

LETTER

doi:10.1038/nature10388

Antibiotic resistance is ancient

Vanessa M. D'Costa^{1,2*}, Christine E. King^{3,4*}, Lindsay Kalan^{1,2}, Mariya Morar^{1,2}, Wilson W. L. Sung⁴, Carsten Schwarz³, Duane Froese⁵, Grant Zazula⁶, Fabrice Calmels⁵, Regis Debruyne⁷, G. Brian Golding⁴, Hendrik N. Poinar^{1,3,4} & Gerard D. Wright^{1,2}

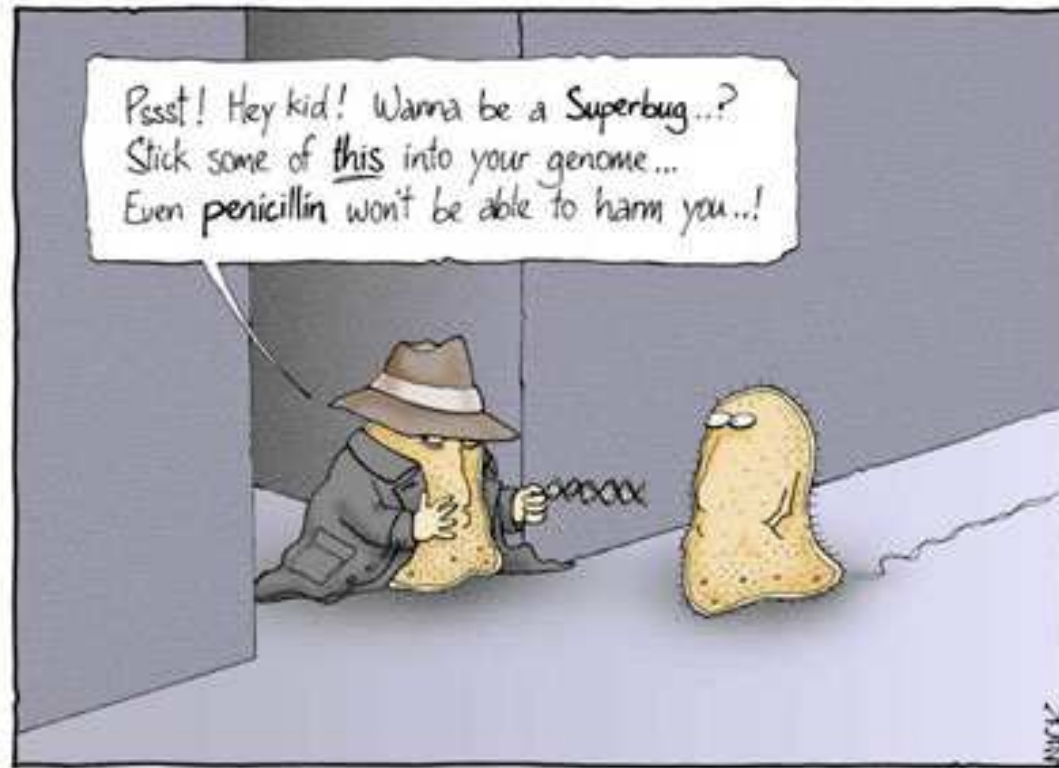
¹Michael G. DeGroot Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5. ²Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5. ³McMaster Ancient DNA Centre, Department of Anthropology, McMaster University, Hamilton, Ontario, Canada, L8S 4L9. ⁴Department of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1. ⁵Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E3. ⁶Yukon Palaeontology Program, Department of Tourism and Culture, Yukon Government, PO Box 2703, Whitehorse, Yukon, Canada, Y1A 2C6. ⁷Muséum National d'Histoire Naturelle, UMR 7206 Eco-anthropologie, 57 rue Cuvier, CP139, 75231 Paris cedex 05, France.

*These authors contributed equally to this work.

NATURE | VOL 477 | 22 SEPTEMBER 2011

Age Brauer

October 3rd, 2011
Journal Club



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

- Modern environmental and human commensal microbial genomes have a much larger concentration of antibiotic resistance genes than has been previously recognized.
- Estimates of the origin of natural product antibiotics range from 2 Gyr to 40Myr ago, suggesting that the resistance should be similarly old.

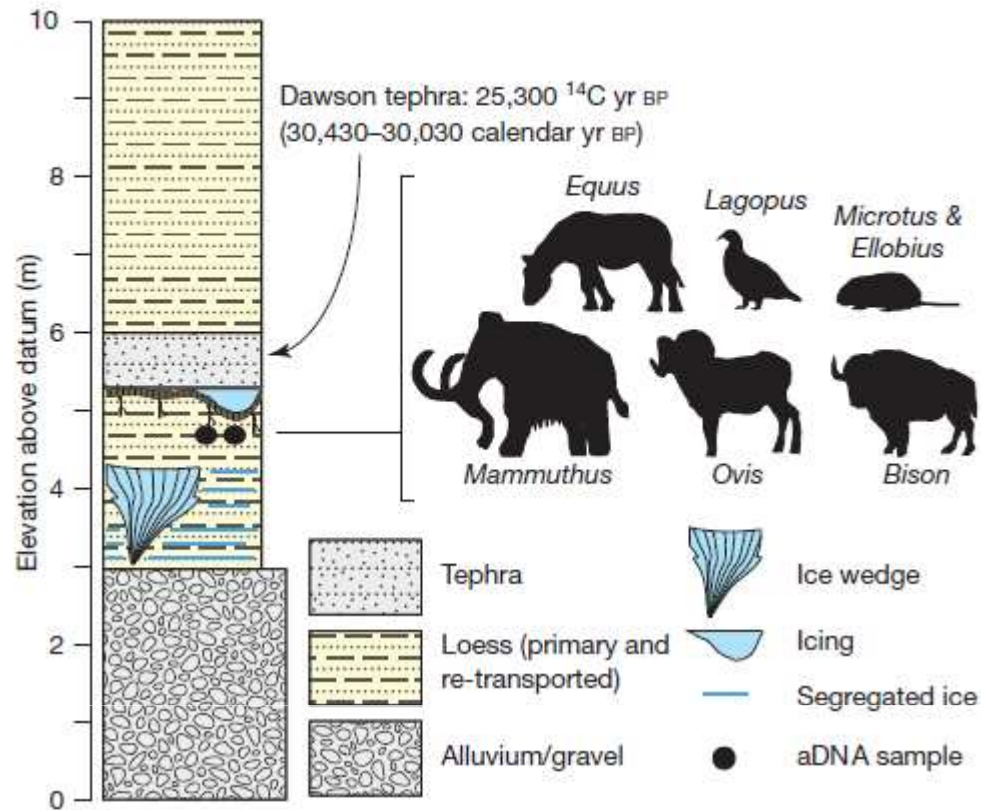


Figure 1 | Stratigraphic profile and location of Bear Creek site. Elevation is given in metres above base of exposure. Permafrost samples from below Dawson tephra were dated to about 30 kyr BP. Preservation of the ice below and above the sample indicates that the sediments have not thawed since deposition. Silhouettes represent mammals and birds identified from ancient DNA sequences that are typical of the regional Late Pleistocene environment. aDNA, ancient DNA.

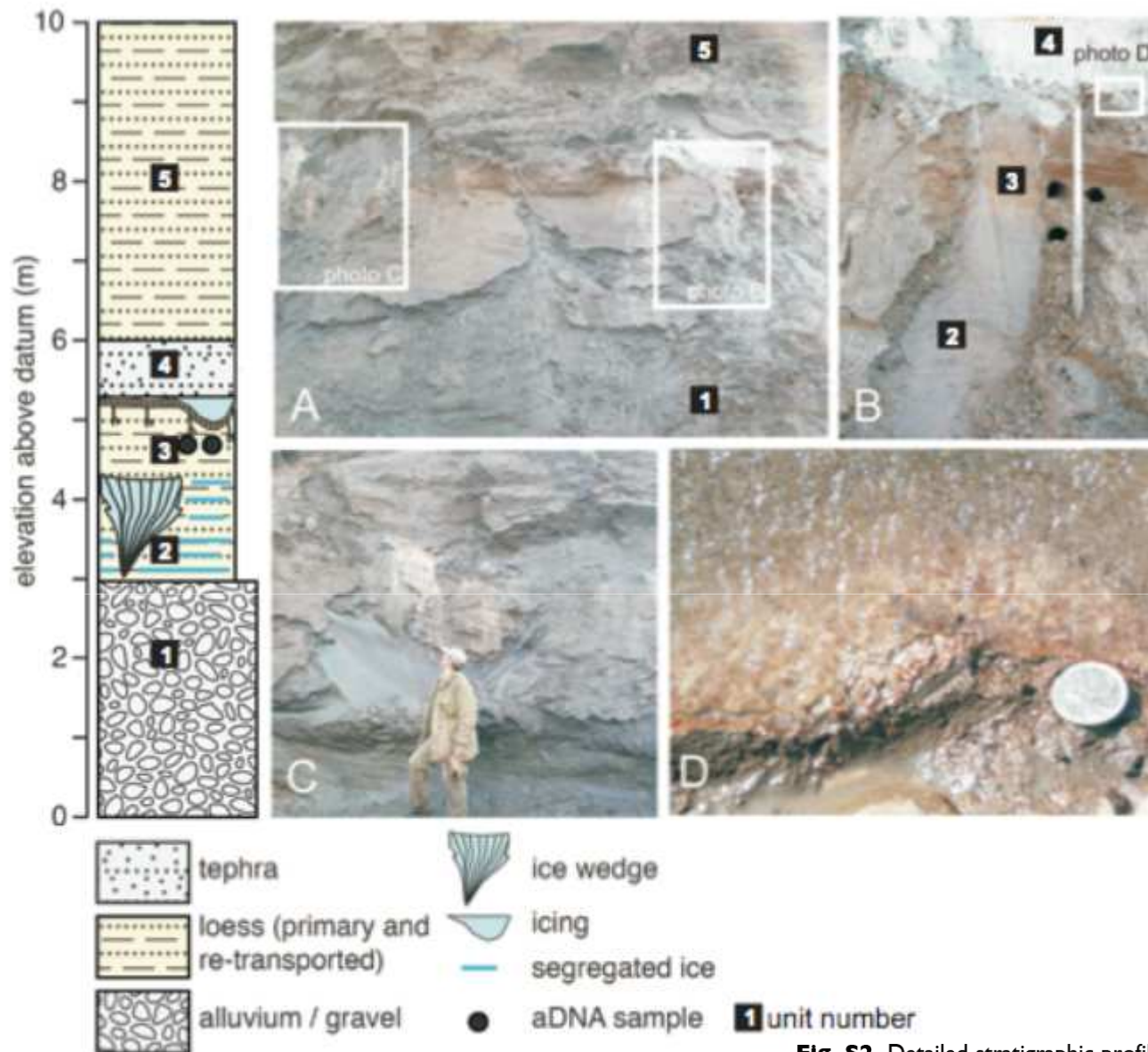


Fig. S2. Detailed stratigraphic profile of Bear Creek. **(A)** Study site with inset boxes of photo B and C showing sample locations. **(B)** Sampling site where two cores were drilled from the paleo-active layer in Unit 3; upper two samples in photo represent the two analyzed core samples (ice axe 80 cm long). **(C)** Ice wedge overlain by Dawson tephra (white sediment in centre of photo), about 5 m lateral to the sampling site (see inset box in A for location). **(D)** Close up of pool ice preserved on surface of Unit 3 (paleo-active layer) including plant macrofossils entombed in the ice.

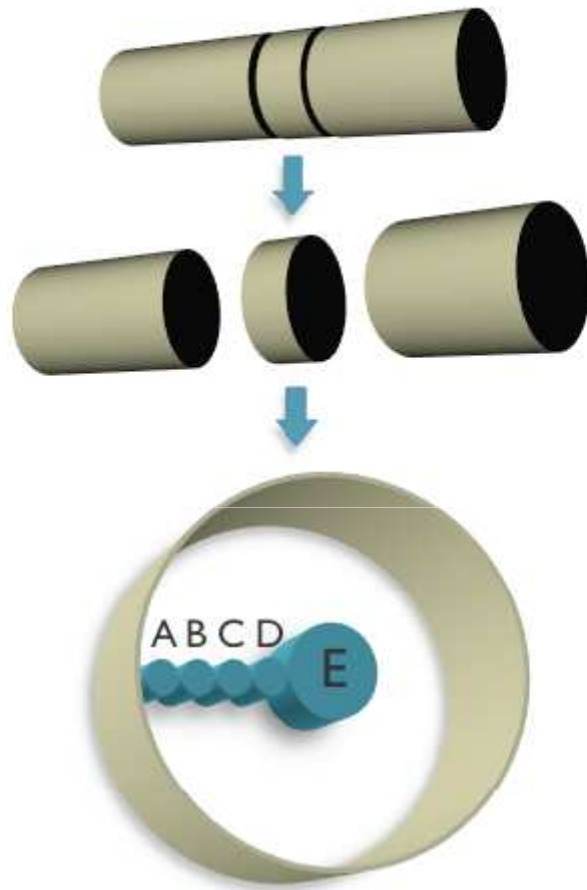


Fig. S3. Subsampling procedure. A disk approximately 5 cm thick was isolated by fracturing the larger core and subsamples A-E taken from the newly exposed surface.

PCR assays

- Vertebrates (12S rRNA)
- Plants (chloroplast *trnL* and *rbcL* genes)
- Bacteria (16S rRNA)
- For the source of contaminants (air, extraction blanks)
- Antibiotic resistance genes with diverse strategies of drug evasion

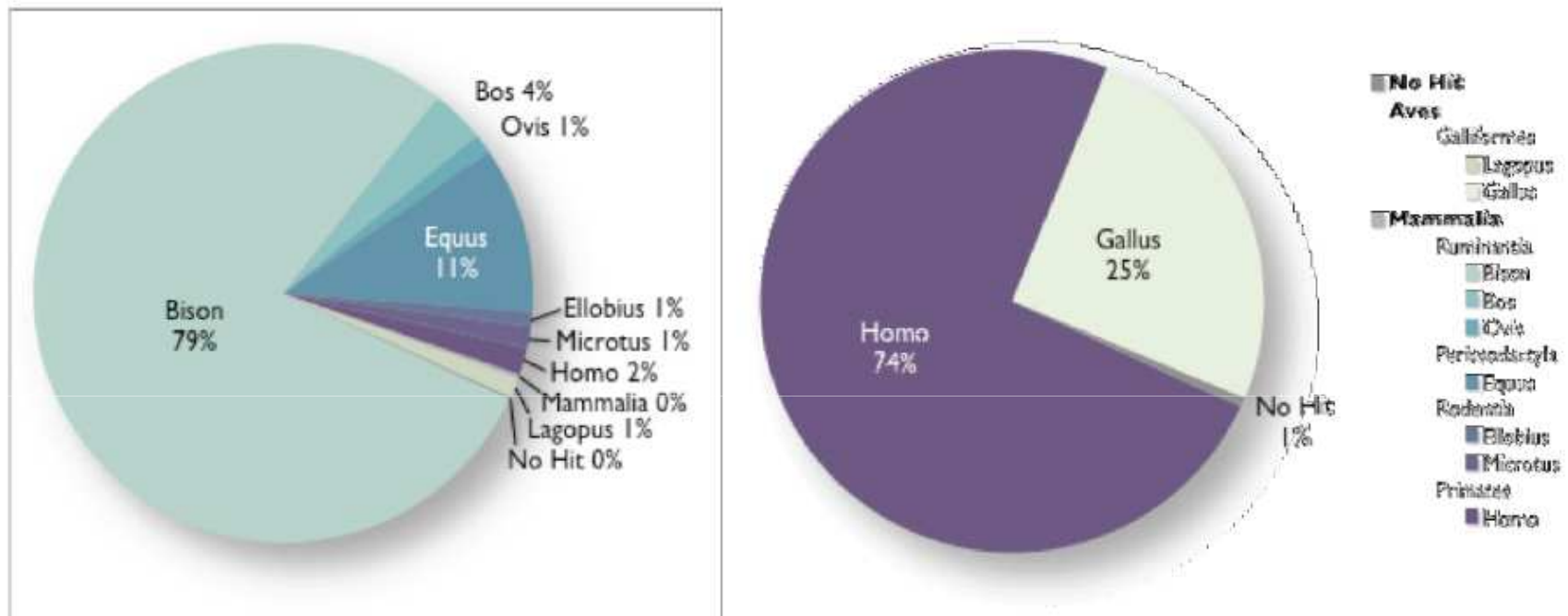


Fig. S6. Top BLAST hits for 12S sequences. Vertebrate sequences in the cores (left) are distinct from those in the contaminant PCR product (right).

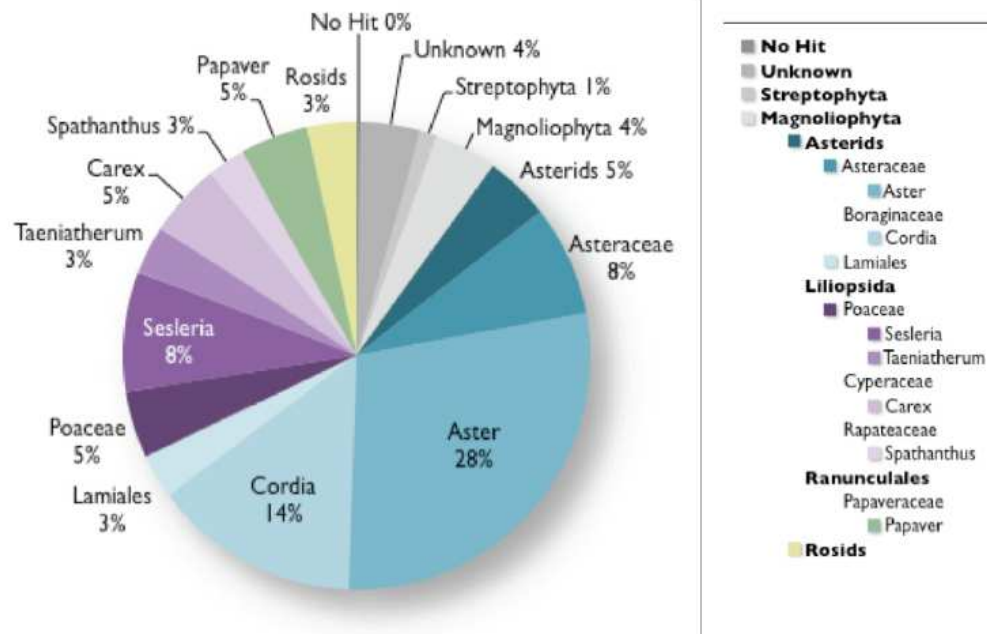


Fig. S7. Top BLAST hits for *rbcl* sequences.

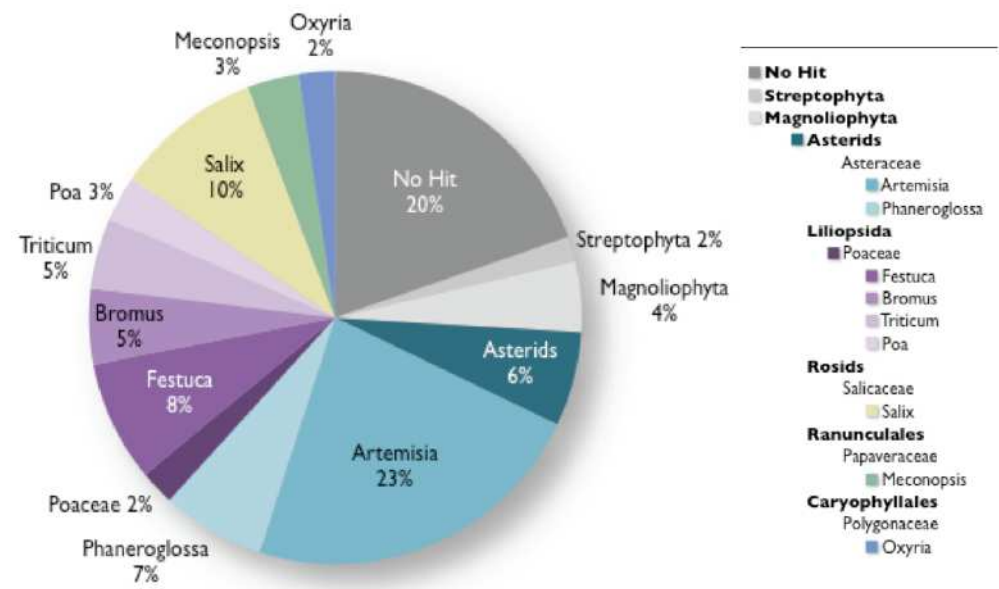
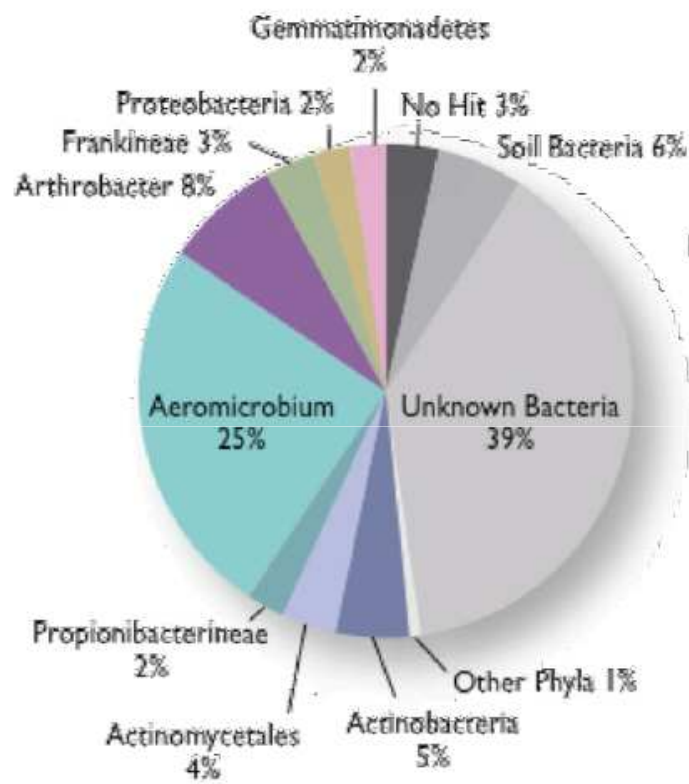
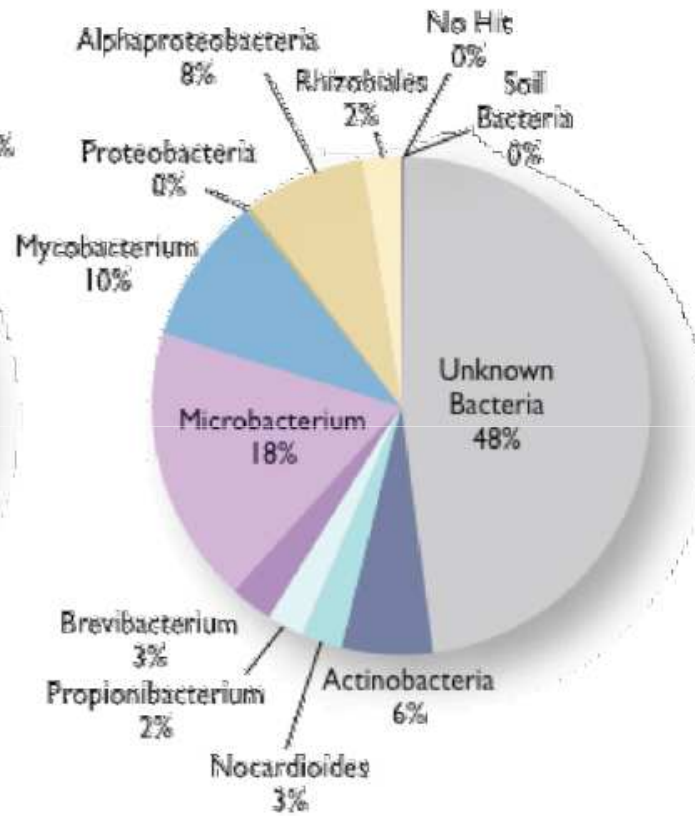


Fig. S8. Top BLAST hits for *trnL* sequences.



Cores



Contaminants



Targets of antibiotic resistance assays

- TetM – resistance to tetracyclin by weakening the interaction between the drug and the ribosome
- VanX – vancomycin resistance, dipeptide hydrolase
- aminoglycoside antibiotic modifying acetyltransferase AAC(3)
- Penicillin inactivating beta-lactamase Bla
- Ribosome methyltransferase Erm – blocks the binding of macrolide, lincosamide and type B streptogramin antibiotics.

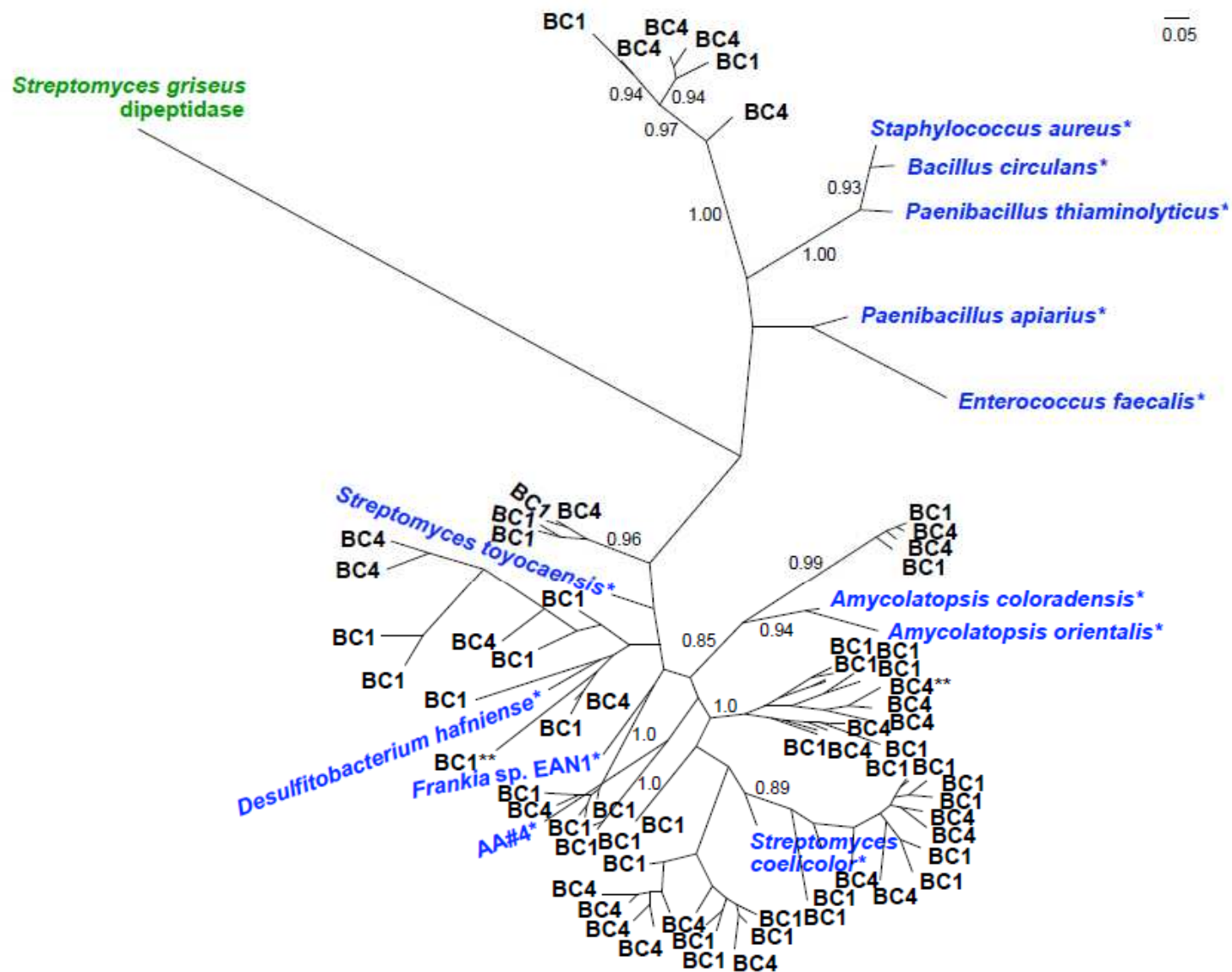


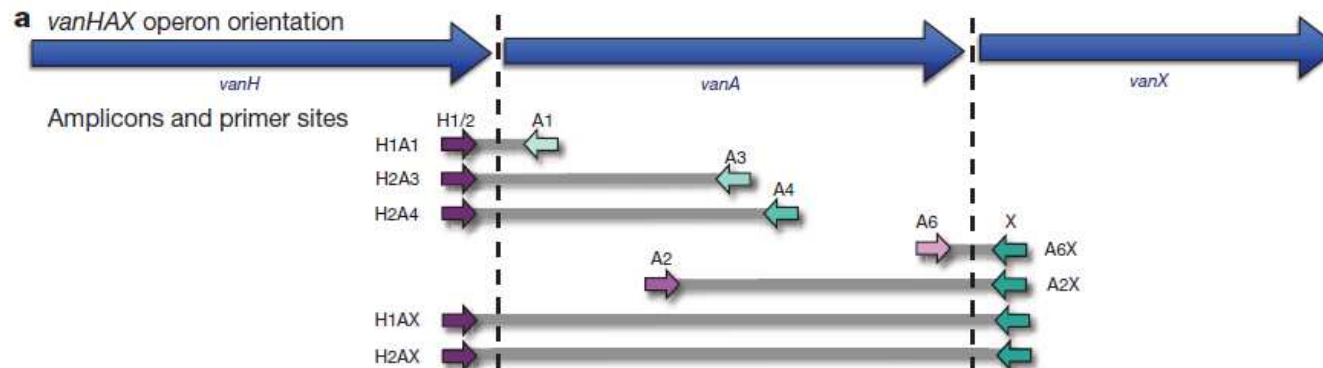
Fig. S10. Genetic diversity of permafrost VanX sequences. Unrooted phylogeny of translated *vanX* sequences. Blue denotes enzymes that are predicted to belong to the VanX family and green denotes those putatively associated with other functions. Permafrost-derived sequences are labeled by the originating core name. Strains that are associated with glycopeptide resistance^{24,38,57-62} are labeled with a single asterisk (*) and those containing putative stop codons but homology throughout with a double asterisk (**). The scale bar represents 0.05 substitutions per site. Posterior probabilities ≥ 0.7 are shown.

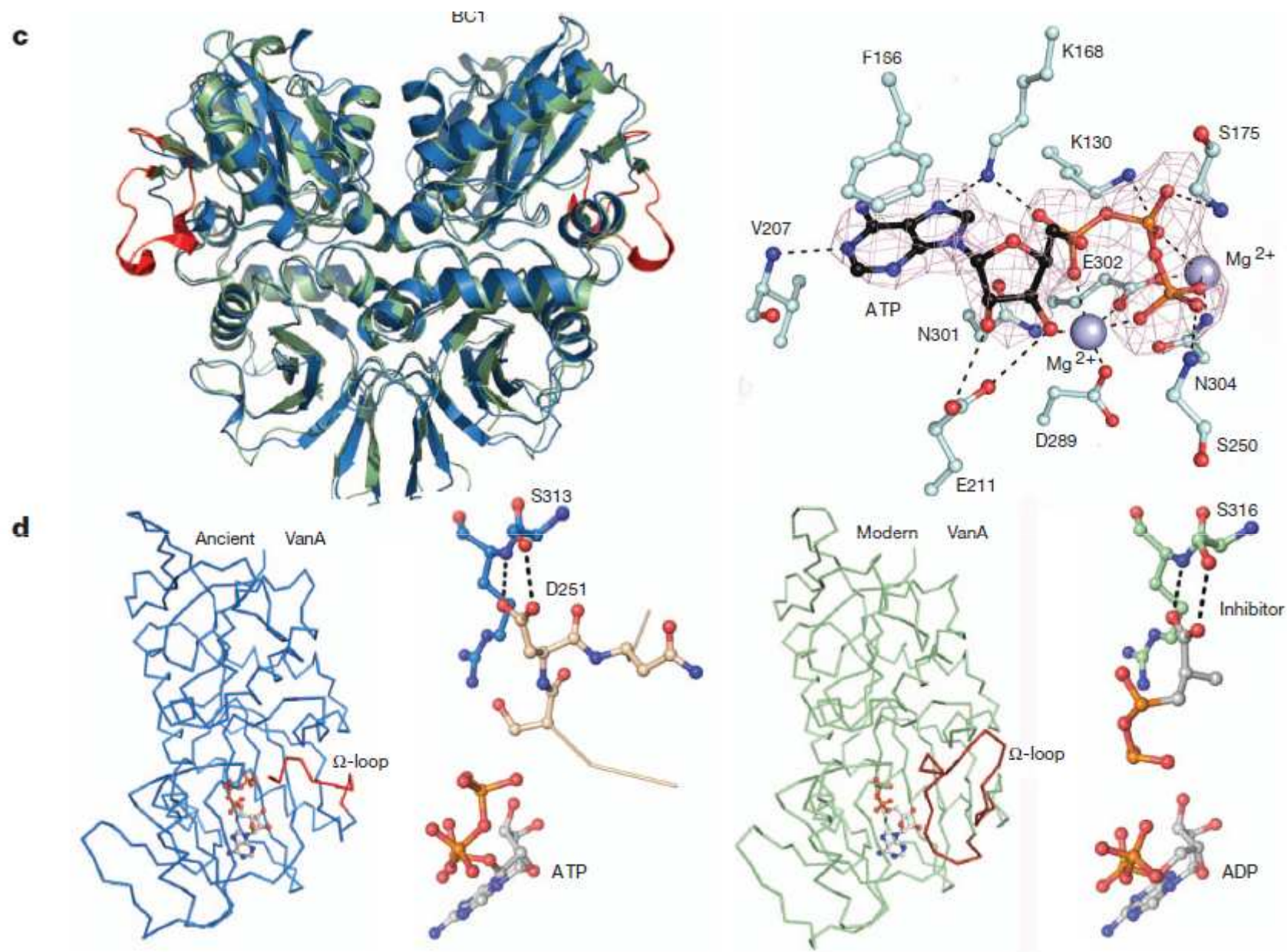
PRSS/PRFX compute the statistical significance of an alignment by aligning the two sequences, and then shuffling the second sequence 200 - 1000 times, and estimating the statistical significance from the distribution of shuffled alignment scores.

Table 1 | *vanHAX* permutation tests

Amplicon	Number	Length (base pairs)	Probability of similarity by chance alone to <i>Streptomyces coelicolor</i> genes		
			<i>vanH</i>	<i>vanA</i>	<i>vanX</i>
H1A1	164	203–213	3.59×10^{-3}	4.39×10^{-17}	0.24
H1A1*	12	209–216	2.83×10^{-3}	8.16×10^{-16}	0.28
H2A3	24	573–605	9.83×10^{-3}	1.27×10^{-54}	0.22
H2A4	79	666–681	4.33×10^{-3}	6.15×10^{-53}	0.18
A6X	159	170–179	0.11	6.87×10^{-8}	5.64×10^{-9}
A6X*	11	176–179	0.04	2.96×10^{-8}	3.63×10^{-9}
A2X	96	735–796	0.11	1.80×10^{-59}	1.35×10^{-6}
HAX†	40	1,173–1,204	5.95×10^{-3}	9.32×10^{-92}	6.47×10^{-7}

*Clones from independent replication in France. †Includes both H1AX and H2AX.





Conclusion

- Antibiotic resistance genes predate our use of antibiotics.
- Antibiotic resistance is an ancient, naturally occurring phenomenon widespread in the environment.