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Exploring Antibiotic Susceptibility, Resistome and Mobilome Structure of Planctomycetes from *Gemmataceae* Family

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Abstract: The family *Gemmataceae* accomodates aerobic, chemoorganotrophic planctomycetes with large genome sizes, is mostly distributed in freshwater and terrestrial environments. However, these bacteria have recently also been found in locations relevant to human health. Since the antimicrobial resistance genes (AMR) from environmental resistome have the potential to be transferred to pathogens, it is essential to explore the resistant capabilities of environmental bacteria. In this study, the reconstruction of in silico resistome was performed for all nine available *gemmata* genomes. Furthermore, the genome of the newly isolated yet-undescribed strain G18 was sequenced and added to all analyses steps. Selected genomes were screened for the presence of mobile genetic elements. The flanking location of mobilizable genomic milieu around the AMR genes was of particular interest since such colocalization may appear to promote the horizontal gene transfer (HGT) events. Moreover the antibiotic susceptibility profile of six phylogenetically distinct strains of *Gemmataceae* planctomycetes was determined.

Keywords: planctomycetes; *Gemmataceae*; antibiotic resistance profile; resistome; mobilome

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1. Introduction

Several decades ago humanity faced the global issue of growing antibiotic resistance of bacterial pathogens in clinic [1–5]. Currently, it applies to all known classes of natural and synthetic compounds. A plausible approach to overcome the issue could be the understanding of resistance through the lens of evolution and ecology and the realization that antibiotic resistance in the clinic in many cases has its origins in environmental microbes [6–8]. Thus, the two documented examples are of *Kluyvera* and *Shewanella* isolates [9,10], which are found free-living in environmental conditions, yet have resistance genes with high similarity to those of pathogens [7]. Environmental microbes are the wellsprings of resistance elements called resistome. The genetic and functional diversity in the resistome is vast and reflects the billions of years of evolution of microbes in close contact with toxic molecules of many origins [6]. The risk inherent within a given resistome is predicated on the genomic context of various antimicrobial resistance (AMR) genes, including their presence within or near the mobile genetic elements [11]. Such colocalization may be the cause for the relatively frequent transfer of such elements to human and animal pathogens [12,13] via horizontal gene transfer (HGT) events. The bacterial mobilome is defined as all detectable mobile genetic elements, including plasmids, integrative conjugative elements (ICEs), transposons, and insertional repeat sequences [11]. Investigation of the resistome-mobilome structures can provide an insight into the mechanisms by which pathogens develop resistance [2].

The objects of current research are planctomycetes of the family *Gemmataceae*. *Gemmataceae* comprised gram-stain-negative, budding bacteria with spherical or ellipsoidal cells, which occur singly, in pairs or are assembled in large rosette-like clusters and dendriform-like structures [14]. These bacteria are characterized by a set of unique features such as elaborate intracellular membrane networks [15], lack of the division protein FtsZ [16] and

large genome sizes (up to 12.5 Mb) [17]. Notably, most of the genome encoded potential remains unknown. Planctomycetes are also known as slow-growing microorganisms that are difficult to isolate and to manipulate in the laboratory [18,19]. Representatives of the family *Gemmataceae* are mostly found in various aquatic habitats [20,21], wetlands [22,23], and soils [24,25]. However recently these microorganisms were also detected in hospital water networks in close proximity to patients [26], in skin microbiota [27], in human stool specimens [28], and in the blood of leukemic aplastic patients with micronodular pneumonia [29]. Furthermore, for a long time gemmatas remained underestimated in clinical specimens by using the routine diagnostic techniques, having mismatches with universal 16S rRNA gene-based primers and probes [30,31]. All these evidences suggested that *gemmata* planctomycetes potentially may behave as opportunistic pathogens [30]. In a recent study the new methodology was developed to determine the *gemmata* bacteremia in the routine screening of human blood samples, which may improve our understanding of the epidemiology of these bacteria [32].

Currently, there are only two studies dedicated to antibiotic susceptibility profiling of *Planctomycetes* [33,34]. The first one was published almost 10 years ago and included several planctomycetes, but only one representative of gemmates—*Gemmata obscuriglobus* [34]. The latter research comprised mostly marine planctomycetes of *Pirellulaceae* family isolated from different macroalgae [33]. Nowadays, there are already 8 described species of the family *Gemmataceae* and 9 full-genomic sequences available at the public databases.

In this study, we performed comparative genomic analysis of all available genomes of *gemmata* planctomycetes with emphasis on the resistome and mobilome structure of these bacteria. Moreover the genome of yet-uncharacterized *gemmata* strain G18 recently isolated in our laboratory was sequenced and analyzed. The antimicrobial susceptibility profiles were determined for five described representatives of various species from family *Gemmataceae* and strain G18. The results obtained in current research provide new data on the pathogenic potential of this group of microorganisms and expand our knowledge about the organization and composition of the resistome in general.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

Five planctomycetes belonging to the family *Gemmataceae*, isolated mostly from aquatic and wetland ecosystems, were used in antibiotic susceptibility tests. *Gemmata obscuriglobus* DSM 5831 [35], *Telmatocola sphagniphila* SP2 [36], *Fimbriiglobus ruber* SP5 [14], *Frigoriglobus tundricola* PL17 [37] and *Limnoglobus roseus* PX52 [38] are all represent type strains of different genus within the studied family and were available at our laboratory collection. One yet undescribed *Gemmata*-like isolate G18 was added to the experiment. The detailed characteristics about the strains are given in Table S1 in Supplementary Materials. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality controls. Planctomycetes were grown in medium M31 containing (L^{-1} distilled water): 0.1 g KH_2PO_4, 20 mL Hutner's basal salts, 1.0 g *N*-acetylglucosamine, 0.1 g peptone and 0.1 g yeast extract; at 26 °C [39]. The reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were cultivated in the Mueller–Hinton media (Agat, Rusia) at 30 °C. Phytagel and agar were used as solidified agents for M31 and MH medium, respectively.

2.2. Antibiotic Susceptibility Tests

The experiment was conducted as described in the article by Godinho et al. [33] The susceptibility of target planctomycetes to different antibiotics was determined using disk diffusion method [40]. The well concentrated suspension of each planctomycete bacterium (OD_{600} between 0.3 and 0.5 A.U.) was used to inoculate the plates. The plates with control strains of *E. coli* and *S. aureus* were incubated overnight and then inspected for zones of inhibition. Due to slow growth rates of planctomycetes almost 7 days of incubation were needed before the measurements. In total, 18 antibiotics (Oxoid, Himedia, Agat) from

various classes and of different target mechanisms were tested. The detailed information is given in Table 1.

2.3. Genome Sequencing and Phylogenomic Analyses

Genomic DNA was isolated from strain G18 using the standard CTAB and phenol-chloroform protocol [41]. For Nanopore sequencing the library was prepared using the 1D ligation sequencing kit (SQK-LSK108, Oxford Nanopore, UK). Sequencing was performed on an R9.4 flow cell (FLO-MIN106) using MinION device. The library preparation with MiSeq Reagent Kit v2 Micro and sequencing on Illumina MiSEQ (2*150 bp read length) platform was done at ReaGen sequencing facility (Moscow, Russia). Hybrid assembly of Illumina and Nanopore reads was performed using Unicycler v.0.4.8 [42] and BWA-MEM2 [43] with subsequent quality comparison in Quast 5.0 [44] and Busco 5.1.2 [45]. Gene search and annotation was performed in Prokka v1.14.6 [46] package against UniProt db [47]. The annotated genome sequence of strain G18 has been deposited at GenBank under the accession number JAGKQQ000000000.

For the phylogenomic analysis, we included the *gemmata* genomes from GTDB as well as genomes of strains SH-PL17 (Genbank accession number CP011271.1), *L. roseus* PX52 (CP042425) and *F. tundricola* PL17 (CP053452) and genome of strain G18 (current study). Recently the genomes of *G. massiliana* Soil9 (LR593886) and *Tuwongella immobilis* MBLW1 (FJ811525) were sequenced and the genome of model organism *G. obscuriglobus* UQM 2246 (LR593888) was resequenced [48]. Those were also included into phylogenomic analysis. The phylogenomic tree was reconstructed based on the comparative sequence analysis of 120 ubiquitous single-copy proteins using the Genome Taxonomy Database toolkit (GTDB-Tk) [49], release 04-RS89 (https://github.com/Ecogenomics/GTDB-Tk, accessed on 15 February 2021) with further decorating in MEGAX [50] applying the maximum-likelihood method with 100 bootstraps and Jukes-Cantor model.

2.4. Prediction of the Antibiotic Resistance Genes and Pan-Resistome Analysis

The antibiotic resistance genes were determined using the Comprehensive Antibiotic Resistance Database (CARD v3.1.1) [51] using loose parameters. CARD is a curated regularly updated resource providing reference DNA and protein sequences of bacterial AMR genes. It is integrated with the software for prediction and resistome analyses such as Resistance Gene Identifier (RGI 5.1.1). Get_homologues [52] software was used for the characterization of pan-resistome. Blastp [53] hits with a minimum of 50% alignment length coverage, 50% identity and an E-value $\leq 1 \times 10^{-5}$ were considered. Clustering was performed based on OrthoMCL [54] algorithm with inflation parameter of 1.5. The pan-resistome was divided into core, soft core, shell, and cloud gene clusters. Core genes are defined as those present in all considered genomes, soft core genes are found in 95% of genomes, shell genes are present in more than 2 genomes and less than 95% of genomes, while cloud genes are found in not more than two genomes.

2.5. Identification of the Mobile Genetic Elements

IS element sequences were founded and classified into IS families using ISsaga pipeline [55] with IS finder database [56]. Potential prophages regions were searched with PHASTER server (http://phaster.ca/, accessed on 15 February 2021) using the settings described in Arndt et al., 2016 [57]. Gene functions were determined by homology with known viral proteins in the NCBI Genbank database and the VirFam package (http://biodev.cea.fr/virfam/, accessed on 15 February 2021) using the settings described in Lopes et al. (2014) [58].

Table 1. Antibiotic susceptibility profile of *Gemmataceae* planctomycetes.

Antibiotic	Class	Target	Disc Potency, µg	Control		*Planctomycetes*							
				S. aureus	***E. coli***	***G. obscuriglobus* DSM 5831**	**'*G. massilina*' IIL30**	***F. tundricola* PL17**	**G_18**	***T. sphagniphila* SP2**	***F. ruber* SP5**	***L. roseus* PX52**	***Z. formosa* A10**
Chloramphenicol	Amphenicol	50S RNA subunit	30	S	S	R	R	R	R	R	R	R	R
Lincomycin	Lincosamides	50S RNA subunit	15	S	R	R	–	S	S	S	R	S	R
Oleandomycin	Macrolide	50S RNA subunit	15	S	R	R	–	–	R	S	–	–	–
Erythromycin	Macrolide	50S RNA subunit	15	S	S	S	S	–	S	–	–	–	–
Gentamicin	Aminoglycosides	30S RNA subunit	10	S	S	S	S	S	S	S	S	R	S
Neomycin	Aminoglycosides	30S RNA subunit	30	S	S	R	–	S	S	R	R	R	S
Streptomycin	Aminoglycosides	30S RNA subunit	10	S	S	R	–	R	S	R	R	R	R
Kanamycin	Aminoglycosides	30S RNA subunit	30	S	S	S	–	S	S	S	S	R	S
Tetracycline	Tetracyclines	30S RNA subunit	30	S	S	S	S	S	S	S	S	S	–
Polymyxin B	Polymyxin	Cell Membrane	300IU	R	S	S	–	S	S	S	S	S	–
Imipenem	Beta-lactams	Cell wall	10	S	S	R	R	S	R	S	–	–	–
Cefotaxime	Beta-lactams	Cell wall	30	S	S	R	–	S	R	S	–	–	–
Ampicillin	Beta-lactams	Cell wall	10	S	S	R	R	R	S	R	R	R	R
Amoxycillin/ Clavulanic acid	Beta-lactams	Cell wall	20/10	R	S	R	–	R	R	R	R	R	–
Fosfomycin	Fosfomycin	Cell wall	200	S	S	R	–	R	R	–	–	R	–
Vancomycin	Glycopeptides	Cell wall	30	S	R	R	R	R	R	R	R	R	–
Novobiocin	Aminocoumarine	DNA gyrase	30	S	S	R	–	R	S	S	R	S	R
Rifampin	Rifamycins	mRNA Transcription	5	S	S	R	R	S	S	–	–	S	–

R—resistant, S—susceptible with the size of diameter >10 mm, – not tested. *Planctomycetes* profiles taken from other studies are indicated in bold.

3. Results and Discussion

3.1. Genomic Properties of Strain G18 and Phylogenomic Analysis

Nanopore sequencing yielded 145,171 reads with a total length of 1.4 Gb (N50~19.9 Kb). The additional round of sequencing on Illumina MiSeq platform generated a total of 3,061,576 paired-end reads, with a mean read length of 150 bp. Both short and long reads were included in a hybrid assembly using Unicycler, resulting in 3 contigs of 8,313,494 bp, 905,837 bp and 8905 bp. Another approach based on BWA-MEM assembler resulted in a single 9,268,081 bp circular contig. However, genome assembly with Unicycler was of better quality and was taken for further analysis. The G+C content of the chromosomal DNA is 65 mol%. A total of 7631 CDSs and 97 tRNA genes were predicted. The genome harbors three copies of rRNA operon. The 16S rRNA gene copies in strain G18 were most similar to that of *Gemmata massiliana* Soil9, showing 98.7% sequence identity. Digital DNA-DNA hybridization (GGDC calculator) value calculated for strain G18 and closest homologue *G. massiliana* Soil9 is 31.2 ± 2.5% while the ANI value is 86%.

Currently, nine full genome sequences of *Gemmataceae* planctomycetes are available in public databases. All planctomycetes of this family are characterized by large genome sizes, which vary between 6.7 Mb in *T. immobilis* MBLW1 [48] and 12.3 Mb in *F. ruber* SP5 [17]. The G+C content range within the *Gemmataceae* is 58–67 mol%. Plasmids are not typical for *Gemmataceae* genomes, only *F. tundricola* PL17 harbor one plasmid of size 25 kb [37]. The number of predicted genes varies from 5233 in *T. immobilis* to 10,640 in *F.ruber*. Notably, only about 50% of all genes could be assigned a function which is low in comparison to other bacteria [48]. The phylogenomic position of all available genomes of *Gemmataceae* planctomycetes are shown on Figure 1.

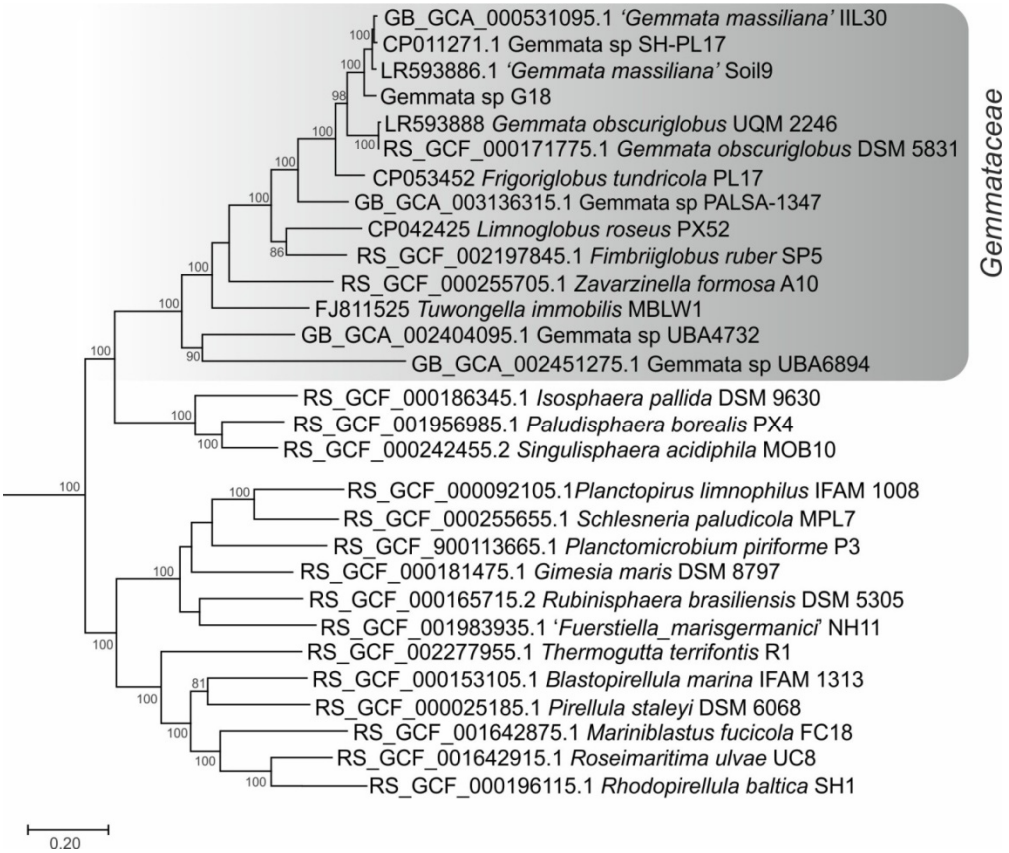

Figure 1. Phylogenomic tree showing the phylogenetic position of strain G18 and *Gemmataceae* planctomycetes based on the comparative sequence analysis of 120 ubiquitous single-copy proteins. The tree was reconstructed using the Genome Taxonomy Database toolkit. The significance levels of interior branch points obtained in maximum-likelihood analysis were determined by bootstrap analysis (100 data re-samplings). Bootstrap values of over 70% are shown. The root (not shown) was composed of genomes of members of the anammox planctomycetes. Bar, 0.2 substitutions per amino acid position.

3.2. Antibiotic Susceptibility Testing

The control tests were performed with *S. aureus* and *E. coli* on Muller-Hinton agar, which is a golden standard for antibiotic susceptibility examinations. However, for *Gemmataceae* planctomycetes the medium M31 was chosen since they require specific growth conditions [33]. Susceptibility profiles of all studied *Gemmataceae* planctomycetes is shown in Table 1.

All *Gemmataceae* planctomycetes were resistant to vancomycin, chloramphenicol, fosfomycin, and amoxycillin/clavulanic acid. Resistance to vancomycin seems to be intrinsic, since in Gram-negative bacteria these molecules are unable to penetrate the outer membrane barrier [59]. Chloramphenicol is a broad-spectrum inhibitor of protein biosynthesis mainly due to the prevention of peptide chain elongation [60]. The *Gemmataceae* planctomycetes enable to circumvent the inhibitory effect because of the high number of efflux pumps encoded in their genomes [34]. Moreover, the specific resistant genes that were absent in *gemmata* genomes but the gene *cfr* were present. Cfr genes produce enzymes which catalyze the methylation of the 23S rRNA subunit at the specific position, which confers resistance to several antibiotics, including chloramphenicol. The similar trend was observed for fosfomycin, which inhibits the MurA enzyme that catalyzes the first committed step in peptidoglycan synthesis [61]. Notably, all pirellula planctomycetes were also resistant to fosfomycin but mainly because of the presence of gene *fosB*, that was not found in *gemmata* genomes [33]. Resistance for amoxycillin/ clavulanic acid involved β-lactamase enzymes that hydrolyze the amide bond of the four-membered β-lactam ring [62]. Several β-lactamases were found in the genomes of *Gemmataceae* members.

Apparently, all studied gemmatas were susceptible to polymyxin B and tetracycline. Tetracycline antibiotics are protein synthesis inhibitors with broad-spectrum activity against bacterial pathogens, viruses, protozoa, and helminths. They are generally believed to bind to the 30S ribosomal subunit and also double-stranded RNAs of random base sequence. However, the exact target sites and mechanisms of action remain subjects of much debate [63]. The mode of action for polymyxins is also controversial. The most common mechanism involves targeting the membranes of Gram-negative bacteria, leading to permeabilized outer and inner membranes and resulting in lysis and cell death. However, there are some studies that explored the ability of polymyxins to bind ribosomes, prevent cell division, and inhibit bacterial respiration [64]. *Pirellulaceae* planctomycetes were shown to have mixed susceptibility profiles for polymyxins [33].

For other antibiotics, resistance appears to be taxon-specific, since the results differed between various strains of gemmatas. Almost all *Gemmataceae* planctomycetes, except *L. roseus*, were susceptible to gentamycin and kanamycin aminoglycoside antibiotics, which inhibit bacterial protein synthesis by binding to 30S ribosomes. On the contrary, recently studied marine planctomycetes of the *Pirellulaceae* family were all resistant to this class of antibiotics [33]. It was hypothesised that they may possess intrinsic resistance mechanisms that protect the cells against their own products, since the enzyme involved in kanamycin biosynthesis was found in the proteome of one of the pirellula planctomycetes [33]. *F. tundricola*, *Z. formosa* and strain G18 were susceptible to neomycin, while strain G18 was also susceptible to streptomycin.

Variable susceptibility profiles were also observed for lincomycin and oleandomycin, which target the 50S RNA subunit. *G. obscuriglobus*, *F. ruber*, and *Z. formosa* have shown resistance to lincomycin. *G. obscuriglobus* and the new isolate G18 were resistant to oleandomycin.

G. massiliana and *G. obscuriglobus* were both resistant to rifampin. Rifampin is one of the most potent and broad spectrum antibiotics against bacterial pathogens and is a key component of anti-tuberculosis therapy [65]. The resistance mechanism has been primarily associated with mutations in the *rpoB* gene encoding the RNA polymerase beta-subunit, reducing the affinity of this drug to the target [34]. *T.sphagniphila*, *L. roseus* and strain G18 were susceptible to novobiocin. Novobiocin is a member of aminocoumarin class, which inhibits the bacterial topoisomerase by binding to the ATP pocket of GyrB [66]. Resistance to novobiocin is predominantly due to the accumulation of point mutations in the gene

gyrB [67]. Different susceptibility patterns were also observed for three beta-lactam antibiotics of different subclasses—imipenem (carbapenems), cefotaxime (cephalosporins) and ampicillin (penicillins). *F. tundricola* and *T. sphagniphila* were susceptible to imipenem and cefotaxime, while strain G18 was susceptible to ampicillin. Interestingly, carbapenemases are currently uncommon but are a source of considerable concern because they are active against oxyimino-cephalosporins, cephamycins and carbapenems [68].

3.3. Assembly of Antibiotic Resistance Genes of Gemmataceae Planctomycetes

The crucial step in analyzing the pool of AMR genes from environmental bacteria is the selection of the most appropriate tool. The vast majority of available software biases toward human-associated microorganisms and highly underestimates the potential impact of environmental resistance reservoirs on pathogens. Here, we tried several approaches, including Resfam, Megares, bldb, sraX and CARD [51,69–72]. The last one was chosen as the most suitable for the analysis of *gemmata* resistance determinants. The whole repertoire of card-out resistance genes from ten *gemmata* genomic sequences was exported for subsequent pan-resistome analysis.

The pan-resistome of *Gemmataceae* planctomycetes was reconstructed based on the clustering of protein-coding sequences of AMR genes into core, soft core, shell, and cloud genomes. A total of 4445 genes were combined into 1350 gene clusters. Core resistome comprised 105 genes (on average 7.8% of each genome) while 148 gene clusters were present in soft core. Accessory resistome contained 326 gene clusters in the shell (27.1% of total gene clusters) and 876 in the cloud (72.9% of total gene clusters) (Figure 2a). CARD category "Resistance mechanism" was represented in core resistome mainly by various multidrug efflux pump systems (59% of all core resistant genes) (Figure 2b). Those are the first line of defense against antibiotics as these pumps decrease the intracellular level of drugs [73]. Bacterial efflux pumps are classified into six structural families: the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxin extrusion (MATE) family, the small multidrug resistance (SMR) family, the resistance-nodulation-cell division (RND) superfamily and the proteobacterial antimicrobial compound efflux (PACE) family [74]. In *Gemmataceae* members the efflux pumps include ABC (26% of all core genes), MFS (13%), RND (19%), and MATE (1%) families. RND and ABC pumps represent tripartite systems that include inner membrane pump, a periplasmic membrane fusion protein and an outer membrane factor. Other pump families normally represent an inner membrane unit and then cooperate with the RND system to deliver substrates across the entire cell envelope [74]. In *Gemmataceae* genomes we were able to find genes, that encoded the whole tripartite ABC and RND systems, but the similarity to already known proteins was low, suggesting that the exact type and protein structure of *Gemmataceae* efflux pumps should be further defined. In a previous study the presence of efflux pumps were shown for two *Pirellulaceae* planctomycetes. However, the number of genes involved in multidrug resistance in *pirellulas* was six for one genome and twenty for another [34]. In comparison the *Gemmataceae* core efflux resistance counts up to 60 genes. Such a difference may occur due to underrepresentation of reference sequences in AMR databases at the moment of analysis of *pirellulas* genomes. The second largest category of resistant mechanisms was a target alteration comprising up to 32% of all core resistance genes (Figure 2b). It includes mutational alteration or enzymatic modification of the antibiotic target, which results in antibiotic resistance.

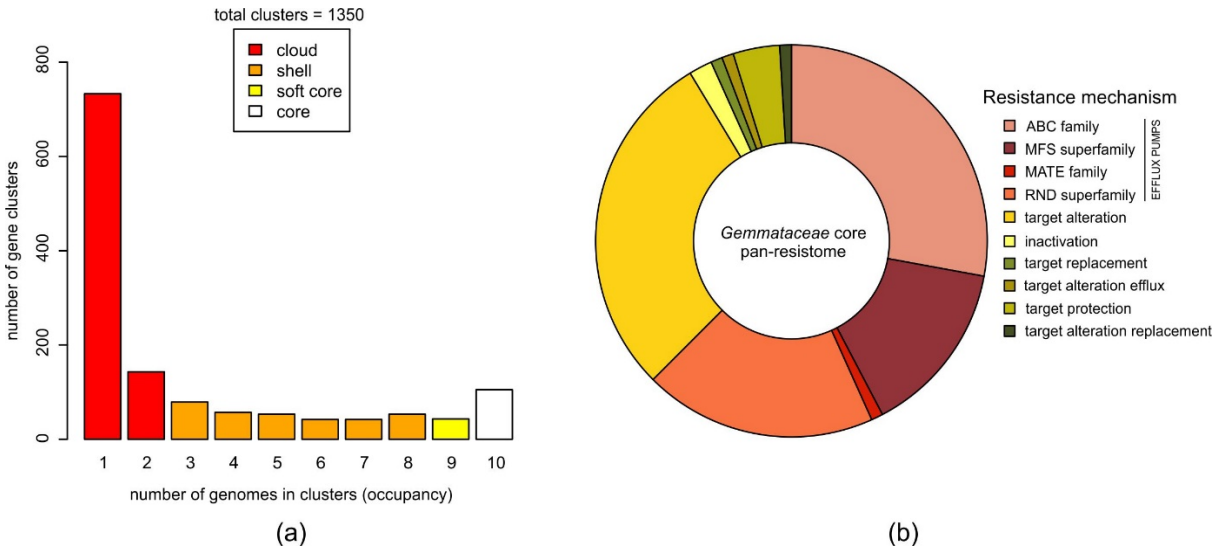

Figure 2. (**a**) Barplot of the pan-resistome matrix. (**b**) Core AMR genes identified among analyzed *Gemmataceae* genomes.

The complete set of CARD genes was also categorized based on resistance potential to various antibiotic classes (Figure 3). As follows from the heatmap, *Gemmataceae* planctomycetes possess a very high antibiotic resistance load. When the antibiotic susceptibility data were coupled to the predicted resistome information, we were able to detect good correspondence between phenotype and genotype for chloramphenicol (phenicol on the heatmap), imipenem (carbapenem), ampicillin and amoxicillin/clavulanic acid (penams).

As was mentioned above, the resistance in these cases could be explained by the presence of specific genes and efflux pumps. However, for some antibiotic classes inconsistencies were found between the phenotypic manifestation and the genomic data. For example, according to heatmap (Figure 3) there are no known genetic determinants responsible for fosfomycin resistance. However, all *gemmata* strains were not susceptible to this antibiotic. Such P+G-(phenotype+/genotype-) findings are significant and may represent a feasible avenue to identify sites of high antibiotic selection pressure [75] or the presence of novel emergent AMR genes. On the contrary, the G+P-profile was also observed for tetracycline and polymyxin B (peptide on the heatmap). The G+P-may be explained by the high proportion of dysfunctional resistance genes (resistance pseudogenes) [75]. These pseudogenes could be defined as stable components of the genome, which was derived by mutation of an ancestral active gene or by ones that endured the mutations eliminating their expression ability [75]. Further in-depth research is needed to accurately assess phenotyp–genotype correlation in *Gemmataceae* planctomycetes.

3.4. The Putative Mobilome of Gemmata Planctomycetes

Insertion sequences (ISs), the smallest and most numerous autonomous transposable elements, are important players in shaping their host genomes [76]. They range in size from 0.7 to 3.5 kbp, generally including a transposase gene encoding the enzyme that catalyzes IS movement. These enzymes are now used to lead classification of ISs into various families. The studied *gemmata* genomes contain from 28 (*T. immobolis*) to 743 (*F. ruber*) transposase genes. IS family distribution is quite variable among these *gemmata* genomes. However, most genomes contain genes of the IS3, IS4, IS5, IS630, IS701, ISAs1 families.

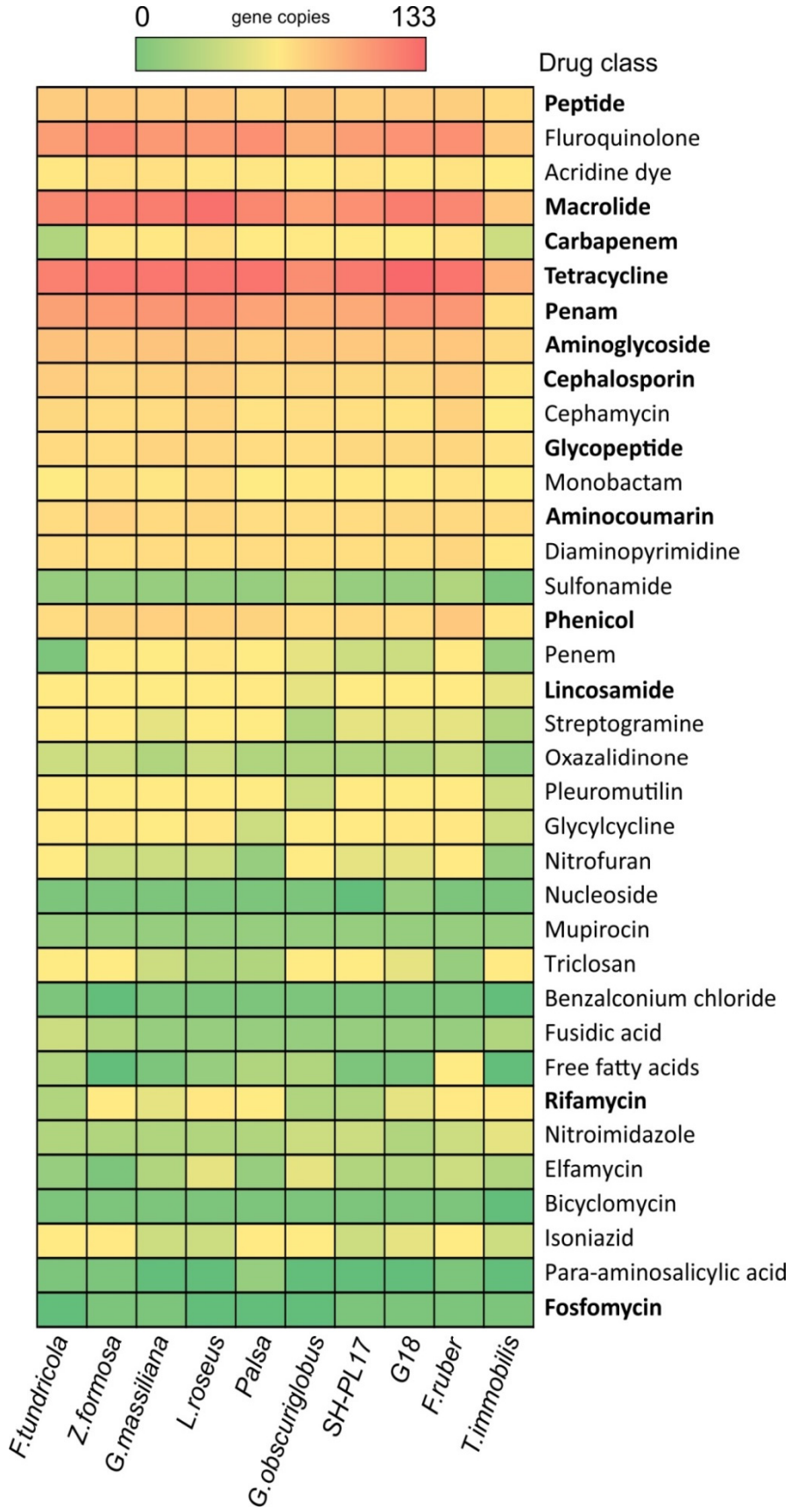

Figure 3. Heat map showing the absolute abundances of drug resistance gene classes identified among the 10 analyzed *Gemmataceae* genomes. Antibiotics that were tested by disk diffusion method are indicated in bold.

Integrative and conjugative elements (ICEs) are widespread mobile DNA that transmit both vertically, in a host-integrated state, and horizontally, through excision and transfer to new recipients. Recent findings have indicated that the main actors of conjugative transfer are not the well-known conjugative or mobilizable plasmids but are integrated mobilizable elements (IMEs) [77,78]. The distribution of IME regions in studied *Gemmataceae* members was as follows: *F. ruber*, 4; *G. obscuriglobus*, 3; strain SH-PL17, 1; *F. tundricola*, 1; strain Palsa, 5; *L. planctonicus*, 1; *G. massiliana*, 2. Interestingly, genomes of *T. immobilis* and strain G18 lacked any ICE elements. Two out of three IME regions of *G. obscuriglobus* were found to contain antibiotic resistance genes that could potentially be transferred to other bacteria. The first IME region contains two genes—*evgS* and *evgA*—that together regulate the efflux proteins of RND and MFS pumps. The last IME region includes gene *mexS*, which indirectly suppresses the RND efflux system. Mutations in *mexS* leads to multidrug resistance.

Integrons are gene-capturing platforms playing a major role in the spread of antibiotic resistance genes [79,80]. The structure of integron includes variable gene cassette array (recombination site *attC* with genes) and a stable platform and contains an integrase (IntI), recombination site (attI) and a promoter. We identified only one complete integron platform in the genome of *Gemmata obscuriglobus*. The number of regions with gene cassettes in the genomes of *Gemmataceae* family members varies from 1 in the genomes of *gemmata* strain SH-PL17 and *L. planctonicus* PX52 to 17 in the genome *of Gemmata obscuriglobus*.

4. Conclusions

Little is known about phenotypic and genotypic antibiotic resistance properties of *Gemmataceae* planctomycetes. Since these microorganisms were found in various biomedical human samples, investigation of these bacteria deserves increased attention. Here we define the antibiotic susceptibility profile of six *Gemmataceae* planctomycetes, revealing resistance to chloramphenicol, fosfomycin and amoxicillin/clavulanic acid. Resistome analysis was performed for 9 publicly available *gemmata* genomes as well as the genome of strain G18 sequenced in this study. The genomic determinants of antibiotic resistance were mainly associated with numerous RND, ABC and MFS efflux pumps. The complete set of genes encoding efflux proteins were identified but demonstrated low similarity to known homologues, suggesting that the exact structure of pump system is worthy of further investigation. Moreover, the inconsistency in phenotypic and genotypic traits of various antibiotic resistance profiles is of particular interest. Such discrepancies may indicate the presence of novel AMR genes. Several AMR genes were found to be associated with ICE regions and potentially could be transferred to other bacteria.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13095031/s1, Table S1: Major characteristics of studied Gemmataceae strains.

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References

1. Martinez, J.L. The Role of Natural Environments in the Evolution of Resistance Traits in Pathogenic Bacteria. *Proc. R. Soc. B Boil. Sci.* **2009**, *276*, 2521–2530. [CrossRef] [PubMed]
2. Wright, G.D. Antibiotic Resistance in the Environment: A Link to the Clinic? *Curr. Opin. Microbiol.* **2010**, *13*, 589–594. [CrossRef] [PubMed]
3. Perry, J.; Waglechner, N.; Wright, G. The Prehistory of Antibiotic Resistance. *Cold Spring Harb. Perspect. Med.* **2016**, *6*, a025197. [CrossRef] [PubMed]
4. D'Costa, V.M.; McGrann, K.M.; Hughes, D.W.; Wright, G.D. Sampling the Antibiotic Resistome. *Science* **2006**, *311*, 374–377. [CrossRef]
5. Cloud-Hansen, K.A.; Handelsman, J.; Wang, H.H.; Davies, J.; Donato, J.; Allen, H.K. Call of the Wild: Antibiotic Resistance Genes in Natural Environments. *Nat. Rev. Microbiol.* **2010**, *8*, 251–259.
6. Surette, M.D.; Wright, G.D. Lessons from the Environmental Antibiotic Resistome. *Annu. Rev. Microbiol.* **2017**, *71*, 309–329. [CrossRef]
7. Forsberg, K.J.; Reyes, A.; Wang, B.; Selleck, E.M.; Sommer, M.O.A.; Dantas, G. The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens. *Science* **2012**, *337*, 1107–1111. [CrossRef]
8. Benveniste, R.; Davies, J. Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 2276–2280. [CrossRef]
9. Poirel, L.; Rodriguez-Martinez, J.M.; Mammeri, H.; Liard, A.; Nordmann, P. Origin of Plasmid-Mediated Quinolone Resistance Determinant QnrA. *Antimicrob. Agents Chemother.* **2005**, *49*, 3523–3525. [CrossRef]
10. Poirel, L.; Kaämpfer, P.; Nordmann, P. Chromosome-Encoded Ambler Class A β-Lactamase of Kluyvera georgiana, a Probable Progenitor of a Subgroup of CTX-M Extended-Spectrum β-Lactamases. *Antimicrob. Agents Chemother.* **2002**, *46*, 4038–4040. [CrossRef]
11. Slizovskiy, I.B.; Mukherjee, K.; Dean, C.J.; Boucher, C.; Noyes, N.R. Mobilization of Antibiotic Resistance: Are Current Approaches for Colocalizing Resistomes and Mobilomes Useful? *Front. Microbiol.* **2020**, *11*, 01376. [CrossRef] [PubMed]
12. Mancino, W.; Lugli, G.A.; van Sinderen, D.; Ventura, M. Mobilome and Resistome Reconstruction from Genomes Belonging to Members of the Bifidobacterium Genus. *Microorganisms* **2019**, *7*, 638. [CrossRef]
13. Martínez, J.L.; Coque, T.M.; Baquero, F. What is a Resistance Gene? Ranking Risk in Resistomes. *Nat. Rev. Genet.* **2015**, *13*, 116–123. [CrossRef]
14. Kulichevskaya, I.S.; Ivanova, A.A.; Baulina, O.I.; Rijpstra, W.I.C.; Damsté, J.S.S.; Dedysh, S.N. Fimbriiglobus Ruber Gen. nov., sp. Nov., a Gemmata-Like Planctomycete from Sphagnum Peat Bog and the Proposal of Gemmataceae Fam. Nov. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 218–224. [CrossRef]
15. Mahajan, M.; Seeger, C.; Yee, B.; Andersson, S.G. Evolutionary Remodelling of the Cell Envelope in Bacteria of the Planctomycetes Phylum. *Genome Biol. Evol.* **2020**, *12*, 1528–1548. [CrossRef]
16. Pilhofer, M.; Rappl, K.; Eckl, C.; Bauer, A.P.; Ludwig, W.; Schleifer, K.-H.; Petroni, G. Characterization and Evolution of Cell Division and Cell Wall Synthesis Genes in the Bacterial Phyla Verrucomicrobia, Lentisphaerae, Chlamydiae, and Planctomycetes and Phylogenetic Comparison with rRNA Genes. *J. Bacteriol.* **2008**, *190*, 3192–3202. [CrossRef]
17. Ravin, N.V.; Rakitin, A.L.; Ivanova, A.A.; Beletsky, A.V.; Kulichevskaya, I.S.; Mardanov, A.V.; Dedysh, S.N. Genome Analysis of Fimbriiglobus ruberSP5T, a Planctomycete with Confirmed Chitinolytic Capability. *Appl. Environ. Microbiol.* **2018**, *84*, 02645-17. [CrossRef]
18. Dedysh, S.N. Cultivating Uncultured Bacteria from Northern Wetlands: Knowledge Gained and Remaining Gaps. *Front. Microbiol.* **2011**, *2*, 184. [CrossRef]
19. Kaboré, O.D.; Godreuil, S.; Drancourt, M. Planctomycetes as Host-Associated Bacteria: A Perspective That Holds Promise for Their Future Isolations, by Mimicking Their Native Environmental Niches in Clinical Microbiology Laboratories. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 519301. [CrossRef] [PubMed]
20. Brümmer, I.-H.; Felske, A.-D.; Wagner-Döbler, I. Diversity and Seasonal Changes of Uncultured Planctomycetales in River Biofilms. *Appl. Environ. Microbiol.* **2004**, *70*, 5094–5101. [CrossRef]
21. Pollet, T.; Tadonléké, R.D.; Humbert, J.F. Spatiotemporal Changes in the Structure and Composition of a Less-Abundant Bacterial Phylum (Planctomycetes) in Two Perialpine Lakes. *Appl. Environ. Microbiol.* **2011**, *77*, 4811–4821. [CrossRef]
22. Ivanova, A.O.; Dedysh, S.N. Abundance, Diversity, and Depth Distribution of Planctomycetes in Acidic Northern Wet-Lands. *Front. Microbiol.* **2012**, *3*, 5. [CrossRef] [PubMed]
23. Dedysh, S.N.; Ivanova, A.A. Planctomycetes in Boreal and Subarctic Wetlands: Diversity Patterns and Potential Ecological Functions. *FEMS Microbiol. Ecol.* **2019**, *95*, fiy227. [CrossRef]
24. Wang, J.; Jenkins, C.; Webb, R.I.; Fuerst, J.A. Isolation of Gemmata-Like and Isosphaera-Like Planctomycete Bacteria from Soil and Freshwater. *Appl. Environ. Microbiol.* **2002**, *68*, 417–422. [CrossRef]

25. Buckley, D.H.; Huangyutitham, V.; Nelson, T.A.; Rumberger, A.; Thies, J.E. Diversity of Planctomycetes in Soil in Relation to Soil History and Environmental Heterogeneity. *Appl. Environ. Microbiol.* **2006**, *72*, 4522–4531. [CrossRef] [PubMed]
26. Aghnatios, R.; Cayrou, C.; Garibal, M.; Robert, C.; Azza, S.; Raoult, D.; Drancourt, M. Draft Genome of Gemmata Massiliana sp. Nov, a Water-Borne Planctomycetes Species Exhibiting two Variants. *Stand. Genom. Sci.* **2015**, *10*, 120. [CrossRef]
27. Kong, H.H.; Oh, J.; Deming, C.; Conlan, S.; Grice, E.A.; Beatson, M.A.; Nomicos, E.; Polley, E.C.; Komarow, H.D.; Murray, P.R.; et al. Temporal Shifts in the Skin Microbiome Associated with Disease Flares and Treatment in Children with Atopic Dermatitis. *Genome Res.* **2012**, *22*, 850–859. [CrossRef]
28. Cayrou, C.; Sambe, B.; Armougom, F.; Raoult, D.; Drancourt, M. Molecular Diversity of the Planctomycetes in the Human Gut Microbiota in France and Senegal. *Apmis* **2013**, *121*, 1082–1090. [CrossRef]
29. Drancourt, M.; Prebet, T.; Aghnatios, R.; Edouard, S.; Cayrou, C.; Henry, M.; Blaise, D.; Raoult, D. Planctomycetes DNA in Febrile Aplastic Patients with Leukemia, Rash, Diarrhea, and Micronodular Pneumonia: FIG 1. *J. Clin. Microbiol.* **2014**, *52*, 3453–3455. [CrossRef] [PubMed]
30. Aghnatios, R.; Drancourt, M. Gemmata Species: Planctomycetes of Medical Interest. *Future Microbiol.* **2016**, *10*, 5.
31. Chouari, R.; Le Paslier, D.; Daegelen, P.; Ginestet, P.; Weissenbach, J.; Sghir, A. Molecular Evidence for Novel Plancto-Mycete Diversity in a Municipal Wastewater Treatment Plant. *Appl. Environ. Microbiol.* **2003**, *69*, 7354–7363. [CrossRef]
32. Christen, J.R.; Edmond, E.; Drancourt, M. Methods for Detecting Gemmata Spp. Bacteremia in the Microbiology Laboratory. *BMC Res. Notes* **2018**, *11*, 4–9. [CrossRef] [PubMed]
33. Godinho, O.; Calisto, R.; Øvreås, L.; Quinteira, S.; Lage, O.M. Antibiotic Susceptibility of Marine Planctomycetes. *Antonie Van Leeuwenhoek* **2019**, *112*, 1273–1280. [CrossRef]
34. Cayrou, C.; Raoult, D.; Drancourt, M. Broad-Spectrum Antibiotic Resistance of Planctomycetes Organisms Determined by Etest. *J. Antimicrob. Chemother.* **2010**, *65*, 2119–2122. [CrossRef] [PubMed]
35. Franzmann, P.D.; Skerman, V.B.D. Gemmata Obscuriglobus, a New Genus and Species of the Budding Bacteria. *Antonie Van Leeuwenhoek* **1984**, *50*, 261–268. [CrossRef] [PubMed]
36. Kulichevskaya, I.S.; Serkebaeva, Y.M.; Kim, Y.; Rijpstra, W.I.C.; Damsté, J.S.S.; Liesack, W.; Dedysh, S.N. Telmatocola Sphagniphila Gen. Nov., sp. Nov., a Novel Dendriform Planctomycete from Northern Wetlands. *Front. Microbiol.* **2012**, *3*, 146. [CrossRef] [PubMed]
37. Kulichevskaya, I.S.; Ivanova, A.A.; Naumoff, D.G.; Beletsky, A.V.; Rijpstra, W.I.C.; Damste, J.S.S.; Mardanov, A.V.; Ravin, N.V.; Dedysh, S.N. Frigoriglobus Tundricola Gen. Nov., sp. Nov., a Psychrotolerantcellulolytic Planctomycete of the Family Gemmataceae from a Littoraltundra Wetland. *Syst. Appl. Microbiol.* **2020**, *43*, 126129. [CrossRef]
38. Kulichevskaya, I.S.; Naumoff, D.G.; Miroshnikov, K.K.; Ivanova, A.A.; Philippov, D.A.; Hakobyan, A.; Rijpstra, W.I.C.; Damsté, J.S.S.; Liesack, W.; Dedysh, S.N. Limnoglobus Roseus Gen. Nov., sp. Nov., a Novel Freshwater Planctomycete with a Giant Genome from the Family Gemmataceae. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 1240–1249. [CrossRef] [PubMed]
39. Staley, J.T.; Fuerst, J.A.; Giovannoni, S.; Schlesner, H. The Order Planctomycetales and the Genera Planctomyces, Pirellula, Gemmata, and Isosphaera. In *The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*; Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H., Eds.; Springer: New York, NY, USA, 1992; pp. 3710–3731. ISBN 978-1-4757-2191-1.
40. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [CrossRef]
41. Wilson, K. Preparation of Genomic DNA from Bacteria. *Curr. Protoc. Mol. Biol.* **2001**, *56*, 2–4. [CrossRef]
42. Wick, R.R.; Judd, L.M.; Gorrie, C.L.; Holt, K.E. Unicycler: Resolving Bacterial Genome Assemblies from Short and Long Sequencing Reads. *PLoS Comput. Biol.* **2017**, *13*, e1005595. [CrossRef]
43. Li, H.; Durbin, R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* **2009**, *25*, 1754–1760. [CrossRef]
44. Gurevich, A.; Saveliev, V.; Vyahhi, N.; Tesler, G. QUAST: Quality Assessment Tool for Genome Assemblies. *Bioinformatics* **2013**, *29*, 1072–1075. [CrossRef]
45. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. BUSCO: Assessing Genome Assembly and Annotation Completeness with Single-Copy Orthologs. *Bioinformatics* **2015**, *31*, 3210–3212. [CrossRef]
46. Seemann, T. Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef] [PubMed]
47. Apweiler, R.; Bairoch, A.; Bougueleret, L.; Altairac, S.; Amendolia, V.; Auchincloss, A.; Argoud-Puy, G.; Axelsen, K.; Baratin, D.; Blatter, M.C.; et al. The Universal Protein Resource (UniProt). *Nucleic Acids Res.* **2009**, *37*, D169–D174.
48. Mahajan, M.; Yee, B.; Hägglund, E.; Guy, L.; Fuerst, J.A.; Andersson, S.G.E. Paralogization and New Protein Architectures in Planctomycetes Bacteria with Complex Cell Structures. *Mol. Biol. Evol.* **2020**, *37*, 1020–1040. [CrossRef]
49. Parks, D.H.; Chuvochina, M.; Waite, D.W.; Rinke, C.; Skarshewski, A.; Chaumeil, P.A.; Hugenholtz, P. A Standardized Bacterial Taxonomy Based on Genome Phylogeny Substantially Revises the Tree of life. *Nat. Biotechnol.* **2018**, *36*, 996–1004. [CrossRef]
50. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis Across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
51. Alcock, B.P.; Raphenya, A.R.; Lau, T.T.Y.; Tsang, K.K.; Bouchard, M.; Edalatmand, A.; Huynh, W.; Nguyen, A.-L.V.; Cheng, A.A.; Liu, S.; et al. CARD 2020: Antibiotic Resistome Surveillance with the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res.* **2020**, *48*, D517–D525. [CrossRef]

52. Contreras-Moreira, B.; Vinuesa, P. GET_HOMOLOGUES, a Versatile Software Package for Scalable and Robust Microbial Pangenome Analysis. *Appl. Environ. Microbiol.* **2013**, *79*, 7696–7701. [CrossRef] [PubMed]
53. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.* **1997**, *25*, 3389–3402. [CrossRef] [PubMed]
54. Li, L.; Stoeckert, C.J.; Roos, D.S. OrthoMCL: Identification of Ortholog Groups for Eukaryotic Genomes. *Genome Res.* **2003**, *13*, 2178–2189. [CrossRef] [PubMed]
55. Varani, A.M.; Siguier, P.; Gourbeyre, E.; Charneau, V.; Chandler, M. ISsaga is an Ensemble of Web-Based Methods for High throughput Identification and Semi-Automatic Annotation of Insertion Sequences in Prokaryotic Genomes. *Genome Biol.* **2011**, *12*, R30. [CrossRef] [PubMed]
56. Siguier, P. ISfinder: The Reference Centre for Bacterial Insertion Sequences. *Nucleic Acids Res.* **2006**, *34*, D32–D36. [CrossRef]
57. Arndt, D.; Grant, J.R.; Marcu, A.; Sajed, T.; Pon, A.; Liang, Y.; Wishart, D.S. PHASTER: A Better, Faster Version of the Phast Phage Search Tool. *Nucleic Acids Res.* **2016**, *44*, W16–W21. [CrossRef]
58. Lopes, A.; Tavares, P.; Petit, M.A.; Guérois, R.; Zinn-Justin, S. Automated Classification of Tailed Bacteriophages According to their Neck Organization. *BMC Genom.* **2014**, *15*, 1027. [CrossRef]
59. Blair, J.M.A.; Webber, M.A.; Baylay, A.J.; Ogbolu, D.O.; Piddock, L.J.V. Molecular Mechanisms of Antibiotic Resistance. *Nat. Rev. Genet.* **2015**, *13*, 42–51. [CrossRef]
60. Schwarz, S.; Kehrenberg, C.; Doublet, B.; Cloeckaert, A. Molecular Basis of Bacterial Resistance to Chloramphenicol and Florfenicol. *FEMS Microbiol. Rev.* **2004**, *28*, 519–542. [CrossRef]
61. Silver, L.L. Fosfomycin: Mechanism and Resistance. *Cold Spring Harb. Perspect. Med.* **2017**, *7*, a025262. [CrossRef]
62. Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y.; Spencer, J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *J. Mol. Biol.* **2019**, *431*, 3472–3500. [CrossRef] [PubMed]
63. Chukwudi, C.U. rRNA Binding Sites and the Molecular Mechanism of Action of the Tetracyclines. *Antimicrob. Agents Chemother.* **2016**, *60*, 4433–4441. [CrossRef] [PubMed]
64. Trimble, M.J.; Mlynarcik, P.; Kola, M.; Hancock, R.E.W. Polymyxin: Alternative Mechanisms of Action. *Cold Spring Harb. Perspect. Med.* **2016**, *6*, a025288. [CrossRef]
65. Sensi, P. History of the Development of Rifampin. *Clin. Infect. Dis.* **1983**, *5*, S402–S406. [CrossRef]
66. Smith, C.G.; Dietz, A.; Sokolski, W.T.; Savage, G.M. Streptonivicin, a New Antibiotic. I. Discovery and Biologic Studies. *Antibiot. Chemother.* **1956**, *6*, 135–142.
67. Fujimoto-Nakamura, M.; Ito, H.; Oyamada, Y.; Nishino, T.; Yamagishi, J.-I. Accumulation of Mutations in both gyrB and parE Genes Is Associated with High-Level Resistance to Novobiocin in Staphylococcus Aureus. *Antimicrob. Agents Chemother.* **2005**, *49*, 3810–3815. [CrossRef]
68. Jacoby, G.A.; Munoz-Price, L.S. Mechanisms of Disease: The New β-Lactamases. *N. Engl. J. Med.* **2005**, *352*, 380–391. [CrossRef]
69. Gibson, M.K.; Forsberg, K.J.; Dantas, G. Improved Annotation of Antibiotic Resistance Determinants Reveals Microbial Resistomes Cluster by Ecology. *ISME J.* **2015**, *9*, 207–216. [CrossRef] [PubMed]
70. Lakin, S.M.; Dean, C.; Noyes, N.R.; Dettenwanger, A.; Ross, A.S.; Doster, E.; Rovira, P.; Abdo, Z.; Jones, K.L.; Ruiz, J.; et al. MEGARes: An Antimicrobial Resistance Database for High throughput Sequencing. *Nucleic Acids Res.* **2017**, *45*, D574–D580. [CrossRef]
71. Naas, T.; Oueslati, S.; Bonnin, R.A.; Dabos, M.L.; Zavala, A.; Dortet, L.; Retailleau, P.; Iorga, B.I. Beta-Lactamase Database (BLDB)–Structure and Function. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 917–919. [CrossRef]
72. Panunzi, L.G. sraX: A Novel Comprehensive Resistome Analysis Tool. *Front. Microbiol.* **2020**, *11*, 52. [CrossRef] [PubMed]
73. Zago, V.; Veschetti, L.; Patuzzo, C.; Malerba, G.; Lleo, M.M. Resistome, Mobilome and Virulome Analysis of Shewanella algae and Vibrio spp. Strains Isolated in Italian Aquaculture Centers. *Microorganisms* **2020**, *8*, 572. [CrossRef] [PubMed]
74. Du, D.; Wang-Kan, X.; Neuberger, A.; Van Veen, H.W.; Pos, K.M.; Piddock, L.J.V.; Luisi, B.F. Multidrug Efflux Pumps: Structure, Function and Regulation. *Nat. Rev. Genet.* **2018**, *16*, 523–539. [CrossRef]
75. Davis, M.A.; Besser, T.E.; Orfe, L.H.; Baker, K.N.K.; Lanier, A.S.; Broschat, S.L.; New, D.; Call, D.R. Genotypic-Phenotypic Discrepancies between Antibiotic Resistance Characteristics of Escherichia Coli Isolates from Calves in Management Settings with High and Low Antibiotic Use. *Appl. Environ. Microbiol.* **2011**, *77*, 3293–3299. [CrossRef]
76. Siguier, P.; Gourbeyre, E.; Chandler, M. Bacterial Insertion Sequences: Their Genomic Impact and Diversity. *FEMS Microbiol. Rev.* **2014**, *38*, 865–891. [CrossRef]
77. Guédon, G.; Libante, V.; Coluzzi, C.; Payot-Lacroix, S.; Leblond-Bourget, N. The Obscure World of Integrative and Mobilizable Elements, Highly Widespread Elements that Pirate Bacterial Conjugative Systems. *Genes* **2017**, *8*, 337. [CrossRef]
78. Delavat, F.; Miyazaki, R.; Carraro, N.; Pradervand, N.; Van Der Meer, J.R. The Hidden Life of Integrative and Conjugative Elements. *FEMS Microbiol. Rev.* **2017**, *41*, 512–537. [CrossRef]
79. Mazel, D. Integrons: Agents of Bacterial Evolution. *Nat. Rev. Genet.* **2006**, *4*, 608–620. [CrossRef]
80. Gillings, M.R. Integrons: Past, Present, and Future. *Microbiol. Mol. Biol. Rev.* **2014**, *78*, 257–277. [CrossRef]