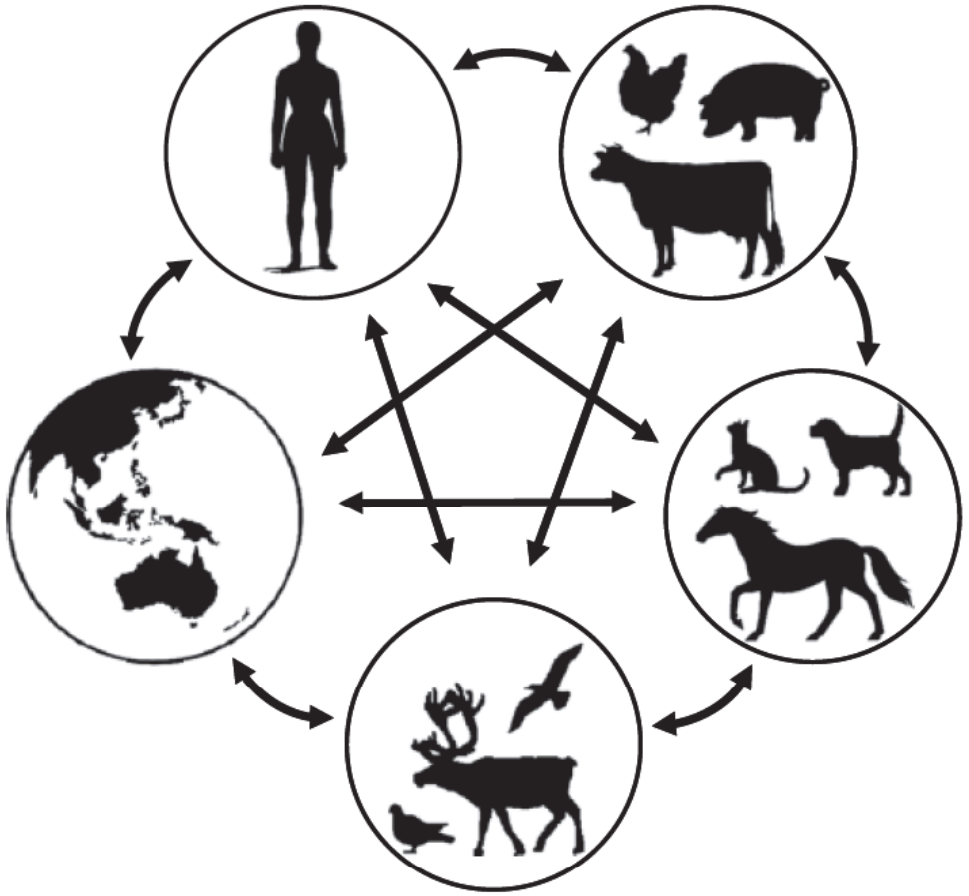
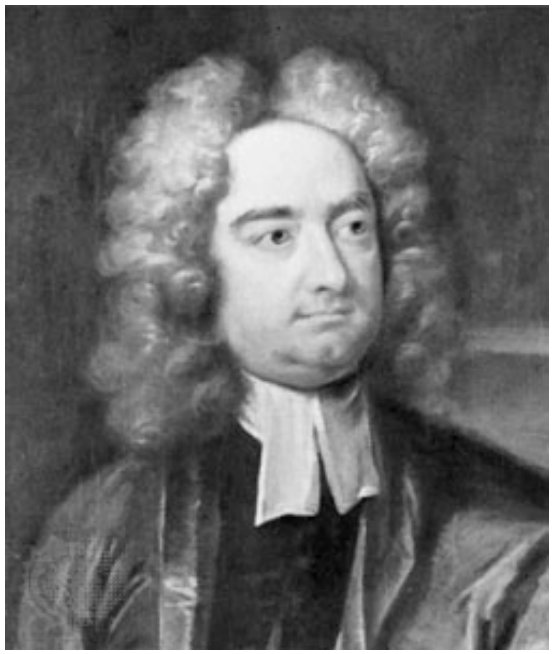


Antibiotic resistant gene flow



"So nat 'ralists observe, a flea
Hath smaller fleas that on him prey,
And these have smaller fleas that bite 'em,
And so proceed ad infinitum. "

Jonathan Swift “On Poetry”

Conclusions

- There is no such thing as “resistance”
- Each antibiotic-bacterial pairing is a separate biological system
- These systems are not independent but interact with each other based on biological epizoological and epidemiological principles
- Promiscuous antibiotic drug use is an important driver tipping the balance in favour of increased density of antibiotic resistance genes

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