Selective pressure of antibiotic pollution

SUPPLEMENTAL MATERIAL

Selective Pressure of Antibiotic Pollution on Bacteria of Importance to Public Health

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Correlation structure of MIC datasets

An implicit assumption in the use of SSDs is that taxa used to derive them represent independent observations drawn from a random distribution (Suter et al. 2002). To minimize the lack of independence between observations, several methods and researchers recommend combining the endpoints from co-generic species (see Suter et al. 2002). Mantel tests (Mantel 1967; Legendre and Legendre 1998) were used to explore the correlation between evolutionary distances and pairwise differences in median MICs between taxa for each antibiotic dataset. Figure 1 shows Mantel correlograms for each antibiotic, along with the Mantel correlation coefficient for the entire dataset in the upper left hand side of each graph (i.e., $R_M$). Grouping data from co-generic species to minimize the lack of independence before SSD derivation seemed advisable for the ciprofloxacin dataset, whereas there did not seem to be a statistical ground for doing so in the erythromycin and tetracycline datasets. In this study we decided to group co-generic species in all datasets in order to apply a consistent methodology in the derivation of SSDs for all three antibiotics that would facilitate the subsequent interpretation and discussion of results.

Supplemental Material, Figure S1. Mantel correlograms describing the correlation between MIC$_{50}$ and evolutionary distance for ciprofloxacin (a), erythromycin (b) and tetracycline (c). Solid dots represent a significant correlation at the corresponding evolutionary distance ($\alpha = 0.05$). Significance tests are based on 5000 permutations.
Supplemental Material, Table S1

Taxa represented in EUCAST MIC datasets. Cross (+) and dash (-) symbols indicate presence or absence of each entry in the given EUCAST MIC antibiotic dataset, respectively. The All-species Living Tree Project names of the subset of species which were represented in the LTP 16S rRNA database are given in the ‘LTP Name’ column. MIC data from co-generic species in each dataset were pooled to derive SSDs.

| Species                              | Ciprofloxacin | Erythromycin | Tetracycline | LTP Name     |
|--------------------------------------|---------------|--------------|--------------|--------------|
| Acinetobacter anitratus              | +             | -            | -            |              |
| Acinetobacter baumanii               | +             | -            | +            | AcnBau29     |
| Acinetobacter lwaffii                | +             | -            | -            | AcnLwo34     |
| Acinetobacter calcoaceticus          | +             | -            | -            | AcnCal77     |
| Acinetobacter spp                    | +             | -            | +            |              |
| Alcaligenes xylosoxidans             | +             | -            | -            |              |
| Bacteroides fragilis                 | +             | -            | -            |              |
| Bacteroides fragilis group           | +             | +            | -            |              |
| Bacteroides ovatus                   | -             | -            | -            | BcdOvat8     |
| Bacteroides thetaiotaomicron         | -             | -            | -            | BcdThe32     |
| Bacteroides vulgatus                 | -             | -            | -            | BcdVulg9     |
| Burkholderia cepacia                 | +             | -            | -            | BurCe154     |
| Bifidobacterium longum               | -             | +            | +            | BifLon13     |
| Bifidobacterium pseudolongum         | -             | -            | +            | BifPseu8     |
| Bifidobacterium thermophilum         | -             | -            | +            |              |
| Campylobacter coli                   | +             | +            | +            | CampCol5     |
| Campylobacter jejuni                 | +             | +            | +            | CampJejun    |
| Chryseobacterium meningosepticum     | +             | -            | -            |              |
| Chryseobacterium spp                 | +             | -            | -            | CitBraak     |
| Citrobacter braakii                  | -             | -            | +            |              |
| Citrobacter freundii                 | -             | +            | -            | CitFre32     |
| Citrobacter koseri                   | -             | -            | +            |              |
| Citrobacter spp                      | +             | -            | +            |              |
| Clostridium difficile                | -             | +            | +            | CloDiff5     |
| Clostridium perfringens              | -             | +            | -            | CloPe172     |
| Enterobacter aerogenes               | +             | -            | +            | EntAero9     |
| Enterobacter agglomerans             | +             | -            | +            | EntClo58     |
| Enterobacter cloacae                 | +             | -            | +            | EntClo52     |
| Enterobacter dissolvens              | +             | -            | +            |              |
| Enterobacter spp                     | +             | -            | +            |              |
| Enterococcus avium                   | -             | -            | -            | EnrAviu7     |
| Enterococcus casseliflavus           | -             | -            | -            | EnrCas20     |
| Enterococcus faecalis                | +             | +            | +            | EnrFa149     |
| Enterococcus faecium                 | +             | +            | +            | EnrFa232     |
| Enterococcus gallinarum              | -             | -            | -            | EnrGall8     |
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### Supplemental Material, Table S1 (continued)

| Species                     | Ciprofloxacin | Erythromycin | Tetracycline | LTP Name  |
|-----------------------------|---------------|--------------|--------------|-----------|
| *Enterococcus hirae*        | -             | +            | +            | EnHir11   |
| *Escherichia coli*          | +             | -            | +            | EschCo52  |
| *Haemophilus influenzae*    | +             | +            | +            | HaeIn157  |
| *Haemophilus parainfluenzae*| +             | +            | +            | HaePara4  |
| *Hafnia alvei*              | +             | -            | +            | HafAlv4   |
| *Helicobacter pylori*       | +             | -            | +            | HelPyl55  |
| *Klebsiella oxytoca*        | +             | -            | +            | KleOxy19  |
| *Klebsiella pneumoniae*     | +             | -            | +            | KlePn101  |
| *Klebsiella spp*            | +             | -            | +            |           |
| *Kluyvera spp*              | -             | +            | +            |           |
| *Legionella pneumophila*    | +             | +            | -            | LgnPne10  |
| *Listeria monocytogenes*    | +             | -            | +            | LstMon13  |
| *Manhemia haemolytica*      | -             | -            | +            | ManHaem8  |
| *Moraxella catharrhalis*    | +             | +            | +            | MorCatar  |
| *Morganella morganii*       | +             | -            | +            | MrgMor25  |
| *Mycobacterium tuberculosis*| +             | -            | -            | MycTube9  |
| *Neisseria gonorrhoeae*     | +             | +            | +            | NeiGono5  |
| *Neisseria meningitidis*    | +             | -            | +            |           |
| *Pasteurella multocida*     | +             | +            | +            | PsuMult6  |
| *Peptostreptococcus spp*    | -             | +            | -            |           |
| *Propionibacterium acnes*   | -             | -            | +            | ProAcn42  |
| *Proteus mirabilis*         | +             | -            | +            | PtsMir19  |
| *Proteus vulgaris*          | +             | -            | +            | PtsVulg9  |
| *Proteus spp*               | -             | -            | +            |           |
| *Providencia rettgeri*      | -             | -            | -            | PrvRett3  |
| *Providencia stuartii*      | +             | -            | -            | PrvStua5  |
| *Providencia spp*           | +             | -            | -            |           |
| *Pseudomonas aeruginosa*    | +             | -            | +            | PseAe290  |
| *Pseudomonas fluorescens*   | +             | -            | -            | PseFl192  |
| *Raoultella spp*            | +             | -            | +            |           |
| *Raoultella spp*            | -             | -            | +            |           |
| *Salmonella enteritidis*    | -             | -            | -            |           |
| *Salmonella paratyphi*      | -             | -            | -            |           |
| *Salmonella typhi*          | -             | -            | -            |           |
| *Salmonella typhimurium*    | -             | -            | -            |           |
| *Salmonella spp*            | +             | -            | +            |           |
| *Serratia liquefaciens*     | +             | -            | +            | SerLiqu3  |
| *Serratia marcescens*       | +             | -            | +            | SerMa116  |
| *Serratia spp*              | +             | -            | +            |           |
| *Shigella sonnei*           | -             | -            | -            | StaAur61  |
| *Staphylococcus aureus*     | +             | +            | +            |           |
| *Staphylococcus aureus MRSA*| +             | +            | +            |           |
| *Staphylococcus aureus MSSA*| +             | +            | +            |           |
| *Staphylococcus auricularis*| +             | -            | -            | StaAuri2  |
| *Staphylococcus capitis*    | +             | +            | -            | StaCap14  |
Supplemental Material, Table S1 (continued)

| Species                                      | Ciprofloxacin | Erythromycin | Tetracycline | LTP Name     |
|----------------------------------------------|--------------|--------------|--------------|--------------|
| *Staphylococcus coagulase negative* MRSE    | +            | +            | +            |              |
| *Staphylococcus coagulase negative* MRSE    | +            | +            | +            |              |
| *Staphylococcus cohnii*                     | +            | -            | -            | StaCohn9     |
| *Staphylococcus epidermidis*                | +            | +            | +            | StaEpi72     |
| *Staphylococcus epidermidis MSSE*           | +            | +            | +            |              |
| *Staphylococcus haemolyticus*               | +            | +            | +            | StaHae18     |
| *Staphylococcus hominis*                    | +            | +            | -            | StaHom26     |
| *Staphylococcus hyicus*                     | +            | +            | -            | StaHyicu     |
| *Staphylococcus intermedius*                | +            | +            | -            | StaInte4     |
| *Staphylococcus lugdunensis*                | +            | -            | -            | StaLugd4     |
| *Staphylococcus saprophyticus*              | +            | +            | +            | StaSap31     |
| *Staphylococcus sciuri*                     | +            | -            | -            | StaSci15     |
| *Staphylococcus simulans*                   | +            | -            | -            | StaSimul     |
| *Staphylococcus warneri*                    | +            | +            | -            | StaWarne     |
| *Staphylococcus xylosis*                    | +            | -            | -            | StaXyl10     |
| *Streptococcus maliophila*                  | +            | -            | +            | SteMa216     |
| *Streptococcus acidominimus*                | +            | -            | -            |              |
| *Streptococcus agalactiae*                  | +            | +            | +            | StpAga25     |
| *Streptococcus anginosus*                   | +            | +            | +            | StpAng35     |
| *Streptococcus bovis*                       | +            | -            | -            |              |
| *Streptococcus constellatus*                | +            | -            | -            | StpCon17     |
| *Streptococcus equinus*                     | +            | -            | -            | StpEqui4     |
| *Streptococcus gordonii*                    | +            | -            | -            | StpGor11     |
| *Streptococcus group C*                     | +            | -            | -            |              |
| *Streptococcus group G*                     | +            | +            | +            |              |
| *Streptococcus intermedius*                 | +            | -            | -            | StpInte8     |
| *Streptococcus milleri*                     | +            | +            | -            |              |
| *Streptococcus mitis*                       | +            | -            | -            | StpMit32     |
| *Streptococcus oralis*                      | +            | +            | +            | StpOra18     |
| *Streptococcus parasanguis*                 | +            | -            | -            | StpPar15     |
| *Streptococcus pneumoniae*                  | +            | +            | +            | StpPne46     |
| *Streptococcus pyogenes*                    | +            | +            | +            | StpPyo39     |
| *Streptococcus salivarius*                  | +            | -            | -            | StpSali6     |
| *Streptococcus sanguinis*                   | +            | -            | -            | StpSan11     |
| *Streptococcus thermophilus*                | +            | +            | +            | StpUber5     |
| *Streptococcus uberis*                      | +            | -            | -            |              |
| *Streptococcus viridans*                    | +            | +            | +            |              |
| Yersinia spp                                 | +            | -            | +            |              |
| **Total**                                    | **91**       | **40**       | **67**       | **79**       |
| **16S rRNA sequences**                      | **63 (70%)** | **28 (70%)** | **43 (64%)** |              |
Supplemental Material, Section S2

Evidence for growth of selected genera in the environment

There is evidence to suggest that several bacteria of importance to public health may, under certain conditions, grow in different environments. Hendricks (1972), for example, showed that some Enterobacteriaceae could grow in water collected downstream of a municipal sewage facility at temperatures as low as 5°C. Gibbs et al. (1997) reported years later the re-growth of faecal coliforms and Salmonella in biosolids and soil ammended with biosolids. The populations of E. coli in manured soils can be very dynamic (Topp et al. 2003) and Inglis et al. (2010) recently showed that Campylobacter can persist for long periods of time in compost, which may suggest cryptic growth. Enterococci have been shown to grow in municipal oxidation ponds (Moriarty et al. 2008), and the facultative intracellular pathogen Listeria monocytogenes has been shown to be widespread in certain catchments (Lyautey et al. 2007) and able to grow in soil suspensions characteristic of certain organic fractions (Sidorenko et al. 2006). Staphylococci, including Staphylococcus aureus, have been isolated from marine water samples (Gunn and Colwell 1983), and methicillin – resistant Staphylococcus aureus was recently isolated from marine water and intertidal sand from beaches on the west coast of the USA (Soge et al. 2009). S. aureus has also been shown to be capable of growth in sterile soil (Liang et al. 1982), suggesting that it might be possible for it to grow in this environment under conditions of low competition. Ayyadurai et al. (2008) recently demonstrated that Yersinia pestis remained viable and fully virulent in humid sand for 40 weeks. Clostridium difficile is widely distributed in the environment (Al Saif and Brazier 1996), and it is not unreasonable to speculate that there may be niches that could
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support its sporulation. Collectively, these studies highlight there is a potential for growth in bacteria of clinical relevance in different environments and under varying biological and physicochemical conditions, even if only during a short temporal window. In the presence of antibiotics, wild-type populations may be inhibited to various extents, increasing the prevalence of resistance. Full references upon which we based our decision to include genera for SSD derivation are given in Table S2.

**Supplemental Material, Table S2**

Bacterial genera represented in the SSDs. References provide either direct evidence of growth in the environment or evidence that suggests that under certain conditions it is possible for growth to occur.

| Genus            | Reference                                      |
|------------------|------------------------------------------------|
| 1 Acinetobacter  | Madigan et al. 2009                           |
| 2 Alcaligenes    | Agerso et al. 2005; Madigan et al. 2009        |
| 3 Burkholderia   | Madigan et al. 2009                           |
| 4 Campylobacter  | Brandl et al. 2004; Inglis et al. 2010         |
| 5 Chryseobacterium | Vandamme et al. 1994                       |
| 6 Citrobacter    | Madigan et al. 2009; Kitts et al. 1994         |
| 7 Clostridium    | A Saif and Brazier 1996; Madigan et al. 2009   |
| 8 Enterobacter   | Hendricks 1972; Rattray et al. 1995; Hernandez et al. 1998 |
| 9 Enterococcus   | Mundt 1961/1963; Desmarais 2002; Moriarty et al. 2008 |
| 10 Escherichia   | Hendricks 1972; Gibbs et al. 1997; Topp et al. 2003; Zaleski et al. 2005 |
| 11 Hafnia        | Jand 2006                                      |
| 12 Klebsiella    | Liang et al. 1982; Zadoks et al. 2011 (and references therein) |
| 13 Kluyvera       | Janda 2006                                     |
| 14 Legionella     | Fliermans et al. 1981                         |
| 15 Listeria       | Sidorenko et al. 2006; Lyautey et al. 2007     |
| 16 Morganella     | Kitts et al. 1994                             |
| 17 Pasteurella    | Bredy and Botzler 1989                        |
| 18 Proteus        | Hendricks 1972                                |
| 19 Providencia    | Kitts et al. 1994                             |
| 20 Pseudomonas    | Madigan et al. 2009                           |
| 21 Raoultella     | Zadoks et al. 2011 (and references therein)    |
| 22 Salmonella     | Liang et al. 1982; Gibbs et al. 1997          |
| 23 Serratia       | Madigan et al. 2009                           |
| 24 Staphylococcus | Liang et al. 1982; Gunn and Colwell 1983; Soge et al. 2009 |
| 25 Stenotrophomonas | Bollet et al. 1995                         |
| 26 Streptococcus  | Gledhill and Casida 1969                      |
| 27 Yersinia       | Sidorenko et al. 2006; Ayyadurai et al. 2008  |
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