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Tetracycline-like resistome of ancient human guts

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ABSTRACT

Tetracyclines were discovered over 70 years ago and their use resulted in the emergence of tetracycline-resistance microorganisms; however, it has been hypothesized that tetracycline-resistance may have originated in the environment, and that determinants were transferred to the human gut microbiota. Ancient microbiomes represent an opportunity to explore the transmission of tetracycline-resistance determinants from the environment to humans. In the present study, tetracycline-like resistomes of three pre-Inca/Inca (10–15th centuries), and five Italian nobility (15–16th centuries) mummies were characterized using high-throughput sequencing. Sequences exhibited low homology to present-day determinants. Results may aid in the understanding of the evolution of tetracycline-resistance.

Introduction

Tetracyclines were discovered over 70 years ago as the first class of broad-spectrum antibiotics, and result from the secondary metabolism of soil microorganisms [1]. Given that tetracycline-resistance has been hypothesized to have an environmental origin, it is feasible to hypothesize that environmental microorganisms have co-evolved resistance to tetracycline in order to avoid auto-toxicity, and/or being targeted by other microorganisms [2]. It remains a matter of further speculation how these genes were then transferred from the environment to the human microbiota. Tetracycline-resistance determinants are usually identified and characterized through PCR amplification [3]; yet, with the increasing availability of high-throughput sequencing technologies, and curated databases, it is possible to characterize tetracycline-resistance determinants altogether [1]. This group of tetracycline-resistance determinants in a sample is known as the tetracycline resistome [1].

While the relative abundance of the global tetracycline-resistome was reported in a previous study, the present study took advantage of the publicly available gut metagenomes of three pre-Inca/Inca (10–15th centuries) and five Italian nobility (15–16th centuries) mummies and further broke down the data to specifically identify potential putative bacterial hosts at the phylum and species level and associated tetracycline-resistance determinants, as well as to provide insights of the evolution of several of the identified determinants [4]. Mining for antibiotic-resistance genes from metagenomic data is complex when characterizing ancient gut microbiomes. High similarity percentage hits (≥97%) are usually searched to avoid false positives; however, it is feasible to hypothesize that sequences predating the antibiotic therapy era may share low similarity percentages to present-day sequences because these may have diversified with the increase use of tetracyclines in the last few decades. Metagenomic data from ancient human guts may provide insights into the identity of earlier tetracycline-resistance determinant sequences. In the present study, it is hypothesized that ancient humans harbored a tetracycline-like resistome that may share resemblance to present-day tetracycline-resistance determinants.

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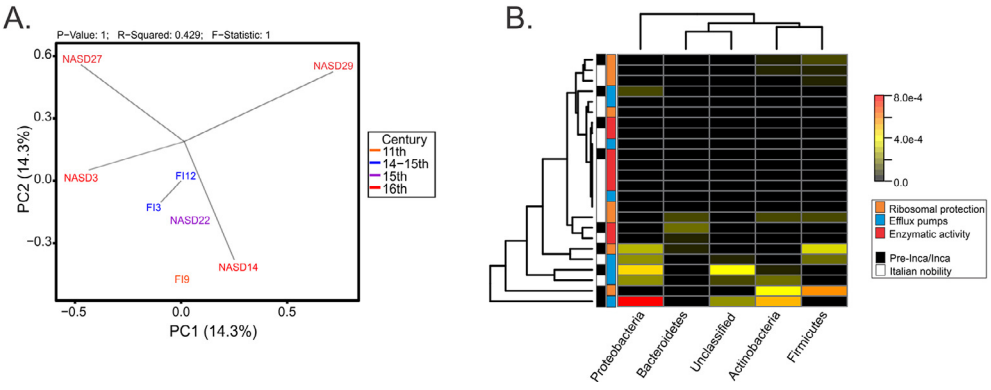

Fig. 1. Analysis of the tetracycline-resistome. Panel A shows PCoA of the beta-diversity based on Euclidean distances of the tetracycline-resistome of three pre-Inca/Inca and five Italian nobility mummies. Panel B shows a heatmap of relative abundances of bacterial phyla harboring tetracycline-resistance-like sequences. Results were curated by mode of action (i.e. ribosomal protection, efflux pumps and enzymatic activity).

Materials and methods

Permission to collect samples from the mummies was acquired from the Department of Paleopathology at the University of Pisa. Description of the pre-Inca/Inca and Italian nobility mummies has been done previously [5–14]. The pre-Inca/Inca mummies, namely FI3, FI9, and FI12, were preserved in funerary crypts and did not go through the process of decomposition aided by soil microorganisms (Supplementary Table 1). Italian nobility mummies, namely NASD3, NASD14, NASD22, NASD27 and NASD29, were preserved in empty spaces and were also not buried (Supplementary Table 1).

DNA was extracted from tissue samples collected from whole internal organs that were removed during the autopsy. Tissue samples were stored aseptically in hermetic plastic containers placed in a dry environment with silica gel at 18–20 °C to reduce any subsequent contamination. All standard precautions for ancient DNA work were employed as described previously [4]. DNA quality and integrity was checked in agarose gels [14].

Sequencing was performed at Molecular Research Laboratory (MRDNA) (www.mrdnalab.com; Shallowater, TX, USA), as described previously [4]. Shotgun metagenomic sequencing, file processing and sequence assembly were also performed as described previously [4,15]. The Comprehensive Antibiotic Resistance Database (CARD) was downloaded from https://card.mcmaster.ca/ for an initial global resistome screening. Briefly, contigs were screened against CARD using the BLASTx tool in CLC Genomics Workbench 9.5.2 (Qiagen, Hilden, Germany) with a minimum e-value cutoff of 1.0 e^{-5}. For tetracycline-resistance determinants, hits were filtered from the global resistome using the filter option in CLC Genomics Workbench 9.5.2. Contigs were extracted using CLC Genomics Workbench 9.5.2., and "reblasted" using BLASTx and the NCBI database as reference. "Reblast" is the process of blasting retrieved hits to a different database. Only hits sharing similarity to present-day tetracycline-resistance determinants, as well as associated groups were considered. The mode of action of each gene was manually curated and classified into efflux pumps, ribosomal protection proteins and enzymatic inactivation proteins. Putative host hits at the phylum level were manually curated and classified as Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. Hits not matching these phyla were grouped as "Unclassified". Data normalization was performed by dividing the total number of determinants by the total number of contigs. A heatmap of the Euclidean distances of normalized values was constructed as described previously [4]. The total number of contigs corresponding to tetracycline-resistance determinants was also used to construct a BIOM table using the script biom convert (http://biom-format.org/documentation/biom_conversion.html). The biom file was then used to construct a Principal Coordinates Analysis (PCoA) using a proprietary script to visualize beta-diversity based on Euclidean distances. For the phylogenetic analyses, contig sequences were converted to protein coding genes (within one frame) using transeq function implemented in EMBOS software. Multiple gene alignment was performed using MAFFT software. Maximum likelihood tree search with non-parametric bootstrapping was performed using RAxML software set at following parameters; 10 randomized parsimony starting trees, fixed empirical substitution matrix (LG), empirical amino acid frequencies from alignment, 8 discrete GAMMA categories, 200 bootstrap replicates.

Results

Before further analyses, authentication of the microbiomes was previously performed using Bayesian microbial source tracker and MapDamage, and no evidence of contamination with modern samples was noted [4]. Sequence information of the mummies is shown in Supplementary Table 2, and include the average read and contig length (bp), and the percentage of assembled reads. Results also show the percentage of the contigs that were classified as any putative antibiotic-resistance gene (Total global-resistome hits), as well as those hits that were specifically classified as part of the tetracycline-resistome (Tetracycline-resistome hits). Overall, the Pre-Inca/Inca mummies had, on average, the highest number of putative antibiotic-resistance genes compared to the Italian nobility mummies (0.83% and 0.37%, respectively), and the highest number of hits classified as putative tetracycline-resistance genes (0.17% and 0.044%, respectively).

PCoA of the beta-diversity of the tetracycline-resistome did not show a significant separation of the samples based on time period (10–16th centuries) (Fig. 1A) or culture (Supplementary Fig. 1). Supplementary Fig. 1 also shows no separation based on time period when modern samples are included. Modern samples included two Amazonian and two modern Italian gut metagenomes, which were also previously characterized [4]. The tetracycline-resistome was then broken down to identify potential putative hosts at both the phylum and species level. At the phylum level, the Proteobacteria contributed the greatest number of sequences classified as efflux pumps; while the Firmicutes contributed the greatest number of sequences classified as having ribosomal protection activity. Only the Bacteroidetes contributed sequences associated with enzymatic inactivation activity in both the Pre-Inca/Inca and Italian nobility mummies (Fig. 1B). No clear separation of the data was noted based on culture or mode of action. When breaking down the data by potential putative hosts at the species level, bacteria identified in the mummies are mostly common inhabitants of the human and other animals gut, including, but not limited to *Bacteroides fragilis*. Many were also bacteria that inhabit both the environment and animal guts including, and included, but were not limited to *Escherichia coli* and *Enterococcus faecalis* (Table 1).

Given that the present study considered putative tetracycline-resistance genes with low identity percentages compared to modern determinants, comparisons between the CARD and NCBI database were performed to exclude any hit with unrelated functions. Expectedly, results between the databases are differing. Results for the pre-Inca/Inca mummies FI3 (Supplementary Table 3), FI9 (Supplementary

Table 1
Tetracycline-like resistome of pre-Inca/Inca (FI3, FI9 and FI12) and Italian nobility mummies (NASD3, NASD14, NASD22, NASD27 and NASD29). Contigs were screened against the Comprehensive Antibiotic-Resistance Database (CARD). Table shows mummy, mode of action, gene, putative bacterial host, number of hits, and the known source.

Mummy	CARD Mode of action	Gene	Putative Hosts (number of hits)	Source [reference]
FI3	Efflux pump	otr(B)	*Streptomyces rimosus* (1)	Soil [21]
	Efflux pump	tet(31)	*Aeromonas salmonicida* (1)	Fish [22]
	Efflux pump	tet(35)	*Vibrio harveyi* (2)	Ocean, marine animals [23]
	Efflux pump	tet(43)	Uncultured bacterium (4)	–
	Ribosomal protection	tet(44)	*Campylobacter fetus* (1)	Sheep, goat and cattle gut [24]
	Efflux pump	tet(A)41	*Serratia marcescens* (1)	Water, soil, plants, insects, animal intestines [25]
	Efflux pump	tet(B)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestines [26]
	Efflux pump	tet(C)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestines [26]
	Efflux pump	tet(H)	*Pasteurella multocida* (1); *Actinobacillus pleuropneumoniae* (1)	Upper respiratory tract of mammals, birds [27]; Swine intestine [28]
	Efflux pump	tet(L)	*Geobacillus stearothermophilus* (2)	Thermophilic habitats, soil, ocean sediment [29]
	Ribosomal protection	tet(M)	*Enterococcus faecalis* (6); *Staphylococcus aureus* (1)	Water, soil, plants, insects, animal intestines [30]; Water, soil, skin, upper respiratory tract, gut mucosa [31]
	Ribosomal protection	tet(S)	*Listeria monocytogenes* (1)	Water, soil, raw vegetables, fecal material [32]
	Ribosomal protection	tet(T)	*Streptococcus pyogenes* (1)	Nasopharynx [33]
	Ribosomal protection	tet(W)	*Bifidobacterium longum* (6); *Butyrivibrio fibrisolvens* (4)	Animal intestine and vagina [34]; Animal intestine [35]
	Enzymatic	tet(X)	*Bacteroides fragilis* (1)	Human intestine [36]
	Efflux pump	tet(Y)	*Pasteurella multocida* (1)	Upper respiratory tract of mammals, birds [27]
FI9	Ribosomal protection	otr(A)	*Streptomyces rimosus* (1)	Soil [37]
	Efflux pump	tet(33)	*Corynebacterium glutamicum* (1)	Soil [38]
	Ribosomal protection	tet(36)	*Bacteroides coprosuis* (1)	Swine manure [39]
	Efflux pump	tet(43)	Uncultured bacterium (10)	–
	Ribosomal protection	tet(44)	*Campylobacter fetus* (1)	Sheep, goat, cattle gut [24]
	Efflux pump	tet(A)	*Pseudomonas aeruginosa* (3); *Vibrio cholerae* (2); *Acinetobacter baumannii* (1)	Water, soil, plants, insects, animal intestine, sewage, hospitals [40]; Brackish riverine, estuarine, coastal waters [41]; Water, soil, hospital environments [43]
	Efflux pump	tet(A)42	*Micrococcus* spp. (1)	Water, dust, soil, human skin, animal, dairy products [42]
	Efflux pump	tet(B)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestine [26]
	Efflux pump	tetA(41)	*Serratia marcescens* (2)	Water, soil, plants, insects, animal intestine [44]
	Efflux pump	tet(C)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestine [26]
	Efflux pump	tet(E)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestine [26]
	Efflux pump	tet(H)	*Actinobacillus pleuropneumoniae* (1)	Swine intestine [28]
	Efflux pump	tet(K)	*Staphylococcus aureus* (1)	Skin, upper respiratory tract, gut mucosa, water [31]
	Ribosomal protection	tet(M)	*Clostridium difficile* (1); *Staphylococcus aureus* (1); *Enterococcus faecalis* (3)	Swine, human, soil, sewage [45]; Skin, upper respiratory tract, gut mucosa, water [31]; Water, soil, plants, insects, animal intestine [30]
	Ribosomal protection	tet(T)	*Streptococcus pyogenes* (2)	Nasopharynx [33]
	Ribosomal protection	tet(W)	*Butyrivibrio fibrisolvens* (4); *Enterococcus faecalis* (2); *Bifidobacterium longum* (10)	Animal intestine [35]; Water, soil, plants, insects, animal intestine [30]; Animal intestine, vagina [34]
	Enzymatic	tet(X)	*Bacteroides fragilis* (5)	Human intestine [36]
	Ribosomal protection	tet(P)	*Clostridium perfringens* (1)	Decaying vegetation, marine sediment, human and animal intestine, insects, soil [46]
FI12	Ribosomal protection	tet(32)	*Clostridiaceae* bacterium (1)	–
	Efflux pump	tet(33)	*Corynebacterium glutamicum* (1)	Soil [38]
	Efflux pump	tet(35)	*Vibrio harveyi* (4)	Ocean, marine animals [23]
	Ribosomal protection	tet(36)	*Bacteroides coprosuis* (1)	Swine manure [39]
	Efflux pump	tetA(39)	*Acinetobacter* sp. (1)	Water, soil, hospital environments [47]
	Efflux pump	tet(43)	Uncultured (3)	–
	Ribosomal protection	tet(44)	*Campylobacter fetus* (2)	Sheep, goat, cattle gut [24]
	Efflux pump	tet(B)	*Neisseria meningitides* (1)	Nasopharynx [48]
	Efflux pump	tet(H)	*Actinobacillus pleuropneumoniae* (1); *Pasteurella multocida* (1)	Swine intestine [28]; Upper respiratory tract of mammals, birds [27]
	Efflux pump	tet(J)	*Proteus mirabilis* (1)	Water, soil, hospital environments, animal intestine [49]
	Ribosomal protection	tet(M)	*Enterococcus faecalis* (2)	Water, soil, plants, insects, animal intestine [30]
	Ribosomal protection	tet(O)	*Campylobacter jejuni* (1)	Animal intestine [50]
	Ribosomal protection	tet(S)	*Listeria monocytogenes* (1)	Water, soil, raw vegetables, fecal material [32]
	Ribosomal protection	tet(T)	*Streptococcus pyogenes* (1)	Nasopharynx [33]
	Efflux pump	tet(V)	*Mycobacterium smegmatis* (2)	Water, soil [51]
	Ribosomal protection	tet(W)	*Butyrivibrio fibrisolvens* (1)	Animal intestine [35]
	Efflux pump	tetA(41)	*Serratia marcescens* (1)	Water, soil, plants, insects, animal intestine [44]
	Efflux pump	tetA	*Pseudomonas aeruginosa* (1)	Water, soil, plants, insects, animal intestine, sewage, hospitals [40]
	Efflux pump	tetA(42)	*Micrococcus* sp. (4)	Water, dust, soil, human skin, animal, dairy products [42]
NASD3	Ribosomal protection	tet(OW)	*Megasphaera elsdenii* (1)	Rumen intestine [52]
NASD14	Efflux pump	tet(35)	*Vibrio harveyi* (1)	Ocean, marine animals [23]
	Efflux pump	tet(K)	*Staphylococcus aureus* (1)	Skin, upper respiratory tract, gut mucosa, water [31]
	Transcription	tet(R)	*Salmonella typhi* (1)	Animal intestine [53]
	Ribosomal protection	tet(W)	*Butyrivibrio fibrisolvens* (1)	Animal intestine [35]
NASD22	Ribosomal protection	otr(A)	*Streptomyces rimosus* (2)	Soil [21]
	Efflux pump	tet(31)	*Aeromonas salmonicida* (1)	Fish [22]

(*continued on next page*)

Table 1 (*continued*)

Mummy	CARD Mode of action	Gene	Putative Hosts (number of hits)	Source [reference]
	Efflux pump	tet(35)	*Vibrio harveyi* (2)	Ocean, marine animals [23]
	Ribosomal protection	tet(36)	*Bacteroides coprosuis* (2)	Swine manure [39]
	Efflux pump	tet(43)	Uncultured bacterium (2)	–
	Efflux pump	tet(A)	*Acinetobacter baumannii* (2)	Water, soil, hospital environments [43]
	Efflux pump	tet(P)	*Clostridium perfringens* (1)	Decaying vegetation, marine sediment, human and animal intestine, insects, soil [46]
	Efflux pump	tet(J)	*Proteus mirabilis* (1)	Water, soil, hospital environments, animal intestine [49]
	Efflux pump	tet(L)	*Geobacillus stearothermophilus* (4)	Soil, hot springs, ocean sediment, and is a cause of spoilage in food products [29]
	Ribosomal protection	tet(Q)	*Bacteroides fragilis* (2)	Human intestine [36]
	Ribosomal protection	tet(S)	*Listeria monocytogenes* (1)	Water, soil, raw vegetables, fecal material [32]
	Ribosomal protection	tet(T)	*Streptococcus pyogenes* (3)	Nasopharynx [33]
	Ribosomal protection	tet(W)	*Bifidobacterium longum* (1); *Butyrivibrio fibrisolvens* (1)	Animal intestine and vagina [34]; Animal intestine [35]
	Efflux pump	tetA(42)	*Micrococcus* sp. (1)	Water, dust, soil, human skin, animal, dairy products [42]
	Efflux pump	tetA(G)	*Acinetobacter baumannii* (1)	Water, soil, hospital environments [43]
NASD27	Ribosomal protection	tet(32)	Clostridiceae bacterium (2)	–
	Efflux pump	tet(33)	*Corynebacterium glutamicum* (1)	Soil [38]
	Ribosomal protection	tet(36)	*Bacteroides coprosuis*(1)	Swine manure [39]
	Efflux pump	tet(43)	Uncultured bacterium (4)	–
	Ribosomal protection	tet(44)	*Campylobacter fetus* (1)	Sheep, goat and cattle gut [24]
	Efflux pump	tet(A)	*Acinetobacter baumannii* (1); *Pseudomonas aeruginosa* (2); *Serratia marcescens* (1)	Water, soil, hospital environments [43]; Water, soil, plants, insects, animal intestine, sewage, hospitals [40]; Water, soil, plants, insects, animal intestine [44]
	Efflux pump	tet(B)	*Escherichia coli* (1)	Water, soil, plants, insects, animal intestine [26]
	Efflux pump	tet(P)	*Clostridium perfringens* (1)	Decaying vegetation, marine sediment, human and animal intestine, insects, soil [46]
	Efflux pump	tet(H)	*Mannheimia haemolytica* (1)	Nasopharynx [54]
	Ribosomal protection	tet(T)	*Streptococcus pyogenes* (1)	Nasopharynx [33]
	Ribosomal protection	tet(W)	*Bifidobacterium longum* (2)	Animal intestine and vagina [34]
	Enzymatic	tet(X)	*Bacteroides fragilis* (3)	Human intestine [36]
	Efflux pump	tet(Z)	*Corynebacterium glutamicum* (2)	Soil [38]
	Efflux pump	tetA(41)	*Serratia marcescens* (1)	Water, soil, plants, insects, animal intestine [44]
	Efflux pump	tetA(42)	*Micrococcus* spp. (3)	Water, dust, soil, human skin, animal, dairy products [42]
NASD29	Enzymatic	tet(X)	*Bacteroides fragilis* (1)	Human intestine [36]

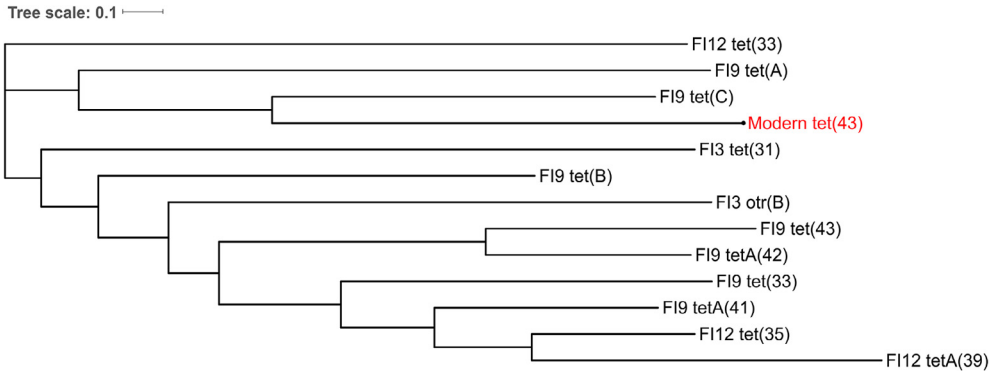

Fig. 2. Phylogenetic analysis of selected putative tetracycline-resistance determinants in the mummies. Modern tet(43) was added to the tree for comparison. Centroids connect samples of same group.

Table 4), and FI12 (Supplementary Table 5) showed a number of putative tetracycline-resistance hits that were classified into the Major Facilitator Superfamily (MFS) when interrogating the NCBI database. This may be due to a number of tetracycline-resistance genes, specifically efflux pumps, belonging to the MFS. Several hits were also classified as elongation factors and those having similar functions. This may be due to some tetracycline-resistance genes sharing homology to genes encoding elongation factors. Similar outcomes were noted for the Italian nobility mummies NASD3 (Supplementary Table 6), NASD14 (Supplementary Table 7), NASD22 (Supplementary Table 8), NASD27 (Supplementary Table 9), NASD29 (Supplementary Table 10). Interestingly, mummies NASD14 and NASD27 showed hits that were specifically classified as tetracycline-resistance genes when performing the reblast with the NCBI database. Pre-Inca/Inca sequences encoding efflux pumps were then selected and a phylogenetic tree was constructed. The phylogenetic analysis showed that the positioning of the sequences is in agreement with these sharing low identities compared to modern counterparts (Fig. 2).

Discussion

Ancient microbiomes are known to harbor putative antibiotic-resistance-like determinants [4,15–17], but little is still known about ancient human gut tetracycline-resistomes [4,15,16]. While the relative abundance of the tetracycline resistome has been reported previously, the study did not break down the data into specific determinants and

the potentially associated putative hosts at the phylum and species level [4]. The advantage of ancient human samples in the study of antibiotic-resistance, including tetracycline-resistance, is that these have not been subjected to the pressure of modern antibiotics; thus, can provide a baseline for the study of the evolution of antibiotic-resistance, which was also not addressed in the previous study [4]. The present study lends further evidence of the human gut as a reservoir of tetracycline-resistance-like determinants prior the antibiotic therapy era. Given the environmental origin of tetracyclines and that microorganisms are constantly co-evolving resistance to prevent auto-toxicity or being targeted by other microorganisms, it is expected that most of the tetracycline-resistance-like sequences identified in the mummified gut remains had low identity percentages to known sequences. A recent paper that applied a similar approach to identify beta-lactamases in ancient environmental and human microbiomes found similar outcomes [16]. This may suggest that the use of tetracycline antibiotics have enabled the evolution and diversification of earlier forms of tetracycline-resistance-like antibiotic genes. This may also be supported by the positioning of the selected sequences in the phylogenetic tree compared to the modern tet(43) counterpart. The positioning of the different selected tetracycline-resistance determinants in the phylogenetic tree may open the opportunity to perform functionality experiments, which may provide evidence of these as potentially new classes of tetracycline-resistance-like determinants, as suggested previously with beta-lactamases [16].

Resistome characterization seems to highly depend on the database interrogated. In many cases, CARD would classify the sequence into specific tetracycline-resistance determinants, while NCBI provided information related to the sequence domains, families, or provided a description associated with sequences having unrelated functions. This suggests that “reblast” against an additional database, and that additional curating may be required to validate results and eliminate false positives with unrelated functions.

The present study showed that many of the identified bacterial putative hosts are inhabitants of the human gut, while others are cosmopolitan and can be found in a variety of environments. While some tetracycline-resistance-like determinants in the present study resembled those in unexpected putative hosts (e.g. *Vibrio harveyi*), it should be noted that results are limited to available databases. In addition, discretion should be applied when interpreting results as many of these tetracycline-resistance determinants are carried in mobile genetic elements. Nevertheless, data are intriguing as it may open the opportunity to hypothesize that earlier forms of tetracycline-resistance genes reached the human gut microbiota by means that remain a matter of further research and support the hypothesis of a divergent (tetracycline) resistome [16,18–20].

Data availability

16S data for the Inca mummies are available in MG-RAST under ID numbers 4644220.3 (descending colon mummy FI9), 4662510.3 (mummy FI3) and 4662511.3 (mummy FI12). 16S data for the Italian mummies are available in MGRAST under ID numbers 4769343.3 (mummy NASD3), 4769345.3 (mummy NASD14), 4769342.3 (mummy NASD22), 4769341.3 (mummy NASD27) and 4769344.3 (mummy NASD29). Shotgun metagenomic data are available in MGRAST under ID numbers 4630170.3 (descending colon mummy FI9), 4629033.3 (mummy FI3), and 4626489.3 (mummy FI12). Shotgun metagenomic data for the Italian mummies are available in MGRAST under ID numbers 4629038.3 (mummy NASD3), 4629034.3 (mummy NASD14), 4629035.3 (mummy NASD22), 4629036.3 (mummy NASD27), and 4629037.3 (mummy NASD29).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.humic.2018.07.001.

References

[1] Thaker M, Spanogiannopoulos P, Wright GD. The tetracycline resistome. Cell Mol Life Sci 2010;67(3):419–31.
[2] Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. Science 2008;321(5887):365–7.
[3] Ng L-K, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 2001;15(4):209–15.
[4] Santiago-Rodriguez TM, Fornaciari G, Luciani S, Toranzos GA, Marota I, Giuffra V, et al. Gut microbiome and putative resistome of inca and italian nobility mummies. Genes 2017;8(11):310.
[5] Fornaciari G. The mummies of the Abbey of Saint Domenico Maggiore in Naples: a preliminary report. Arch Antrop Etnol 1985;115:21.
[6] Pollina L, Tornaboni D. Pulmonary and hepatic pathologies in the series of mummies of S, Domenico Maggiore at Naples (XVI century).
[7] Fornaciari G, Bruno J, Corcione N, Tornaboni D, Castagna M. Un cas de tumeur maligne primitive de la region naso-orbitaire dans une momie de la basilique de S. Domenico Maggiore à Naples (XVIe siècle). Advances in Paleopathology. In: Proceedings of the VII European Meeting of the Paleopathology Association. Lyon; 1988. p. 65–9.
[8] Gaeta R, Ventura L, Fornaciari G. Il tumore di Ferdinando Orsini, duca di Gravina di Puglia (+1549). Atti del 50° Congrsso Nazionale della Società Italiana di Storia della Medicina. Palermo 2015.
[9] Gaeta R, Ventura L, Fornaciari G. The cutaneous cancer of ferdinando orsini. Jama Dermatol 2017;153(7):643.
[10] Gaeta R, Giuffra V, Fornaciari G. Cancer in the Renaissance court of Naples. Lancet Oncol 2017;18(8):e432.
[11] Fornaciari G. Histology of ancient soft tissue tumors: a review. Int J Paleopathol 2017.
[12] Marchetti A, Pellegrini S, Bevilacqua G, Fornaciari G. K-RAS mutation in the tumour of Ferrante I of Aragon, King of Naples. Lancet 1996;347(9010). 1272-nil.
[13] Ottini L, Falchetti M, Marinozzi S, Angeletti LR, Fornaciari G. Gene-environment interactions in the pre–Industrial Era: the cancer of King Ferrante I of Aragon (1431–1494). Hum Pathol 2011;42(3):332–9.
[14] Ciranni R, Fornaciari G. Juvenile cirrhosis in a 16th century Italian mummy. Current technologies in pathology and ancient human tissues. Virchows Arch 2004;445(6):647–50.
[15] Santiago-Rodriguez TM, Fornaciari G, Luciani S, Dowd SE, Toranzos GA, Marota I, et al. Gut microbiome of an 11th century A.D. Pre-columbian andean mummy. PLoS One 2015;10(9). e0138135.
[16] Rascovan N, Telke A, Raoult D, Rolain JM, Desnues C. Exploring divergent antibiotic resistance genes in ancient metagenomes and discovery of a novel beta-lactamase family. Environ Microbiol Rep 2016.
[17] D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, et al. Antibiotic resistance is ancient. Nature 2011;477(7365):457–61.
[18] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010;8(4):251–9.
[19] Graham DW, Knapp CW, Christensen BT, McCluskey S, Dolfing J. Appearance of beta-lactam resistance genes in agricultural soils and clinical isolates over the 20(th) Century. Sci Rep 2016;6:21550.
[20] D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, et al. Antibiotic resistance is ancient. Nature 2011;477(7365):457–61.
[21] Petkovic H, Cullum J, Hranueli D, Hunter IS, Peric-Concha N, Pigac J, et al. Genetics of *Streptomyces rimosus*, the oxytetracycline producer. Microbiol. Mol. Biol. Rev. 2006;70(3):704–28.
[22] Kamble R. *Aeromonas salmonicida* furunculosis in an adult male. Int J Curr Microbiol App Sci 2015;4(2):59–63.
[23] Austin B, Zhang XH. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. Lett Appl Microbiol 2006;43(2):119–24.
[24] Wagenaar JA, van Bergen MA, Blaser MJ, Tauxe RV, Newell DG, van Putten JP. *Campylobacter fetus* infections in humans: exposure and disease. Clin Infect Dis 2014;58(11):1579–86.
[25] Thompson SA, Maani EV, Lindell AH, King CJ, McArthur JV. Novel tetracycline resistance determinant isolated from an environmental strain of *Serratia marcescens*. Appl Environ Microbiol 2007;73(7):2199–206.
[26] Blount ZD. The unexhausted potential of *E. coli*. Elife 2015;4.
[27] Wilson BA, Ho M. *Pasteurella multocida*: from zoonosis to cellular microbiology. Clin Microbiol Rev 2013;26(3):631–55.
[28] Menzel A, Beyerbach M, Siewert C, Gundlach M, Hoeltig D, Graage R, et al. *Actinobacillus pleuropneumoniae* challenge in swine: diagnostic of lung alterations by infrared thermography. BMC Vet Res 2014;10(1):199.
[29] Burgess S, Flint S, Lindsay D, Cox M, Biggs P. Insights into the *Geobacillus stearothermophilus* species based on phylogenomic principles. BMC Microbiol 2017;17(1):140.

[30] Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. Microbiol Mol Biol Rev 2012;76(4):685–706.
[31] Enright MC, Witte W. Epidemiology of MRSA and MSSA. In: Ala' Aldeen DAA, editor. Staphylococcus aureus Molecular and Clinical Aspects. Great Britain: Horwood Publishing Limited; 2004. p. 31–53.
[32] Linke K, Rückerl I, Brugger K, Karpiskova R, Walland J, Muri-Klinger S, et al. Reservoirs of *Listeria* species in three environmental ecosystems. Appl Environ Microbiol 2014;80(18):5583–92.
[33] Patterson M. Streptococcus pyogenes, other streptococci, and enterococcus. Streptococcus. Med Microbiol 1996.
[34] Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, et al. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. Proc Natl Acad Sci 2002;99(22):14422–7.
[35] Melville CM, Scott KP, Mercer DK, Flint HJ. Novel tetracycline resistance gene, tet (32), in the *Clostridium*-related human colonic anaerobe K10 and its transmission in vitro to the rumen anaerobe Butyrivibrio fibrisolvens. Antimicrob Agents Chemother 2001;45(11):3246–9.
[36] Wexler HM. *Bacteroides*: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 2007;20(4):593–621.
[37] Petković H, Cullum J, Hranueli D, Hunter IS, Perić-Concha N, Pigac J, et al. Genetics of *Streptomyces rimosus*, the oxytetracycline producer. Microbiol Mol Biol Rev 2006;70(3):704–28.
[38] Kirchner O, Tauch A. Tools for genetic engineering in the amino acid-producing bacterium *Corynebacterium glutamicum*. J Biotechnol 2003;104(1–3):287–99.
[39] Whitehead TR, Cotta MA, Collins MD, Falsen E, Lawson PA. *Bacteroides coprosuis* sp. nov., isolated from swine-manure storage pits. Int J Syst Evol Microbiol 2005;55(6):2515–8.
[40] Frimmersdorf E, Horatzek S, Pelnikevich A, Wiehlmann L, Schomburg D. How *Pseudomonas aeruginosa* adapts to various environments: a metabolomic approach. Environ Microbiol 2010;12(6):1734–47.
[41] Almagro-Moreno S, Taylor RK. *Cholera*: environmental reservoirs and impact on disease transmission. Microbiol Spectr 2013;1(2).
[42] Kocur M, Kloos WE, Schleifer K-H. The genus *Micrococcus*. The Prokaryotes. Springer; 2006. p. 961–71.
[43] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21(3):538–82.
[44] Hejazi A, Falkiner F. Serratia marcescens. J Med Microbiol 1997;46(11):903–12.
[45] Warriner K, Xu C, Habash M, Sultan S, Weese S. Dissemination of *Clostridium difficile* in food and the environment: significant sources of *C. difficile* community-acquired infection? J Appl Microbiol 2017;122(3):542–53.
[46] Matches JR, Liston J, Curran D. *Clostridium perfringens* in the environment. Appl Microbiol 1974;28(4):655–60.
[47] Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. Microbes Environ 2011;26(2):101–12.
[48] Rouphael NG, Stephens DS. *Neisseria meningitidis*: biology, microbiology, and epidemiology. *Neisseria meningitidis*. Springer 2012:1–20.
[49] Drzewiecka D. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol 2016;72(4):741–58.
[50] Bronowski C, James CE, Winstanley C. Role of environmental survival in transmission of *Campylobacter jejuni*. FEMS Microbiol Lett 2014;356(1):8–19.
[51] Ofer N, Wishkautzan M, Meijler M, Wang Y, Speer A, Niederweis M, et al. Ectoine biosynthesis in *Mycobacterium smegmatis*. Appl Environ Microbiol 2012;78(20):7483–6.
[52] Stanton TB, Humphrey SB. Isolation of tetracycline-resistant *Megasphaera elsdenii* strains with novel mosaic gene combinations of tet (O) and tet (W) from swine. Appl Environ Microbiol 2003;69(7):3874–82.
[53] Andino A, Hanning I. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. Sci World J 2015;2015.
[54] Klima CL, Alexander TW, Hendrick S, McAllister TA. Characterization of *Mannheimia haemolytica* isolated from feedlot cattle that were healthy or treated for bovine respiratory disease. Can J Vet Res 2014;78(1):38–45.