EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 July 2013
EMA/291760/2013

Use of glycylcyclines in animals in the European Union: development of resistance and possible impact on human and animal health

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

An agency of the European Union

Table of Contents

1. Introduction

The global emergence and steady increase in bacteria that are resistant to multiple antimicrobials has become a global public health threat (Carlet 2012). Treatment options for infections caused by multidrug-resistant (MDR), extensively-resistant (XDR) or pandrug-resistant bacteria (PDR) (Magiorakos, Srinivasan et al. 2012) are very limited because there are very few or no effective antimicrobials available. In addition, infections with resistant bacteria are often associated with higher morbidity and mortality, higher costs, and longer length of hospital stay (Cosgrove 2006).

The lack of an effective antimicrobial armamentarium has led to the use of older antimicrobials or the development of new compounds that belong to "old classes" of antimicrobials. The glycylcyclines are a new antimicrobial subgroup developed from an older class of antimicrobials, the tetracyclines. Glycylcyclines were derived in the 1990s from minocycline in an effort to overcome tetracycline resistance (Zhanel, Homenuik et al. 2004). Tigecycline is the only glycylcycline currently in clinical use and was authorised in 2006 in the EU for the treatment of complicated skin and soft tissue and abdominal infections in humans. Tigecycline has a broad antimicrobial spectrum. It is also effective against certain multidrug-resistant bacteria e.g. extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL), carbapenem-resistant *Enterobacteriaceae* (CRE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and multi-drug resistant *Acinetobacter* species (Zhanel, Homenuik et al. 2004). Regrettably there are already reports of emerging resistance to tigecycline, especially among *Enterobacteriaceae* and *Acinetobacter* species, only a few years after the antimicrobial was introduced for clinical use (Sun, Cai et al. 2013).

Bacteria are often able to develop resistance against new antimicrobial agents relatively quickly if the antimicrobial in question shares a similar structure or mechanism of action with older agents, to which resistance is already widespread (Chopra and Roberts 2001). This is a potential concern in case of tigecycline although organisms resistant to older tetracyclines are usually susceptible to tigecycline. Tetracyclines have been widely used for decades both in humans and animals as well as in agriculture (Zhanel, Homenuik et al. 2004), and they are still one of the most used antimicrobials in food animals in the EU (ESVAC 2012). Extensive use of tetracyclines have led to widespread resistance to this class of antimicrobials, with the involvement of several types of mobile resistance determinants (Zhanel, Homenuik et al. 2004).

In order to maintain tigecycline efficacy, the European Commission (EC) has requested the EMA to provide scientific advice on the impact of use in animals of glycylcyclines or related substances on human health and to assess whether restricting the use of glycylcyclines in veterinary medicine would impact on the development of tigecycline resistance in bacteria which can cause disease in humans. This review summarises the information on glycylcyclines, mainly tigecycline, with special reference to the above mentioned aspects (European Commission 2013).

2. Characteristics of glycylcyclines in relation to tetracyclines

Chlortetracycline was the first tetracycline antimicrobial discovered in 1945. This was followed by tetracycline in 1953, doxycycline in 1967 and minocycline in 1972. Two first antimicrobials are products of *Streptomyces aureofaciens*, whilst doxycycline and minocycline are semisynthetic molecules. Tetracyclines prevent bacterial protein synthesis and are considered as bacteriostatic drugs, although a bactericidal action has been shown for some organisms (Norcia, Silvia et al. 1999).

Tetracyclines can be devided into three generations: the first include naturally occurring antibiotics such as tetracycline, oxytetracycline and chlortetracycline; the second generation includes doxycycline and minocycline which are semisynthetic antimicrobials, are more lipophilic, and are absorbed from gastrointestinal tract better than older molecules; the third generation includes glycylcyclines, a novel extended-spectrum class of antimicrobials developed in 1990s to overcome resistance to conventional tetracyclines (Chopra and Roberts 2001). Tigecycline was the first glycylcycline approved for clinical use in 2006 (Giamarellou and Poulakou 2011).

Chemically tigecycline is a 9-glycylamino-tetracycline, a semisynthetic derivative of minocycline (Olson, Ruzin et al. 2006). Tigecycline binds to the A site of the 30S subunit as well as interacts with residues of H34 ribosomal subunit (Olson, Ruzin et al. 2006). This leads to prevention of protein synthesis by blocking incorporation of amino acid residues into elongating peptide chain (Doan, Fung et al. 2006). Binding of tigecycline to ribosomes is five times higher than that of older tetracyclines. This feature makes tigecycline less affected by ribosomal protection mechanisms caused by the majority of tetracycline resistance determinants. Another feature of tigecycline is a long side chain which prevents binding of tigecycline to many tetracycline efflux and transporter systems (Sun, Perng et al. 2012). For these reasons, tigecycline activity is not affected by major mechanisms of tetracycline resistance (Zhanel, Homenuik et al. 2004).

Tetracycline resistance mechanisms are numerous and widely spread both in Gram-negative and Gram positive bacteria. Most of the tetracycline efflux systems cause resistance to tetracycline but not to minocycline or glycylcyclines. The exception is an efflux protein coded by *tet*(B) gene, the substrate of which include also minocycline in addition to older tetracyclines, but not glycylcyclines. However, in vitro studies have shown that mutations in *tet*(A) or *tet*(B) genes can lead to increased minimum inhibitory concentrations (MICs) to glycylcyclines, indicating that resistance to glycylcyclines may develop over time from tetracycline determinants (Chopra and Roberts 2001). Ribosomal protection has wider substrate specificity compared to tetracycline efflux proteins. Ribosomal protection is mediated by genes coding cytoplasmic proteins that confer resistance to first and second generation tetracyclines (Chopra and Roberts 2001). Different tetracycline resistance mechanisms can be harboured at the same time by the bacterial cell.

All tetracyclines are broad-spectrum antimicrobials covering most aerobic and anaerobic Gram-positive and Gram-negative species, as well as atypical organisms such as *Chlamydia*, *Mycoplasma*, *Rickettsiae*, *Spirochetes*, rapidly growing mycobacteria and many protozoa. Minocycline and doxycycline MICs against different pathogens are usually lower compared to older tetracyclines (Zhanel, Homenuik et al. 2004). Tigecycline has a similar antimicrobial spectrum to minocycline, although the MICs of tigecycline are usually lower against many *Enterobacteriaceae* species (Zhanel, Homenuik et al. 2004). In the EU, tigecycline susceptibility breakpoint varies between 0.25 to 1 µg/ml depending on bacterial genera with *Enterobacteriaceae* species having the highest breakpoint and *Streptococcus* the lowest (www.eucast.org). Tigecycline antimicrobial spectrum covers, among others, the following genera: *Staphylococcus*, *Enterococcus*, *Streptococcus*, most *Enterobacteriaceae* and non-fermenting Gram-negative rods such as *Acinetobacter* spp. The last two groups include extended spectrum betalactamase and carbapenemase producing isolates. Among anaerobes tigecycline is active against *Clostridium* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., *Prevotella* spp., and *Bacteroides fragilis* group. Tigecycline has been reported to be effective in vitro against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and quinolone-resistant *Streptococcus pneumoniae*. The species which are inherently resistant to tigecycline include *Proteus*, *Morganella* and *Providencia* species and *Pseudomonas aeruginosa* (Zhanel, Homenuik et al. 2004). Bacterial kill profiles with tigecycline were the most prominent in fluoroquinolone-resistant *S. pneumoniae* (>6 log total reduction within 18 hours), whilst only 1.5 and 1.2 log reductions were observed against MRSA and VRE isolates within 18 hours (Garrison and Nuemiller 2007). Apart from *S.*

pneumoniae and some other species, the effect of tigecycline is usually bacteriostatic. The AUC/MIC ratio has been considered as the most important pharmacodynamic parameter to predict the efficacy of tigecycline (Falagas, Karageorgopoulos et al. 2009).

3. The use of tigecycline in humans

Tigecycline is authorised in the EU for treatment of complicated skin and soft tissue infections (excluding diabetic foot infection) and complicated intra-abdominal infections in adults (EMA 2011). Tigecycline is also authorised for the treatment of community-acquired pneumonia in the USA (FDA 2006). In addition to the above authorised indications, tigecycline is also used in some off-label indications such as hospital-acquired pneumonia (HAP), including ventilator associated pneumonia (VAP), diabetic foot infections, rescue therapy for infections due to multidrug-resistant pathogens, urinary tract infections (UTIs), and refractory *Clostridium difficile* infections (Stein and Babinchak 2013).

Tigecycline is administered intravenously. The approved dosing regimen consists of a 100 mg loading dose followed by 50 mg every 12 hours infused over 30 to 60 minutes. Relatively low peak serum concentrations of tigecycline (0.6 to 0.9 µg/ml) have been reported in the steady state after the standard dosing scheme. Tigecycline has a large volume of distribution (7 to 9 l/kg), which reflects extensive distribution into tissues. The site to serum mean $AUC_{0\text{-}24h}$ ratios are from few to several hundred in lung tissue, colon, gallbladder and bile, but in bone, synovial or cerebrospinal fluid less than one. In peritonitis, the concentration of tigecycline corresponds to 44 to 54% of the serum values in the peritoneal fluid. The plasma protein binding of tigecycline ranges from 71 to 89%. Reported systemic clearance of tigecycline varies from 0.2 to 0.3 l/(h*kg) (Falagas, Karageorgopoulos et al. 2009). Tigecycline mean elimination half-life is 37 to 38 hours. Tigecycline is excreted mainly unchanged via the faeces and to a lesser extent via urine. It is estimated that less than 20% of tigecycline is metabolised before excretion. The main metabolites are a glucuronide, an N-acetylmetabolite and a tigecycline epimer, which have no antimicrobial activity. Tigecycline dosage adjustment is necessary in case of severe hepatic failure (Falagas, Karageorgopoulos et al. 2009).

In clinical trials tigecycline has been as effective as its comparators (vancomycin-aztreonam or imipenem-cilastatin combinations) for the treatment of complicated skin and soft tissue infections as well as complicated intra-abdominal infections (Doan, Fung et al. 2006). Conditions associated with bacteraemia can have questionable response to tigecycline therapy (McGovern, Wible et al. 2013).

A recent systematic meta-analysis revealed that tigecycline treatment can be more often associated with increased risk for mortality or non-cure rates even in approved indications (Prasad, Sun et al. 2012). Similar results were observed in another meta-analysis which reported higher overall mortality and increased risk for therapeutic failure with tigecycline than comparators (Yahav, Lador et al. 2011). Tigecycline was less effective than comparators in a meta-analysis conducted by Tasina and the co-workers (Tasina, Haidich et al. 2011), but as effective as comparators in a meta-analysis by Cai *et al*. (Cai, Wang et al. 2011). The results of these two meta-analyses also reported numerically higher mortality with tigecycline than with comparators, but detected differences were not statistically significant (Cai, Wang et al. 2011; Tasina, Haidich et al. 2011). In order to minimize the risk of fatal outcome in susceptible or severely ill patients, in the summary of product characteristics (SmPC) of tigecycline (EMA 2011), it is recommended to use tigecycline only when other treatment options are not available and warnings have been included about the mortality findings, the need to closely monitor patients and institute alternative antibacterial therapy if super infection occurs.

In addition tigecycline is more often associated with adverse effects than comparators (Cai, Wang et al. 2011; Tasina, Haidich et al. 2011; Yahav, Lador et al. 2011). Most common adverse effects with tigecycline are gastrointestinal disturbances such as vomiging and nausea, but there is also evidence that serious adverse events – of which some can lead to discontinuation of the therapy – are more often related to tigecycline therapy compared with other antimicrobial regimens (Yahav, Lador et al. 2011).

Despite all the limitations described above, tigecycline is among very few alternatives for the treatment of infections caused by multi drug-resistant bacteria (Carlet 2012).

4. Acquired resistance to glycylcyclines

4.1 General aspects

To date there have been no findings to support the involvement of tetracycline efflux pumps in clinical resistance to tigecycline (Zhanel, Homenuik et al. 2004), although *Enterobacteriaceae* isolates with tetracycline resistance mechanism show moderate, usually 2 to 4 fold, increase in tigecycline MICs compared to wild type isolates. This is species dependent (Fritsche, Strabala et al. 2005), and has also been observed in *Acinetobacter* species (Insa, Cercenado et al. 2007). Acquired clinical resistance to tigecycline is (so far) mediated by broad-spectrum multidrug-resistance efflux pumps that belong to the RND (Resistance Nodulation Division) or MATE (the multidrug and toxic compound extrusion) family of secondary multidrug transporters, which operate by a proton motive force. RND efflux systems are very common in Gram-negative species. Often they confer low-level resistance to many drugs, but mutations or other changes in genes regulating efflux systems may lead to overexpression of the system and consequently high-level resistance to antimicrobials (Fernandez and Hancock 2012). The emergence of nonspecific efflux systems that are capable of pumping several drug classes is a major concern. Intrinsic resistance to tigecycline is also due to endogenous non-specific efflux pumps, MexAB-Opr(M) and AcrAB, in *P. aeruginosa*, *Proteus* and *Morganella* species (McIntosh 2012). Substrates of these efflux systems are numerous and include, for example, several antimicrobials, biocides, detergents, and dyes (Fernandez and Hancock 2012).

4.2. Tigecycline resistance mechanisms

4.2.1 Enterobacteriaceae

A study published in 2000 reported reduced glycylcycline susceptibility in two *Salmonella* isolates (*S. choleraesuis*, *S. typhimurium*) of veterinary origin, which carried a *tet*A(A) tetracycline resistance determinant. Reduced susceptibility was observed especially to early glycylcyclines, DMG-MINO and DMG-DMDOT (two early investigational glycylcyclines), but less to tigecycline. Cloning experiments proved that *tet*A(A) gene coded efflux system was responsible for increased MICs to glycylcyclines due to mutations in specific areas of the gene (Tuckman, Petersen et al. 2000). The same study reported that laboratory-derived mutants of *Salmonella* with *tet*A(B) class efflux having decreased glycylcycline susceptibility.

Another study observed that combination of *ram*R mutation, a positive regulator of AcrAB efflux, together with Tn1721 carrying the *tet*(A) gene, was linked with tigecycline resistance as well as elevated MICs to ciprofloxacin and chloramphenicol in *Salmonella* Hadar (Hentschke, Wolters et al. 2010), although the patient from whom the bacterial strain was isolated did not have previous exposure to tigecycline. In later studies overexpression of RND efflux systems AcrAB and AcrEF have been reported to confer resistance to tigecycline in *Salmonella* (Horiyama, Nikaido et al. 2011).

In 2004 tigecycline was demonstrated to be a target molecule for AcrAB and AcrEF multidrug efflux pumps of *E. coli* (Hirata, Saito et al. 2004). Overexpression of the efflux was reported to be due to *mar*A transcriptional activator (Keeney, Ruzin et al. 2008). In 2005 *Klebsiella pneumoniae* isolates showing reduced susceptibility to tigecycline were demonstrated to also harbour the AcrAB transporter together with its activator, *ram*A (Ruzin, Visalli et al. 2005). In isolates with reduced tigecycline susceptibility *ram*A is constitutively overexpressed, leading to increased expression of AcrAB efflux. The same mechanism applies to tigecycline resistance in *Enterobacter cloacae* (Keeney, Ruzin et al. 2007). In *K. pneumoniae* (Ruzin, Immermann et al. 2008) other regulators such as *Sox*S (Bratu, Landman et al. 2009), *ram*R (Hentschke, Christner et al. 2010) or *rar*A (Veleba and Schneiders 2012) may also be involved in addition to *ram*A (Bratu, Landman et al. 2009). Enhanced activity of endogenous efflux SdeXY-HasF correlates with tigecycline, ciprofloxacin and cefpirome resistance in *S. marcescens* (Hornsey, Ellington et al. 2010).

4.2.2 Acinetobacter spp.

Overexpression of the AdeABC multidrug efflux pump is responsible for tigecycline resistance in *A. baumannii-calcoaceticus* complex (Ruzin, Keeney et al. 2007). The AdeABC efflux system is controlled by the two-component signaling system via *ade*R and *ade*S genes. Insertion of an IS(ABA-1) element in the *ade*S gene possibly leads to overexpression of AdeABC (Ruzin, Keeney et al. 2007). Mutations also in *ade*B are associated with reduced susceptibility to tigecycline in *Acinetobacter* (Peleg, Adams et al. 2007). AdeFGH overexpression due to *ade*L (Coyne, Rosenfeld et al. 2010) and AdeIJK (Damier-Piolle, Magnet et al. 2008) have also been encountered with tigecycline resistance in *Acinetobacter* spp. These efflux pumps are non-specific, converting resistance to many antimicrobials such as beta-lactams, chloramphenicol, tetracyclines and fluoroquinolones in addition to tigecycline. Recently, other efflux-pump regulation mechanisms have been linked with tigecycline resistance in *Acinetobacter* species (Sun, Perng et al. 2012).

4.2.3 Other species

Other bacterial species with efflux-pump mediated tigecycline resistance include *Burkholderia cepacia*-complex (Rajendran, Quinn et al. 2010) and *S. aureus*. In *S. aureus* tigecycline resistance has been acquired in laboratory conditions by exposing bacterial cells to increasing concentrations of tigecycline in serial passage (McAleese, Petersen et al. 2005). Further investigations revealed that resistance was due to novel MATE family efflux pump, mep(A) – mepRAB.

Tigecycline resistance due to enzymatic inactivation was described in 2005 (Moore, Hughes et al. 2005). TetX is a flavin-dependent mono-oxygenase protein affecting the first and second-generation tetracyclines. Its activity targets also tigecycline by modifying it into 11a-hydroxytigecycline. It was first described in *Bacteroides fragilis*, but because molecular oxygen is needed for its function, the clinical significance of *tetX* gene in *Bacteroides* spp. has been questioned (Moore, Hughes et al. 2005). *TetX1* and *tetX2* are homologues of *tetX* in *Bacteroides* spp. A recent study reported that tigecycline resistance was correlated in *Bacteroides* species with the presence of a *tetQ* determinant and to a lesser extent, with a *tetX1* determinant (Bartha, Soki et al. 2011). These genes are often carried by the CTnDOT determinant, which is a conjugative transposon. Although the role of these tetracycline resistance determinants in tigecycline resistance in *Bacteroides* spp. is as yet not understood, the presence of genes possibly mediating the tigecycline resistance highlights the potential spread of tigecycline resistance (Bartha, Soki et al. 2011). *TetX* gene has been described also in a *Sphingobacterium* sp. isolate. The chromosomal G+C percentage content of *tetX* is similar to that of *Sphingobacterium* indicating that this aerobic Gram-negative soil bacteria or related species could be an ancestral source of *tetX* instead of *Bacteroides* (Ghosh, Sadowsky et al. 2009). *TetX* gene along

with other tetracycline determinants have been found in soil from swine production facilities (Wu, Qiao et al. 2010) in activated sludge of sewage treatment plants (Zhang, Zhang et al. 2009) as well as from aerosols of swine and dairy farms and human medical clinics (Ling, Pace et al. 2013). Until recently, tetX determinants were believed to be not widespread in clinical isolates, but a recent study from Sierra Leone surprisingly reported the presence of *tet*X gene or its homologues in clinical isolates of *E. cloacae*, *E. coli, K. pneumoniae*, and other members of *Enterobacteriaceae* as well as *Delftia acidovorans Comamonas testosteroni* and *Pseudomonadaceae*, although tigecycline had not been used (Leski, Bangura et al. 2013) . Whether this gene is as widespread in other countries and whether the gene has a role in tigecycline resistance in these bacterial species needs to be confirmed.

4.3 Emergence of tigecycline resistance and resistance surveillance

So far, tigecycline resistance is mostly described in bacteria isolated from infections in humans, but this may be because tigecycline susceptibility is seldom tested for veterinary isolates. A Portuguese study reported the presence of ten tetracycline and minocycline resistant enterococci showing tigecycline non-susceptibility that were derived from humans, chicken meat and swine mainly before human use of tigecycline was introduced (Freitas, Novais et al. 2011). Another study performed in Lithuania using EUCAST epidemiological cut-off values reported 2% tigecycline resistance among 105 enterococcal isolates collected from cattle, swine and poultry (Ruzauskas 2009).

Many reports regarding the development of tigecycline resistance in human pathogens have been published (Sun, Perng et al. 2012). Tigecycline resistance can develop quickly during the tigecycline treatment, especially during monotherapy, in *A. baumannii* (Reid, Grim et al. 2007), *Enterococcus faecalis* (Werner, Gfrorer et al. 2008), *Enterobacter hormaechei* (Daurel, Fiant et al. 2009), *Klebsiella pneumoniae* (Rodriguez-Avial, Rodriguez-Avial et al. 2012; Spanu, De Angelis et al. 2012), and *E.coli* (Spanu, De Angelis et al. 2012). The development of tigecycline resistance has also been reported during fluoroquinolone treatment (Hornsey, Ellington et al. 2010) which was due to emergence of *ram*A mediated AcrAB up-regulation. The same mechanism confers resistance to fluoroquinolones (Fernandez and Hancock 2012). The capability of fluoroquinolones for selecting cross-resistance to tigecycline is of high concern.

Tigecycline susceptibility rates and its comparators have been monitored systemically for example in the Tigecycline Evaluation and Surveillance Trial (TEST) for several years (www.testsurveillance.com). In general the results of this program have suggested that tigecycline susceptibility rates in different pathogens have maintained satisfactory results (Hawser, Bouchillon et al. 2012; Mayne and Dowzicky 2012). According to many reports 95-100% of investigated isolates are susceptible to tigecycline depending on bacterial species. For instance, investigation of global collection of more than 5000 bacterial strains from hospitalized patients during 2000 to 2004 resulted in that all enterococci, streptococci, staphylococci and *E. coli* were inhibited by 2 µg /ml or less of tigecycline. However, already at that time, 3% of *Klebsiella* spp. and *Acinetobacter* spp., and 5% of *Enterobacter* spp. had tigecycline MIC > 2 µg/ml indicating the presence of less susceptible population (Fritsche, Sader et al. 2005). Since then, significant changes in MICs of tigecycline have been observed in a global surveillance program in *A. baumannii* isolates collected in 2004 to 2009 (Morfin-Otero and Dowzicky 2012) and among vancomycin-resistant enterococci in Taiwan (Tsai, Liao et al. 2012), although only 1.4% of enterococci showed reduced susceptibility to tigecycline in this particular study. In the Asia-Pacific region, a tigecycline surveillance programme also detected a shift towards higher tigecycline MICs in *K. pneumoniae* and *S. marcescens* (Hawser, Bouchillon et al. 2012). Very high tigecycline resistance rates have been reported among multidrug-resistant subpopulations such as ESBL or carbapenemase producing *A. baumannii* and *Enterobacteriaceae* (Sun, Cai et al. 2013), although this

can partly be due to methodological differences in susceptibility testing. The comparison between the different reports can be challenging also due to differences in used breakpoints.

5. Veterinary use of antimicrobial agents related with glycylcycline resistance

Tetracyclines have several therapeutic indications for the treatment of infections in food and companion animal species. In food producing species, including horses, usually the first generation tetracyclines are used whilst in small companion animals the second generation tetracyclines are preferred. Therapeutic indications in animals include respiratory infections, skin and soft tissue infections, peritonitis, metritis and enteric infections. Tetracyclines are also used for treating infections in aquatic species and honeybee. In food animal species the treatment is often group treatment via the drinking water or feed aiming for cure (treatment or methaphylaxis) or prophylaxis of the disease during an outbreak (Giguere, Prescott et al. 2006). Tetracyclines have also been used for growth promotion in food animal species but concerns related emerging resistance lead to a withdraw of the use of tetracyclines from the list of authorized growth promoters in many European countries in 1972–1974 (Cogliani, Goossens et al. 2011), since then the EU has phased out the use of antibiotics as growth promoters (Commission 2003). However, the use of tetracyclines for growth promotion is still allowed in many countries including the USA (Chopra and Roberts 2001).

Although antimicrobial resistance have impaired the usefulness of tetracyclines, they are still widely used in animal husbandry. According to the latest European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report tetracyclines are the most sold antimicrobial agents, expressed in mg per Population Correction Unit (PCU), for veterinary use in EU countries corresponding to 39% of the total use of antimicrobials for food producing animals (ESVAC 2012). As shown in Table 1, there is a wide variation in tetracycline use in veterinary medicine between the countries, probably indicating differences in medication practices, production types, and animal health care programmes. The reasons behind the variation on veterinary antimicrobial consumption should be investigated.

Another group of antimicrobials possibly associated with the selection of tigecycline resistance is fluoroquinolones. The fluoroquinolone use accounts for 2% of the total use of antimicrobials for food producing animals (ESVAC 2012). There are no similar data on the antimicrobial use in companion animals in the EU.

There are currently no tigecycline-containing products authorised for the veterinary use in the EU. For food-producing animals, tigecycline cannot be used because of the lack of Maximum Residue Limit values. In principle, tigecycline could be used for companion animal species since *the Cascade* rule allows the use of human-approved drugs in certain circumstances (Official Journal of the European Communities 2001). In dogs, cats and horses similar problems with multidrug-resistant organisms to those in human medicine have been described with increasing frequency. For example, ESBL-producing multidrug-resistant *E. coli* (Ewers, Bethe et al. 2012), multidrug-resistant *A. baumannii* (Endimiani, Hujer et al. 2011) and methicillin-resistant staphylococci (Catry, Van Duijkeren et al. 2010) are common in these species. For these reasons there is a need for new drugs in companion animal medicine. Tigecycline is one such option and has been suggested as an alternative for the treatment of infections caused by (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP) in veterinary dermatology (Papich 2012). In horses tigecycline might be useful for the treatment of septicaemia in foals (K. Baptiste, personal communication). The magnitude of the use of tigecycline in veterinary medicine due to *the Cascade* is not known. Some Member States, for example Finland, have banned the use of tigecycline for animals by national legislation (www.finlex.fi, Act 847/2008).

Table 1. Percentages of sales for food-producing animals (including horses), in mg per population correction unit (mg/PCU) of tetracyclines, by country, for 2010 (ESVAC 2012)

Country	% Tetracyclines
Austria	59%
Belgium	25%
Czech Republic	40%
Denmark	31%
Estonia	13%
Finland	14%
France	47%
Hungary	56%
Iceland	10%
Ireland	36%
Latvia	24%
Lithuania	19%
Netherlands	51%
Norway[1]	4.1%
Portugal	41%
Slovenia	13%
Spain	40%
Sweden[1]	8%
United Kingdom	46%
Average 19 countries	**39%**

[1]Sales of antimicrobial VMPs for farmed fish not included; fish not included in PCU.

6. Discussion

As the result of widespread use of tetracyclines, different types of mobile tetracycline resistance genes are common in veterinary and human pathogens and commensals (Kumar and Varela 2012) as well as in the environment (Zhang, Zhang et al. 2009; Wu, Qiao et al. 2010; Ling, Pace et al. 2013). Bacteria carrying tetracycline resistance determinants often have higher MICs to tigecycline compared to wild-type isolates although MICs are usually lower than clinical breakpoint. The exception is *TetX*, which confers resistance also to tigecycline, but the clinical relevance of this determinant in tigecycline resistance has not been confirmed. Therefore acquired tigecycline resistance is mainly due to chromosomal mutations in regulatory genes of various RND multidrug efflux pumps. RND mechanisms are ubiquitous in Gram-negative bacteria and they are capable of converting resistance to several antimicrobial classes. Wild-type RND efflux system slightly elevate the MICs of several antimicrobials, but when mutations occur the over expression of the system leads to clinically relevant resistance. Therefore bacterial species harbouring multidrug efflux pumps or tetracycline resistance determinants could act as a source of pre-population of which the tigecycline resistance will rise. Tigecycline resistance can be selected by both, tigecycline and fluoroquinolones.

The possible impact of veterinary use of tetracyclines or fluoroquinolones on the tigecycline resistance is difficult to estimate. Although tetracycline resistance in animal pathogens is common, tigecycline susceptibilities are hardly ever reported in bacteria of veterinary origin. This may be due to the fact

that tigecycline is not routinely tested for veterinary isolates. Tigecycline resistance has appeared quickly parallel with tigecycline use in humans. If tigecycline or other glycylcyclines were authorised for veterinary use, a similar trend may be noted in veterinary bacteria. Consequently this could compromise the usefulness of tigecycline in human clinical medicine, especially where zoonotic bacteria are concerned.

In many studies ESBL-producing *Enterobacteriaceae* strains from veterinary and human specimens have been shown to be identical or closely-related (Ewers, Bethe et al. 2012). The same applies to *A. baumannii* (Endimiani, Hujer et al. 2011) and MRSA (Catry, Van Duijkeren et al. 2010). Tigecycline resistance has been reported in all these organisms. Although tigecycline resistance is not known to spread horizontally, mutated strains can be further selected under the pressure of antimicrobials increasing the likelihood for transmission of resistant strains between the animals and man.

7. Conclusions

- Tigecycline is used in human medicine for the treatment of complicated skin and soft-tissue infections and complicated intra-abdominal infections, often as one of the last resort drugs.
- Tigecycline is not authorised for use in animals, nor is any other glycylcycline.
- Tigecycline is suggested in the veterinary literature as one of the alternatives for treatment of methicillin-resistant staphylococcal infections in dogs and cats but there are no authorised products available and the extent of *the Cascade* use of tigecycline is not known.
- Reports from human medicine concerning treatment failures and tigecycline resistance are increasing, especially in multidrug-resistant *Enterobacteriaceae* and *Acinetobacter* species.
- Clinically relevant tigecycline resistance is currently mediated via unselective RND (Resistance Nodulation Division) resistance efflux systems that are converting resistance to many types of antimicrobials. These are not spread horizontally. Tetracycline resistance mechanisms often cause mildly elevated MICs to tigecycline. Tetracycline resistance determinants are spread horizontally.
- Tigecycline resistance is selected mainly by tigecycline use, but also other antimicrobials such as fluoroquinolones may be associated with the selection of tigecycline resistance.
- Tigecycline resistance has been described in bacterial genera which are not species-specific. Many of these bacteria can be transferred between animals and humans.
- Overall decrease of use of antimicrobials in animals, especially of tetracyclines and fluoroquinolones, might help to decrease the transmission of resistance from animals to humans of relevance for tigecycline, but with the information available the effect of such reduction cannot be quantified.

8. Recommended Risk Management options:

8.1. Approval of new applications for veterinary products containing tigecycline

For tigecycline, which is currently not approved for use in veterinary medicine, only limited needs for use in dogs and cats have been identified.

The impact on human health from use of tigecycline in animals is dependent on the amount used and is therefore thought to be limited.

The use of tigecycline has been proposed as one of the treatment alternatives for dogs and cats for infections caused by multidrug resistant bacteria, which are increasing in incidence. As yet there is no reported use of glycylcyclines in food animal species and no clear need identified in these species. There are no maximum residue limits (MRLs) established for tigecycline therefore the antimicrobial agent cannot be used under *the Cascade* for food producing species (article 11 of Directive 2001/82/EC of the European Parliament and of the Council relating to veterinary medicinal products).

8.2 Tetracyclines

Tetracyclines are the most widely used veterinary antimicrobials (ESVAC 2012). In some food producing animal species they are a first line therapy, and steps to reduce exposure in food animals would possibly increase the use of critically important antimicrobials (CIA) such as macrolides or fluoroquinolones. Although the use of tetracyclines in animals may select for “pre-resistance” mechanisms to tigecycline, such determinants are already widespread in both animal and human bacterial species. Prudent use of tetracyclines is strongly suggested in agreement with guidelines for appropriate use in animals. Tetracyclines, as all antimicrobials, should be used mainly for the treatment of infections; prophylactic use should be discouraged.

8.3 Fluoroquinolones

In addition to tigecycline resistance emerging due to tigecycline use, tigecycline resistance can also be selected as a result of exposure to fluoroquinolones. Recommendations are already in place to limit the extent and restrict the use of fluoroquinolones in food animal species (EMA/CVMP/SAGAM 2007). This should be extended to companion animals.

8.4 Resistance surveillance

Since animal bacteria are not routinely tested for tigecycline, tigecycline resistance surveillance among animal bacteria could be considered in order to get better information of the current resistance situation.

8.5 Overall conclusion on risk management options for tigecycline use in animals

Taking into account the importance of glycylcyclines for public health and the potential for emergence of multidrug-resistance, restrictions in the use of glycylcyclines in animals is recommended. At present no need for any approval of a veterinary medicinal product containing tigecycline is foreseen. Should, in the future, a need for such medicinal products for animals be identified, authorisations could be considered on the basis of a positive benefit risk assessment where the risk for transfer of resistance to humans is included in the assessment. In light of available information on the need for tigecycline in humans and the estimated risk level it appears unlikely that a benefit/risk balance for tigecycline containing products for veterinary use would be found positive.

Overall decrease in the use of antimicrobials in veterinary medicine, especially of tetracyclines and fluoroquinolones, might help decrease the possibility of transmission of tigecycline resistance between animals and man, but because of the lack of data the impact of such a reduction cannot be quantified.

In dogs and cats the extent of the need to use tigecycline in circumstances where no veterinary-authorised alternative antimicrobial is available cannot be assessed. Further information on the extent

and reasons behind the tigecycline use due to *the Cascade* principle in companion animals is needed to conclude on the measures to be taken for use of tigecycline in these animal species.

9. References

Bartha, N. A., J. Soki, et al. (2011). "Investigation of the prevalence of tetQ, tetX and tetX1 genes in Bacteroides strains with elevated tigecycline minimum inhibitory concentrations." Int J Antimicrob Agents **38**(6): 522-525.

Bratu, S., D. Landman, et al. (2009). "Correlation of the expression of acrB and the regulatory genes marA, soxS and ramA with antimicrobial resistance in clinical isolates of Klebsiella pneumoniae endemic to New York City." J Antimicrob Chemother **64**(2): 278-283.

Cai, Y., R. Wang, et al. (2011). "Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease." Antimicrob Agents Chemother **55**(3): 1162-1172.

Carlet, J. J., V.; Harbarth, S.; Voss, A.; Goossens, H.; Pittet, D.; (2012). "Ready for a world without antibiotics? The Pensières Antibiotic Resistance. Call to Action." Antimicrobial Resistance & Infection Control **1**(11).

Catry, B., E. Van Duijkeren, et al. (2010). "Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health." Epidemiol Infect **138**(5): 626-644.

Chopra, I. and M. Roberts (2001). "Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance." Microbiol Mol Biol Rev **65**(2): 232-260 ; second page, table of contents.

Cogliani, C., H. Goossens, et al. (2011). "Restricting antimicrobial use in food animals: lessons from Europe." Microbe **6**(6): 274.

Commission, E. (2003). Regulation (EC) No 1831/2003 of the European Parliament and of the Council replacing Directive 70/524/EEC on additives in feeding-stuffs. . http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2003R1831:20050325:EN:PDF.

Cosgrove, S. E. (2006). "The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs." Clin Infect Dis **42 Suppl 2**: S82-89.

Coyne, S., N. Rosenfeld, et al. (2010). "Overexpression of resistance-nodulation-cell division pump AdeFGH confers multidrug resistance in Acinetobacter baumannii." Antimicrob Agents Chemother **54**(10): 4389-4393.

Damier-Piolle, L., S. Magnet, et al. (2008). "AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in Acinetobacter baumannii." Antimicrob Agents Chemother **52**(2): 557-562.

Daurel, C., A. L. Fiant, et al. (2009). "Emergence of an Enterobacter hormaechei strain with reduced susceptibility to tigecycline under tigecycline therapy." Antimicrob Agents Chemother **53**(11): 4953-4954.

Doan, T. L., H. B. Fung, et al. (2006). "Tigecycline: a glycylcycline antimicrobial agent." Clin Ther **28**(8): 1079-1106.

EMA (2011). Tygacil SPC. http://www.emea.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000644/WC500044508.pdf.

EMA/CVMP/SAGAM (2007). Public statement on the use of (fluoro)quinolones in food-producing animals in the european union: development of resistance and impact on human and animal health. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2009/10/WC500005152.pdf.

Endimiani, A., K. M. Hujer, et al. (2011). "Acinetobacter baumannii isolates from pets and horses in Switzerland: molecular characterization and clinical data." Journal of Antimicrobial Chemotherapy.

ESVAC (2012). European Medicines Agency. Sales of veterinary antimicrobial agents in 19 EU/EEA countries in 2010 (EMA/88728/2012). http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500133532.

European Commission (2013). Request for advice on the impact on public health and animal health of the use of antibiotics in

animals. http://www.ema.europa.eu/docs/en_GB/document_library/Other/2013/04/WC500142070.pdf.
Ewers, C., A. Bethe, et al. (2012). "Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective." Clin Microbiol Infect **18**(7): 646-655.
Falagas, M. E., D. E. Karageorgopoulos, et al. (2009). "Clinical significance of the pharmacokinetic and pharmacodynamic characteristics of tigecycline." Curr Drug Metab **10**(1): 13-21.
FDA (2006). Tygacil Prescribing Information. http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021821s021lbl.pdf.
Fernandez, L. and R. E. Hancock (2012). "Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance." Clin Microbiol Rev **25**(4): 661-681.
Freitas, A. R., C. Novais, et al. (2011). "Non-susceptibility to tigecycline in enterococci from hospitalised patients, food products and community sources." Int J Antimicrob Agents **38**(2): 174-176.
Fritsche, T. R., H. S. Sader, et al. (2005). "Potency and spectrum of tigecycline tested against an international collection of bacterial pathogens associated with skin and soft tissue infections (2000-2004)." Diagn Microbiol Infect Dis **52**(3): 195-201.
Fritsche, T. R., P. A. Strabala, et al. (2005). "Activity of tigecycline tested against a global collection of Enterobacteriaceae, including tetracycline-resistant isolates." Diagn Microbiol Infect Dis **52**(3): 209-213.
Garrison, M. W. and J. J. Nuemiller (2007). "In vitro activity of tigecycline against quinolone-resistant Streptococcus pneumoniae, methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci." Int J Antimicrob Agents **29**(2): 191-196.
Ghosh, S., M. J. Sadowsky, et al. (2009). "Sphingobacterium sp. strain PM2-P1-29 harbours a functional tet(X) gene encoding for the degradation of tetracycline." J Appl Microbiol **106**(4): 1336-1342.
Giamarellou, H. and G. Poulakou (2011). "Pharmacokinetic and pharmacodynamic evaluation of tigecycline." Expert Opin Drug Metab Toxicol **7**(11): 1459-1470.
Giguere, S., J. F. Prescott, et al. (2006). Antimicrobial therapy in veterinary medicine, Blackwell Publishing.
Hawser, S. P., S. K. Bouchillon, et al. (2012). "Trending 7 years of in vitro activity of tigecycline and comparators against Gram-positive and Gram-negative pathogens from the Asia-Pacific region: Tigecycline Evaluation Surveillance Trial (TEST) 2004-2010." Int J Antimicrob Agents **39**(6): 490-495.
Hentschke, M., M. Christner, et al. (2010). "Combined ramR mutation and presence of a Tn1721-associated tet(A) variant in a clinical isolate of Salmonella enterica serovar Hadar resistant to tigecycline." Antimicrob Agents Chemother **54**(3): 1319-1322.
Hentschke, M., M. Wolters, et al. (2010). "ramR mutations in clinical isolates of Klebsiella pneumoniae with reduced susceptibility to tigecycline." Antimicrob Agents Chemother **54**(6): 2720-2723.
Hirata, T., A. Saito, et al. (2004). "Effects of efflux transporter genes on susceptibility of Escherichia coli to tigecycline (GAR-936)." Antimicrob Agents Chemother **48**(6): 2179-2184.
Horiyama, T., E. Nikaido, et al. (2011). "Roles of Salmonella multidrug efflux pumps in tigecycline resistance." J Antimicrob Chemother **66**(1): 105-110.
Hornsey, M., M. J. Ellington, et al. (2010). "Emergence of AcrAB-mediated tigecycline resistance in a clinical isolate of Enterobacter cloacae during ciprofloxacin treatment." Int J Antimicrob Agents **35**(5): 478-481.
Insa, R., E. Cercenado, et al. (2007). "In vitro activity of tigecycline against clinical isolates of Acinetobacter baumannii and Stenotrophomonas maltophilia." J Antimicrob Chemother **59**(3): 583-585.
Keeney, D., A. Ruzin, et al. (2007). "RamA, a transcriptional regulator, and AcrAB, an RND-type efflux pump, are associated with decreased susceptibility to tigecycline in Enterobacter cloacae." Microb Drug Resist **13**(1): 1-6.
Keeney, D., A. Ruzin, et al. (2008). "MarA-mediated overexpression of the AcrAB efflux pump results in decreased susceptibility to tigecycline in Escherichia coli." J Antimicrob Chemother **61**(1): 46-53.
Kumar, S. and M. F. Varela (2012). "Biochemistry of bacterial multidrug efflux pumps." Int J Mol Sci **13**(4): 4484-4495.

Leski, T. A., U. Bangura, et al. (2013). "Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone." Int J Antimicrob Agents.

Ling, A. L., N. R. Pace, et al. (2013). "Tetracycline resistance and class 1 integron genes associated with indoor and outdoor aerosols." Environ Sci Technol **47**(9): 4046-4052.

Magiorakos, A. P., A. Srinivasan, et al. (2012). "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance." Clin Microbiol Infect **18**(3): 268-281.

Mayne, D. and M. J. Dowzicky (2012). "In vitro activity of tigecycline and comparators against organisms associated with intra-abdominal infections collected as part of TEST (2004-2009)." Diagn Microbiol Infect Dis **74**(2): 151-157.

McAleese, F., P. Petersen, et al. (2005). "A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived Staphylococcus aureus mutants to tigecycline." Antimicrob Agents Chemother **49**(5): 1865-1871.

McGovern, P. C., M. Wible, et al. (2013). "All-cause mortality imbalance in the tigecycline phase 3 and 4 clinical trials." Int J Antimicrob Agents **41**(5): 463-467.

McIntosh, E. D. (2012). "Efflux: how bacteria use pumps to control their microenvironment." Handb Exp Pharmacol(211): 153-166.

Moore, I. F., D. W. Hughes, et al. (2005). "Tigecycline is modified by the flavin-dependent monooxygenase TetX." Biochemistry **44**(35): 11829-11835.

Morfin-Otero, R. and M. J. Dowzicky (2012). "Changes in MIC within a global collection of Acinetobacter baumannii collected as part of the Tigecycline Evaluation and Surveillance Trial, 2004 to 2009." Clin Ther **34**(1): 101-112.

Norcia, L. J., A. M. Silvia, et al. (1999). "Studies on time-kill kinetics of different classes of antibiotics against veterinary pathogenic bacteria including Pasteurella, Actinobacillus and Escherichia coli." J Antibiot (Tokyo) **52**(1): 52-60.

Official Journal of the European Communities (2001). Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products.

Olson, M. W., A. Ruzin, et al. (2006). "Functional, biophysical, and structural bases for antibacterial activity of tigecycline." Antimicrob Agents Chemother **50**(6): 2156-2166.

Papich, M. G. (2012). "Selection of antibiotics for meticillin-resistant Staphylococcus pseudintermedius: time to revisit some old drugs?" Vet Dermatol **23**(4): 352-360, e364.

Peleg, A. Y., J. Adams, et al. (2007). "Tigecycline Efflux as a Mechanism for Nonsusceptibility in Acinetobacter baumannii." Antimicrob Agents Chemother **51**(6): 2065-2069.

Prasad, P., J. Sun, et al. (2012). "Excess deaths associated with tigecycline after approval based on noninferiority trials." Clin Infect Dis **54**(12): 1699-1709.

Rajendran, R., R. F. Quinn, et al. (2010). "Efflux pumps may play a role in tigecycline resistance in Burkholderia species." Int J Antimicrob Agents **36**(2): 151-154.

Reid, G. E., S. A. Grim, et al. (2007). "Rapid development of Acinetobacter baumannii resistance to tigecycline." Pharmacotherapy **27**(8): 1198-1201.

Rodriguez-Avial, C., I. Rodriguez-Avial, et al. (2012). "Klebsiella pneumoniae: development of a mixed population of carbapenem and tigecycline resistance during antimicrobial therapy in a kidney transplant patient." Clin Microbiol Infect **18**(1): 61-66.

Ruzauskas, M. V., M.; Siugzdiniene, R.; Suziedeliene, E.; Seputiene, V.; Daugelavicius, R.; Zienius, D.; Sengaut, J.; Pavilonis, A. (2009) "Antimicrobial resistance of Enterococcus spp. isolated from livestock in Lithuania." Veterinarski Arhiv **79**, 439-449.

Ruzin, A., F. W. Immermann, et al. (2008). "Real-time PCR and statistical analyses of acrAB and ramA expression in clinical isolates of Klebsiella pneumoniae." Antimicrob Agents Chemother **52**(9): 3430-3432.

Ruzin, A., D. Keeney, et al. (2007). "AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in Acinetobacter calcoaceticus-Acinetobacter baumannii complex." J Antimicrob Chemother **59**(5): 1001-1004.

Ruzin, A., M. A. Visalli, et al. (2005). "Influence of transcriptional activator RamA on expression of multidrug efflux pump AcrAB and tigecycline susceptibility in Klebsiella pneumoniae." Antimicrob Agents Chemother **49**(3): 1017-1022.

Spanu, T., G. De Angelis, et al. (2012). "In vivo emergence of tigecycline resistance in multidrug-resistant Klebsiella pneumoniae and Escherichia coli." Antimicrob Agents Chemother **56**(8): 4516-4518.

Stein, G. E. and T. Babinchak (2013). "Tigecycline: an update." Diagn Microbiol Infect Dis **75**(4): 331-336.

Sun, J. R., C. L. Perng, et al. (2012). "A truncated AdeS kinase protein generated by ISAba1 insertion correlates with tigecycline resistance in Acinetobacter baumannii." PLoS One **7**(11): e49534.

Sun, Y., Y. Cai, et al. (2013). "The emergence of clinical resistance to tigecycline." Int J Antimicrob Agents **41**(2): 110-116.

Tasina, E., A. B. Haidich, et al. (2011). "Efficacy and safety of tigecycline for the treatment of infectious diseases: a meta-analysis." Lancet Infect Dis **11**(11): 834-844.

Tsai, H. Y., C. H. Liao, et al. (2012). "Trends in susceptibility of vancomycin-resistant Enterococcus faecium to tigecycline, daptomycin, and linezolid and molecular epidemiology of the isolates: results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) study, 2006 to 2010." Antimicrob Agents Chemother **56**(6): 3402-3405.

Tuckman, M., P. J. Petersen, et al. (2000). "Mutations in the interdomain loop region of the tetA(A) tetracycline resistance gene increase efflux of minocycline and glycylcyclines." Microb Drug Resist **6**(4): 277-282.

Veleba, M. and T. Schneiders (2012). "Tigecycline resistance can occur independently of the ramA gene in Klebsiella pneumoniae." Antimicrob Agents Chemother **56**(8): 4466-4467.

Werner, G., S. Gfrorer, et al. (2008). "Tigecycline-resistant Enterococcus faecalis strain isolated from a German intensive care unit patient." J Antimicrob Chemother **61**(5): 1182-1183.

Wu, N., M. Qiao, et al. (2010). "Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in China." Environ Sci Technol **44**(18): 6933-6939.

Yahav, D., A. Lador, et al. (2011). "Efficacy and safety of tigecycline: a systematic review and meta-analysis." J Antimicrob Chemother **66**(9): 1963-1971.

Zhanel, G. G., K. Homenuik, et al. (2004). "The glycylcyclines: a comparative review with the tetracyclines." Drugs **64**(1): 63-88.

Zhang, T., M. Zhang, et al. (2009). "Tetracycline resistance genes and tetracycline resistant lactose-fermenting Enterobacteriaceae in activated sludge of sewage treatment plants." Environ Sci Technol **43**(10): 3455-3460.