In vitro antibacterial activity of telithromycin against *Enterococcus* spp. isolated from China

Yanpeng Xiong

Shenzhen University

Junwen Chen

Shenzhen University

Xiang Sun

Shenzhen University

Guangjian Xu

Shenzhen University

Peiyu Li

Shenzhen University

Qiwen Deng

Shenzhen University

Zhijian Yu

Shenzhen University

Zhong Chen

Shenzhen University

Jinxin Zheng (✉ jinxinzheng@fudan.edu.cn)

Shenzhen University

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Abstract

Background: Enterococci has resistant to a wide range of antimicrobials, and the treatment of enterococcal infection has always been an issue of concern. This study aimed to explore the new ketolides antimicrobials-telithromycin, against the planktonic cells and biofilms of enterococci. The minimum inhibitory concentration (MIC) of telithromycin was determined. The sequence types (STs) and genotypes of resistance to erythromycin in enterococci were detected. Furthermore, the effect of telithromycin against the biofilms of enterococci were investigated.

Results: A total of 280 *Enterococcus faecalis* and 122 *Enterococcus faecium* isolates were collected from individual inpatients in China. Telithromycin showed excellent activity against the *E. faecalis* and *E. faecium* strains no matter sensitive or resistant to erythromycin with *erm A*, *erm B* or *erm C*, with the MIC₅₀ at 2 µg/mL and 4 µg/mL, respectively. The predominant STs of *E. faecalis* isolates were ST16, ST30, ST179, and ST18, ST78, ST80 were the predominant STs of *E. faecium* isolates. Moreover, 87.1% (135/158) and 80.4% (41/51) isolates of the predominant STs carried the *erm* genes in *E. faecalis* and *E. faecium* isolates, respectively. The subinhibitory concentration of telithromycin (at 1/4× and 1/8× MICs) significantly inhibited the biofilm formation of 16 *E. faecalis* isolates. Telithromycin (at 8× MIC) indicated the removal effect on the established biofilms of 8 *E. faecalis* isolates, and combined with ampicillin eradicated more biofilms than telithromycin or ampicillin alone.

Conclusion: Telithromycin showed an excellent activity against the planktonic cells of *E. faecalis* and *E. faecium*, and against the biofilms of *E. faecalis*.

Background

Enterococci are common nosocomial infections recognized as opportunistic pathogens

and form biofilms, being associated with urinary tract infection, wound infection, intra-abdominal and pelvic regions infection, and bloodstream infection [1-3]. *Enterococcus faecalis* is the most common species of *enterococcus*, accounting for 85% to 90% of human enterococcal infections [4]. *Enterococcus faecium* accounts for most of the rest of infections induced by enterococci [5]. According to previous reports, biofilms are recognized as a major cause of nosocomial infections, accounting for more than 80% microbial infection, and both of *E. faecalis* and *E. faecium* are able to form biofilms [1, 6]. Compared to planktonic cells, enterococci in biofilms are more extremely resistant to antimicrobial agents [7]. Moreover, enterococci have emerged increasingly resistant to antimicrobials, and the treatment of enterococcal infection has always been an issue of concern. Furthermore, it has been discovered that *enterococcus* frequently resistant to oxazolidinones, glycopeptides, aminoglycosides, β-lactams, and macrolides [8, 9]. Therefore, there is an urgent need to assess any new therapeutic alternative treatment for enterococcal infections.

Telithromycin (HMR3647), known as a member of ketolides antibiotic, was approved by the US Food and Drug Administration (FDA) for the treatment of community-acquired pneumonia (CAP), acute exacerbations of chronic bronchitis (AECB), and acute maxillary sinusitis (AMS) [10]. The resistance to macrolide-lincosamide-streptogramin B (MLSB) in enterococci based on mono- or bimethylation of 23S rRNA and its target sites modification (A2058 or A2509) on domain V [11-13]. Methylation identified as usually dominated by the acquisition of *erm* genes, which encode methyltransferases is specific adenine residues of N6-dimethylation in the conserved region of rRNA [14, 15]. However, telithromycin has one more target site modification (A725) on domain III compared with MLSB antibiotics, including erythromycin, and resulting in affinity increased for rRNA and impairing the function of methylate, and telithromycin is capable of remaining bound and active [15, 16].

In this study, we examined the in vitro antibacterial activity of telithromycin against enterococcal clinical isolates collected from inpatients in Shenzhen Nanshan People's Hospital and compared with other antimicrobials. The effect of telithromycin against the biofilms of *E. faecalis* isolates was also explored in this study.

Results

In vitro activity of telithromycin against enterococci isolates

The *E. faecalis* isolates (n=280) were retrospectively collected from urine (n=129, 46.1%), tracheal secretions (n=48, 17.1%), blood (n=29, 10.4%), bile (n=19, 6.8%), phlegm (n=9, 3.2%) and others sources (n=46, 16.4%) (**Fig. S1. A**). A total of 122 *E. faecium* isolates were also collected from urine (n=59, 48.4%), tracheal secretions (n=20, 16.4%), blood (n=16, 13.1%), bile (n=12, 9.8%) and other sources (n=15, 12.3%) (**Fig. S1. B**). The enterococcal isolates were highly resistant to erythromycin (ERY) and common tetracycline antibiotics, including tetracycline (TET), doxycycline (DOX), minocycline (MIN), and were more sensitive to vancomycin (VAN), nitrofurantoin (NIT), and linezolid (LZD) (**Table 1** and **Table 2**).

Telithromycin showed an excellent activity against the *E. faecalis* and *E. faecium* strains, with the MIC₅₀/MIC₉₀ at the 2/8 µg/mL and 4/8 µg/mL, respectively (**Table 1** and **Table 2**). It should be noted that the *E. faecalis* isolates were susceptible to ampicillin (274/275) (**Table 1**), on the contrary, the *E. faecium* isolates exhibited high resistance to ampicillin (98/112) (**Table 2**). *E. faecalis* and the *E. faecium* isolates had a higher MIC₅₀/MIC₉₀ to ERY (>256/>256 µg/mL and >256/>256 µg/mL). It was noteworthy that 212 *E. faecalis* isolates with ERY MIC ≥ 8 µg/mL (resistance rate reached at 75.7%), but only 33 isolates with telithromycin MIC ≥ 8 µg/mL (**Table 1**).

In vitro activity of telithromycin with ERY-specific resistance genes

To examine the effect of ERY-specific resistance genes on susceptibility of telithromycin in *E. faecalis* and *E. faecium* isolates, PCR assays were performed to detect the expression of *erm A*, *erm B*, *erm C* (Table 3 and Table 4). The data showed that telithromycin MIC_{50/90} values of 2/8 µg/mL and 4/8 µg/mL were obtained respectively in *E. faecalis* and *E. faecium* isolates with the ERY-specific resistance genes, suggesting that the presence of resistance genes may affect telithromycin sensitivity of enterococcal isolates. However, the ERY sensitivity rate of enterococcal isolates harboring *erm A*, *erm B* or *erm C* genes reached a lower level at 0-10% (Table 3 and Table 4).

MICs of telithromycin and ERY to MLST and carriage of *erm* genes

MLST was used to determine the ST distribution of the *E. faecalis* and *E. faecium* isolates (Table S1 and Table S2). The present research detected forty-four STs in *E. faecalis* isolates, with the predominant STs being ST16, ST30 and ST179, and 44 isolates of new ST that were not recorded in MLST database. Moreover, sixteen STs in *E. faecium* isolates, with the predominant STs being ST18, ST78 and ST80, and 14 isolates of new ST were found in this study.

The distribution of telithromycin and ERY MICs with the predominant STs in *E. faecalis* and *E. faecium* isolates as shown in Table 5 and Table 6. The data indicated that 26.6% (42/158), 9.5% (15/158), 47.5% (75/158), 16.4% (26/158) and 15.7% (8/51), 2% (1/51), 23.5% (12/51), 58.8% (30/51) of the *E. faecalis* and *E. faecium* isolates with telithromycin MIC ≤1, 2, 4, ≥ 8 µg/mL, respectively. However, the ERY non-sensitivity rate of the *E. faecalis* and *E. faecium* isolates with the predominant STs reached a higher level at 98.7% (156/158) and 94.1% (48/51), respectively. Moreover, 87.1% (135/158) and 80.4% (41/51) isolates of the predominant STs carried the *erm* genes in *E. faecalis* and *E. faecium* isolates, respectively (Table 5 and Table 6). Our results demonstrate that telithromycin may exert more excellent in vitro antimicrobial activity against enterococcus than ERY.

The effect of telithromycin against the biofilms of *E. faecalis* isolates

To determine the inhibitory effect of telithromycin on biofilms of sixteen *E. faecalis* isolates, biofilm assays were performed in 96 well microtiter plates by detecting the OD570 after crystal violet staining, and 1/2×, 1/4× and 1/8× MICs were used to in this study. As shown in Fig.1, telithromycin dramatically inhibited the biofilm formation of sixteen *E. faecalis* isolates that MIC values as listed in Table S3. Moreover, we investigated the eradicated effect of telithromycin on established biofilms of 4 *E. faecalis* isolates and compared with common antimicrobials of AMP, VAN, LZD (Fig. S2). The data showed that telithromycin and AMP were capable of eradicating the established biofilms of *E. faecalis*, but VAN and LZD could not. Thus, we further determined the eradicating effect of telithromycin combined with AMP on the established biofilms of 8 *E. faecalis* isolates, suggesting that telithromycin exhibited robust synergistic effect with AMP (Fig. 2).

Discussion

Enterococci species are commensals bacteria in human gastrointestinal tract with the ability to cause various nosocomial infections. They are not only equipped with various intrinsic antimicrobial resistances, but also are able to acquire mutation and/or new resistance genes [17, 18]. Telithromycin (HMR3647), known as a novel ketolide antimicrobial agent applied for treatment of respiratory infection [19]. Wolter N *et al.* identified telithromycin resistance in *Streptococcus pneumoniae* is associated with a deletion in the crucial sequence of rRNA methylation induced by *erm B* [20]. Uzun B *et al.* reported that telithromycin resistance in erythromycin-resistant isolates, but all erythromycin-susceptible isolates were also susceptible to telithromycin [21]. However, the treatment of telithromycin for enterococci infection has been studied around the world, and its effect is better than those of the first and second generation macrolides [22]. In this study, our data suggested that the *E. faecalis* and *E. faecium* isolates had a lower MIC₅₀/MIC₉₀ of telithromycin at the 2/8 µg/mL and 4/8 µg/mL, respectively. However, ERY MIC₅₀/MIC₉₀ was >256/>256 µg/mL and the resistance rate was reached at more than 75% in both of the *E. faecalis* and *E. faecium* isolates. Since increasing evidence has suggested that the prevalence of AMP, VAN, and LZD resistance in enterococci around the world [18, 23, 24]. Our results were consistent with that the emergence of AMP resistance among *E. faecium* isolates in our hospital (resistance rate at 87.5%). However, those of ERY-resistant and ERY-non-resistant *E. faecalis* isolates were highly sensitive to AMP, VAN and LZD. These results suggest that telithromycin is more effective than ERY, and both antibiotics of VAN and LZD may be more suitable for the treatment of enterococcal infections in our hospital.

The macrolide-lincosamide-streptogramin B resistance phenotype widely appears in erythromycin resistance clinical strains. Weisblum B *et al.* demonstrated that erythromycin resistance in *Staphylococcus aureus* is section of the macrolide-lincosamide-streptogramin B resistance phenotype after erythromycin was used in clinical practice [25]. This phenotype was determined by *erm* gene that product an erythromycin resistance methylase, inducing methylation of specific adenosine residues on the 23S rRNA, which causes resistance of newly synthesized ribosomes to macrolide-lincosamide-streptogramin B antimicrobials [14, 15]. Sule Celik *et al.* described that the emergence of high-level erythromycin resistance may lead to poorer prognosis, more severe recurrence, and higher mortality, in enterococcal infections [26].

In this study, *erm* genes were examined and revealed that *erm B* gene was the most common resistance genes in ERY-resistant isolates of *E. faecalis*. However, both of *erm B* and *erm C* gene were detected, which are the most common resistance of genes in *E. faecium* isolates. These results suggest that *in vitro* antibacterial activity of telithromycin is associated with ERY-specific resistance genes.

Recombination is involved in genetic variation of resistance and virulence determinants, which might promote hospital adaptation among enterococcus bacterial including *E. faecalis* and the *E. faecium* [27, 28]. Ruiz *et al.* investigated that ST16 is composition of hospital-adapted strains, suggest that most continual alteration in clinical isolates of enterococcal, and promoting to generate multiple-resistance, consequently [27]. In the present study, forty-four STs were detected in *E. faecalis* isolates, with the absolutely predominant STs being ST16 and ST179, and they are belonging to clonal complex of

CC16. Thus, the treatment of antibiotics for *E. faecalis* infection should choose reasonably according to the characteristics of CC16. Moreover, sixteen STs were determined in *E. faecium* isolates, with the positively predominant STs being ST18 and ST78. Comparison of other STs, the susceptibility of predominant STs to telithromycin is not well in enterococci isolates. Furthermore, the carriage of *erm* genes in enterococcal isolates with the predominant STs were confirmed, suggesting that the sensitivity of isolates of predominant STs to telithromycin may be affected by *erm* genes in enterococci.

Increasing evidence has manifested that biofilms formation is playing a crucial role in enterococcal infection [29]. *E. faecalis* is capable of forming biofilms on inanimate and living surfaces, and it promotes tolerance and reduces sensitivity to environmental influences or antimicrobial measures [30, 31]. Thus, targeting biofilms formation of *E. faecalis* can potentially contribute to the treatments for enterococcal infection. As previously described, telithromycin is able to decrease and eradicate *Staphylococcus aureus* biofilms [32]. Our results showed that telithromycin dramatically inhibited the biofilm formation of sixteen *E. faecalis* isolates, and exhibited excellent eradicated effect combined with AMP on the established biofilms of eight *E. faecalis* isolates. However, telithromycin decreased biofilms formation of *E. faecalis* isolates was not dose-dependent. Further investigation is needed to characterize the inhibitory effect of telithromycin on biofilms formation of *E. faecalis*.

Conclusion

In summary, telithromycin showed robust in vitro antimicrobial activity against enterococci isolated clinically from Shenzhen Nanshan People's Hospital with lower MIC_{50/90} values than ERY. Moreover, telithromycin exhibited excellent activity of *E. faecalis* and *E. faecium* isolates with the ERY-specific resistance genes at MIC_{50/90} values of 2/8 µg/mL and 4/8 µg/mL. We further demonstrated that telithromycin could repress *E. faecalis* biofilms formation, eradicate the established biofilms and exhibit robust synergistic effect combined with AMP. These results suggest that telithromycin is an efficient antimicrobial agent for *enterococcus*.

Methods

Bacterial isolates and growth condition

A total of 280 non-duplicate *E. faecalis* and 122 non-duplicate *E. faecium* strains were isolated from individual patients at Shenzhen Nanshan People's Hospital from 2011-2015. Bacterial isolates were determined by standard methods using a VITEK 2 system (Biomérieux, Marcy l'Etoile, France). *E. faecalis* ATCC29212 and OG1RF (ATCC 47077) were tested as quality control strains. The isolates were cultured in TSB (Oxoid, Basingstoke, UK) at 37°C with a shaker at 220 rpm. The antimicrobials telithromycin (TEL), erythromycin (ERY), ampicillin (AMP), vancomycin (VAN), tetracycline (TET), doxycycline (DOX), minocycline (MIN), ciprofloxacin (CIP), nitrofurantoin (NIT), linezolid (LZD), tedizolid (TED) and tetracycline (TEC) were purchased from MCE (Princeton, NJ).

All procedures involving human participants were approved by the ethics committee of Shenzhen Nanshan People's Hospital, in accordance with the ethical standards of Shenzhen University and the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. For this type of study, formal consent is not required.

Antimicrobials susceptibility testing

Antimicrobial susceptibilities of enterococcus to several clinical antibiotics, including TEL, ERY, AMP, VAN, TET, DOX, MIN, CIP, NIT, LZD, TED and TEC were tested

with the VITEK 2 system. The minimum inhibitory concentration (MIC) of AMP, VAN, TEL and ERY were identified by the broth macrodilution method according to the CLSI guidelines. As the breakpoint of TEL against enterococci have not been established, we referred to the value of TEL against *Staphylococcus aureus* (MIC ≤ 1 µg/mL, 2 µg/mL, ≥ 4 µg/mL) based on the 2019 CLSI guidelines. We employed five MIC levels for TEL in the antimicrobial susceptibility analysis MIC ≤ 1, 2, 4, ≥ 8 µg/mL.

PCR analyses

DNA was extracted from all clinical enterococcal isolates in lysis buffer and as templates for PCR according to the manufacturer's instructions (Takara Bio Inc., Japan). All of the microbial DNA served as templates were stored at -20°C. PCR analysis was performed to detect *ermA*, *ermB*, and *ermC* genes as described previously. The primer pairs used for PCR amplification were as follows: *ermA*: sense, 5'-TCTAAAAAGCATGTAAAAGAAA-3' and antisense 5'-CGATACTTTTGTAGTCCTTC-3'; *ermB*: sense, 5'-CCGTTTACGAAATTGGAACAGGTAAAGGGC-3' and antisense 5'-GAATCGAGACTTGAGTGTGC-3'; *ermC*: sense, 5'-GCTAATATTGTTAAATCGTCAATTCC-3' and antisense 5'-GGATCAGGAAAAGGACATTTTAC-3'.

Multilocus sequence typing

MLST was performed to analyze the genotypes of the enterococcal isolates. Seven pairs of housekeeping genes: *gdh*, *gyd*, *pstS*, *gki*, *aroE*, *xpt* and *yqjL* for *E. faecalis*, and *atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS* and *adk* for *E. faecium* were amplified by PCR. The purified PCR products were sequenced, and results were submitted to the MLST database (<http://efaecalis.mlst.net/> and <http://efaecium.mlst.net/>) for comparison, and the sequence types (STs) of enterococci were determined. The primer pairs used for PCR amplification as listed in **Table S4** and **Table S5** have been described previously [33].

Inhibition and eradication of *E. faecalis* biofilms

As previously described [34], the *E. faecalis* isolates were diluted 1:200 and cultivated in 200 µl tryptic soy broth (TSB) medium at 37 °C, and inoculated into 96 polystyrene microtiter plates with TSBG (TSB+0.5% glucose) containing the indicated concentrations of telithromycin. After incubation for 24 hours, biofilm biomasses were stained with crystal violet and stained biofilms were detected with an optical density (OD570). For eradication assays of established biofilms, the *E. faecalis* isolates were cultivated in tryptic soy broth (TSB) medium at 37 °C for 24h to form matured biofilms, and then treated with antimicrobials (8× MIC) for 48h with the medium replaced daily, the biofilm biomasses were stained and detected. The results were collected from three independent experiments.

Statistical analysis

Statistical analyses were performed with the SPSS software (version 19.0) and GraphPad Prism software (version 5.0). Results are showed as mean ± SD. The *p*-value <0.05 was regarded as statistically significant.

Abbreviations

MICs: Minimum inhibitory concentrations; MLST: Multilocus sequence typing; STs: Sequence types; TEL: telithromycin; ERY: erythromycin; AMP: ampicillin; VAN: vancomycin; TET: tetracycline; DOX: doxycycline MIN: minocycline; CIP: ciprofloxacin; NIT: nitrofurantoin; LZD: linezolid; TED: tedizolid; TEC: tetracycline; PCR: Polymerase chain reaction

Declarations

Ethics approval and consent to participate

All procedures involving human participants were approved by the ethics committee of Shenzhen Nanshan People's Hospital, in accordance with the ethical standards of Shenzhen University and the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. For this type of study, formal consent is not required.

Consent for publication

Not Applicable.

Availability of data and materials

All data supporting the conclusion described in this study are included within the article.

Competing interests

The authors declare that there is no competing interest.

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Authors' Contributions

YX conducted the PCR analyses, MLST analyze, inhibition and eradication of *E. faecalis* biofilms, drafted the manuscript. JC collected the bacterial isolates,

performed the antimicrobials susceptibility tests and gene manipulation. XS participated in the gene manipulation, MLST analyze, inhibition of *E. faecalis* biofilms. GX, PL participated in the collection of bacterial isolates, MLST analyze, inhibition and eradication of *E. faecalis* biofilms. ZY, QD participated in the collection of bacterial isolates, inhibition and eradication of *E. faecalis* biofilms. ZC, JZ designed the study, analyzed the experimental data and revised the manuscript.

all authors have read and approved the manuscript.

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Tables

Table 1 In vitro antibacterial activity of TEL compared with various antibiotics against *E. faecalis* isolates.

Antibiotic	No. isolates	Resistance rate (%)	MIC (μg/mL) breakpoints	NO.	TEL MIC (μg/mL)						ERY MIC (μg/mL)			
					≤0.5	1	2	4	≥8	MIC ₅₀ /MIC ₉₀	≤0.5	1-4	≥8	MIC ₅₀ /MIC ₉₀
Total	280	-	-	-	116	7	27	97	33	2/8	10	58	212	>256/>256
Ampicillin	275	0.4	≤8	274	130	6	26	83	29	2/8	8	55	211	>256/>256
			≥16	1	0	0	0	1	0	4/4	0	0	1	-
Vancomycin	280	0	≤4	278	115	7	27	96	33	2/8	10	58	210	>256/>256
			8-16	2	1	0	0	1	0	0.125/4	0	0	2	>256/>256
			≥32	0	0	0	0	0	0	-	0	0	0	-
Tetracycline	276	83.7	≤4	39	33	3	1	2	0	0.625/1	7	17	15	4/>256
			8	6	3	0	0	2	1	0.625/4	1	1	4	128/>256
			≥16	231	79	4	25	91	32	4/8	2	39	190	>256/>256
Doxycycline	280	78.9	≤4	40	36	1	2	1	0	0.0625/0.125	7	19	14	2/>256
			8	19	7	2	3	6	1	2/4	1	2	16	128/>256
			≥16	221	73	4	22	90	32	4/8	2	37	182	>256/>256
Minocycline	280	73.2	≤4	43	37	2	2	2	0	0.625/2	7	21	15	2/>256
			8	32	11	0	9	9	3	2/4	1	5	26	>256/>256
			≥16	205	68	5	16	86	30	4/8	2	32	171	>256/>256
Ciprofloxacin	252	26.6	≤1	151	68	3	11	51	18	2/8	5	25	121	>256/>256
			2	34	22	1	2	8	1	0.125/4	1	12	21	128/>256
			≥4	67	24	3	14	20	6	2/8	2	12	53	>256/>256
Nitrofurantoin	254	1.2	≤32	247	102	6	26	84	29	2/8	9	52	186	>256/>256
			64	4	2	0	0	2	0	0.5/4	0	0	4	>256/>256
			≥128	3	3	0	0	0	0	0.03/0.25	0	2	1	4/>128
Linezolid	280	5.4	≤2	214	87	6	21	75	25	2/8	9	44	161	>256/>256
			4	51	23	1	6	17	4	2/4	1	11	39	>256/>256
			≥8	15	6	0	0	5	4	4/8	0	3	12	>256/>256
Tedizolid	279	-	≤0.5	239	97	7	25	81	29	2/8	9	45	185	>256/>256

Note: MIC, minimum inhibitory concentration; TEL, telithromycin; ERY, erythromycin;

Table 2 In vitro antibacterial activity of TEL compared with various antibiotics against *E. faecium* isolates.

Antibiotic	No. isolates	Resistance rate (%)	MIC (µg/mL) breakpoints	No.	TEL MIC (µg/mL)					MIC ₅₀ /MIC ₉₀	ERY MIC (µg/mL)			
					≤0.5	1	2	4	≥8		≤0.5	1-4	≥8	MIC ₅₀ /MIC ₉₀
Total	122	-	-	-	25	2	12	34	49	4/8	6	12	104	>256/>256
Ampicillin	112	87.5	≤8	14	11	0	0	3	0	0.063/4	1	6	7	8/>128
			≥16	98	13	2	12	29	42	4/8	5	6	87	>128/>256
Vancomycin	120	0.0	≤4	116	23	2	12	31	48	4/8	6	11	99	128/>256
			8-16	4	2	0	0	2	0	0.063/4	0	1	3	8/>256
			≥32	0	0	0	0	0	0	-	0	0	0	-
Teicoplanin	115	0.9	≤8	114	24	2	12	33	43	4/8	6	12	96	>128/>256
			16	0	0	0	0	0	0	-	0	0	0	-
			≥32	1	0	0	0	0	1	8	0	0	1	>256/>256
Tetracycline	119	46.2	≤4	45	11	1	1	16	16	4/8	1	6	38	>128/>256
			8	19	3	0	1	3	12	8/8	2	0	17	>256/>256
			≥16	55	10	1	10	14	20	4/8	3	5	47	>256/>256
Doxycycline	120	38.3	≤4	62	15	1	4	22	20	4/8	4	8	50	>128/>256
			8	12	1	0	4	1	6	4/8	0	0	12	>256/>256
			≥16	46	9	1	4	8	24	8/8	2	4	40	>128/>256
Minocycline	120	27.5	≤4	66	16	1	4	22	23	4/8	4	8	54	>128/>256
			8	21	3	1	6	4	7	2/8	1	1	19	>256/>256
			≥16	33	6	0	2	7	18	8/8	1	3	29	>128/>256
Ciprofloxacin	113	9.7	≤1	29	10	1	7	8	3	2/4	1	5	23	>256/>256
			2	4	1	0	0	1	2	4/8	1	0	3	>256/>256
			≥4	80	13	1	5	24	37	4/8	3	7	70	>128/>256
Nitrofurantoin	114	57.0	≤32	11	2	0	3	2	4	4/8	1	1	9	>256/>256
			64	38	6	1	5	12	14	4/8	1	4	33	>256/>256
			≥128	65	16	1	4	18	26	4/8	4	7	54	>128/>256
Linezolid	122	2.4	≤2	116	22	2	12	32	48	4/8	6	10	100	>128/>256
			4	3	2	0	0	1	0	0.063/4	0	1	2	8/>256
			≥8	3	1	0	0	1	1	4/8	0	1	2	>256/>256

Note: MIC, minimum inhibitory concentration; TEL, telithromycin; ERY, erythromycin;

Table 3 In vitro activity of TEL against *E. faecalis* isolates with ERY-specific resistant genes.

Erythromycin		No.	TEL MIC (µg/mL)						ERY MIC (µg/mL)			
resistance genes			≤0.5	1	2	4	≥8	MIC50 /MIC90	≤0.5	1-4	≥8	MIC50 /MIC90
Total		280	116	7	27	97	33	2/8	10	58	212	>256/>256
ermA	+	11	1	0	0	6	4	4/8	0	0	11	>256/>256
	-	269	115	7	27	91	29	2/8	10	58	201	>256/>256
ermB	+	188	39	4	23	92	30	4/8	1	8	179	>256/>256
	-	92	77	3	4	5	3	0.625/2	9	50	33	2/>256
ermC	+	0	0	0	0	0	0	-	0	0	0	-
	-	280	116	7	27	97	33	2/8	10	58	212	>256/>256

Note: MIC, minimum inhibitory concentration; TEL, telithromycin; ERY, erythromycin; +, positive; -, negative;

Table 4 In vitro activity of TEL against *E. faecium* isolates with ERY-specific resistant genes

Erythromycin		No.	TEL MIC (µg/mL)						ERY MIC (µg/mL)			
resistance genes			≤0.5	1	2	4	≥8	MIC50 /MIC90	≤0.5	1-4	≥8	MIC50 /MIC90
Total		122	25	2	12	34	49	4/8	6	12	104	≥0.25-256
ermA	+	12	2	0	2	3	5	4/8	0	2	10	128/>256
	-	110	23	2	10	31	44	4/8	6	10	94	>128/>256
ermB	+	40	2	0	3	21	14	4/8	0	2	38	128/>256
	-	82	23	2	9	13	35	4/8	6	10	66	>128/>256
ermC	+	56	5	2	7	10	32	8/8	0	1	55	>256/>256
	-	66	20	0	5	24	17	4/8	6	11	49	>128/>256

Note: MIC, minimum inhibitory concentration; TEL, telithromycin; ERY, erythromycin; +, positive; -, negative

Table 5 TEL MIC values of predominant STs in *E. faecalis* isolates with carrying *erm B* gene

		No. (%)	TEL MIC (µg/mL)						ERY MIC (µg/mL)				<i>ermB</i>
ST			≤0.5	1	2	4	≥8	MIC ₅₀ /MIC ₉₀	≤0.5	1-4	≥8	MIC ₅₀ /MIC ₉₀	
ST16		78 (27.9)	18	3	9	36	12	4/8	0	6	72	>256/>256	67
ST30		8 (2.9)	7	0	1	0	0	0.125/2	0	2	6	128/>256	6
ST179		72 (25.7)	14	0	5	39	14	4/8	2	7	63	>256/>256	62

Note: MIC, minimum inhibitory concentration; TEL, telithromycin; ERY, erythromycin

Table 6 TEL MIC values of predominant STs in *E. faecium* isolates with carrying *erm B* and *erm C* genes.

		No. (%)	TEL MIC (µg/mL)						ERY MIC (µg/mL)				<i>ermB</i>	<i>ermC</i>
ST			≤0.5	1	2	4	≥8	MIC ₅₀ /MIC ₉₀	≤0.5	1-4	≥8	MIC ₅₀ /MIC ₉₀		
ST18	18 (12.3)	3	0	0	0	15	8/8		0	1	17	>256/>256	1	17
ST78	26 (21.3)	4	1	0	9	12	4/8		3	1	22	128/>256	16	3
ST80	7 (5.7)	0	0	1	3	3	4/8		0	0	7	>128/>128	4	0

Figures

Figure 1
Telithromycin inhibiting the biofilm formation of 16 *E. faecalis* isolates. (A) and (B) The four isolates of per group were treated with TEL at 1/2 $\times$, 1/4 $\times$ and 1/8 $\times$ MICs, respectively. All of the 8 isolates with the TEL MIC = 8 μ g/mL. (C) and (D) The four isolates of per group were treated with TEL at 1/2 $\times$, 1/4 $\times$ and 1/8 $\times$ MICs, respectively. All of the 8 isolates with the TEL MIC $\leq$ 0.5 μ g/mL. The data presented was the average of three independent experiments (mean $\pm$ SD). Statistical significance between groups was calculated by Student's t-test. CTRL, control group; *Compared to CTRL, P<0.05.

Figure 2

Telithromycin alone or combined with ampicillin eradicating the established biofilms of 8 *E. faecalis* isolates. The total of 8 isolates formed mature biofilms for 24 h, then treated with TEL, or combined with AMP at 8× MICs for 48h. The data presented was the average of three independent experiments (mean ± SD). Statistical significance between groups was calculated by Student's t-test. CTRL, control group; TEL, telithromycin; AMP, ampicillin; *P<0.05.

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