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## Antibiotic-Resistant *Enterococcus* Species in Marine Habitats: A Review

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### Abstract

Antibiotic-resistant *Enterococcus* (ARE) are among leading causes of nosocomial infections worldwide. *Enterococcus* spp. are ubiquitous in sewage, which can contaminate surface waters via many pathways, providing a route of exposure for humans. This review focuses on ARE in marine and estuarine habitats, including marine animals. Phylogenetic confirmation of the genus *Enterococcus* and intermediate or full resistance to clinically relevant antibiotics were inclusion criteria. The proportion of resistant isolates varied greatly among antibiotics, for example, 24.2% for ampicillin and 2.4% for vancomycin. The water column contained the highest proportion of ARE observations (18.8%), followed by animal feces and tissues (14.8%), sediment (9.4%), and sand (2.0%). The proportion of multidrug-resistant isolates was the greatest in animal tissue and fecal samples, followed by water and sediments. This review indicates that clinically relevant ARE are present in marine/estuarine habitats and that animals may be important reservoirs.

### Keywords

antibiotic resistance; enterococci; marine; estuarine; review

### Introduction

Antibiotic-resistant bacteria and their genes are among the most recalcitrant issues in infectious disease. Pathogens once thought nearly vanquished, such as *Mycobacterium tuberculosis* and *Neisseria gonorrhoea*, are reemerging in antibiotic-resistant strains that are difficult to treat, whereas others formerly considered relatively unimportant opportunists such as *Enterococcus* species have acquired genes to combat multiple antibiotics, even those of last resort such as vancomycin (VRE) [1,2]. As a result, ARE are among the leading causes of nosocomial infections [3] and have been recently listed as a serious threat to public health [4]. *Enterococcus* species, including the most clinically important members of the

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genus, *Enterococcus faecalis* and *Enterococcus faecium*, are normal flora of the human gastrointestinal tract, and are thus ubiquitous in domestic sewage [5,6]. Sewage can contaminate surface and groundwater via many pathways, including inadequate treatment of sewage, spills due to infrastructure malfunction or overload, and land or lagoon disposal of animal waste [7,8], providing a route of exposure for humans who ingest or otherwise come in contact with contaminated water [8].

Recent reports indicate that antibiotics are over-prescribed worldwide [9–11]. For example, in the United States nearly 300 million antibiotic prescriptions annually (equivalent to more than 800 prescriptions per 1000 individuals) were prescribed for outpatient care alone [11]. An estimated 30% of those antibiotic prescriptions were unnecessary or inappropriate because they were prescribed for respiratory conditions for which they were not indicated (e.g. viral infections, bronchitis, asthma and allergy, nonsuppurative otitis media) or the antibiotic prescribed did not adhere to treatment guidelines (e.g.  $\beta$ -lactams are first-line therapy for common bacterial infections yet azithromycin is the most commonly prescribed antibiotic) [10,11]. Table 1 provides a summary of the most commonly prescribed antibiotic classes (along with the example antibiotics for each) used in the treatment of infections caused by various gram-positive organisms, including *Enterococcus*.

This review focuses on antibiotic resistance in *Enterococcus* in marine and estuarine ecosystems. Currently *Enterococcus* species are the only fecal indicator bacteria recommended for monitoring the sanitary quality of marine and brackish waters in the U.S [5]. Furthermore, recent review articles have summarized available literature on antibiotic-resistant bacteria and their genes in wastewater and freshwaters [12,13], but marine environments have received less attention. This review focuses on antibiotic-resistant *Enterococcus* species isolated from marine and estuarine ecosystems; specifically those that displayed intermediate or full resistance to antibiotics used in human medicine or animal husbandry [14,15](Table 2). Habitats sampled within these ecosystems included water, sediment, sand, and marine animals (e.g. feces and tissues). Many publications on antibiotic-resistant bacteria in the environment include isolates with low-level resistance in the data and/or do not confirm the identity of isolates. Such practices can exaggerate the potential relevance of findings to human health, and therefore we used resistance level and phylogenetic confirmation as criteria for inclusion in the study.

### Literature Search Criteria

We searched PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>) and Web of Science (<https://clarivate.com/webofsciencegroup/solutions/web-of-science/>) data-bases for articles published between 2009 and 2019 and containing the following keywords: enterocc\* AND antibiotic resistan\* (OR antibiotic susceptible OR antimicrobial resistan\*) AND marine (OR sea OR salt OR estuarine OR brackish). Three hundred ten unique articles were first examined to verify that studies measured antibiotic-resistant *Enterococcus* spp. in marine or estuarine habitats. Two hundred eighty-eight articles were eliminated due to at least one of the following reasons: (1) studies were carried out in wastewater and/or hospital setting, (2) food items were tested, and (3) the antimicrobial effect of extracts/chemicals was tested. The remaining 22 articles [16–37] were further examined to ensure that (1) confirmation of

genus and/or species was carried out using either biochemical (e.g. API20Strep, matrix-assisted laser desorption ionization time of flight mass spectrometry) or phylogenetic (e.g. DNA sequencing, quantitative PCR or [q]PCR) methods and (2) antibiotics with clinical application for *Enterococcus* species were used at recommended concentrations and isolates displayed either intermediate or full resistance, as described in Performance Standards for Antimicrobial Susceptibility Testing Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14,15] or as evidenced by presence of appropriate genes (e.g. *vanA*, *vanB*). Some publications did not discriminate between intermediate and full resistance; therefore, we have grouped them into a single category. Three publications were removed because of failure to confirm genus/species [35] or use of inappropriate antibiotics and/or antibiotic concentrations [18,30]. Within the remaining 19 articles, certain antibiotics tested were either inappropriate for *Enterococcus* (e.g. clindamycin) or were tested at concentrations insufficient to isolate organisms with intermediate or full resistance [17,20,23–26,31,33], and these partial data sets were also removed from our analysis.

### Characteristics of included studies

A summary of studies containing location, habitat (estuarine/marine waters and/or sediments and animal tissue and/or fecal samples) and antibiotics tested is provided in Table 2. Study locations were distributed worldwide, including Australia, Europe, North America, Southeast Asia, South America, and India (Table 2). The primary isolation method (e.g. selective-differential media supplemented with antibiotics or not) is also included. Seven of 19 studies (36.8%) relied on media supplemented with antibiotics for isolation of bacteria. Utilization of media supplemented with antibiotics can bias the results by increasing the likelihood of selecting the resistant organisms and can therefore affect the calculated resistance frequency. Although we did not separate studies based on isolation method when calculating the proportion of resistant isolates, we note this issue particularly in the case where small sample size (number of studies) results in a high proportion of resistant isolates.

In many studies isolates were tested against multiple antibiotics, yielding a total of 9099 observations on 1769 unique isolates. Because many studies did not report results on an isolate-by-isolate basis, comparisons across antibiotics are reported on a per-observation basis. When the proportion of isolates resistant to a given antibiotic is reported, the calculation is simply the number of resistant to the given antibiotic divided by the total number tested against the antibiotic. Although many studies tested multiple antibiotics against multiple isolates, the multiple antibiotics were not necessarily tested against a single isolate to generate multidrug-resistant (MDR) data. The MDR comparisons were performed for studies that explicitly tested multiple antibiotics on single, confirmed *Enterococcus* isolates.

Studies most frequently tested *Enterococcus* for resistance to ampicillin, followed by erythromycin, tetracycline, vancomycin, chloramphenicol, gentamicin, streptomycin, and ciprofloxacin (Table 2). Less than 50% of studies tested resistance to quinupristin-dalfopristin, teicoplanin, linezolid, levofloxacin, nitrofurantoin, rifampicin, sulfamethoxazole-trimethoprim (Table 2). Resistance to daptomycin, kanamycin,

norfloxacin, penicillin, and tigecycline was tested in only one study each (Table 2). At least one study reported resistance to ampicillin, erythromycin, tetracycline, gentamicin, ciprofloxacin, quinupristin-dalfopristin, streptomycin, vancomycin, chloramphenicol, levofloxacin, rifampicin, sulfamethoxazole-trimethoprim, daptomycin, norfloxacin and penicillin (Table 2), whereas resistance to linezolid, nitrofurantoin, and tigecycline was not observed.

### Relationship of habitat and *Enterococcus* species to antibiotic resistance

The proportion of antibiotic-resistant *Enterococcus* isolates varied widely by habitat (Table 3). For example, of the 1133 isolates tested for ampicillin susceptibility across all habitats, 24.2% were resistant, but the proportion of *Enterococcus* isolated from water that was resistant to ampicillin was 37.6%. A smaller proportion of isolates from animal fecal and tissue samples (17.4%), as well as sediment samples (9.7%) were ampicillin resistant. A greater proportion of *Enterococcus* isolates were resistant to erythromycin (29.9%) compared to ampicillin, and followed a different pattern of habitat distribution, as isolates from animal samples were most frequently resistant (37.4%), followed closely by isolates from water (36.6%), while sediment isolates were least frequently resistant (9.7%). One publication [23] reported data from water and oysters so that the source of isolates could not be distinguished, and therefore they were not accounted for in Table 3.

Vancomycin resistance was much less common than resistance to ampicillin or erythromycin (2.5% of all isolates tested); but was observed most frequently in isolates from water (4.4%) followed by animal samples (3.3%). No vancomycin-resistant *Enterococcus* were isolated from sediment (n = 98) or sand (n = 421). One study tested for vancomycin resistance in water and sand, finding 3.1% to be resistant, but the proportion of isolates from each habitat was not specified [31]. Considering all isolates (n = 1769) tested against all antibiotics (n = 9099 observations), isolates from water were most frequently resistant (18.8%) followed by those from animals (14.8%), sediment (9.4%), and finally sand (2.0%). It should be noted that 95% of observations on sand isolates were for vancomycin resistance, which was rare in all habitats, thus the frequency of antibiotic resistance in sand is artificially low. The observed frequency of resistance in various habitats is influenced by the fact that studies varied in terms of habitats sampled and antibiotics tested, for example, the mean number of antibiotics used in water habitats was 5.1, that in sediment 7.3, that in animals 10.7, and that in sand 2.7.

Eight different *Enterococcus* spp. were isolated across all studies including *E. avium*, *E. casseliflavus*, *E. dispar*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. hirae*. Figure 1 shows a compilation of antibiotic resistance by species across all studies. A bar denoting a particular species indicates that resistance to that antibiotic was observed in at least one study. *E. faecium* and *E. faecalis* displayed resistance to 14 and 13 antibiotics, respectively, in at least one study, and sulfamethoxazole was the only antibiotic to which all *E. faecium* and *E. faecalis* were sensitive (Figure 1). *E. casseliflavus* and *E. durans* isolates displayed resistance to 9 antibiotics each, but each species was susceptible to a different group of antibiotics. *E. casseliflavus* was susceptible to daptomycin, penicillin, rifampicin, streptomycin, sulfamethoxazole and teicoplanin, while *E. durans* was sensitive to

ciprofloxacin, daptomycin, gentamicin, levofloxacin, rifampicin, and streptomycin. *E. gallinarum* and *E. hirae* were resistant to eight and nine antibiotics each, *E. dispar* to five, and *E. avium* to three antibiotics (Figure 1). All eight *Enterococcus* species displayed resistance to erythromycin, followed by tetracycline (seven species), then ampicillin, gentamicin, quinupristin-dalfopristin and vancomycin (six species each). *E. avium* and *E. dispar* were the only species that were completely susceptible to ampicillin and quinupristin-dalfopristin, while *E. durans* and *E. hirae* were the only species completely susceptible to gentamicin (Figure 1).

### MDR enterococci

We considered resistance to three or more antibiotics to represent multidrug resistance. Of 17 studies that tested individual isolates against at least three antibiotics, ten reported a total of 102 MDR *Enterococcus* isolates [16,17,19,20,24,25,27–29,34]. Within these ten studies, only one study reported resistance to either eight [17] or seven antibiotics [16], with one [24] and three studies [24,25,34] reporting resistance to six and five antibiotics, respectively. Resistance to four or three antibiotics was reported more frequently, in six [16,19,24,25,28,29] and seven [16,17,19,20,25,29,34] studies, respectively. Multidrug resistance profiles most commonly included resistance to erythromycin and tetracycline, followed by ampicillin, ciprofloxacin, and quinupristin-dalfopristin.

The great majority of the 102 confirmed MDR *Enterococcus* isolates (83.3%) came from animal fecal and/or tissues sample [16,17,19,25,28,29], followed by water (8.8%) [20,24,27]. The proportion of MDR isolates among all *Enterococcus* isolated from animal samples was 9.9%, while that from the water was 3.1%. The proportion of MDR isolates from sediments were described in only one study, but comprised 7.8% of all MDR identified and 6.5% of all *Enterococcus* isolated from this habitat [34]. The comparatively high frequency of MDR isolates in animal samples was probably influenced by the comparatively high number of antibiotics used in the studies in which animal samples were tested. Of the 102 isolates, speciation was performed on 98, and the majority (59.2%) were identified as *E. faecium*, followed by *E. faecalis* (27.6%). MDR *E. gallinarum* and *E. hirae* were detected less frequently (4.1% and 5.1%, respectively), with only a singular MDR isolate each of *E. casseliflavus*, *E. dispar* and *E. durans*. Even though isolates from water were most frequently resistant to at least one antibiotic, animal samples from seabirds, whales and clams were the most common source of MDR strains (and in particular *E. faecium*).

### Conclusions

Our review of the relevant literature indicates that ARE are widespread in marine environments, but their observed distribution across habitats varied widely. The proportion of ARE observations was the greatest in water column, followed by animals (fecal and tissue samples) and sediment/sand and was not consistent across antibiotics, for example, the proportion of ampicillin resistant isolates in water samples (37.6%) was more than twice that of any other habitat. Among different *Enterococcus* species, *E. faecium* and *E. faecalis* were resistant to the greatest number of different antibiotics. Erythromycin was the only antibiotic to which each species showed resistance in at least one study. Resistance to vancomycin was

observed infrequently, and it was mainly associated with isolates from the water column and animals. Similarly, MDR isolates were detected most frequently in marine animals, suggesting they may be an important source and/or reservoir of these strains in these environments.

While we were able to synthesize the available information on ARE in marine habitats to draw some preliminary conclusions, our review also identified many knowledge gaps which should be addressed in future studies to aid in more accurate risk assessment for exposure to ARE in aquatic environments. The most common issue identified resides with study design and methodological considerations. No selective-differential medium is infallible, and all produce a certain percentage of false-positive or false-negative results, even widely used formulations that are a part of standard methods such as mEI [38]. Confirmation of the phylogenetic identity of ARE isolates is of paramount importance due to the fact that the frequency of false-positive isolates can be much higher when antibiotics are used in the primary isolation medium, and to the public health implications of the topic. Another important methodological consideration is the antibiotic tested and the resistance level reported. Standardized protocols, such as CLSI [14] and EUCAST [15], provide clinically relevant antibiotics for many different genera, as well as suggestions for interpretation of the resulting data. *Enterococcus* spp. antibiotic resistance has three tiers (sensitive, intermediate, and full resistance) with the fully resistant phenotype carrying the most public health implications. Despite the availability of these guidelines, many studies used either inappropriate antibiotics, tested below the intermediate resistance level, or failed to discriminate between intermediate and full-level resistance. Addressing these shortcomings by standardizing methodological approaches to studying antibiotic-resistant bacteria in the environment will enable better management of ARE and other antibiotic-resistant bacteria and allow for more accurate risk assessment.

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## Appendix

Papers of particular interest, published within the period of review, have been highlighted as:

\* of special interest

\*\* of outstanding interest

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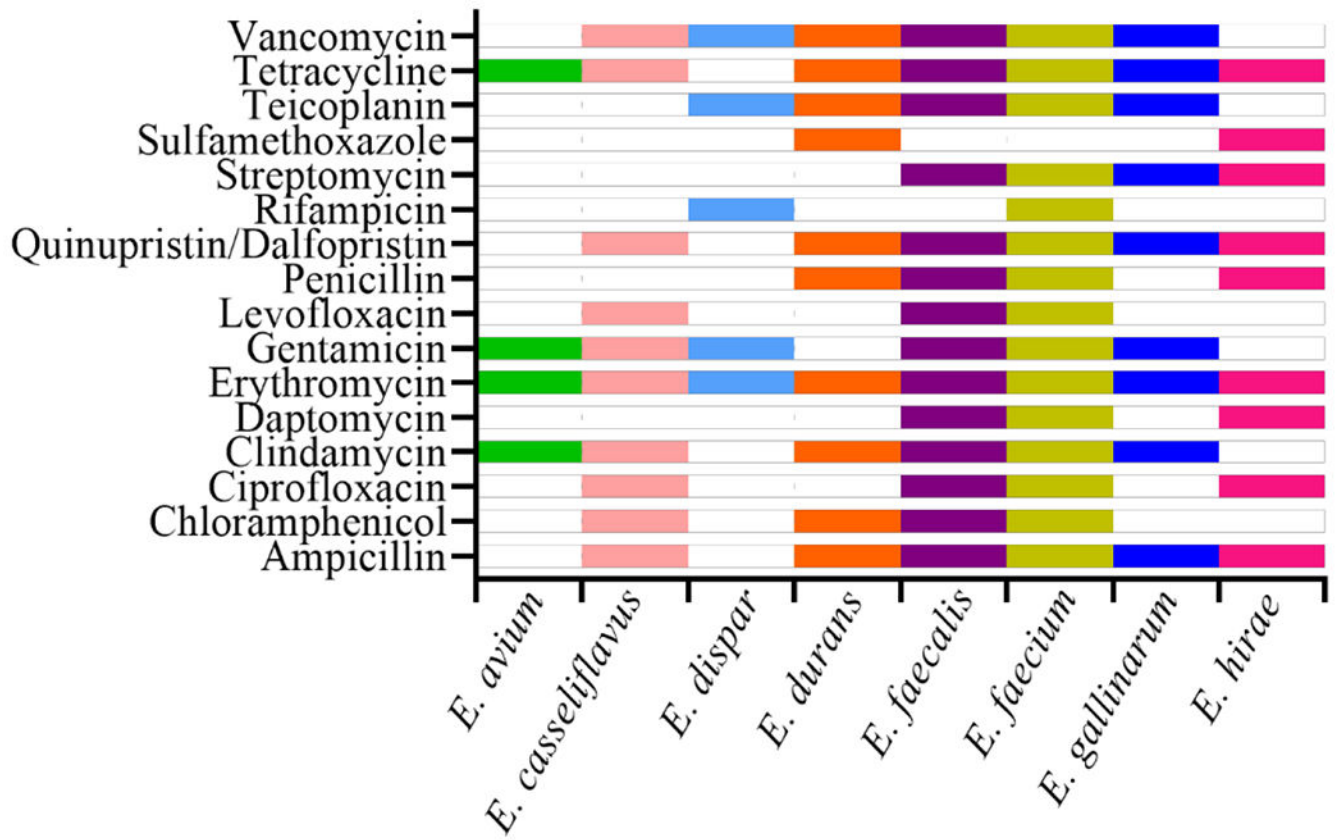
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**Figure 1.** Antibiotic resistance observations in various *Enterococcus* spp. across all studies. A bar denotes resistance to a particular antibiotic observed in at least one isolate of a given species. Antibiotics to which no resistance was observed in any species are omitted.

**Table 1.**

Most prescribed/used antibiotic classes for infections caused by *Enterococcus* species.

Classification	Examples	Usage	Activity spectrum	Comments	References
Glycopeptides	Vancomycin teicoplanin telavancin	Serious infections of blood, watery diarrhea, skin infections, endocarditis, bone and joint infections, and meningitis	<i>C. difficile</i> , <i>E. faecalis</i> , <i>S. aureus</i>	Prescriptions worldwide	[39]
Quinolones	Ciprofloxacin lomefloxacin norfloxacin ofloxacin	Treat conditions associated with bacteria including lower respiratory tract infections, skin infections and urinary tract infections.	<i>Streptococcus</i> spp., <i>P. aeruginosa</i> , <i>S. aureus</i>	Widely used in human, animal husbandry and veterinary medicine	[40]
Macrolides	Erythromycin azithromycin clarithromycin	Used to treat infections of the respiratory tract, whooping cough, diphtheria syphilis, ear, intestine, gynecological, urinary tract, and skin infections.	<i>Enterococcus</i> spp., <i>M. pneumoniae</i> , <i>V. trachomatis</i> , <i>L. pneumophila</i> , <i>C. jejuni</i> , <i>B. pertussis</i>	The most commonly prescribed antibiotics worldwide used in human and veterinary medicine	[41]
Sulfonamides	Sulfisoxazole sulfadiazine	Treat urinary tract infections and parasitic disease	<i>S. typhi</i> , <i>Serratia</i> , <i>Enterobacter</i> , <i>Pneumocystis</i> spp. and some protozoa, such as <i>Toxoplasma</i> and <i>Plasmodia</i>	Widely used in clinical and veterinary medicine to treat bacterial and protozoal infections	[42]
Tetracyclines	Tetracycline	Treat many different bacterial infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems.	Gram-positive bacilli, <i>Clostridium</i> and <i>Nocardia</i> spp. and gram-negative bacteria such as, <i>Chlamydia</i> , <i>Mycoplasmas</i> , <i>Rickettsia</i> , <i>Vibrio</i> , <i>Helicobacter</i> spp. and protozoan parasites.	Tetracyclines retain important roles in both human and veterinary medicine globally	[43]
Lipopeptides	Daptomycin	Complicated skin infection and MRSA	<i>E. faecalis</i> , <i>E. faecium</i> , <i>S. aureus</i> ,	Used in clinical and veterinary medicine	[44]
Aminoglycosides	Rifampicin gentamicin amikacin neomycin	Treat several types of bacterial infections, including tuberculosis, leprosy and other bacterial infections.	Gram-positive cocci (except for some enterococci), <i>H. influenzae</i> , <i>N. gonorrhoeae</i> , <i>N. meningitidis</i> , <i>Legionella</i> spp., <i>L. monocytogenes</i> , <i>M. tuberculosis</i>	Used in clinical and veterinary medicine worldwide	[45]
Lincosamides	Lincomycin, clindamycin, pirlimycin	Used to treat severe bacterial infections	<i>Streptococcus</i> , <i>Staphylococcus</i> and <i>Mycoplasma</i> spp.	Rarely used due to toxicity and adverse effects. Used for patients that are allergic to penicillin. First-choice use antibiotic in veterinary.	[46]
Beta-lactams	Penicillin, amoxicillin, cephalosporins, carbapenems, aminopenicillins	Treatment of bacterial infections caused by susceptible organisms.	<i>Streptococcus</i> spp., <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>Enterococcus</i> spp., <i>P. mirabilis</i> , <i>E. coli</i>	Used in clinical and veterinary medicine worldwide	[47]
Oxazolidinones	Linezolid, eperzolid	Treatment of pneumonia, skin infections, and infections that are resistant to other antibiotics	<i>E. faecium</i> , <i>S. aureus</i> , <i>S. agalactiae</i> , <i>S. pneumoniae</i> , <i>S. pyogenes</i>	Used in clinical and veterinary medicine	[48]

Table 2.

Study characteristics and antibiotic resistance tested among the studies.

Reference	Location	Habitat	Antibiotic included in primary isolation *	Antibiotics tested	Antibiotic resistance reported
[34]	Italy	Sediments	None	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, quinupristin/dalfopristin, streptomycin, tetracycline, vancomycin	Chloramphenicol, ciprofloxacin, erythromycin, quinupristin/dalfopristin, tetracycline,
[22]	Italy	Water and sediment	Tetracycline (10 mg/L) or erythromycin (20 mg/L) or ampicillin (20 mg/L), or linezolid (10 mg/L), or levofloxacin (20 mg/L), or chloramphenicol (40 mg/L) or gentamicin (250 mg/L) or streptomycin (1000 mg/L)	Ampicillin, chloramphenicol, erythromycin, gentamicin, levofloxacin, linezolid, streptomycin, tetracycline	Ampicillin, erythromycin, tetracycline
[21]	Italy	Sediments	None	Ampicillin, erythromycin, gentamicin, tetracycline	None
[16]	Portugal	Gilthead seabream	Vancomycin (4 mg/L)	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline, vancomycin	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline, vancomycin,
[32]	Poland	Water and sediment	None	Ampicillin, ciprofloxacin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline, vancomycin	Ampicillin, ciprofloxacin, erythromycin, levofloxacin, gentamicin, quinupristin/dalfopristin, streptomycin, tetracycline
[25]	Portugal	Echinoderms	None	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline, vancomycin	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, teicoplanin, tetracycline, vancomycin
[26]	India	Water	None	Ampicillin, clindamycin, erythromycin, penicillin, tetracycline	Ampicillin
[33]	Italy	Sediment	None	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, linezolid, tetracycline, vancomycin	Ampicillin, erythromycin, gentamicin, tetracycline
[17]	Portugal	Gilthead seabream	None	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline, vancomycin	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, quinupristin/dalfopristin, streptomycin, teicoplanin, tetracycline
[23]	Venezuela	Water	None	Ampicillin, ampicillin/sulbactam, penicillin, sulfamethoxazole	Ampicillin, ampicillin/sulbactam, penicillin, sulfamethoxazole
[29]	Brazil	Sea turtles, sea birds, mammals (Minke and, Humpback whale, Risso's dolphin)	None	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nitrofurantoin, norfloxacin, rifampicin, streptomycin, tetracycline, vancomycin	Ciprofloxacin, erythromycin, norfloxacin, rifampicin, tetracycline

Reference	Location	Habitat	Antibiotic included in primary isolation*	Antibiotics tested	Antibiotic resistance reported
[20]	Malaysia	Water and sediment	None	Ampicillin, chloramphenicol, erythromycin, tetracycline	Ampicillin, chloramphenicol, erythromycin, tetracycline
[37]	Italy	Sand	Tetracycline (10 mg/L) or erythromycin (20 mg/L) or ampicillin (20 mg/L), or linezolid (10 mg/L), or levofloxacin (20 mg/L), or chloramphenicol (40 mg/L) or gentamicin (250 mg/L) or streptomycin (1000 mg/L)	Ampicillin, erythromycin, gentamicin, tetracycline, vancomycin	Erythromycin, tetracycline, vancomycin
[24]	United States	Water	Ampicillin (50 mg/L), or cefotaxime (25 mg/L), or ciprofloxacin (0.1 mg/L), or erythromycin (20 mg/L), or streptomycin (50 mg/L), or sulfamethoxazole (250 mg/L), or tetracycline (18 mg/L)	Ampicillin, ciprofloxacin, erythromycin, streptomycin, sulfamethoxazole, tetracycline	Ampicillin, ciprofloxacin, erythromycin, streptomycin, sulfamethoxazole, tetracycline
[28]	Australia	Silver gull	Vancomycin (32 mg/L)	Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nitrofurantoin, quinupristin/dalopristin, rifampicin, teicoplanin, tetracycline, tigecycline, vancomycin	Ampicillin, erythromycin, gentamicin, rifampicin, teicoplanin, vancomycin
[19]	Italy	Venus clam	None	Ampicillin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, quinupristin/dalopristin, streptomycin, teicoplanin, tetracycline, vancomycin	Ampicillin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, quinupristin/dalopristin, streptomycin, tetracycline
[27]	Italy	Water	None	Ampicillin, clindamycin, erythromycin, gentamicin, tetracycline, vancomycin	Ampicillin, clindamycin, erythromycin, gentamicin, tetracycline, vancomycin
[31]	Puerto Rico	Water and sand	Vancomycin (6 mg/L)	Vancomycin	Vancomycin
[36]	Puerto Rico	Water and sand	Tetracycline (16 mg/L) or vancomycin (20 mg/L)	Vancomycin	Vancomycin

\* Denotes the type and concentration of antibiotics used to select for resistant *Enterococcus* spp.

**Table 3.** Proportion of antibiotic-resistant *Enterococcus* spp. (%) by habitat and total isolates tested against each antibiotic.

	Number of isolates tested and percent resistant (%)					
	Water	Sediment	Sand	Animals	All habitats	
Ampicillin	495 (37.6%)	299 (9.70%)	5 (0%)	334 (17.4%)	1133 (24.2%)	
Chloramphenicol	150 (0.67%)	149 (7.39%)	N/A <sup>a</sup>	432 (0.93%)	731 (2.2%)	
Ciprofloxacin	81 (38.3%)	98 (8.16%)	N/A	288 (31.3%)	467 (27.6%)	
Daptomycin	74 (0%)	N/A	N/A	46 (43.5%)	120 (16.7%)	
Erythromycin	495 (36.6%)	299 (9.70%)	5 (80%)	334 (37.4%)	1133 (29.9%)	
Gentamicin	272 (20.6%)	214 (0.93%)	5 (0%)	334 (2.69%)	825 (8.1%)	
Levofloxacin	120 (3.33%)	14 (0%)	N/A	46 (21.7%)	180 (7.8%)	
Linezolid	120 (0%)	64 (0%)	N/A	48 (0%)	232 (0%)	
Nitrofurantoin	N/A	N/A	N/A	64 (0%)	64 (0%)	
Norfloxacin	N/A	N/A	N/A	62 (12.9%)	62 (12.9%)	
Penicillin	112 (0%)	N/A	N/A	N/A	112 (0%)	
Quinupristin-dalfopristin	66 (54.5%)	48 (22.9%)	N/A	272 (17.3%)	386 (24.4%)	
Rifampicin	N/A	N/A	N/A	64 (60.9%)	64 (60.9%)	
Streptomycin	127 (6.30%)	62 (9.68%)	N/A	334 (2.99%)	523 (4.6%)	
Sulfamethoxazole	7 (100%)	N/A	N/A	N/A	7 (100%)	
Teicoplanin	74 (0%)	N/A	N/A	272 (3.68%)	346 (2.9%)	
Tetracycline	495 (13.1%)	299 (19.4%)	5 (100%)	334 (26.9%)	1133 (19.2%)	
Tigecycline	N/A	N/A	N/A	2 (0%)	2 (0%)	
Vancomycin	479 (4.35%)	98 (0%)	421 (0%)	334 (3.29%)	1332 (2.4%)	

<sup>a</sup>N/A: No isolates obtained from that combination of habitat and antibiotic