Interaction networks, ecological stability, and collective antibiotic tolerance in polymicrobial infections

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Polymicrobial infections constitute small ecosystems that accommodate several bacterial species. Commonly, these bacteria are investigated in isolation. However, it is unknown to what extent the isolates interact and whether their interactions alter bacterial growth and ecosystem resilience in the presence and absence of antibiotics. We quantified the complete ecological interaction network for 72 bacterial isolates collected from 23 individuals diagnosed with polymicrobial urinary tract infections and found that most interactions cluster based on evolutionary relatedness. Statistical network analysis revealed that competitive and cooperative reciprocal interactions are enriched in the global network, while cooperative interactions are depleted in the individual host community networks. A population dynamics model parameterized by our measurements suggests that interactions restrict community stability, explaining the observed species diversity of these communities. We further show that the clinical isolates frequently protect each other from clinically relevant antibiotics. Together, these results highlight that ecological interactions are crucial for the growth and survival of bacteria in polymicrobial infection communities and affect their assembly and resilience.

microbiology | systems biology | antibiotics | infection

Bacteria in a polymicrobial infection form an ecosystem that can be more than the sum of its parts. Interactions between microbes can alter the severity of infections (1, 2) and complicate treatments (3–6). Still, the interplay between microbes in clinical environments remains poorly understood, as clinical isolates are commonly investigated in isolation. This leads to the implicit assumption that microorganisms in polymicrobial infections grow and can be treated independently of each other. Ecological interactions occur when organisms affect each other's proliferation or survival. Mechanistically, such interactions can be due to competition for the same nutrient (7), excretion of metabolites (cross-feeding) (8), or production of other molecules that specifically harm or help other community members (9–12) and signaling molecules (13). The quantitative analysis of ecological interactions between microbes can shed light on the emergent properties of the infection ecosystem, its microbial interaction network (14–17), its stability, and its resilience to perturbations.

Polymicrobial urinary tract infections (UTIs) in elderly patients consist of relatively simple bacterial communities (Fig. 1*A*) (6). However, the role of ecological interactions between these bacteria (3, 5, 18) largely remains obscure. As the communities are typically caused by only up to five isolates (6), it is feasible to systematically investigate all interactions. This is in contrast to other ecosystems that are generally too complex to map the network of all interactions (15, 19). Due to the lack of comprehensive experimental data on interactions, quantitative studies of microbial ecosystems have so far relied on theoretical modeling (15, 20, 21) or sequencing methods (22) or have focused on incomplete subcommunities (23). Indeed, only a few studies have experimentally quantified ecological interactions in different microbial ecosystems on a larger scale (23–26). Importantly, these interactions can potentially alter the efficacy of drug treatments as bacteria can protect others from the effect of antibiotics (27), in the simplest case by just degrading the drug (13, 28, 29). Thus, understanding the effects of ecological interactions on bacterial growth and antibiotic efficacy is crucial for elucidating the nature of polymicrobial infections.

Results

Systematic Quantification of Ecological Interactions. We systematically quantified all pairwise interactions between 72 bacterial isolates derived from 23 elderly persons showing symptoms of UTI which cultured polymicrobial communities. Each community isolated from the same host contained three to five distinct isolates (Fig. 1*A* and *SI Appendix*) (6). To identify key ecological properties of polymicrobial communities, we used a high-throughput approach; we measured the maximum growth yield (optical density) and the growth rate of all isolates in artificial urine medium (AUM). The medium was conditioned by growing single isolates for 48 h, recovering the supernatant, and replenishing it with nutrients (Fig. 1*B* and *SI Appendix*). We quantified interactions using the parameter $\varepsilon = \log(N_c/N_u)$; here, N_c and N_u are the growth yield in conditioned and in unconditioned medium, respectively; growth rate interactions are described analogously. Thus, a positive interaction ($\varepsilon > 0$) corresponds to an increase in growth compared with the reference medium (e.g., due to cross-feeding), and a negative interaction ($\varepsilon < 0$) corresponds to a decrease (Fig. 1 *C* and

Significance

In many infections, multiple microbial species are present simultaneously. Such polymicrobial infections can be viewed as small microbial ecosystems. Do bacteria in these communities interact with each other? If so, do these interactions affect the stability of the ecosystem, in particular, when antibiotics are present? We focus on urinary tract infections and demonstrate that there are ample ecological interactions between different bacterial species, both in the presence and absence of antibiotics. We further show that they crucially affect ecosystem stability and resilience to environmental perturbations such as antibiotics. Understanding the nature of these polymicrobial communities can point toward ways of disrupting infection ecosystems, which could potentially be used as a new strategy to eradicate infective communities.

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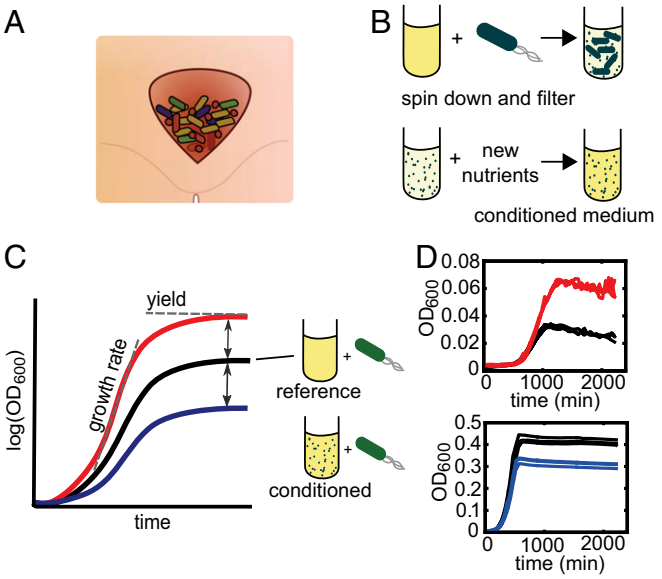

Fig. 1. Quantifying ecological interactions between bacteria isolated from polymicrobial UTIs. (*A*) Schematic illustrating a bladder environment infected with a community consisting of different bacterial species. (*B*) Preparation of conditioned medium for interaction experiments. Bacteria were grown in AUM for 48 h, followed by centrifugation, filtration, and replenishment with fresh nutrients. (*C*) Interaction measures from growth in conditioned medium. The growth in the conditioned medium is compared with the growth in the reference medium (black trace) (*SI Appendix*) for each of the 72 isolates. For positive interactions (red trace), the growth yield in conditioned medium (N_c) exceeds the growth in the reference medium (N_u), $\varepsilon = \log(N_c/N_u) > 0$. For negative interactions (blue trace), the growth in the conditioned medium (N_c) is exceeded by the growth in the reference medium (N_u), $\varepsilon = \log(N_c/N_u) < 0$. (*D*) Examples of growth curves in triplicate of *Enterococcus faecalis* (*Upper*) and *Citrobacter koseri* (*Lower*) in medium conditioned by *C. koseri* (colored traces) and reference medium (black traces). The growth of *E. faecalis* is positively affected by the conditioned medium (red curve above black curve), whereas *C. koseri* is negatively affected by medium conditioned by another isolate from the same species (blue curve below black curve).

D and *SI Appendix*, Fig. S1). Replenishing the conditioned medium with nutrients allowed us to disentangle the negative interactions. Relatively weak negative interactions [$\log(0.6) = -0.51 < \varepsilon < \log(1) = 0$] are likely due to resource overlap, and stronger negative interactions are likely due to warfare, e.g., via bacteriocins. To validate our interaction measurements using conditioned medium, we performed complementary assays—spot-on-lawn assays and coculture assays—which showed a good agreement with the conditioned medium assays (*SI Appendix*, Fig. S2).

We observed many positive and negative interactions between isolates from polymicrobial UTIs. When we used a stringent threshold for detecting significant interactions (*SI Appendix*), most interactions were neutral, but 18% of all possible interactions were positive, and nearly 40% of these led to a greater than twofold increase in growth yield ($\varepsilon > 0.7$) (*SI Appendix*, Fig. S1*A*). Similarly, 23% of all possible interactions were negative; most of these (78%) were relatively weak [$\log(0.6) = -0.51 > \varepsilon > \log(0.8) = -0.22$] (*SI Appendix*, Fig. S1). Bacterial interactions also affected the lag phase; many of these interactions led to a slight shortening of the lag phase (*SI Appendix*, Fig. S1 *D* and *E*). The frequent occurrence of interactions highlights the limitations of investigating individual bacteria in isolation when tackling polymicrobial infections.

Interactions Are Largely Determined by Phylogeny. Statistical analysis of the global network of ecological interactions revealed key features of interspecific relationships. Clustering the isolates by phylogenic distance (*SI Appendix*) showed that the interactions between pairs of isolates are largely determined by their respective genera, as the phylogenetic clusters coincide with similarly clustered interactions in the interaction matrix (*SI Appendix*, Figs. S1*C* and 2 *A* and *B*). This observation supports the view that these ecological interactions cluster based on evolutionary relatedness (30). Within-genus interactions are predominantly negative or neutral (Fig. 2*B* and *SI Appendix*, Fig. S3 and Table S2), consistent with the paradigm that closely related isolates tend to have negative interactions, often due to resource overlap (31). Among the clearest features in the interaction network is that enterococci and staphylococci benefit from many other genera but mostly do not affect the others in return (Fig. 2 *A* and *B*). In contrast, *Proteus mirabilis* affects many other genera negatively and is also often negatively affected by others, confirming previous reports (32). Few

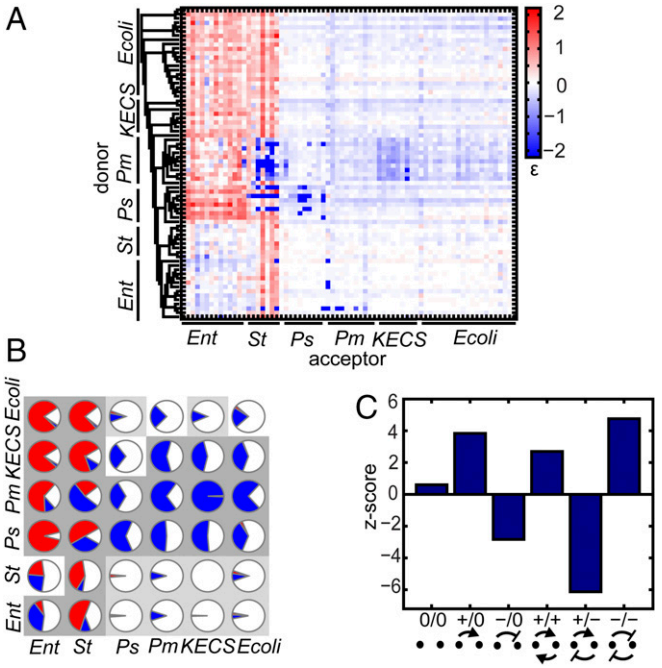

Fig. 2. The global UTI interaction network reflects species phylogeny and is enriched for competition but depleted of exploitation. (*A*) Pairwise interaction matrix depicting the interactions in yield (maximum reached OD_{600}) of 72 UTI isolates in conditioned medium prepared from these same isolates; the interaction measure is $\varepsilon = \log(N_c/N_u)$ (*SI Appendix*). The acceptors (columns) are grown in the conditioned medium of the donors (rows). Interactions cluster according to phylogeny, as can be seen from the 16S rDNA phylogenetic tree on the left. The isolates are symmetrically ordered on the horizontal and vertical axes. Ecoli, *E. coli*; Ent, *Enterococcus* spp. (*E. faecalis* and *E. faecium*); KECS, *Klebsiella* spp. (*K. pneumoniae*, *K. oxytoca*), *Enterobacter cloaca*, *Citrobacter koseri*, *Serratia liquefaciens*, and *Pantoea* sp4; Pm, *P. mirabilis*; Ps, *Pseudomonas* spp. (*P. aeruginosa*, *P. fluorescens*); St, *Staphylococcus* spp. (*S. aureus*, *S. haemolyticus*, *S. capitis*); one isolate belonging to *Morganella morganii* is located between *Pseudomonas* and *P. mirabilis*. The lower left to upper right diagonal depicts the self-interactions. (*B*) Statistical analysis of phylogenetically clustered interactions. Donors are ordered on the vertical axis and acceptors on the horizontal axis. Red and blue correspond to respective positive and negative interactions, respectively; dark and light gray backgrounds depict significant over- or underrepresentation of the most prominent interactions compared with randomized matrices. A white background depicts nonsignificant over- or underrepresentation (*SI Appendix*). Statistics on *Morganella morganii* are omitted due to small sample size ($n = 1$). (*C*) Statistical analysis of all two-node motifs in the measured interaction network. Reciprocal interactions with the same sign (–/– and +/+) and commensalism (+/0) are overrepresented, whereas amensalism (–/0) and exploitation (+/–) are depleted. *Z* scores were calculated based on the comparison with an ensemble of random networks preserving the degree of distribution (*SI Appendix*).

individual isolates display interactions that deviate from the general trends for the genera (Fig. 2 *A* and *B* and *SI Appendix*, Fig. S1*C*). Hence, these data indicate that most ecological interactions in these polymicrobial communities are conserved. This suggests that despite the high potential for genotypic diversity within these groups, these ecological interactions are deep-branching and possibly ancestral.

The interaction strength distributions differ qualitatively between the genera. Specifically, the strength distribution of outgoing negative interactions is either unimodal, peaking between $\varepsilon = \log(1) = 0$ and $\varepsilon \approx \log(0.8) = -0.22$ and dropping to zero around $\varepsilon \approx \log(0.6) = -0.51$ (for *Escherichia coli*, *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Citrobacter*), or bimodal with a second peak near zero (for *P. mirabilis* and *Pseudomonas*) (*SI Appendix*, Fig. S4). The interaction parameter $\varepsilon = \log(0.6)$ corresponds to complete resource overlap, while lower values indicate active growth-inhibiting mechanisms (*SI Appendix*).

Reciprocal Interactions, in Particular Competition, Are Enriched in the Interaction Network. It is a fundamental question if microbes in polymicrobial infections predominantly cooperate or compete with each other (4). To systematically identify such interaction patterns between pairs of isolates, we compared the occurrence of two-node subnetworks in the measured interaction network to that in an ensemble of randomized networks that preserve the degree distribution (*SI Appendix*, *SI Methods*). This analysis revealed that reciprocal interactions are generally enriched: Competitive (−/−) and cooperative (+/+) interactions are significantly overrepresented in the measured network (−/−, z score 4.76, $P < 10^{-5}$; +/+, z score 2.70, $P = 0.007$) (Fig. 2*C* and *SI Appendix*), an interesting similarity to *Streptomyces* communities in soil (24). The enrichment of competitive interactions supports the general predominance of competitive interactions in ecosystems (23). In addition, exploitive (+/−) interactions are extremely underrepresented (z score −6.13, $P < 10^{-9}$) (Fig. 2*C* and *SI Appendix*). Mutual interactions within each genus are generally enriched for competition and amensalism and depleted of cooperation and commensalism (*SI Appendix*, Fig. S3 and Table S2), supporting the paradigm that competition is the dominant interaction type between closely related isolates (33, 34); the sole exception is *Staphylococcus* (*SI Appendix*, Fig. S3 and Table S2). Overall, this analysis shows that a predominance of reciprocal interactions, in particular competition, is a hallmark of the polymicrobial UTI ecosystem.

Communities Are More Enriched for Competition. We next asked if the complete communities from each host contained ecological signatures that point to specific community structures by investigating the distribution of isolates in these communities and the interactions among these isolates. To address this question, we included only isolates from communities that were complete, i.e., those for which all isolates from one host were in our studied panel of 72 isolates (Fig. 3*B* and *SI Appendix*, Fig. S5*A* and Table S1). The distribution of genera in the communities derived from individual hosts was not random: enterococci and *E. coli* occurred together significantly more frequently than would be expected from random occurance ($P = 0.004$) (*SI Appendix*, Fig. S5*B*), in accordance with previous reports (35). Thus, these likely do not reflect random invasion events by UTI strains from the gut (36). Compared with random isolate assemblies, the complete host communities further tended to be enriched for negative interactions (Fig. 3*A* and *SI Appendix*, Fig. S5*C*) and competition, and depleted of exploitation and cooperation (Fig. 3*A* and *SI Appendix*). These trends for increased competition in communities from the same site are opposite those for *Streptomyces* communities on soil grains (24) but are consistent with findings on *Bacillus* spp. from lagoons (26) and the microbiome (15). The predominance of competitive interactions between coexisting isolates in UTIs defies the naive expectation that bacteria in these communities are selected based on their ability to cooperate.

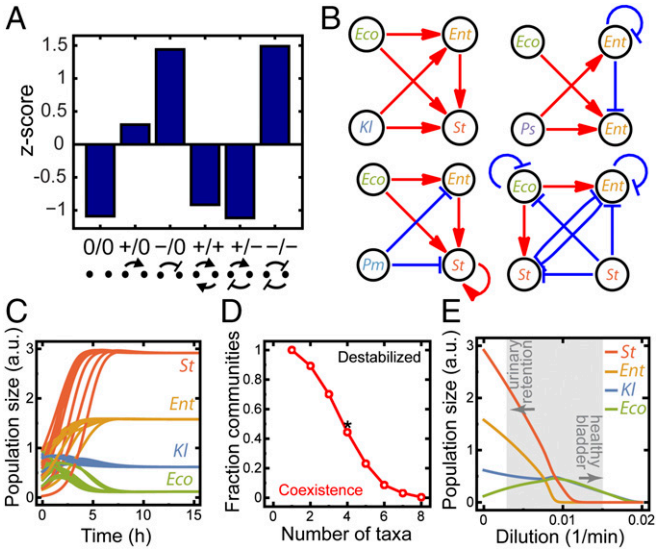

Fig. 3. Polymicrobial UTI communities are often ecologically stable, and the number of taxa per community is close to the limit of stability. (*A*) Interactions within complete communities compared with interactions in an ensemble of randomized communities (*SI Appendix*). Competitive interactions (−/−) are slightly enriched, while exploitation (+/−) is slightly depleted in communities. No cooperative interactions (+/+) occur in the communities (*SI Appendix*). (*B*) Intracommunity microbial interactions in four stable communities. Each depicted complete community consists of all taxa that were isolated from one host. Red arrows indicate positive interactions; blue arrows indicate negative interactions. Eco, *E. coli*; other abbreviations are as in Fig. 2. (*C*) Examples of population dynamics for one of the stable communities with one fixed point in the absence of voiding. Lines of the same color show independent simulations starting from different initial conditions (*SI Appendix*); the initial population size and inoculation order do not affect the final population size. (*D*) Fraction of stable randomly assembled communities as a function of the number of taxa in a community. The stability of the communities drops with increasing number of taxa. The observed number of four to five taxa is close to the typical number of taxa of randomly assembled stable communities. The black asterisk indicates the number of taxa and fraction of observed coexisting communities. (*E*) Steady-state population size for different UTI species as a function of the effective dilution rate for a stable community (*C* and *SI Appendix*). The effective dilution rate (mimicking repeated voiding of the bladder) markedly affects the stability of the community. The gray band shows the voiding patterns ranging from a healthy bladder to a bladder with an increased postvoid residual urine volume (*SI Appendix*, Fig. S9).

Ecologically Stable Communities Can Be Destabilized by Targeted Invaders. To elucidate the underlying principles and dynamics of UTI community assembly, we next investigated the ecological stability of the complete communities (*SI Appendix*, Table S1). To this end, we generalized an ecological logistic growth model to capture interactions at the level of both growth rate and yield (*SI Appendix*). All parameters entering this model are constrained by our quantitative interaction data (Fig. 2*A* and *SI Appendix*, Fig. S1*C*). Further, the stability of communities in this model restricted to growth yield interactions only corresponds to predictions of the well-established Lotka–Volterra–Gause model for interacting species (*SI Appendix*). For simplicity, we assumed that interactions are additive when more than two isolates are present. This model thus provides a theoretical description of population dynamics in polymicrobial communities and makes quantitative predictions for the stability and dynamics of arbitrary assemblages of isolates.

UTI communities from the same host are often stable and can assemble in any order (Fig. 3*C* and *SI Appendix*, Fig. S6). We found that four of eight complete communities consisting of four different isolates have at least one stable fixed point (attractor) in which all isolates coexist according to the predictions of the model,

whereas the other four complete communities do not allow for stable coexistence (*SI Appendix*, Fig. S5*A*). While two stable communities can be assembled isolate-by-isolate in any order (i.e., all intermediate communities are stable), two of the four communities that allow for stable coexistence had an additional unstable fixed point. This implies that some initial population densities are prohibited for stable community formation with all four members, as they lead to the loss of at least one community member. We experimentally tested the stability of these eight communities in coculture experiments by the serial transfer of the communities. All community members were maintained for two of the four predicted stable communities. In contrast, all other communities consisted of only two remaining community members; four of these were predicted to be unstable, and two were predicted to reach the stable fixed point in 99% and 84% of the cases, respectively. Therefore, the model successfully predicted whether or not all isolates in the community can coexist stably in six of the eight communities, 75% of cases (*SI Appendix*, Fig. S7). The presence of a single attractor in which all community members coexist implies that the community is resilient: It returns to this attractor and thus maintains coexistence even upon large perturbations, such as caused by a short antibiotic treatment. The fraction of communities that is predicted to allow for stable coexistence is not significantly different from that of randomly assembled communities ($P \sim 0.9$, 1,000 sets of eight randomly assembled communities) (Fig. 3*D* and *SI Appendix*). Overall, these results suggest that many possible combinations of UTI isolates could, in principle, form stable communities.

It is of key relevance for probiotic treatments if stable communities can be invaded and potentially destabilized by newly arriving benign bacteria. We thus described invasion attempts by adding a few individuals of invading bacteria and calculated the ensuing community dynamics. We found that many randomly chosen invading UTI strains can coexist with the complete host communities (on average 45%), but in a considerable fraction (~30%) one of the original isolates is replaced by the invader. About 15% of invasion attempts fail, resulting in the loss of the invader. In less than 10% of cases the invader can destabilize the community, leading to the loss of more than one community member (*SI Appendix*, Table S3). Thus, invaders are often incorporated in the community or switch sides with community members, suggesting that UTI communities are dynamically stable. Benign bacteria could thus potentially replace pathogenic community members.

The Stability of the Communities Declines with Increasing Number of Taxa. Our ecological model makes a prediction for the maximum number of different taxa in a stable UTI community. The relation between stability and the number of community members is at the heart of a long-standing debate (17, 37). According to our model, the stability of UTI communities declines with increasing number of randomly chosen UTI isolates (*SI Appendix*). Starting with pairs of strains, 90% of communities with two members are stable. We observe the same fraction of stable pairs in a corresponding Lotka–Volterra–Gause model (*SI Appendix*, Fig. S8). For a community with four members 50% of the communities are stable, but this fraction drops to around 25% for five community members and falls below 2% for a community with eight members (Fig. 3*D*). The predicted relatively high fraction of stable communities for a community of four or five isolates agrees with the number of different isolates derived from polymicrobial UTIs (6) and suggests that larger communities may be unsustainable.

Community stability also depends on the voiding and refilling of the bladder, which removes and effectively dilutes the UTI bacteria. When this effect is included in the ecological model, all bacteria are washed out if the effective dilution rate exceeds a critical value (*SI Appendix*) (38). In our model, *E. coli* is consistently among the last two strains that can persevere at the highest dilution rates (Fig. 3*E* and *SI Appendix*, Fig. S9). This is interesting, since uropathogenic *E. coli* are known to attach to the bladder and gut lining to prevent washing out in vivo (39, 40). Here, in agreement with earlier findings (38), we show that their inherent relatively larger growth rate already seems to give them a considerable advantage for growth and survival under these conditions. For lower dilution rates, communities with an increasing number of different taxa are stably maintained. This effect can explain the higher incidence of polymicrobial UTIs in elderly men who have an increased bladder "dead volume" which effectively reduces the dilution rate (Fig. 3*E* and *SI Appendix*, Fig. S9) (41) and contributes to shifting the female versus male incidence ratio from 30:1 to 2:1 in the elderly (42). Indeed, using plausible estimates for the effective dilution rate in our model, infections in individuals with urinary retention allow the stable coexistence of polymicrobial communities, while those in healthy individuals tend to be clonal (Fig. 3*E* and *SI Appendix*, Fig. S9). Overall, these results indicate that ecological interactions and dilution together determine the stability of polymicrobial communities and constrain their maximum size.

Bacterial Interactions Affect Antibiotic Tolerance. The fact that UTIs are often treated with antibiotics (43) raises the question if ecological interactions alter the efficacy of antibiotics. We therefore measured the effects of media conditioned by 14 different donor isolates on all 72 acceptor isolates in the presence of two different antibiotics that are commonly used for the treatment of UTIs: trimethoprim-sulfamethoxazole (44, 45) and nitrofurantoin (46). The donor isolates were selected to represent the diversity of the genera in our dataset. We assessed the fold change in antibiotic tolerance by comparing the concentrations of antibiotics that sustained growth in the conditioned medium and in the unconditioned reference medium (Fig. 4 *A* and *B* and *SI Appendix*, Fig. S10*A*).

The clinical isolates often protect each other from antibiotics. We observed >3.5-fold increases in the tolerance to trimethoprim-sulfamethoxazole for 25% of tested interactions but decreases of

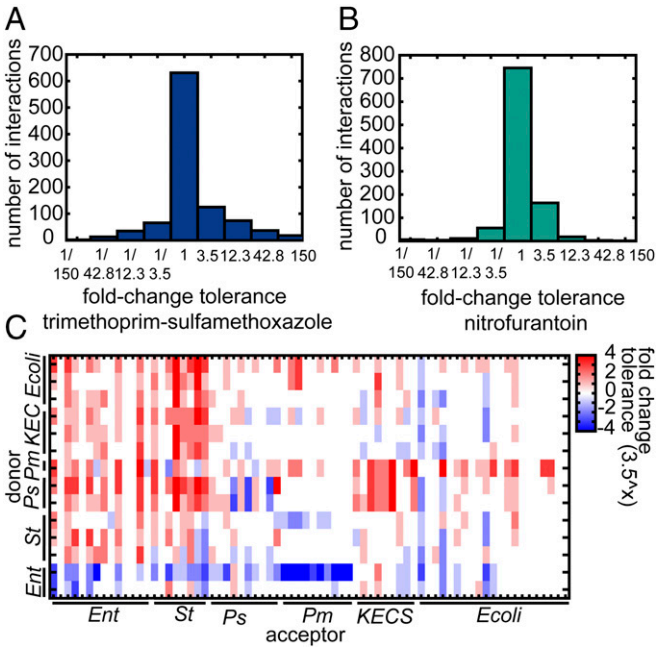

Fig. 4. Bacteria in polymicrobial UTIs frequently protect each other from antibiotics. (*A*) Histogram of the change in tolerance of 72 isolates in 14 conditioned media to the trimethoprim-sulfamethoxazole combination (*SI Appendix*, Fig. S10*A*). (*B*) As in *A*, but for nitrofurantoin. (*C*) Interaction matrix depicting the effect on the tolerance to the trimethoprim-sulfamethoxazole combination of 14 conditioned media on 72 isolates in conditioned medium (*SI Appendix*). Abbreviations are as in Fig. 2.

the same magnitude in only 12% of cases (Fig. 4*A* and *SI Appendix*, Fig. S10*A*); this trend was similar for nitrofurantoin (18% and 8%, respectively) (Fig. 4*B* and *SI Appendix*, Fig. S10*A*). The protection effects were often correlated with positive interactions in the absence of antibiotics. However, 13% of the protective interactions for trimethoprim-sulfamethoxazole were neutral in the absence of antibiotics, whereas for nitrofurantoin 9% of these protective interactions were silent in the environment without antibiotics (*SI Appendix*, Fig. S10 *B* and *C*). Notably, the protective effect on *Enterococcus* spp. and *Staphylococcus* spp. corresponds with their positive incoming interactions in the absence of antibiotics (Figs. 2*B* and 4*C* and *SI Appendix*, Fig. S10*E*). Interestingly, *Enterococcus* spp. returned the favor by decreasing the antibiotic tolerance of several other isolates (Fig. 4*C* and *SI Appendix*, Fig. S10*E*). *P. mirabilis* predominantly protected other isolates from antibiotics (Fig. 4*C* and *SI Appendix*, Fig. S10*E*), while this species mostly harmed others in the absence of antibiotics (Fig. 2*B*). *P. mirabilis* produces ammonia and increases the pH of the medium; its protective effect is thus consistent with reports that ammonia and an increased pH in the environment decrease the efficacy of antibiotics (47–49). We further found that drug tolerance increased more strongly the less resistant the strain was to the drug (Pearson's $\rho = -0.25$ for trimethoprim-sulfamethoxazole and $\rho = -0.33$ for nitrofurantoin, $P < 0.01$ for both antibiotics), and the increase in tolerance to both antibiotics was correlated ($\rho = 0.24$, $P < 0.01$). Thus, UTI communities exhibit hallmarks of collective drug tolerance (29, 50): The presence of community members tends to protect other isolates from antibiotics, with the most sensitive strains experiencing the greatest benefit.

Communities may be assembled such that collective antibiotic tolerance is boosted. We found that isolates from the same community tend to protect each other from antibiotics slightly more frequently than isolates from other communities, while the fraction of interactions that increase antibiotic sensitivity is smaller within communities than between communities (*SI Appendix*, Fig. S10*D*). Thus, negative interactions on antibiotic tolerance in communities are underrepresented, in contrast to the interaction between community members in the absence of antibiotics, where negative interactions are overrepresented. Taken together, these data show that ecological interactions affect the growth and survival of the community members in the absence and presence of antibiotics.

Discussion

We elucidated the ecological interaction network of bacteria from elderly persons diagnosed with polymicrobial UTIs. In contrast to the gut microbiome (15), the number of different taxa in a UTI community is relatively small (6), and most of the isolates are readily cultured in the laboratory (Fig. 1*D*) (51). This enabled us to measure all pairwise interactions within these infection communities and establishes polymicrobial communities as a fruitful and relatively simple model system that enables a uniquely quantitative approach to elucidating key principles of ecology.

Understanding the ecology of polymicrobial interactions may provide opportunities for new treatment strategies. We found that UTI communities can be destabilized by a targeted invader bacterium, although the odds of destabilization are smaller than the odds for the coexistence of the invader in the community. Quantitative measurements of ecological interaction networks and ecological models as presented here may therefore guide the design of synthetically modified strains that can destabilize the infection community (52).

Although our standardized high-throughput measurements allowed us to investigate the bacterial interactions in great detail, this study has the general limitations of any reductionist approach in that it cannot capture the full complexity of the host environment. Specifically, the composition of the host's urine can vary in time and between hosts (53); it will thus deviate from our standardized AUM, which allows us to reproducibly measure the bacterial interactions. In addition, as donor and acceptor bacteria were not in physical contact in our assay, additional contact-mediated interactions (54) may affect in vivo interactions. Moreover, higher-order interactions between more than two bacteria could potentially affect community stability (20), and the complexity of such interactions may limit the predictability of simple pairwise models (55). However, a recent study found that pairwise interactions largely predict the population dynamics of larger communities (56).

In vivo, UTIs could be further complicated by host–pathogen interactions (57–59) and the microbiogeography (60, 61). Specifically, bacteria are known to live planktonically in the bladder (62), but they may also attach to or invade the bladder lining (43). In this case, the spatial structure and cell–cell contact in the community may affect the outcome of the interactions (63–65). Moreover, cooperative interactions such as the active degradation of antibiotics by other community members [e.g., by β-lactamases (28)] may also play a role. Future studies are needed to investigate these additional factors that can affect bacterial interactions in the host and illuminate the underlying molecular mechanisms, in particular by identifying the molecules excreted by the different bacteria and the gene-regulatory responses they trigger in other bacteria. For instance, it is striking that the Gram-positives (*Enterococcus* and *Staphylococcus*) are generally more positively affected by others than the Gram-negatives, which experience more negative interactions. Also, the interactions are conserved among the genera, which seems to suggest that the interactions between UTI isolates are caused by their core metabolism and signaling functions. In addition, it will be interesting to elucidate the underlying mechanisms of the observed antibiotic tolerance, in particular since it does not necessarily correlate with the general persistence or tolerance to antibiotics that occurs with slower growth (66). Therefore, to shed light on whether the interactions between bacteria derived from polymicrobial infections are preserved in vivo and on the additional contributions from interactions between host and microbes, an exciting challenge will be to extend this ecological approach to in vivo models of UTIs and other polymicrobial infections. In conclusion, this work shows that polymicrobial infective communities can and should be viewed as small ecosystems, as the emergent properties in these ecosystems can dictate the growth and survival of the individual bacterial community members.

Materials and Methods

UTI Isolates and Growth Medium. UTI isolates were collected anonymously in a previous study (6). Therefor no ethical approval or informed consent was required. Seventy-two isolates derived from 23 hosts, including eight complete communities isolated from eight hosts, were cultured in AUM (51).

Conditioned Medium Preparation. Spent medium was prepared by culturing isolates in AUM, followed by centrifugation and filtering. To prepare conditioned medium, the AUM spent medium fraction was mixed with AUM medium. The spent medium fraction was replenished with AUM constituents, such that the concentration in the final conditioned medium ranged from 0.6× (for constituents that were consumed in the spent medium) to 1× (for constituents that were not consumed in the spent medium) of the concentration in AUM.

Growth Measurements. Each isolate was incubated for 36 h in one well of a 96-well plate containing 200 μL of prewarmed medium. Cultures were inoculated by ∼0.2 μL from a thawed overnight culture stored at −80 °C. The plates were incubated at 30 °C and shaken at 720 rpm in aerobic conditions. OD_{600} was recorded every ∼44 min.

Statistical Network Analysis and Community Analysis. The enrichment or depletion of subgraphs in the UTI network was quantified with respect to ensembles of randomized global networks or host communities. Different randomized ensembles were used depending on the specific problem. Using the obtained distributions, we calculated the random expectation and the corresponding *P* value (two-tailed) of observing a given pair of genera in the original data.

Theoretical Model of Ecological Interactions. We developed a minimal theoretical model that is based on established models (67, 68) of logistic growth in liquid batch culture without spatial structure, which is parameterized by our measurements. This model is used to determine both the dynamics of the growing bacterial populations and the number of isolates in steady state.

Additional information is provided in *SI Appendix*.

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