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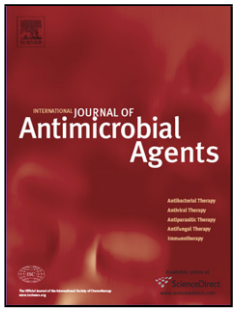

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Non-susceptibility to tigecycline in enterococci from hospitalised patients, food products and community sources

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ABSTRACT

In this study, the in vitro activity of tigecycline against 1140 enterococci collected from humans, food products, animals and the environment in Portugal (1996–2008) was analysed. Ten isolates (seven *Enterococcus faecalis* and three *Enterococcus* spp.) non-susceptible to tigecycline (minimum inhibitory concentrations of 0.5–1.0 mg/L), which were also resistant to tetracycline and minocycline, were mostly observed in samples collected before the introduction of tigecycline in the therapeutic arsenal. The *E. faecalis* isolates were recovered from hospitalised patients (*n* = 2; ST319/CC2 and ST34), healthy humans (*n* = 2; ST21/CC21), chicken meat (*n* = 1; ST260) as well as from two swine samples. The remaining isolates were also recovered from chicken meat (*n* = 1; *Enterococcus gallinarum*) and swine (*n* = 2; *Enterococcus hirae* and *Enterococcus* spp.). Recovery of enterococcal isolates with reduced susceptibility to tigecycline among different reservoirs, including animals for food consumption, suggests that selection of tigecycline-resistant isolates by antibiotics other than tigecycline might occur in non-clinical settings.

1. Introduction

The most clinically relevant Gram-positive pathogens, i.e. meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multidrug-resistant (MDR) pneumococci, have increasingly acquired resistance to most antimicrobial agents, making the nosocomial infections they cause difficult to treat [1]. Against this background, there is a clear need for therapeutic alternatives.

Glycylcyclines are novel tetracycline analogues with improved ribosomal binding-site affinity that show activity against a broad spectrum of bacteria, including those resistant to tetracycline [2,3]. Tigecycline (9-*t*-butylglycylamido-minocycline), the first glycylcycline approved for clinical use by the US Food and Drug Administration (FDA) in 2005 and registered by the European Medicines Agency in May 2006, is recommended to treat complicated skin and intra-abdominal infections caused by several Gram-negative and Gram-positive organisms, including MDR *Enterococcus faecalis* and *Enterococcus faecium*. The World Health Organization (WHO) has recently highlighted the relevance of tigecycline as one of the last therapeutic options in several human infections [4]. A few resistant *E. faecalis* isolates showing minimum inhibitory concentrations (MICs) close to the epidemiological cut-off value (ECOFF) for this antibiotic have been reported in large clinical trials, although the mechanism of resistance remains to be characterised [5,6].

To our knowledge, the activity of tigecycline against enterococcal isolates recovered from the community has not previously been studied. Moreover, there are no data regarding the activity of this antibiotic against enterococcal isolates from Portugal, one of the countries with the highest prevalence of VRE (http://www.earss.rivm.nl).

The aim of this study was to evaluate the in vitro activity of tigecycline, an antibiotic of last resort to treat severe human infections, against enterococci from different sources.

2. Methods

A total of 1140 enterococcal isolates collected during 1996–2008 were studied, most of which have been included in previous studies by our group [7]. The isolates were recovered from the following sources: (i) 294 clinical isolates from different patients at six hospitals in different cities in northwestern, central and eastern Portugal (1996–2008); (ii) 208 isolates from faecal samples of healthy human volunteers living in the north and centre of the country (2001–2004); (iii) 288 isolates from swine faeces and environmental samples recovered in six piggeries and one slaughterhouse from the north, centre and south of Portugal (1997–2007); (iv) 234 isolates from raw poultry products corresponding to 93 chicken lots and 6 turkey lots from ten different commercial brands and purchased at two different butcher shops in the Porto area (1999–2001); and (v) 116 isolates from hospitals wastewaters and from the estuary of the Douro River in Porto city (2001–2003).

Clonal relatedness was established in specific cases by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) as described elsewhere (http://www.mlst.net) [7]. Species identification and vancomycin and tetracycline resistance gene detection were performed by multiplex polymerase chain reaction (PCR) assays [8].

Susceptibility testing was performed by the standard agar dilution or disk diffusion methods following Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. For tigecycline (Wyeth Pharmaceuticals, Havant, UK), cation-adjusted Mueller–Hinton II agar (bioMérieux, Marcy-l'Étoile, France) was always freshly prepared and the antibiotic was incorporated into the media a few hours prior to use to avoid oxidation [10]. When borderline to resistance MIC values were obtained, susceptibility assays were repeated five times to evaluate the reproducibility of the results. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as controls for susceptibility tests. The susceptibility breakpoint value was determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which establishes clinical criteria for interpretation of tigecycline activity in enterococci (susceptible ≤0.25 mg/L and resistant >0.5 mg/L) (http://www.eucast.org; last update January 2011). This committee also recommends an ECOFF (MIC ≤ 0.25 mg/L) to detect enterococci expressing acquired resistance mechanisms to tigecycline (http://217.70.33.99/Eucast2/SearchController) [11]. According to these guidelines, isolates showing MICs ≥ 0.5 mg/L were considered non-susceptible to tigecycline.

3. Results

Among 294 clinical isolates and 846 non-clinical isolates, enterococci were identified as *E. faecium* (*n* = 485), *E. faecalis* (*n* = 405) and *Enterococcus* spp. (*n* = 250). In total, 884 isolates (78%) were resistant to three or more antibiotics from different groups, 796 isolates (70%) showed resistance to tetracycline and 270 isolates (24%) were resistant to vancomycin (83% *vanA*, 16% *vanC1* and 1% *vanB*). Most studied isolates (>99%) were susceptible to tigecycline, showing MICs ranging from ≤0.03

mg/L to 0.25 mg/L (Table 1). In agreement with other studies, the median MIC_{50} and MIC_{90} values (MICs for 50% and 90% of the organisms) for enterococci from different origins were 0.125 mg/L and 0.25 mg/L, respectively [12,13]. Moreover, *E. faecalis* isolates showed higher MIC_{50} values than *E. faecium* and *Enterococcus* spp. isolates (0.25 mg/L vs. 0.125 mg/L) as previously reported [13,14]. A difference in tigecycline activity was not observed in isolates from different sources or with different antibiotic resistance phenotypes.

Ten tetracycline-resistant isolates recovered from hospitalised and non-hospitalised humans, food products and animals (7 *E. faecalis,* 1 *Enterococcus hirae*, 1 *E. gallinarum* and 1 *Enterococcus* spp.; MICs 32 mg/L to >256 mg/L) were also resistant to minocycline (MICs 32–64 mg/L) and showed non-susceptibility to tigecycline, with MICs ranging from 0.5 mg/L (*n* = 7, of which 3 contained *tetM* and 1 contained *tetM* + *tetL*) to 1 mg/L (*n* = 3; 2 *tetM*) (Table 2). *Enterococcus faecalis* were obtained from: (i) hospitalised patients [*n* = 2 isolates recovered in 2002; the new sequence type 319 (ST319) belonging to clonal complex 2 (CC2) and ST34]; (ii) healthy humans (*n* = 2 isolates from 2001; same PFGE type identified as ST21/CC21); (iii) chicken meat (*n* = 1; ST260 from 1999); and (iv) swine (*n* = 2 isolates from 2006–2007; not typed by MLST). The remaining enterococcal isolates (1999–2007) were recovered from chicken meat (1 *E. gallinarum*) and swine (1 *E. hirae* and 1 *Enterococcus* spp.). Previous studies have also described a few *E. faecalis* clinical isolates exhibiting MICs against tigecycline of 0.5 mg/L [6,12,15] and 1 mg/L (by broth microdilution) [5].

4. Discussion

To our knowledge, the only enterococcal isolate showing an MIC value against tigecycline higher than the EUCAST clinical breakpoint was a clinical German *E. faecalis* strain belonging to the prevalent ST6 (CC2) from which resistance was associated with antibiotic treatment comprising multiple courses of different drugs besides tigecycline [5]. In this study, we report the lack of susceptibility to tigecycline among *E. faecalis* from hospitalised patients, healthy humans, chicken meat and swine mainly collected before the introduction of tigecycline in clinical practice. The data suggest that a selection process of tigecycline-resistant isolates might occur in non-clinical settings involving antibiotics other than tigecycline.

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Competing interests

None declared.

Ethical approval

Not required.

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Table 1

In vitro activity of tigecycline against enterococci isolated from different species and sources

Species	Source	Type	No. of isolates tested	No. of isolates inhibited at tigecycline MIC (mg/L) of: [a]						MIC_{50} (mg/L)	MIC_{90} (mg/L)
				≤0.03	0.0625	0.125	0.25	0.5	1.0		
Enterococcus faecalis	ATCC 29212	Control [b]			X	X					
Staphylococcus aureus	ATCC 29213	Control [b]				X	X				
Enterococcus faecium (*n* = 485)	Hospitalised patients	VRE	112	2	36	49	25			0.125	0.25
		VSE	18		5	10	3			0.125	0.25
	Healthy humans	VRE	6		1	3	2			0.125	0.25
		VSE	80	3	23	42	12			0.125	0.25
	Swine	VRE	7		2	4	1			0.125	0.25
		VSE	98	1	17	50	30			0.125	0.25
	Poultry	VRE	46	3	4	26	13			0.125	0.25
		VSE	42	1	7	23	11			0.125	0.25
	Environment	VRE	26		9	15	2			0.125	0.25
		VSE	50	1	12	23	14			0.125	0.25
E. faecalis (*n* = 405)	Hospitalised patients	VRE	37		5	15	17			0.125	0.25
		VSE	123	2	20	38	61	**2**		0.25	0.25
	Healthy humans	VRE	0								

		VSE	100	1	7	24	66	**1**	**1**	0.25	0.25
	Swine	VRE	1				1			N/D	N/D
		VSE	52	2	3	8	37	**2**		0.25	0.25
	Poultry	VRE	5	1		2	2			N/D	N/D
		VSE	59	1	1	21	35	**1**		0.25	0.25
	Environment	VRE	3		1	2				N/D	N/D
		VSE	25		5	12	8			0.125	0.25
Enterococcus spp. (*n* =	Hospitalised	VRE	2			2				N/D	N/D
250)	patients	VSE	2			2				N/D	N/D
	Healthy humans	VRE	0								
		VSE	22		3	9	10			0.125	0.25
	Swine	VRE	0								
		VSE	130	4	10	53	61	**1** [c]	**1** [d]	0.125	0.25
	Poultry	VRE	25		5	12	8			0.125	0.25
		VSE	57	3	9	28	16		**1** [e]	0.125	0.25
	Environment	VRE	0								
		VSE	12		3	7	2			0.125	0.25

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC for 50% and 90% of the organisms, respectively; VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci; N/D, not determined due to the small number of isolates.

[a] Numbers in bold correspond to the 10 isolates showing MIC values of 0.5–1.0 mg/L (non-susceptibility).

[b] The MICs of tigecycline varied between 0.125 mg/L and 0.25 mg/L for *S. aureus* ATCC 29213 and 0.06 mg/L and 0.125 mg/L for *E. faecalis* ATCC 29212 control strains, which is consistent with data reported by other groups [5,12].

[c] Swine isolate identified as *Enterococcus hirae.*

[d] Swine isolate that could not be identified by the multiplex polymerase chain reaction (PCR) assays used, which includes *E. faecium*, *E. faecalis*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *E. hirae* and *Enterococcus durans*.

[e] Poultry isolate identified as *E. gallinarum.*

Table 2

Features of tigecycline-non-susceptible *Enterococcus* isolates

Species	Source	Year	PFGE	MLST	*tet* [a]	Antibiotic resistance profiles [MIC (μg/mL) or S/R]						
						TIG	TET	MIN	ERY	CIP	STR	GEN
E. faecalis	Healthy human	2001	A	ST21 (CC21)	M	0.5	32	64	S	S	R	S
E. faecalis	Healthy human	2001	A	ST21 (CC21)	M	1	64	64	S	S	R	S
E. faecalis	Chicken retail product	1999	B	ST260 (CS)	M	0.5	64	64	S	S	S	S
E. faecalis	Piggery 1 (waste lagoon)	2006	N/D	N/D	–	0.5	128	32	R	S	S	R
E. faecalis	Piggery 1 (liquid manure)	2007	N/D	N/D	–	0.5	>256	64	R	S	R	S
E. faecalis	Hospitalised patient	2002	C	ST319 (CC2) [b]	–	0.5	>256	64	R	S	R	S
E. faecalis	Hospitalised patient	2002	D	ST34	M	0.5	128	64	S	S	S	S
E. gallinarum	Chicken retail product	1999	N/D	N/A	M	1	128	32	S	S	S	S
E. hirae	Piggery 1 (solid manure)	2006	N/D	N/A	M, L	0.5	>256	32	S	S	S	S
Enterococcus spp.	Piggery 2 (liquid manure)	2007	N/D	N/A	–	1	64	32	S	R	S	S

PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; MIC, minimum inhibitory concentration; S, susceptible; R, resistant; TIG, tigecycline; TET, tetracycline; MIN, minocycline; ERY, erythromycin; CIP, ciprofloxacin; STR, high-level resistance to streptomycin; GEN, high-level resistance to gentamicin; ST, sequence type; CC, clonal complex; CS, singleton; N/D, not determined; N/A, not applicable.

[a] *tet* genes sought by polymerase chain reaction (PCR) included *tetM*, *tetL*, *tetO*, *tetK*, *tetS* and *tetX*.

[b] ST319 is a novel sequence type clustering in CC2 by eBURST analysis.