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Determining the prevalence, identity and possible origin of bacterial pathogens in soil

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Summary

Soil biomes are vast, exceptionally diverse and crucial to the health of ecosystems and societies. Soils also contain an appreciable, but understudied, diversity of opportunistic human pathogens. With climate change and other forms of environmental degradation potentially increasing exposure risks to soilborne pathogens, it is necessary to gain a better understanding of their ecological drivers. Here we use the *Galleria mellonella* insect virulence model to selectively isolate pathogenic bacteria from soils in Cornwall (UK). We find a high prevalence of pathogenic soil bacteria with two genera, *Providencia* and *Serratia,* being especially common. *Providencia alcalifaciens*, *P. rustigianii, Serratia liquefaciens* and *S. plymuthica* strains were studied in more detail using phenotypic virulence and antibiotic resistance assays and whole-genome sequencing. Both genera displayed low levels of antibiotic resistance and antibiotic resistance gene carriage. However, *Serratia* isolates were found to carry the recently characterized metallo-β-lactamase *bla*SPR-1 that, although not conferring high levels of resistance in these strains, poses a potential risk of horizontal transfer to other pathogens where it could be fully functional. The *Galleria* assay can be a useful approach to uncover the distribution and identity of pathogenic bacteria in the environment, as well as uncover resistance genes with an environmental origin.

Introduction

Soil ecosystems are a vital part of the earth's biosphere, ensuring environmental and human health through regulating air and water quality and food production (Wall *et al*., 2015). Soil function, including biogeochemical cycling (Beare *et al*., 1995), plant health (Berendsen *et al*., 2012) and the provision of natural resources such as antibiotics (Crits-Christoph *et al*., 2018), is underpinned by rich microbial diversity (Fierer and Jackson, 2006; Vos *et al*., 2013; Wall *et al*., 2015). However, a minority of species comprising the soil microbiome can also cause opportunistic infections in plants, animals and humans (Bultman *et al*., 2013; Steffan *et al*., 2018). Dozens of bacterial genera containing species able to infect humans are present in soils (Berg *et al*., 2005). These include gut-associated species originating from human and agricultural contamination (Santamaría and Toranzos, 2003), but also many species native to soil habitats with largely unknown, and probably non-mammalian host-associated life histories. Although of potential importance to human health, relatively little is known about the prevalence and identity of soilborne pathogenic bacteria, especially when compared to waterborne pathogens.

The lack of basic knowledge on the ecology of pathogenic bacteria in the vast soil biome is problematic, particularly because climate change is expected to favour the growth and prevalence of pathogens infecting warm-blooded hosts (Hellberg and Chu, 2016). Exposure risks are likely to increase as well, with more frequent extreme weather events resulting in increased aerosolization (Chen *et al*., 2014), run-off (Patz *et al*., 2008) and flooding (Dayrit *et al*., 2018). Moreover, pervasive pollution with biocides, metals and antibiotics can select for antimicrobial resistance (AMR) in environmental pathogens (Wellington *et al*., 2013), limiting treatability of infections. The recent finding of a positive correlation between temperature and AMR in human pathogens suggests that

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climate change and the AMR crisis could even interact (MacFadden *et al*., 2018).

A major challenge when surveying bacterial pathogens is that both culture-based and culture-independent approaches usually screen only for a subset of species only, and that they are often unable to differentiate pathogenic from non-pathogenic strains. In this study, we employ a recently developed approach that is not dependent on molecular markers or growth requirements specific to a subset of known pathogens, but instead only screens for the phenotype of interest: pathogenicity. By directly injecting environmental samples into *Galleria mellonella*, a well-established model for mammalian infection (Brennan *et al*., 2002; Wand *et al*., 2011), any bacteria able to cause disease can be selectively enriched at 37°C *in vivo* (Hernandez *et al*., 2019). Infectious clones can subsequently be isolated from the larvae and phenotypically and genetically characterized. Using this approach on sediment and water samples, both common, human-associated pathogens (such as *Escherichia coli*), as well as free-living pathogen species not previously known to occur in the United Kingdom (such as *Vibrio injenensis*), could be detected (Hernandez *et al*., 2019).

Here we use the *Galleria* enrichment approach to identify and determine the prevalence of virulent bacteria that could potentially represent human pathogens in a range of soil environments in Cornwall (United Kingdom). For selected isolates belonging to the most commonly encountered *Providencia* and *Serratia* species, we characterize AMR and virulence determinants phenotypically and genomically and discuss their relation to known clinical lineages.

Results

Prevalence and diversity of pathogenic bacteria in soils sampled in Cornwall (United Kingdom)

Forty soil samples were collected from randomly selected, easily accessible locations near Penryn, Cornwall, UK, 2018 (Tables S1 and S2, Fig. S1). Sampling types included suburban soils under grass or tree cover, as well as seven samples from an area contaminated with mine waste. A *Galleria mellonella* bioassay was used to screen soil microbial communities for pathogenic bacteria (Hernandez *et al*., 2019). Concentrated soil washes were directly injected into 10 *Galleria* each to assess percent mortality, after which they were incubated for 24 h at 37°C. A total of 29 (73%) out of 40 samples resulted in >5/10 *Galleria* mortality and were classified as potentially harbouring pathogenic bacteria (Fig. 1A; Fig. S2).

Mortality was not found to be affected by soil type using three broad categories 'grass cover', 'tree cover' and 'metal contaminated' (Kruskal-Wallis rank sum test, $\chi^2 = 0.55061$, $p > 0.75$) (Fig. S3). For seven grassland sites, apart from a standard sample taken at ≥5 cm depth, a paired sample was also taken from the grass roots above. When comparing these paired bulk soil and rhizosphere samples, the latter samples displayed significantly elevated *Galleria* mortality (paired *t*-test, $t = 2.5205$, $p < 0.05$), with six out of seven rhizosphere communities causing 100% mortality (Fig. S4). By mainly using bulk soil samples in this study, the incidence of pathogenic bacteria is thus underestimated. However, by mostly sampling deeper underground, we can be confident that 'true' soil bacteria were isolated and not bacteria from other sources more ephemerally present on the surface. As bulk soil is probably more similar among broad sample types than is rhizosphere soil, this could in part explain the lack of variation in *Galleria* mortality among soil types.

To identify the pathogens' responsible for mortality, the 29 soil wash samples resulting in >50% mortality were reinjected, and larvae were incubated and dissected to isolate bacterial clones from hemocoel (Fig. S2). Clones were classified into 1 of 10 morphological types (data not shown) and at least one of each morphotype present in each hemocoel extraction was isolated. A total of 66 hemocoel extractions were performed, with at least two clones being isolated from each extraction in all but three instances (Table S3, Fig. S5). In total, 155 clones were isolated and for each, the proportion of dead *Galleria* (out of 10) was assayed after injecting $\sim 10^5$ CFU and incubation for 24 h at 37°C. Injection of 115 clones (72.4%) resulted in >50% *Galleria* mortality, with 94 clones (60.6%) causing 100% mortality (Fig. 1B, Fig. S2). We note that the inoculum CFU used must have influenced to some degree what clones were isolated. For instance, the important human pathogen *Staphylococcus aureus* is usually injected in *Galleria* at a higher inoculum (e.g. 2×10^6 CFU) (Sheehan *et al*., 2019); our lower inoculation number thus could mean that this type of pathogen would be missed.

A subset of pathogenic clones, originating from different samples and representing a range of colony morphologies, were selected for 16s rRNA sequencing (Table 1, Fig. S2). A variety of species were identified, including an *Aeromonas hydrophila* clone from the metal-contaminated site with very high virulence ($LT_{50} < 10$ h). *Aeromonas hydrophila* is known as a highly virulent pathogen of fish (Cipriano *et al*., 1984), but it can also cause skin and soft-tissue infections in humans after wound exposure to freshwater (Gold and Salit, 1993) or cause gastro-intestinal infection after consumption of infected fish or chicken (Praveen *et al*., 2016). Several *Bacillus*

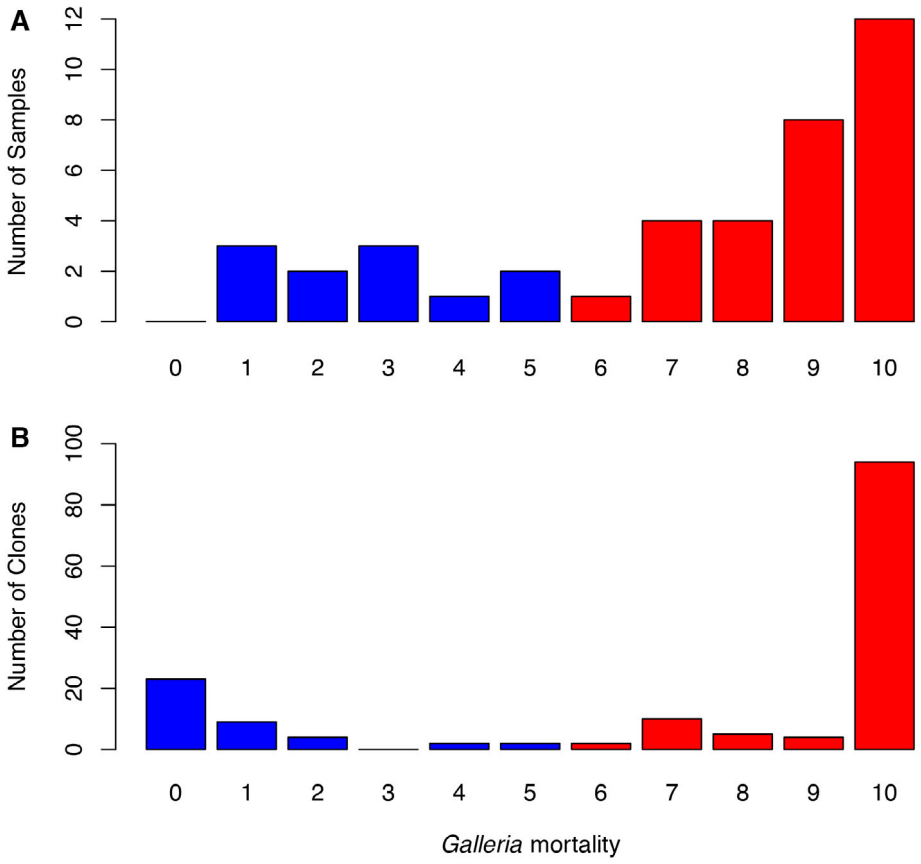

Fig 1. *Galleria* mortality after injection with soil washes or clones isolated from infections after soil wash injections.
A. *Galleria* mortality after inoculation with microbial communities extracted from soil. Ten larvae were inoculated with 10 µl of concentrated soil washes from 40 samples and incubated at 37°C for 24 h after which mortality was scored. Red bars: samples classified as potentially harbouring pathogens, blue bars: samples classified as not likely to harbour pathogens.
B. *Galleria* mortality after inoculation with clones isolated from *Galleria* inoculations with microbial communities extracted from soil (see A). Red bars: isolates classified as pathogenic, blue bars: isolates not classified as pathogenic.

clones in the *B. cereus* group were identified. This group is primarily known for insect pathogens (Raymond *et al*., 2010) but representatives can also cause infection in humans (Bottone, 2010). Most clones belonged to two genera, *Serratia* and *Providencia* (26 and 10 of 46 virulent clones identified respectively) (Table 1). Both genera were found in all sampling locations (data not shown). A subset of isolates was characterized in detail through antibiotic susceptibility testing, construction of *Galleria* survival curves and whole genome sequencing.

Providencia alcalifaciens *and* P. rustigianii

Ten *Providencia* isolates were tested for resistance to 13 different antibiotics using Clinical and Laboratory Standards Institute (CLSI) protocols (Table 2). All were resistant to azithromycin, but mostly displayed intermediate susceptibility. Five strains representing five distinct AMR profiles were whole genome sequenced (Table S4). No AMR genes could be detected in these strains via *in silico* analysis (Fig. S6). A maximum-likelihood tree constructed from the core alignment affirmed that four soil isolates belong to the species *P. alcalifaciens* (Fig. 2; Table S4). The four isolates occupy a long branch with the most genetically similar strain isolated from a human gut. Another isolate belongs to the related species *P. rustigianii* (C83.1) (Hickman-Brenner *et al*., 1983) (Fig. 2; Table S4). A more detailed *Galleria* mortality assay was performed for four *P. alcalifaciens* isolates and the *P. rustigianii* clone, following the mortality of 20 larvae after inoculation with 10^3 CFU. All clones caused 100% mortality within 20 h, with a mean LT_{50} of 13.4 h (Fig. 4A). Isolates displayed indistinguishable virulence (pairwise comparisons using log-rank test on survival fitted by Kaplan–Meier method, $p > 0.25$ for all comparisons). Only a single virulence gene, *gmhA* involved in LPS biosynthesis (Bauer *et al*., 1998), was detected in the four *P. alcalifaciens* isolates (Fig. S7).

Serratia liquefaciens *and* S. plymuthica

Clones identified as *Serratia* were also tested for resistance to 13 antibiotics. Ten of 21 clones were susceptible to all antibiotics, with 11 clones showing (intermediate) resistance to two antibiotics per strain at most (Table 2). A total of eight distinct resistance profiles could be distinguished, and a representative of each was selected for whole genome sequencing (Table S5). A whole genome maximum-likelihood tree revealed that the soil isolates are representatives of two distinct *Serratia* species. Most

Table 1. Identity based on 16S rRNA sequencing of selected clones causing >50% *Galleria* mortality.

Site	Clone	Genus	% Match (bp used)	Closest 16S match	% coverage (*E*-value)	GenBank accession no.
A1	A11.2	*Serratia*	98.6 (783)	*S. liquefaciens*	100 (0)	MN619709
	A12.3	*Serratia*	99.61 (776)	*S. liquefaciens*	100 (0)	MN619710
A2	A21.2	*Serratia*	99.42 (518)	*S. liquefaciens*	100 (0)	MN619711
A6	A61.2	*Aeromonas*	100 (840)	*A. hydrophila*	100 (0)	MN619712
	A63.1	[a]	99.78 (451)		100 (0)	MN619713
	A63.2	*Serratia*	99.51 (822)	*S. liquefaciens*	100 (0)	MN619714
A7	A72.3	[a]	99.51 (408)		100 (0)	MN619718
	A73.3	*Serratia*	99.76 (822)	*S. liquefaciens*	100 (0)	MN619719
C1	C11.2	*Serratia*	98.94 (659)	*S. liquefaciens*	100 (0)	MN619720
	C12.2	*Serratia*	98.96 (672)	*S. liquefaciens*	100 (0)	MN619721
	C13.1	*Serratia*	98.78 (655)	*S. liquefaciens*	100 (0)	MN619722
	C13.2	*Serratia*	99.24 (785)	*S. liquefaciens*	100 (0)	MN619723
C3	C31.1	*Bacillus*	100 (664)	*B. cereus* group	100 (0)	MN619724
	C31.2	*Bacillus*	100 (767)	*B. cereus* group	100 (0)	MN619725
	C32.1	*Serratia*	99.7 (670)	*S. liquefaciens*	100 (0)	MN619726
C5	C51.1	*Serratia*	99.85 (654)	*S. liquefaciens*	100 (0)	MN619727
C7	C72.3	*Bacillus*	99.85 (666)	*B. cereus* group	100 (0)	MN619732
C8	C81.1	*Serratia*	98.47 (653)	*S. liquefaciens*	100 (0)	MN619733
	C83.1	*Providencia*	100 (831)	*P. alcalifaciens*[b]	100 (0)	MN619736
G1	G1R1.1	*Serratia*	99.85 (654)	*S. liquefaciens*	100 (0)	MN619741
	G1S1.2	*Serratia*	99.85 (653)	*S. liquefaciens*	100 (0)	MN619742
G2	G2S1.1	*Serratia*	100 (655)	*S. nematodiphila*	100 (0)	MN619743
G3	G3R1.1	*Serratia*	99.7 (670)	*S. liquefaciens*	100 (0)	MN619744
	G3S1.1	*Serratia*	99.85 (653)	*S. liquefaciens*	100 (0)	MN619745
	G3S1.3	*Bacillus*	100 (664)	*B. cereus* group	100 (0)	MN619746
G4	G4R1.1	*Providencia*	99.69 (654)	*P. alcalifaciens*	99 (0)	MN619747
G5	G5R1.1	*Serratia*	99.55 (660)	*S. liquefaciens*	100 (0)	MN619748
	G5R1.3	*Bacillus*	99.74 (775)	*B. cereus* group	100 (0)	MN619749
	G5R2.1	*Bacillus*	99.85 (672)	*B. cereus* group	100 (0)	MN619750
	G5R4.2	*Serratia*	99.85 (653)	*S. liquefaciens*	100 (0)	MN619751
G7	G7R1.1	*Serratia*	99.65 (570)	*Serratia* spp.	100 (0)	MN619757
	G7R2.2	*Serratia*	99.84 (613)	*S. liquefaciens*	100 (0)	MN619758
	G7S1.2	*Serratia*	99.54 (655)	*S. liquefaciens*	100 (0)	MN619759
P1	P11.1	*Providencia*	99.88 (849)	*P. alcalifaciens*	100 (0)	MN619760
	P11.2	*Providencia*	100 (832)	*P. alcalifaciens*	100 (0)	MN619761
	P12.2	*Bacillus*	100 (667)	*B. cereus* group	100 (0)	MN619762
	P13.1	*Bacillus*	99.55 (880)	*B. cereus* group	100 (0)	MN619763
	P13.2	*Serratia*	98.93 (654)	*S. liquefaciens*	100 (0)	MN619764
	P14.1	*Providencia*	100 (652)	*P. alcalifaciens*	99 (0)	MN619765
P5	P51.1	*Providencia*	100 (653)	*P. alcalifaciens*	99 (0)	MN619766
	P51.2	*Providencia*	99.85 (656)	*P. alcalifaciens*	100 (0)	MN619767
	P52.1	*Providencia*	99.88 (828)	*P. alcalifaciens*	100 (0)	MN619768
	P53.1	*Providencia*	100 (680)	*P. alcalifaciens*	99 (0)	MN619769
	P54.1	*Bacillus*	99.89 (884)	*B. cereus* group	100 (0)	MN619770
	P54.2	*Providencia*	100 (666)	*P. alcalifaciens*	99 (0)	MN619771
SO	SO1.1	*Serratia*	99.64 (828)	*Serratia* spp.	100 (0)	MN619772

[a]Clones where no closest match is indicated had multiple genus hits.
[b]Based on subsequent WGS, this clone was identified as *P. rustigianii*.
For site information, see Tables S1 and S2. BLAST results were sorted by total score, coverage, *E*-value and percentage identity, and the top results were recorded.

of the isolates (7/8) were observed as two clonal lineages nested among the broad diversity of *S. liquefaciens* comprising strains of both environmental and clinical origin (Fig. 3). A single isolate (G7R1.1) was found to form a long branch extending from within *S. plymuthica*.

Four AMR resistance genes were identified in most *S. liquefaciens* isolates (Fig. S8). The first is a beta lactamase with high similarity to *bla-C* in *Mycobacterium* (>98% AA similarity), potentially responsible for the observed instances of (intermediate) phenotypic resistance to beta-lactam antibiotics (Table 2). The second are genes with high (∼80% AA) similarity to *oqxB*; an efflux pump conferring resistance to fluoroquinolones and benzalkonium chloride and are prevalent in *S. marcescens* clinical isolates (Saralegui *et al*., 2020). Most interestingly is the identification of *blaSPR-1*, a metallo-β-lactamase (MBL), which confer carbapenem resistance originally characterized in *S. proteamaculans* (Vella *et al*., 2013) (∼80% AA similarity). As resistance to carbapenems in bacterial pathogens is of grave concern

Table 2. Antimicrobial susceptibility profiles.

	FOX	CPD	W	TZP	TGC	CAZ	C	AMC	ATM	CIP	CN	ETP	AZM	# clones
Providencia alcalifaciens	S	S	S	S	S	S	S	S	S	S	S	S	R	4
	S	S	S	S	I	S	S	I	S	S	S	S	R	2
	S	S	S	S	I	S	S	I	S	S	S	I	R	1
	S	S	S	S	I	S	S	S	S	S	S	S	R	1
	S	S	S	S	S	S	S	I	S	S	S	S	R	1
Typical	$S^{1,2}$	S^{1}	U^{1}	S^{1}	-	S^{1}	U^{1}, $I^{2,}$ S^{3}	U^{1}	$S^{1,2}$	$S^{1,2}$	$S^{1,2,3}$	-	R^{1}	
P. rustigianii	S	S	S	S	S	S	S	S	S	S	S	S	R	1
Typical	S^{1}	S^{1}	S^{1}	S^{1}	-	S^{1}	U^{1}, S^{3}	U^{1}	S^{1}	S^{1}	$S^{1,3}$	-	R^{1}	
Serratia liquefaciens	S	S	S	S	S	S	S	S	S	S	S	S	S	10
	S	S	S	S	S	S	S	I	S	S	S	S	S	5
	I	S	S	S	S	S	S	S	S	S	S	S	S	1
	S	S	S	R	S	S	S	R	S	S	S	S	S	1
	S	S	S	S	S	S	S	R	S	S	S	S	S	1
	S	S	S	S	S	I	S	I	S	S	S	S	S	1
	S	R	S	S	S	S	S	R	S	S	S	S	S	1
Typical	$I\text{-}R^{4}$	S^{4}	S^{5}	$S^{4,6}$, $S\text{-}I^{7}$	S^{5}	$S^{4,5}$	$S^{4,5}$	$I\text{-}R^{4}$, $R^{5,7}$	$S^{4,5}$	$S^{4,5,7}$	$S^{4\text{-}7}$	$S^{4,5}$	$R^{4,5}$	
S. plymuthica	I	I	S	S	S	S	S	S	S	S	S	S	S	1
Typical	R^{8}	-	-	-	-	-	S^{8}	S^{8}	-	-	-	-	$I\text{-}R^{8}$	

CLSI antibiotic susceptibility against 13 antibiotics. Each row represents a distinct resistance profile. The number of isolated conforming to each profile is indicated on the right. Beneath the AMR profiles we found are previously reported resistance profiles for the species based on the following references: (i) Stock and Wiedemann (1998), (ii) Wang *et al*. (2014), (iii) Chander *et al*. (2006), (iv) Stock *et al*. (2003b), (v) Mahlen (2011), (vi) Engelhart *et al*. (2003), (vii) Traub (2000) and (viii) Stock *et al*. (2003a). Antibiotics used: Cefoxitin (FOX), Cefpodoxime (CPD), Trimethoprim (W), Piperacillin/Tazobactam combination (TZP), Tigecycline (TGC), Ceftazidime (CAZ), Chloramphenicol (C), Amoxicillin/Clavulanic acid combination (AMC), Aztreonam (ATM), Ciprofloxacin (CIP), Gentamicin (CN), Ertapenem (ETP), Azithromycin (AZM). S: susceptible, R: resistant, I: intermediate resistance, U: unclear, –: no data available.

(O'Neill, 2016), the presence of this MBL is of significance. The mode of action of *bla*SPR-1 is unlike that of known MBLs due to unusual predicted active site residues (Hou *et al*., 2014). We report, for the first time, that *bla*SPR-1 is found across a range of *Serratia* species (but not *S. marcescens*) from both environmental and clinical sources (Fig. S8), suggesting that this chromosomal gene is ancestral to this group of predominantly environmental organisms.

The *S. liquefaciens* isolates displayed indistinguishable levels of virulence in *Galleria* (Fig. 4B); all clones tested caused 100% *Galleria* mortality within 20 h, with a mean LT_{50} of 16.6 h (Fig. 4B). The *S. plymuthica* strain (G7R1.1) was markedly less virulent (pairwise comparisons using log-rank test on survival fitted by Kaplan–Meier method, $p < 0.05$) (Fig. 4B). Several flagella genes with potential roles in virulence were present in all *Serratia* genomes (Fig. S9).

Discussion

This study uncovered a high prevalence of virulent bacteria that could represent human pathogens in soils. Most isolates displaying virulence in the *Galleria* insect model were found to be *S. liquefaciens* and *P. alcalifaciens*. Neither species is likely to be associated with human or livestock faecal contamination as they were consistently encountered in subsurface samples taken across a range of non-agricultural sites. *Providencia alcalifaciens* can be found in the environment but is mainly associated with gastrointestinal tract of humans and animals (Müller, 1986) and can cause traveller's diarrhoea (Yoh *et al*., 2005), occasionally leading to outbreaks (Murata *et al*., 2001). However, a paucity of *P. alcalifaciens* genomes deposited in public databases prevented genomic comparison between human pathogens and soil isolates identified in this study. *Providencia rustigianii* is not known to cause disease in humans.

Serratia liquefaciens has been sampled from a range of hosts and environments including humans, animals, plants and food products (Petersen and Tisa, 2013). *Serratia liquefaciens* can be isolated from many human body sites and comprises both commensal and pathogenic strains, which can cause blood stream infections and pneumonia, occasionally leading to outbreaks (Mahlen, 2011). A much smaller number of human infections are caused by *S. plymuthica* (Mahlen, 2011) and the lower virulence of the isolate in this study compared to *S. liquefaciens* is consistent with this observation. The lack of genomic differentiation between isolates from both clinical and environmental sources observed here is consistent with a highly generalist lifestyle, as has also been

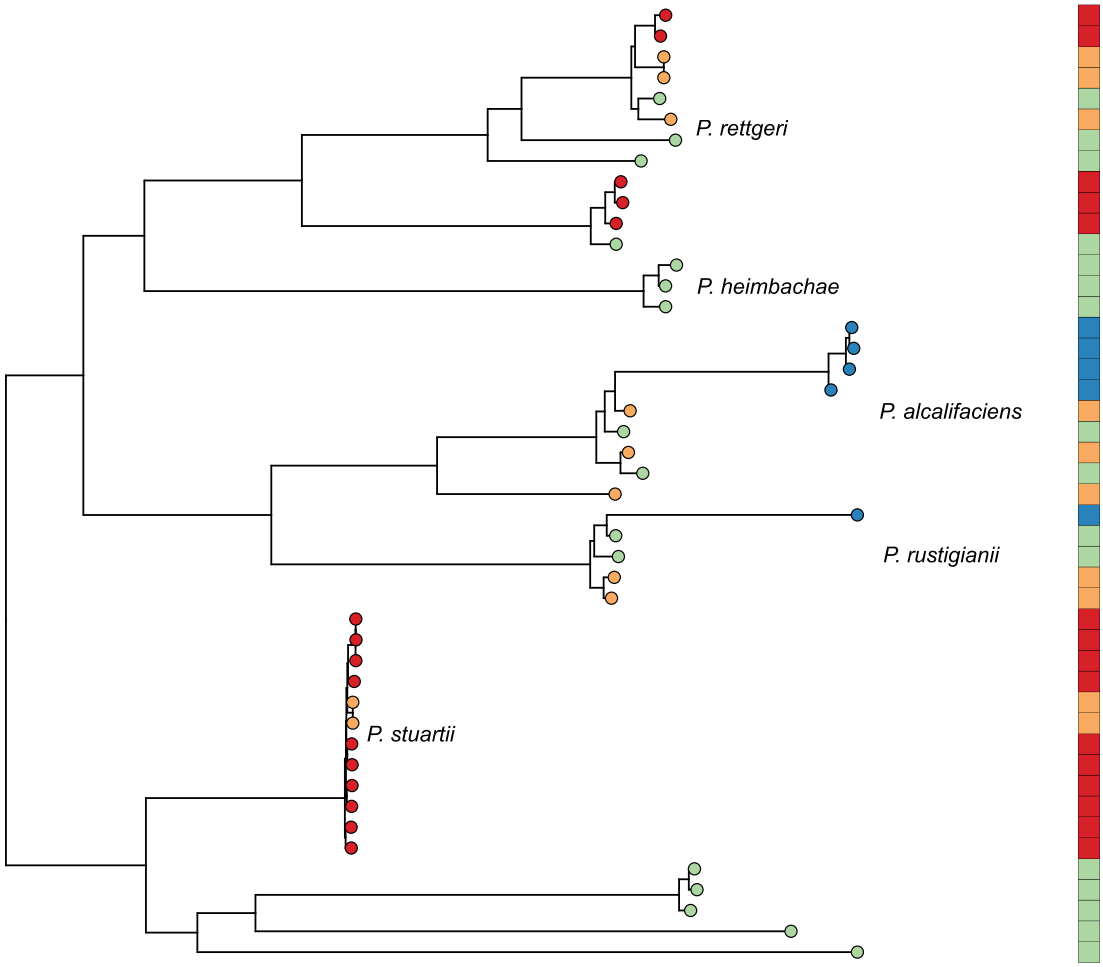

Fig 2. Maximum likelihood core genome phylogeny of the genus *Providencia*.
Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was constructed using variant sites present in >95% of samples reads from reads mapped against reference genome *Providencia rustigianii* NCTC6933. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

found in other human pathogens such as *Klebsiella pneumoniae* (Wyres and Holt, 2018) and *Campylobacter jejuni* (Sheppard *et al*., 2014).

Both the *Serratia* and *Providencia* genera contain pathogens of insects and nematodes, including entomopathogenic species, where nematode-bacteria mutualisms aid insect predation (Jackson *et al*., 1995; Somvanshi *et al*., 2006; Park *et al*., 2011; Petersen and Tisa, 2013; Grimont *et al*., 1979, Galac & Lazzaro, 2011). It is likely that isolates from both species in this study lead a similar lifestyle. The high antibiotic susceptibility and paucity of AMR genes in both sets of isolates is consistent with an environmental lifestyle lacking human contact. This raises the question how these widespread, putative Ecdysozoan pathogens occasionally emerge as opportunistic human pathogens. Two possible scenarios could explain the dispersed distribution of human pathogenic isolates across the phylogenies for both genera (Méric *et al*., 2018). In the first, traits resulting in a broader host range (i.e. enabling human infection) could independently originate in different strains or be acquired through horizontal gene transfer. In the second, all (or most) strains in principle are capable of infecting humans but through a lack of contact most do not (and therefore remain generally susceptible to antibiotics). More detailed genome-wide association studies coupling phylogenetic information to infection studies (e.g. Yahara *et al*., 2017; Méric *et al*., 2018) will be needed to distinguish between these hypotheses.

While omics-based studies are highly useful for high-throughput surveys of AMR genes (e.g. Zhang *et al*., 2019), isolate-based studies have the advantage of being able to unequivocally link AMR gene carriage to host genomic background. The discovery of *bla*SPR-1 being widespread in *S. liquefaciens* and *S. plymuthica* strains isolated from both the environment and clinic is of

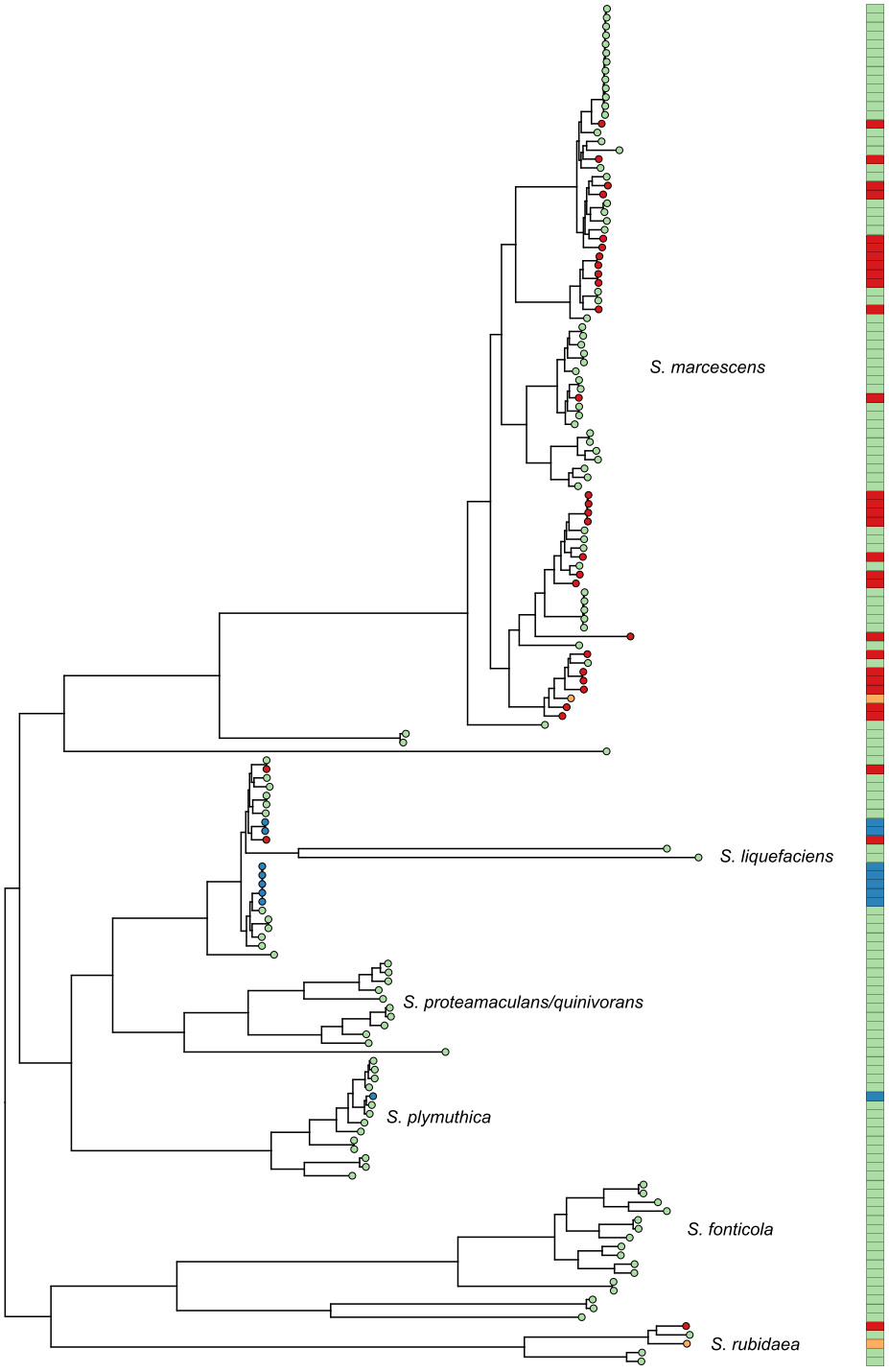

Fig 3. Maximum likelihood core genome phylogeny of the genus *Serratia*.
Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was constructed using variant sites present in >95% of samples reads from reads mapped against reference genome *Serratia liquefaciens* ATCC 27592. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

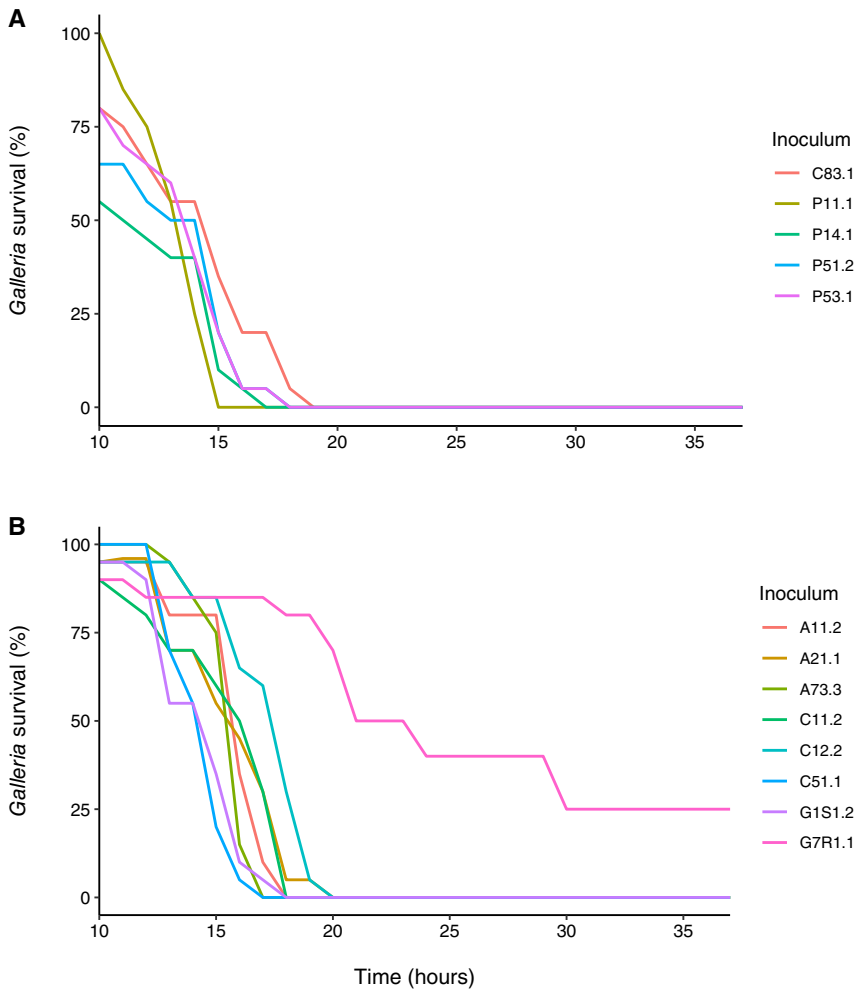

Fig 4. *Galleria mellonella survival curves (n=20) for A: Providencia alcalifaciens and P. rustigianii (C83.1) and B: Serratia liquefaciens and S. plymuthica (G7R1.1). One isolate of each AMR susceptibility profile (Table 2) is represented. (One exception is the omission of one of the Providencia alcalifaciens resistance types, as C83.1 was originally identified as P. alcalifaciens using 16S sequencing and wrongly taken as a representative for a fifth P. alcalifaciens susceptibility type).*
One isolate of each AMR susceptibility profile (Table 2) is represented. (One exception is the omission of one of the *Providencia alcalifaciens* resistance types, as C83.1 was originally identified as *P. alcalifaciens* using 16S sequencing and wrongly taken as a representative for a fifth *P. alcalifaciens* susceptibility type.)

note. Our findings suggest this potentially important resistance gene is ancestral to this *Serratia* clade and is of environmental origin. Although not conferring high phenotypic resistance in our isolates, it is conceivable that this gene could be horizontally transferred to other pathogenic species in a clinical context conferring carbapenem resistance in this different genomic background. For instance, *Kluyvera ascorbata* produces a β-lactamase identified as the progenitor of a clade of *bla*CTX-Ms conferring only low levels of resistance, but when expressed in *E. coli* this same gene results in high level resistance (Humeniuk *et al*., 2002).

Uncovering the links between environmental reservoirs on one hand and human carriage and infection on the other is crucial to understand exposure risk. Exposure risks to soilborne pathogens are generally poorly understood, with some exceptions. In tropical countries, *Burkholderia pseudomallei* primarily infects through skin contact (Limmathurotsakul *et al*., 2013) but can also cause gastrointestinal infections through ingestion with water contaminated with soils (Chen *et al.,* 2015) or respiratory infections following aerosolization (especially after extreme weather events) (Chen *et al*., 2015). In higher latitude countries, soil contact during sports (Vally *et al*., 2004) and gardening (Den Boer *et al*., 2007) are known to occasionally result in bacterial infections of the skin and of wounds, and food can form another route of exposure to soilborne pathogens (Santamaría and Toranzos, 2003; Bultman *et al*., 2013).

We hope low-cost, high-throughput *Galleria* assays will be adopted for more detailed investigations into the ecology of pathogens in soil and other environments. For instance, spatially, temporally and ecologically explicit sampling could reveal the importance of edaphic properties (Bultman *et al*., 2013) and vegetation (Berg *et al*., 2005) in determining pathogen prevalence and identity in soils. It can at the same time serve as a tool uncovering the distribution of known and novel AMR genes in environmental bacteria, which could eventually find their way to other pathogen species.

Experimental procedures

Soil sample collection

A total of 40 soil samples were collected from three randomly chosen, accessible locations in-and-around Penryn, Cornwall, UK, in 2019 (Tables S1 and S2, Fig. S1). For taking 26 urban soil samples from around Penryn Campus and Falmouth, the topsoil was removed to a minimum of 5 cm depth, and exposed soil was sampled using a metal spoon sterilized with 70% ethanol into a universal tube (30 ml, Thermo Scientific). A further seven rhizosphere samples (paired with seven bulk soil samples) were collected by scraping soil from plant roots (<5 cm depth). Finally, we collected seven soil samples from the banks of the Carnon River which runs through a mine heritage site with high levels of heavy metal contamination (Vos *et al*., 2019).

Sample processing

All soil samples were brought to the lab on the same day as collection for further processing in a Category II extraction hood. Bacterial extraction methods were based on Hernandez *et al*. (2019). Briefly, 10 g of each sample was weighed out and homogenized in a new universal tube in 10 ml of sterile M9 buffer solution (12.8 g l^{-1} Na_2HPO_4–$7H_2O$; 3 g l^{-1} KH_2PO_4; 0.5 g l^{-1} NaCl; 0.1 g l^{-1} NH_4Cl) via vortexing (Stuart® Vortex) at maximum speed for 2 min. This solution was centrifuged at 500 r.p.m. (56 g) for 15 min in a Heraeus Megafuge 40R centrifuge (Thermo Scientific) to separate solid matter from the buffer containing bacteria. The supernatant was poured into a new sterile universal tube and centrifuged at 3500 rpm (2739 g) for a further 30 min to pellet bacteria. The supernatant was discarded, and the pellet containing bacteria re-suspended in 1 ml of M9 buffer and stored at 4°C. All centrifugation steps were performed at 4°C.

Galleria mellonella *assay for whole soil communities*

Boxes of *Galleria mellonella* larvae in the last instar before pupation were obtained from UK Waxworms (www.uk-waxworms.co.uk) and stored at 4°C prior to injection. All larvae were used within 2 weeks of purchase. All concentrated soil wash samples were assayed using 10 *Galleria* per sample. Larvae were placed in a petri dish and kept on ice for a minimum of 10 min prior to injection in order to render the larvae immobile and unconscious. A Hamilton 50 µl syringe (model 80 950) with a 0.3 × 13 mm needle (BD Microlance 3, Becton Dickinson, Plymouth, UK) was used to inject larvae with 10 µl of concentrated whole soil community into the last left proleg. New sterile needles were used for injection of each sample, and the syringe was cleaned between sample injections with 70% ethanol and sterile M9 buffer. Two negative controls were used for each experiment: a no-injection control (n = 10) to control for background larvae mortality and a buffer control (n = 10) in which larvae were injected with 10 µl of M9 buffer to control for impact of physical trauma. After injection, Petri dishes were incubated at 37°C and morbidity and mortality were recorded after 24 h. Larvae were considered infected if they expressed dark pigmentation (melanization) after inoculation. Larvae were scored as dead if they did not respond to touch stimuli by blunt sterile forceps.

Galleria mellonella *assay for individual clones*

For those samples that caused mortality in five or more *Galleria* out of 10, the assay was repeated the next day using stored concentrated soil wash. In addition to the two controls described above, a third control was added where larvae were injected with filter-sterilized samples (0.22 µm syringe filters; Thermo Fisher, Waltham, MA, USA) to account for any toxic chemicals present in the sample (Hernandez *et al*., 2019). None of the controls resulted in significant mortality. Larvae were monitored regularly post-injection to obtain individuals that were clearly infected (grey) but not dead (black) to extract hemocoel and isolate infecting bacteria. A minimum of two infected larvae were selected per sample and placed in an Eppendorf tube on ice for 30 min prior to extraction. The site of dissection was sterilized with 70% ethanol. Sterile micro-scissors were used to remove the last left proleg, and a drop of hemocoel was allowed to exude from the larvae before being collected with a pipette and diluted in 400 µl M9 (Hernandez *et al*., 2019). *Galleria* were immediately euthanised by freezing at −80°C.

Hemocoel extractions were plated on LB agar (Miller; 10 g Tryptone, 5 g yeast extract, 10 g NaCl and 15 g agar per litre of ddH^2O) and incubated overnight at 37°C. Distinct colony morphologies were identified, and a minimum of one of each morphotype was purified and made into a freezer stock. Overnight cultures of each clone were diluted in M9 buffer to 10^5 CFU and injected into 10 *Galleria* as described above. After incubation for a period of 12 h, mortality was recorded, and clones causing mortality in five or more larvae were deemed potentially pathogenic and selected for further study. Selected clones were injected in 20 *Galleria* at 10^3 CFU to record larval morbidity and mortality hourly after an initial 10 h period for a total of 37 h, allowing for construction of survival plots and calculation of the LT_{50}.

16s rRNA Sanger sequencing

Genomic DNA was extracted using a 'DNeasy® UltraClean® Microbial Kit' (QIAGEN). Each extracted DNA sample was amplified using the following PCR protocol and reagents. Twenty-five microlitre of Green master mix (DreamTaq, Thermo Scientific™), 5 μl of primer mix (consisting of 16S27f: 5′-AGAGTTTGATYMTGGCTCAG-3′ and 16S1492r: 5'-TACCTTGTTAYGACTT-3′) at 4.5 μM concentration, 0.5 μl of BSA, 18.5 μl of PCR-grade water and 1 μl of the extracted DNA. Thermocycler used was a Veriti 96-well thermal cycler (Applied Biosystems). Thermocycler protocol was one cycle of 95°C for 5 min, 30 cycles of 95°C for 30 s, 48°C for 30 s and 72°C for 90 s, then one cycle of 72°C for 10 min. Samples were then held at 4°C until collection. PCR product was run on a 1% agarose gel with 5 μl ETBR per 100 ml for 35 min at 120 V. Gels were imaged in a Syngene G: Box alongside positive and negative controls and a DNA ladder. PCR product was purified using a NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel). The concentration of purified PCR product was quantified using Fluorometric Quantification with a Qubit™ dsDNA HS assay kit in a Qubit® 2.0 flurometer (Invitrogen) and diluted appropriately using PCR-grade H_2O. DNA was sent for sequencing at Eurofins Genomics (NGS-Laboratory, Anzinger Str. 7a, 85,560, Ebersberg, Germany) using the 'TubeSeq' service with the 16S27f primer. Sequences were trimmed in MEGA-X version 10.0.5 (Kumar *et al*., 2018) and poor-quality reads were discarded. Nucleotide BLAST (Altschul *et al*., 1990) was used to identify sequences.

Antimicrobial resistance profiling

Resistance of selected clones was measured using the CLSI disk diffusion method for antimicrobial susceptibility testing (Wayne, 2011), along with the following reference strains: *E. coli* (ATCC® 25922™ and ATCC® 35218™) and *Klebsiella pneumoniae* (ATCC® 700603™). Overnight cultures of each strain and clone were diluted using 0.85% NaCl solution to 0.5 McFarland standard based on optical density at 600 nm. Clones were swabbed on Muller-Hilton agar ensuring an even spread. Thirteen different antibiotic discs were added on top of the cultures, and plates were incubated at 37°C for 16–20 h. The time between starting dilutions and placing the prepared plates in the incubator was kept to a maximum of 15 min to minimize cell growth. Antibiotics used were Oxoid™ antimicrobial susceptibility disks (Thermo Scientific™): Cefoxitin (30 μg), Cefpodoxime (10 μg), Trimethoprim (5 μg), Piperacillin/tazobactam combination (36 μg), Tigecycline (15 μg), Ceftazadime (10 μg), Chloramphenicol (30 μg), Amoxycillin/clavulanic acid combination (30 μg), Aztreonam (30 μg), Ciprofloxacin (5 μg), Gentamicin (10 μg), Ertapenem (10 μg) and Azithromycin (15 μg). Inhibition zone diameters were compared to the relevant CLSI Clinical Breakpoint Tables (v.9.0, 2019), and scored as sensitive, resistant, or intermediately resistant. Susceptibility profiles were interpreted after species designation via whole-genome sequencing.

Whole genome sequencing

DNA isolation, Illumina HiSeq sequencing and basic bioinformatics were performed through the MicrobesNG programme in Birmingham, UK. Vials containing beads inoculated with liquid culture were washed with extraction buffer containing lysostaphin and RNase A, incubated for 25 min at 37°C. Proteinase K and RNase A were added and incubated for 5 min at 65°C. Genomic DNA was purified using an equal volume of SPRI beads and resuspended in EB buffer. DNA was quantified in triplicates with the Quantit dsDNA HS assay in an Eppendorf AF2200 plate reader. Genomic DNA libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol with the following modifications: two nanograms of DNA instead of one were used as input, and PCR elongation time was increased to 1 min from 30s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche LightCycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250 bp paired end protocol. European Nucleotide Archive accession numbers are listed in Tables S4 and S5.

Bioinformatics analyses

All informatics analyses were performed on MRC CLIMB cloud computing infrastructure (Connor *et al*., 2016). A reference dataset collected from the RefSeq genome database (Pruitt *et al*., 2007) (accessed 1 Nov 2019) by identifying all of the available genomes for genus *Serratia* (667) and genus *Providencia* (78). The *Serratia* dataset contained a high degree of redundancy and, for ease of analysis, was sub-sampled to 225 samples by the removal of additional draft *S. marcescens* genomes. All samples were mapped against the appropriate reference genomes (*P. rustigianii* NCTC6933 and *S. liquefaciens* ATCC 27592) and variants called using established SNP-calling pipeline snippy (https://github.com/tseemann/snippy). The resulting alignments were used as inputs to IQ-TREE (Nguyen *et al*., 2015). Phylogenetic trees were reconstructed using a General Time Reversible substitution model, a gamma distribution of rate variation among sites and the proportion of invariant sites were estimated.

The phylogeny was reconstructed using 1000 ultrafast bootstraps further optimized using a hill-climbing nearest neighbour interchange search. For virulence and AMR gene detection assemblies were passed to ABRicate (Seemann, 2018) using the Resfinder (Zankari *et al*., 2012) and VFDB (Chen *et al*., 2015) databases using 75% length identity and 75% sequence identity as a cut-off. Average nucleotide Identity was calculated using FASTANI on default parameters (Jain *et al*., 2018).

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Bauer, B.A., Stevens, M.K., and Hansen, E.J. (1998) Involvement of the *Haemophilus ducreyi gmhA* gene product in lipooligosaccharide expression and virulence. *Infect Immun* **66**: 4290–4298.

Beare, M.H., Coleman, D.C., Crossley, D.A., Hendrix, P. F., and Odum, E.P. (1995). A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *In The significance and regulation of soil biodiversity*, (5–22). Dordrecht, Netherlands: Springer.

Berendsen, R.L., Pieterse, C.M., and Bakker, P.A. (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* **17**: 478–486.

Berg, G., Eberl, L., and Hartmann, A. (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol* **7**: 1673–1685.

Bottone, E.J. (2010) *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* **23**: 382–398.

Brennan, M., Thomas, D.Y., Whiteway, M., and Kavanagh, K. (2002). Correlation between virulence of Candida albicans mutants in mice and Galleria mellonella larvae. *FEMS Immunology & Medical Microbiology*, **34** (2), 153–157.

Bultman, M.W., Fisher, F.S., and Pappagianis, D. (2013) The ecology of soil-borne human pathogens. In *Essentials of Medical Geology*, Netherlands: Springer, pp. 477–504.

Chander, Y., Goyal, S.M., and Gupta, S.C. (2006) Antimicrobial resistance of *Providencia* spp. isolated from animal manure. *Vet J* **172**: 188–191.

Chen, L., Zheng, D., Liu, B., Yang, J., and Jin, Q. (2015) VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. *Nucleic Acids Res* **44**: D694–D697.

Chen, N.-T., Chen, M.-J., Guo, C.-Y., Chen, K.-T., and Su, H.-J. (2014) Precipitation increases the occurrence of sporadic legionnaires' disease in Taiwan. *PLoS One* **9**: e114337.

Chen, P.S., Chen, Y.S., Lin, H.H., Liu, P.J., Ni, W.F., Hsueh, P.T., *et al.* (2015). Airborne transmission of melioidosis to humans from environmental aerosols contaminated with B. pseudomalle. *PLoS Negl Trop Dis*, **9**(6), p.e0003834

Cipriano, R.C., Bullock, G.L., and Pyle, S.W. (1984) Aeromonas Hydrophila *and Motile Aeromonad Septicemias of Fish*, (134). Falls Church, VA: US Fish and Wildlife Publications.

Connor, T.R., Loman, N.J., Thompson, S., Smith, A., Southgate, J., Poplawski, R., *et al*. (2016) CLIMB (the Cloud Infrastructure for Microbial Bioinformatics): an online resource for the medical microbiology community. *Microb Genom* **2**: e000086.

Crits-Christoph, A., Diamond, S., Butterfield, C.N., Thomas, B.C., and Banfield, J.F. (2018) Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis. *Nature* **558**: 440–444.

Dayrit, J.F., Bintanjoyo, L., Andersen, L.K., and Davis, M.D. P. (2018) Impact of climate change on dermatological conditions related to flooding: update from the International Society of Dermatology Climate Change Committee. *Int J Dermatol* **57**: 901–910.

Den Boer, J.W., Yzerman, E.P.F., Jansen, R., Bruin, J.P., Verhoef, L.P.B., Neve, G., and Van der Zwaluw, K. (2007). Legionnaires' disease and gardening. *Clinical microbiology and infection*, **13**(1), 88–91.

Engelhart, S., Saborowski, F., Krakau, M., Scherholz-Schlösser, G., Heyer, I., and Exner, M. (2003) Severe *Serratia liquefaciens* sepsis following vitamin C infusion treatment by a naturopathic practitioner. *J Clin Microbiol* **41**: 3986–3988.

Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci U S A* **103**: 626–631.

Galac, M.R., and Lazzaro, B.P. (2011) Comparative pathology of bacteria in the genus Providencia to a natural host, *Drosophila melanogaster*. *Microbes Infect* **13**: 673–683.

Gold, W., and Salit, I. (1993) *Aeromonas hydrophila* infections of skin and soft tissue: report of 11 cases and review. *Clin Infect Dis* **16**: 69–74.

Grimont, P.A., Grimont, F., and Lysenko, O. (1979) Species and biotype identification of Serratia strains associated with insects. *Curr Microbiol* **2**: 139–142.

Hellberg, R.S., and Chu, E. (2016) Effects of climate change on the persistence and dispersal of foodborne bacterial pathogens in the outdoor environment: a review. *Crit Rev Microbiol* **42**: 548–572.

Hernandez, R.J., Hesse, E., Dowling, A.J., Coyle, N.M., Feil, E.J., Gaze, W.H., and Vos, M. (2019) Using the wax moth larva *Galleria mellonella* infection model to detect emerging bacterial pathogens. *PeerJ* **6**: e6150.

Hickman-Brenner, F., Farmer, J.d., Steigerwalt, A., and Brenner, D. (1983) Providencia rustigianii: a new species in the family Enterobacteriaceae formerly known as Providencia alcalifaciens biogroup 3. *J Clin Microbiol* **17**: 1057–1060.

Hou, C.-F.D., Phelan, E.K., Miraula, M., Ollis, D.L., Schenk, G., and Mitić, N. (2014) Unusual metallo-β-lactamases may constitute a new subgroup in this family of enzymes. *Am J Mol Biol* **4**: 11–15.

Humeniuk, C., Arlet, G., Gautier, V., Grimont, P., Labia, R., and Philippon, A. (2002) β-Lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. *Antimicrob Agents Chemother* **46**: 3045–3049.

Jackson, T., Wang, H., Nugent, M.J., Griffin, C.T., Burnell, A. M., and Dowds, B.C. (1995) Isolation of insect pathogenic bacteria, *Providencia rettgeri*, from *Heterorhabditis* spp. *J Appl Bacteriol* **78**: 237–244.

Jain, C., Rodriguez-R, L.M., Phillippy, A.M., Konstantinidis, K.T., and Aluru, S. (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* **9**: 1–8.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* **35**: 1547–1549.

Limmathurotsakul, D., Kanoksil, M., Wuthiekanun, V., Kitphati, R., deStavola, B., Day, N.P., and Peacock, S.J. (2013) Activities of daily living associated with acquisition of melioidosis in northeast Thailand: a matched case-control study. *PLoS Negl Trop Dis* **7**: e2072.

MacFadden, D.R., McGough, S.F., Fisman, D., Santillana, M., and Brownstein, J.S. (2018) Antibiotic resistance increases with local temperature. *Nat Clim Chang* **8**: 510–514.

Mahlen, S.D. (2011) Serratia infections: from military experiments to current practice. *Clin Microbiol Rev* **24**: 755–791.

Méric, G., Mageiros, L., Pensar, J., Laabei, M., Yahara, K., Pascoe, B., *et al*. (2018) Disease-associated genotypes of the commensal skin bacterium *Staphylococcus epidermidis*. *Nat Commun* **9**: 1–11.

Müller, H. (1986) Occurrence and pathogenic role of Morganella-Proteus-Providencia group bacteria in human feces. *J Clin Microbiol* **23**: 404–405.

Murata, T., Iida, T., Shiomi, Y., Tagomori, K., Akeda, Y., Yanagihara, I., *et al*. (2001) A large outbreak of foodborne infection attributed to *Providencia alcalifaciens*. *J Infect Dis* **184**: 1050–1055.

Nguyen, L.-T., Schmidt, H.A., Von Haeseler, A., and Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* **32**: 268–274.

O'Neill, J. (2016) Tackling drug-resistant infections globally: final report and recommendations. In *Review on Antimicrobial Resistance*. London, UK: HM Government and Welcome Trust.

Park, H.W., Kim, Y.O., Ha, J.-S., Youn, S.H., Kim, H.H., Bilgrami, A.L., and Shin, C.S. (2011) Effects of associated bacteria on the pathogenicity and reproduction of the insect-parasitic nematode Rhabditis blumi (Nematoda: Rhabditida). *Can J Microbiol* **57**: 750–758.

Patz, J.A., Vavrus, S.J., Uejio, C.K., and McLellan, S.L. (2008) Climate change and waterborne disease risk in the Great Lakes region of the US. *Am J Prev Med* **35**: 451–458.

Petersen, L.M., and Tisa, L.S. (2013) Friend or foe? A review of the mechanisms that drive Serratia towards diverse lifestyles. *Can J Microbiol* **59**: 627–640.

Praveen, P.K., Debnath, C., Shekhar, S., Dalai, N., and Ganguly, S. (2016) Incidence of Aeromonas spp. infection in fish and chicken meat and its related public health hazards: a review. *Vet World* **9**: 6.

Pruitt, K.D., Tatusova, T., and Maglott, D.R. (2007) NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* **35**: D61–D65.

Raymond, B., Johnston, P.R., Nielsen-LeRoux, C., Lereclus, D., and Crickmore, N. (2010) *Bacillus thuringiensis*: an impotent pathogen? *Trends Microbiol* **18**: 189–194.

Santamaría, J., and Toranzos, G.A. (2003) Enteric pathogens and soil: a short review. *Int Microbiol* **6**: 5–9.

Saralegui, C., Ponce-Alonso, M., Pérez-Viso, B., Moles Alegre, L., Escribano, E., Lázaro-Perona, F., *et al*. (2020) Genomics of *Serratia marcescens* isolates causing outbreaks in the same pediatric unit 47 years apart: position in an updated phylogeny of the species. *Front Microbiol* **11**: 451.

Seemann, T. (2018) ABRicate. GitHub https://github.com/tseemann/abricate.git.

Sheehan, G., Dixon, A., and Kavanagh, K. (2019) Utilization of *Galleria mellonella* larvae to characterize the development of *Staphylococcus aureus* infection. *Microbiology* **165**: 863–875.

Sheppard, S.K., Cheng, L., Meric, G., De Haan, C.P., Llarena, A.K., Marttinen, P., *et al*. (2014) Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals. *Mol Ecol* **23**: 2442–2451.

Somvanshi, V.S., Lang, E., Sträubler, B., Spröer, C., Schumann, P., Ganguly, S., *et al*. (2006) Providencia vermicola sp. nov., isolated from infective juveniles of the entomopathogenic nematode Steinernema thermophilum. *Int J Syst Evol Microbiol* **56**: 629–633.

Steffan, J., Brevik, E., Burgess, L., and Cerdà, A. (2018) The effect of soil on human health: an overview. *Eur J Soil Sci* **69**: 159–171.

Stock, I., Burak, S., Sherwood, K.J., Grüger, T., and Wiedemann, B. (2003b) Natural antimicrobial susceptibilities of strains of 'unusual' *Serratia* species: *S. ficaria*, *S. fonticola*, *S. odorifera*, *S. plymuthica* and *S. rubidaea*. *J Antimicrob Chemother* **51**: 865–885.

Stock, I., Grueger, T., and Wiedemann, B. (2003a) Natural antibiotic susceptibility of strains of *Serratia marcescens* and the *S. liquefaciens* complex: *S. liquefaciens* sensu stricto, *S. proteamaculans* and *S. grimesii*. *Int J Antimicrob Agents* **22**: 35–47.

Stock, I., and Wiedemann, B. (1998) Natural antibiotic susceptibility of *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens* and *P. rustigianii* strains. *J Med Microbiol* **47**: 629–642.

Traub, W.H. (2000) Antibiotic susceptibility of *Serratia marcescens* and *Serratia liquefaciens*. *Chemotherapy* **46**: 315–321.

Vally, H., Whittle, A., Cameron, S., Dowse, G.K., and Watson, T. (2004). Outbreak of Aeromonas hydrophila

wound infections associated with mud football. *Clinical infectious diseases*, **38**(8), 1084–1089.
Vella, P., Miraula, M., Phelan, E., Leung, E.W., Ely, F., Ollis, D.L., *et al*. (2013) Identification and characterization of an unusual metallo-β-lactamase from *Serratia proteamaculans*. *J Biol Inorg Chem* **18**: 855–863.
Vos, M., Sibleyras, L., Lo, L.K., Hesse, E., Gaze, W.H., and Klümper, U. (2019) Zinc can counteract selection for ciprofloxacin resistance. *bioRxiv*, **367**(3), 780981.
Vos, M., Wolf, A.B., Jennings, S.J., and Kowalchuk, G.A. (2013) Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol Rev* **37**: 936–954.
Wall, D.H., Nielsen, U.N., and Six, J. (2015) Soil biodiversity and human health. *Nature* **528**: 69–76.
Wand, M.E., Müller, C.M., Titball, R.W., and Michell, S.L. (2011). Macrophage and Galleria mellonella infection models reflect the virulence of naturally occurring isolates of B. pseudomallei, B. thailandensis and B. oklahomensis. *BMC microbiology*, **11**(1), 11.
Wang, X., Wang, J., Hao, H., Qiu, L., Liu, H., Chen, S., *et al*. (2014) Pathogenic *Providencia alcalifaciens* strain that causes fatal hemorrhagic pneumonia in piglets. *Curr Microbiol* **68**: 278–284.
Wayne, P. (2011) *Performance Standards for Antimicrobial Susceptibility Testing*, Wayne, PA: Clinical and Laboratory Standards Institute.
Wellington, E.M.H., Boxall, A.B.A., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., *et al*. (2013) The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis* **13**: 155–165.
Wyres, K.L., and Holt, K.E. (2018) *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr Opin Microbiol* **45**: 131–139.
Yahara, K., Méric, G., Taylor, A.J., de Vries, S.P., Murray, S., Pascoe, B., *et al*. (2017) Genome-wide association of functional traits linked with C ampylobacter jejuni survival from farm to fork. *Environ Microbiol* **19**: 361–380.
Yoh, M., Matsuyama, J., Ohnishi, M., Takagi, K., Miyagi, H., Mori, K., *et al*. (2005) Importance of *Providencia* species as a major cause of travellers' diarrhoea. *J Med Microbiol* **54**: 1077–1082.
Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., *et al*. (2012) Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* **67**: 2640–2644.
Zhang, L., Calvo-Bado, L., Murray, A., Amos, G., Hawkey, P., Wellington, E., and Gaze, W. (2019) Novel clinically relevant antibiotic resistance genes associated with sewage sludge and industrial waste streams revealed by functional metagenomic screening. *Environ Int* **132**: 105120.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Sampling maps.

Maps indicating the locations of sampling sites.

A. Falmouth, Cornwall, UK.

B. University of Exeter, Cornwall campus, Penryn, UK.

C. Carnon River near Bissoe, Cornwall, UK.

Fig. S2. Effect of soil sample type on *Galleria* mortality.

Broad soil type categories 'grass cover' (n = 25), 'tree cover' (n = 7) and 'metal contaminated' (n = 7) did not have a differential effect on *Galleria* mortality (Kruskal-Wallis rank sum test, $\chi^2 = 0.55061$, $p > 0.75$).

Fig. S3. Comparison of paired bulk and rhizosphere soil samples on *Galleria* mortality.

Paired rhizosphere soil and bulk soil samples were collected at seven sites. There was a significant difference in *Galleria* mortality for the two sample types (paired t-test., $t = 2.5205$ and $p < 0.05$) with six grass root communities causing 100% mortality. Samples from one site (G5) caused the lowest mortality for both treatments (shown as outliers).

Fig. S4. The number of clones isolated from *Galleria* hemocoel from each soil community that caused >50% *Galleria* mortality following injection of 10^5 CFU. Indicated in red are clones that caused >50% *Galleria* mortality, and incubation at 37°C for 12 h. Those in blue caused ≤50% mortality. No clones could be isolated from samples P0 and P3 that initially showed high mortality. For sampling site information see Table S2.

Fig. S5. Maximum likelihood core genome phylogeny of the genus *Providencia*. Bars show the presence (red) or absence (white) of antimicrobial genes identified using the resfinder database using 75% length identity and 75% sequence identity as a cut-off. Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was constructed using variant sites present in >95 % of samples reads from reads mapped against reference genome *Providencia rustigianii* NCTC6933. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

Fig. S6. Maximum likelihood core genome phylogeny of the genus *Providencia*. Bars show the presence (red) or absence (white) of virulence genes identified using the abricate database using 75% length identity and 75% sequence identity as a cut-off. Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was constructed using variant sites present in >95% of samples reads from reads mapped against reference genome *Providencia rustigianii* NCTC6933. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

Fig. S7. Maximum likelihood core genome phylogeny of the genus *Serratia*. Bars show the presence (red) or absence (white) of antimicrobial genes identified using the resfinder database using 75% length identity and 75% sequence identity as a cut-off. Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was

constructed using variant sites present in >95% of samples reads from reads mapped against reference genome *Serratia liquefaciens* ATCC 27592. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

Fig. S8. Maximum likelihood core genome phylogeny of the genus *Serratia*. Bars show the presence (red) or absence (white) of virulence genes identified using the abricate database using 75% length identity and 75% sequence identity as a cut-off. Tips and bars are coloured on the source of the respective isolates: current study = blue, environmental origin = green, human infection = red, human asymptomatic carriage = orange (isolates of unknown origin or human isolates of unknown status were not included). The tree was constructed using variant sites present in >95% of samples reads from reads mapped against reference genome *Serratia liquefaciens* ATCC 27592. Phylogenetic reconstruction was performed using a GTR+G+I model in IQ-TREE.

Table S1. Sampling site information.

Table S2. Sampling site details.

Table S3. Numbers of clones isolated from 66 *Galleria* hemocoel extractions.

Table S4. *Providencia* genome information.

Table S5. *Serratia* genome information.