On the other hand, evolving Day 20 TOBR to piperacillin also led to a partial resensitization (MICTOB of Day 20 TOBR versus Day 40 TOBRPIPR, p0.05) but not as much as it did when Day 20 TOBR was evolved to ciprofloxacin (MICTOB of Day 40 TOBRPIPR versus Day 40 TOBRCIPR, p0.01) and LB (MICTOB of Day 40 TOBRPIPR versus Day 40 TOBRLB, p0.05). After 5 days of subsequent piperacillin adaptation (Day 25 CIPRPIPR), the MICCIP was significantly different than that of Day 20 CIPR (p0.001), while this was not the case after 5 days of subsequent tobramycin (p = 1.00) or LB (p = 0.57) adaptation. then result in multidrug–resistant P. aeruginosa cultures that are resistant to both piperacillin and tobramycin. During subsequent tobramycin adaptation, the decrease in the MICCIP from Day 20 CIPR to Day 40 CIPRTOBR (p0.01) was marginally more than the decrease in the MICCIP from Day 20 CIPR to Day 40 CIPRPIPR (p0.05) during subsequent piperacillin adaptation. When Day 20 TOBR was evolved to ciprofloxacin, partial resensitization occurred (MICTOB of Day 20 TOBR versus Day 40 TOBRCIPR, p10–5), and the MICTOB of Day 40 TOBR–CIPR fell to a comparable level as that of Day 40 TOBRLB (p = 0.98) (Fig 3A [middle]). On the other hand, when Day 20 PIPR was evolved to ciprofloxacin, the resulting cultures became resensitized to piperacillin (Fig 3A [top], p0.05), and the MICPIP declined to levels comparable to those of the initially susceptible cultures (MICPIP of Day 1 PIPR versus Day 40 PIPRCIPR, p = 0.80), indicative of a full resensitization.. In the second type of drug–order±specific effects, prior adaptation to a first drug reduces the rate of subsequent adaptation to a second drug (such that the endpoint level of resistance to that second drug is lower compared to the amount of resistance developed when the Day 0 Ancestor is directly evolved to that second drug). Thus, regardless if ciprofloxacin adaptation occurred before or after tobramycin adaptation, ciprofloxacin adaptation led to piperacillin collateral sensitivity. We observed that evolution first to piperacillin reduces the rate of subsequent evolution to tobramycin (Fig 2D and 2E). Interestingly, we observed no cases in which prior drug adaptation led to an enhancement in the rate of adaptation to a second drug. In a contrasting example, we also found it interesting that while ciprofloxacin adaptation also led to collateral sensitivity of tobramycin, subsequent piperacillin adaptation did not cause the MICTOB to return to baseline levels (Fig 2F) in the manner in which subsequent tobramycin adaptation returned the MICPIP to baseline values (Fig 2C). That is, the MICTOB of Day 40 PIPRTOBR was less than that of Day 20 TOBR (Fig 3B, p0.05). All the cases of collateral sensitivity that were observed occurred during ciprofloxacin treatment whereby ciprofloxacin adaptation resulted in a lower MIC of piperacillin or tobramycin compared to baseline levels (S4 Fig). First, adaptation to ciprofloxacin starting from the Day 0 Ancestor resulted in collateral sensitivity to both piperacillin (Fig 2C; S4A Fig [right], p0.01) and .(tobramycin (Fig 2F; S4A Fig [left], p0.0001