TITLE: Differential susceptibility to tetracycline, oxytetracycline and doxycycline of the calf pathogens Mannheimia haemolytica and Pasteurella multocida in three growth media

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Title: Differential susceptibility to tetracycline, oxytetracycline and doxycycline of the calf pathogens *Mannheimia haemolytica* and *Pasteurella multocida* in three growth media.

Running Title: Potency of tetracyclines for *M. haemolytica* and *P. multocida* in CAMHB, FBS and RPMI

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Abstract:

For clinical isolates of bovine *Mannheimia haemolytica* and *Pasteurella multocida,* this study reports: minimum inhibitory concentration (MIC) differences for tetracycline, oxytetracycline and doxycycline between cation-adjusted Mueller Hinton broth (CAMHB), foetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI) medium. MICs were determined according to CLSI standards and additionally using five overlapping sets of two-fold dilutions. *Matrix effect*: (a) free drug MICs and minimum bactericidal concentrations (MBC) for all drugs were significantly higher in FBS than in CAMHB for both pathogens ($p < 0.001$); (b) MICs and MBCs were higher for CAMHB and FBS compared to RPMI for *P. multocida* only. Net growth rate for *P. multocida* in CAMHB was significantly slower than in FBS and higher than in RPMI, correlating to MIC and MBC ranking. *Drug effect*: doxycycline MICs and MBCs were significantly lower ($p < 0.001$) in both CAMHB and FBS than tetracycline and oxytetracycline for both pathogens. Only for *M. haemolytica* were oxytetracycline MIC and MBC significantly lower than tetracycline, precluding the use of tetracycline to predict oxytetracycline susceptibility in this species. Determining potencies of tetracyclines in a physiological medium, such as FBS, is proposed, when the objective is correlation with pharmacokinetic data for dosage determination.

Keywords: *Mannheimia haemolytica*, *Pasteurella multocida*, minimum inhibitory concentration (MIC), Oxytetracycline, Doxycycline

Introduction:

The bovine pathogens *Mannheimia haemolytica* and *Pasteurella multocida* have been specifically linked to cases of bovine calf pneumonia (Davies *et al*., 2004; Griffin *et al*., 2010; Welsh *et al*., 2004). The high prevalence of these infections has necessitated the widespread use in veterinary medicine of tetracyclines, especially oxytetracycline and doxycycline. Susceptibility to these AMDs is most commonly measured using the minimum inhibitory concentration (MIC). Standard methodologies have been published by the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI). Adoption of these procedures ensures inter-laboratory and international dissemination of generated data to common standards (Papich, 2014).

Although useful for ensuring comparability of data between laboratories, the standardised methods have limitations of accuracy. As discussed by Mouton *et al.* (2018), the use of MIC based on a single MIC determination is not sufficient for purposes of dosage determination when combined with PK/PD data. First, as MICs are based on a two-fold dilution series, the *a priori* inaccuracy may approach 100% for a single isolate. In the current study, the inaccuracy was reduced to less than 20% by use of five overlapping two-fold dilutions series (Aliabadi and Lees, 2001; Sidhu *et al.*, 2011). Secondly, the physiological relevance of *in vitro* methods using artificial media, such as cation-adjusted Mueller-Hinton broth (CAMHB), has been questioned for some drug classes, including tetracyclines and macrolides. For these classes, MICs for most pathogens are markedly dependent on the growth medium (Brentnall *et al.*, 2012; Buyck *et al.*, 2012; Dorey *et al.*, 2016; Lees *et al.*, 2015, 2016; Toutain *et al.*, 2017). For the same drug and similar testing conditions (inoculum size and incubation time), the differences in MIC (on a free-concentration basis) between the afore-mentioned

media could be related solely to rates of bacterial growth and death in each medium (Mouton and Vinks, 2005).

Dalhoff (2018) commented that the impact of media protein on AMD activity is multi-faceted, influencing cell permeability to the AMD and growth of the organism. Using physiological fluids, such as foetal bovine serum (FBS) and inflammatory exudate or an equivalent designed for eukaryotic cell culture, such as Roswell Park Memorial Institute (RPMI) medium, may provide useful, alternatives to those broths, which are formulated not to mimic conditions *in vivo* but to facilitate bacterial growth *in vitro* (Buyck et al., 2012).

When established clinical breakpoints are not available for a given AMD, those available for structurally related members of the same drug class have been used. For example, when information on the efficacy of oxytetracycline is not available, tetracycline has been used to represent other drugs of the same class. As culture sensitivity testing panels may only include tetracycline and/or doxycycline, this study compared MICs and MBCs of tetracycline, oxytetracycline and doxycycline for two calf pneumonia pathogens.

The objective was to identify, for six isolates each of *M. haemolytica* and *P. multocida* in three matrices (CAHMB, FBS and RPMI), if the growth medium, based on comparative static growth curves, impacts on susceptibility and MIC. MICs and MBCs were determined using two-fold standardised dilution series (Clinical and Laboratory Standards Institute CLSI, 2013) but also using five overlapping two-fold dilution series. A secondary objective was to identify whether usingfive overlapping two-fold dilution series impacts on tetracycline as an appropriate susceptibility benchmark for oxytetracycline.

Materials and Methods

Selection and storage of bacterial strains

Six strains each of *M. haemolytica* and *P. multocida*, previously shown to grow logarithmically in MHB and FBS, were recovered from -70°C storage (medium glycerol:milk:water, 20:10:70). These strains were clinical isolates derived from non-related cases of calf pneumonia within the UK; they had been used in a previous study and were known to be sensitive to oxytetracycline (Lees et al., 2015). Strains were stored at -70°C in brain heart infusion (BHI) broth containing 25% glycerol for the duration of the study.

Culture methods

Bacteria were cultured in BHI broth or CAMHB (CM0405, Oxoid, UK) or as static cultures on BHI agar (1.5% bacteriological agar [LP0011, Oxoid, UK]) or Mueller-Hinton agar (CM0337, Oxoid, UK); all were prepared according to the manufacturer's guidelines, unless otherwise stated. Agar cultures were incubated statically (HeraCell incubator, Heraeus, UK) and broth cultures were incubated with shaking at 150 rpm (Incu-shaker mini, Benchmark, UK), both at 37°C.

Antimicrobial drug preparation and storage

Stock drug solutions of tetracycline hydrochloride (#10460264, Fisher Scientific, UK) and oxytetracycline hydrochloride (#O5875, Sigma, UK) were prepared to concentrations of 10 mg/mL in deionized water and doxycycline monohydrate (#15580594, Fisher scientific, UK) was prepared to 2 mg/mL in ethanol. Concentrations refer to base molecules. All solutions were filter sterilised using a 0.22 µm syringe filter. Weighing of drug powders was adjusted according to the potency calculations outlined in the CLSI guidelines (CLSI, 2013). Aliquots

of 1 mL were stored in amber microcentrifuge tubes at – 20°C.

Determination of MIC and MBC

MICs were determined in accordance with CLSI standards (CLSI, 2013). The CLSI two-fold dilution series (0.0625 – 32 µg/mL) method was adapted; four additional overlapping dilution series (0.04375 – 22.4, 0.05 – 25.6, 0.05625 – 28.8, 0.0625 – 32, 0.075 – 38.4 µg/mL) were used to improve the accuracy of MIC and MBC measurements (Sidhu *et al,.* 2011). Dilutions of AMDs were prepared in broths (CAMHB and RPMI) or FBS at the aforementioned concentrations. In FBS, free drug fractions were calculated from protein binding data, using values of 31% for tetracycline (Ziv and Sulman, 1972; Riviere and Papich, 2009), 50% for oxytetracycline (Brentnall *et al.,* 2013; Pilloud, 1973) and 92% for doxycycline (Riviere and Papich, 2018). For RPMI, MICs could be determined for *P. multocida* only after supplementation with 0.1 M phosphate, pH 6.8, according to the method previously described (Sun and Clinkenbeard, 1998). *M. haemolytica* MIC could not be determined in RPMI, as it could not be grown without adding a proportion of FBS of at least 0.1%. MIC tests were repeated a minimum of three times, on separate days, and mean MIC values were calculated.

MBC was determined by a spot-plate method. A 10 µL sample from each well, equal to and exceeding the MIC, was spotted onto a Mueller-Hinton agar plate and incubated overnight at 37°C. Plates were inspected for growth and MBC was recorded as the point at which no growth occurred.

Growth curves:

Static growth curves of *P. multocida* were performed in each of the three growth media. Each strain was grown overnight (14-16h) in BHI broth at high-density logarithmic growth. A 100 μL aliquot of the suspension was transferred into 5 mL of either FBS, CAMHB or RPMI (supplemented with 0.1 M phosphate, pH 6.8). Each inoculated medium was then incubated at 37ºC in a shaking incubator at 150 rpm. Samples were taken at 0, 1, 2, 4, 8, 24 h and viable cell counts performed using a spot-plate method, in which a ten-fold dilution series was prepared and three 10 μL drops were spotted onto a Mueller-Hinton agar plate. Following drying and overnight incubation, colonies were counted and counts adjusted for the dilution factor.

Statistical analyses

MIC and MBC are reported as geometric means and standard deviations. Concentration data were transformed to compensate for the doubling dilution series by ln(2) transformation prior to statistical analysis, and presented graphically on an ordinate axis with a ln(2) base (2-fold increments). Differences between MIC and MBC values were identified following analysis of variance (ANOVA) and, when appropriate, Tukey *post-hoc* analysis of significance for each of the variables using the software R (open source (https://www.r-project.org/). Data were also converted to reflect the traditional testing approach, using 2-fold dilution series (0.25, 0.5, 1, 2, 4, 8, 16, 32 μg/mL) and subjected to the same statistical analysis to determine whether any significant differences would have been detected, had overlapping dilutions not been used.

Growth rates were evaluated by comparing log10 bacterial counts for each medium at each time point and testing the effect of time x medium interaction (linear mixed effect model with Tukey *post-hoc* analysis in R).

Results

Matrix effect

Following correction of FBS values for protein binding, there were highly significant differences between media in geometric mean MIC and MBC values for *P. multocida* for all drugs, tetracycline, oxytetracycline and doxycycline (Table 1, Fig.1). Compared to MICs determined in CAMHB (the standard CLSI-proposed medium for determination of MIC for *P. multocida*) MICs in FBS were significantly higher with ratios (FBS:CAMHB) of 6.7:1, 7.0:1 and 1.3:1 for tetracycline, oxytetracycline and doxycycline, respectively. For tetracycline and oxytetracycline, MICs in RPMI were significantly lower than those determined in both FBS and CAMHB. In RPMI, MICs for tetracycline were 5.4x, and for oxytetracycline 3.4x lower than in CAMHB. Consequently, ratios FBS:RPMI, of 36.1:1 for tetracycline and 23.8:1 oxytetracycline were even higher than FBS:CAMHB ratios.

Inter-strain variability in MBCs was greater than MIC variability for each drug in each medium. However, the order of potency (most to least) for MBCs was the same as MICs, namely RPMI>CAMHB>FBS for all drugs, and MBC ratios FBS:CAMHB and FBS:RPMI exceeded unity but were smaller in magnitude than corresponding MIC ratios.

For *M. haemolytica* and all tetracyclines, MICs were significantly higher in FBS (corrected for protein binding) than in CAMHB. Thus, FBS:CAMHB ratios were 10.5:1, 7.7:1, and 1.7:1, respectively, for tetracycline, oxytetracycline and doxycycline. As with *P. multocida*, there was greater inter-strain variability in MBCs compared to MICs. However, MBCs were again higher in FBS compared to CAMHB for tetracycline and oxytetracycline. In summary, for both pathogens, the growth medium exerted a highly significant ($p < 0.001$) impact on MICs and MBCs for all drugs (Figure 1).

183 *Influence of matrix on bacterial growth rate*

184 The rate and magnitude of bacterial growth in the absence of drugs was determined using
185 static growth curves. Comparison of the three media indicated that the support of growth of
186 six isolates of *P. multocida* was consistently higher in FBS compared with CAMHB (Figure
187 2). Thus, bacterial counts were significantly higher from 8 to 24 h ($p < 0.01$) for FBS. RPMI
188 (supplemented with 0.1M phosphate, pH 6.8) was relatively poor in supporting the growth
189 of *P. multocida,* compared with both FBS and CAMHB. Bacterial counts were significantly
190 higher for the latter two media than with RPMI at all time points after inoculation ($p < 0.05$).
191 Therefore, the medium providing the highest bacterial growth rate (FBS) had highest MIC
192 and MBC values for these tetracyclines, whilst the medium with lowest growth rate (RPMI)
193 had the lowest MICs and MBCs.

194 *Method effect*

195 Differences in drug potency/efficacy between tetracycline, oxytetracycline and doxycycline
196 were explored by comparing MICs and MBCs obtained in CAMHB, FBS and RPMI using
197 five overlapping sets of doubling dilutions (Fig. 3). Using this adapted method, for *P.*
198 *multocida*, in RPMI only, tetracycline MICs and MBCs were significantly lower ($P < 0.001$)
199 than those for oxytetracycline. Both CAMHB and FBS showed no significant difference
200 between MICs for tetracycline and oxytetracycline. For *M. haemolytica*, tetracycline MICs,
201 determined using five overlapping sets of doubling dilutions in both CAMHB and FBS, were
202 significantly higher ($p < 0.001$) than those for oxytetracycline. MBC values were again
203 significantly higher ($p < 0.001$) for tetracycline than for oxytetracycline in FBS. Doxycycline
204 MICs and MBCs were significantly lower ($p < 0.001$) across both strains and all media.

205

When MICs were determined using the traditional 2-fold dilution series (0.25, 0.5, 1, 2, 4, 8, 16, 32 µg/mL) and applying the same statistical analyses (Supplementary Table and Figures S1 and S2), there were no significant differences between the MICs for tetracycline and oxytetracycline against *M. haemolytica* in CAMHB, whereas the 5-dilution series revealed statistically significant differences between all three drugs. For *P. multocida,* however*,* the 2-fold dilution series gave the same conclusion as the 5-overlapping dilution series, namely that doxycycline was significantly more potent than tetracycline and oxytetracycline, whilst tetracycline and oxytetracycline did not differ significantly.

Discussion:

This study evaluated if growth matrix exerted a significant effect on MICs and MBCs for three tetracyclines against the bovine pathogens, *P. multocida* and *M. haemolytica* and, if so, by what underlying mechanism. A second objective was to identify if, using a method of increased accuracy for MIC determination, namely five-overlapping dilution series, tetracycline MICs are indicative of those for oxytetracycline.

Comparison of FBS and CAMHB for MIC and MBC determination

The literature cites many examples of differences in MIC measured, on the one hand, in broths using the internationally recognised CLSI or EUCAST standards and, on the other, determinations made in physiological fluids such as serum or eukaryotic media such as RPMI. Brentnall *et al*. (2012, 2013) determined oxytetracycline MIC in calf serum against a single isolate of *M. haemolytica.* They reported a six-fold higher serum MIC than in broth. These studies were confirmed and extended to six bovine isolates each of both *M. haemolytica* and *P. multocida* (Lees , 2016). Increased MIC values of oxytetracycline with serum:MHB ratios of 25.2:1 and 27.4:1, respectively, before correction for protein binding, and ratios of the order of 6-8:1 for free drug concentration were obtained. Subsequently

Lees *et al.* (2017) reported a free fraction serum MIC:broth ratio for oxytetracycline against *P. multocida* of pig origin of 6.30:1.These data are corroborated by the results of this study.

Differences in MIC between serum and broths are not limited to *P. multocida* and *M. haemolytica* or to calf and pig pathogens. Comparing MICs for a range of tetracyclines in broth and 50% broth: 50% serum (both mouse and human serum) for *S. pneumoniae* and *S. aureus* revealed increased MICs in the serum:broth mixed matrix compared with broth (Honeyman *et al*., 2015).For 12 tetracyclines and 10 strains of *S. aureus,* increased MICs were obtained in the presence of serum and, for seven of these compounds, the increase was in the range of 8- to 128-fold. Honeyman *et al*. (2015) did not correct for protein binding in their study but, as they explored multiple tetracyclines under the same conditions, if protein binding were the only influencing factor it would be predicted that MIC proportional differences would be obtained consistently. They reported variability in MIC ratios between organisms and between drugs, demonstrating unequivocally that factors other than protein binding impact markedly on numerical values of MIC.

Matrix-dependent factors influence MICs either through direct interaction with the AMD or indirectly through an influence on microorganism growth rate . Indeed, using the minimal model of MIC, as reported by Mouton and Vinks (2005), growth rate is a major factor influencing the numerical value of MIC, when other conditions are equal. A recent study by Dorey and Lees (2017) quantified 14 biochemical constituents in calf serum and CAMHB and, despite considerable variation in each, none of the differences explained the substantial differences in MIC. Barbour (2014) suggests that these factors may differ between subjects of differing ages and health status, further impacting on the matrix effect. The present data substantiate earlier findings that unidentified factors affecting bacterial growth rate exert significant effects on MIC.

Many studies have shown that inoculum size can exert profound effects on MIC (Dorey *et al*., 2016, 2017; Illambas *et al*., 2013). Although the EUCAST and CLSI standards dictate a starting inoculum count, there is limited literature exploring the effect of growth rate and the bacterial burden over time.

The strains selected for this study were previously shown to grow logarithmically in both FBS and CAMHB. However, comparing growth curves in the absence of AMD in this study, maximal viable cell counts after 8 and 24h incubation were higher for FBS than CAMHB, which in turn was higher than RPMI. The capacity to support bacterial growth, correlating with numerical MIC values, suggests that bacterial growth rate, and therefore bacterial burden achieved, is one and possibly the principal factor determining matrix MIC and MBC differences. This might be attributable to the higher challenge to drug activity with higher bacterial counts with FBS and, conversely, the lower bacterial counts with RPMI providing a lesser challenge to drug inhibitory action.

Whatever the underlying cause of matrix-based potency differences, the present data unequivocally indicate that other matrix-specific factors influence measured MICs, possibly through differences in bacterial growth or death rates. Mouton and Vinks (2005) presented an equation for calculation of MIC, based on several input factors, including growth and kill rates and this model is consistent with the present results, indicating that reducing the net growth rate decreases correlatively with the MIC, other factors being equal.

Tetracycline as a surrogate for susceptibility testing of oxytetracycline

The standards for determination of MIC and MBC rely on the unproven assumption that, in the absence of defined breakpoints for a given drug, other drugs within the same class will have equal potency. This assumption should be questioned; it is a fundamental principle of pharmacology that two agonist (or antagonist) drugs of differing chemical structures (even

very minor differences) acting at the same site (on the same receptor or enzyme) will almost invariably have differing potencies. MICs may differ by several orders of magnitude, as a consequence of differing pharmacodynamic factors; including efficacy (*in vitro* killing rate), potency (differing concentrations to achieve a given *in vitro* killing rate) and sensitivity of the concentration/effect relationship. Moreover, as previously discussed, other biochemical factors that are matrix dependent may also be consequential, even when the AMDs share similar antimicrobial actions and physico-chemical properties. As MIC breakpoints are used in conjunction with pharmacokinetic data to predict dosage regimens, it is essential to allow for pharmacodynamic as well as pharmacokinetic differences between drugs of a single class. This study investigated whether tetracycline, the prototypic drug of the class, can be used as a surrogate representative for oxytetracycline.

This study evaluated the impact of using five overlapping 2- fold dilution series, compared to the widely used single 2-fold dilution series. For *M. haemolytica,* analysis of the data by the traditional methodology indicated no significant potency differences between the three drugs, when tested in CAMHB. In contrast, the data obtained from the five overlapping 2-fold dilution series revealed small but significant differences between tetracycline and oxytetracycline. This implies that standard testing methods may not be sufficiently sensitive to identify small but nevertheless significant potency differences between AMDs of the same class for some bacterial species. Therefore, it is possible that the use of tetracycline as a surrogate for oxytetracycline is inappropriate, due to the limited discriminatory power of the susceptibility assay (single 2-fold dilution series). However, this was not always the case. For *P. multocida,* in both the five overlapping dilution series and the single 2-fold dilution series, it is concluded that tetracycline and oxytetracycline did not differ significantly in potency.

In summary, the five overlapping 2-fold dilution series provides a more accurate MIC determination for single or small numbers of isolates. Additionally, it provides a method for identifying minor differences in drug potency that would otherwise not be revealed using standard methods. The assumption that tetracycline is representative of oxytetracycline does not hold true for *M. haemolytica* in a biologically relevant context. It is conluded that prediction of dosages for clinical use, based on traditional *in vitro* MIC and MBC measurements, is insufficiently accurate and might therefore potentially lead to sub-optimal dosing regimens. To ensure relevance and accuracy of MIC measurements for clinical therapeutic decisions, it is concluded that they should be determined in physiological fluids such as FBS. Whilst FBS may not be representative of all biological fluids (e.g. interstitial fluid or inflammatory exudate) it is likely to be more so than CAMHB (Brentnall *et al.,* 2012, 2013; Dorey and Lees 2017; Dorey *et al.,* 2017).

An important challenge, arising from the present study, is how to standardise estimates of AMD potency (MIC and MBC) in biological fluids such as FBS. It is suggested that future studies should examine the reproducibility of MIC / MBC testing with different FBS batches, possibly from different animal breeds, animals of differing age and in healthy versus diseased animals. The use of FBS is one means of ensuring that serum is not already primed for the organisms being studied, as antibodies are not transferred to the foetus, due to their inhibition by the synepitheliochorial placenta (Borghesi *et al*., 2014). However, a study by Reiche *et al.* (1980), demonstrated that the degree of protein binding of chloramphenicol was greater in adult cattle compared to calves, highlighting an important consideration when performing studies in FBS. Moreover, protein concentrations and various co-factors may vary in FBS obtained from different sources, e.g. different breeds or even countries. Nevertheless, if the level of variation is known, it can be accounted for. A next step can then be more precise and accurate determination of pharmacodynamic indices in biologically

relevant fluids and their application in dosage estimation. Whilst these variations must be determined experimentally, they are likely to be much smaller than the marked differences between FBS and CAMHB reported in thisstudy.

The use of the five-overlapping 2-fold dilution series in this study limits the potential for inaccuracy in MIC measurement to no more than 20% for each isolate. The small number of isolates used, six for each organism, requires confirmation using a larger number of wild-type environmental isolates; future studies will seek to expand on this facet of the work.

Conclusions:

The results presented in this paper indicate a significant effect of growth matrix on MICs and MBCs of three tetracyclines for two cattle pathogens. These findings indicate that the determination of *in vitro* pharmacodynamic values, and their subsequent application to dosage regimen prediction, may require the use of a physiologically relevant growth medium to more accurately predict drug action *in vivo*. The sole reliance on broths as growth media may, for the tetracycline class of drugs, lead to sub-optimal therapeutic drug choice, reduced clinical efficacy and increased resistance selection. Further studies are now required to further optimise the use of alternative growth matrices for determination of *in vitro* pharmacodynamics for this drug class.

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conceived the original idea and supervised the project. All authors have read and approved the final manuscript.

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Table 1:
Geometric mean free drug concentration (µg/mL) MIC, MBC and standard deviation (SD, n=6) for tetracycline, oxytetracycline and doxycycline, measured in CAMHB, FBS and RPMI for *P. multocida* and *M. haemolytica*.
N/A= not applicable

P. multocida	**Tetracycline**		**Oxytetracycline**		**Doxycycline**	
Medium	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CAMHB	0.38 (0.15)	1.14 (1.07)	0.34 (0.11)	1.27 (0.85)	0.18 (0.13)	0.53 (0.45)
FBS	2.53 (1.42)	4.95 (1.80)	2.38 (0.87)	3.21 (1.83)	0.24 (0.09)	0.54 (0.12)
RPMI	0.07 (0.02)	0.22 (0.03)	0.10 (0.03)	0.35 (0.09)	N/A	N/A
M. haemolytica	**Tetracycline**		**Oxytetracycline**		**Doxycycline**	
Medium	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CAMHB	0.52 (0.18)	1.38 (0.80)	0.35 (0.14)	1.58 (0.99)	0.31 (0.05)	0.86 (0.47)
FBS	5.46 (0.93)	9.38 (4.70)	2.68 (0.68)	5.03 (1.49)	0.53 (0.13)	0.99 (0.28)

Figure 1. MIC and MBC comparisons between CAMHB, FBS and RPMI for tetracycline, oxytetracycline and doxycycline

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (µg/mL) for tetracycline, oxytetracycline and doxycycline, measured in CAMHB, FBS and RPMI for *M. haemolytica* and *P. multocida* after protein-binding correction. *P < 0.001 (analysis of variance with Tukey *post-hoc* analysis). MIC and MBC determinations were based on 5-overlapping sets of doubling dilutions to increase accuracy.

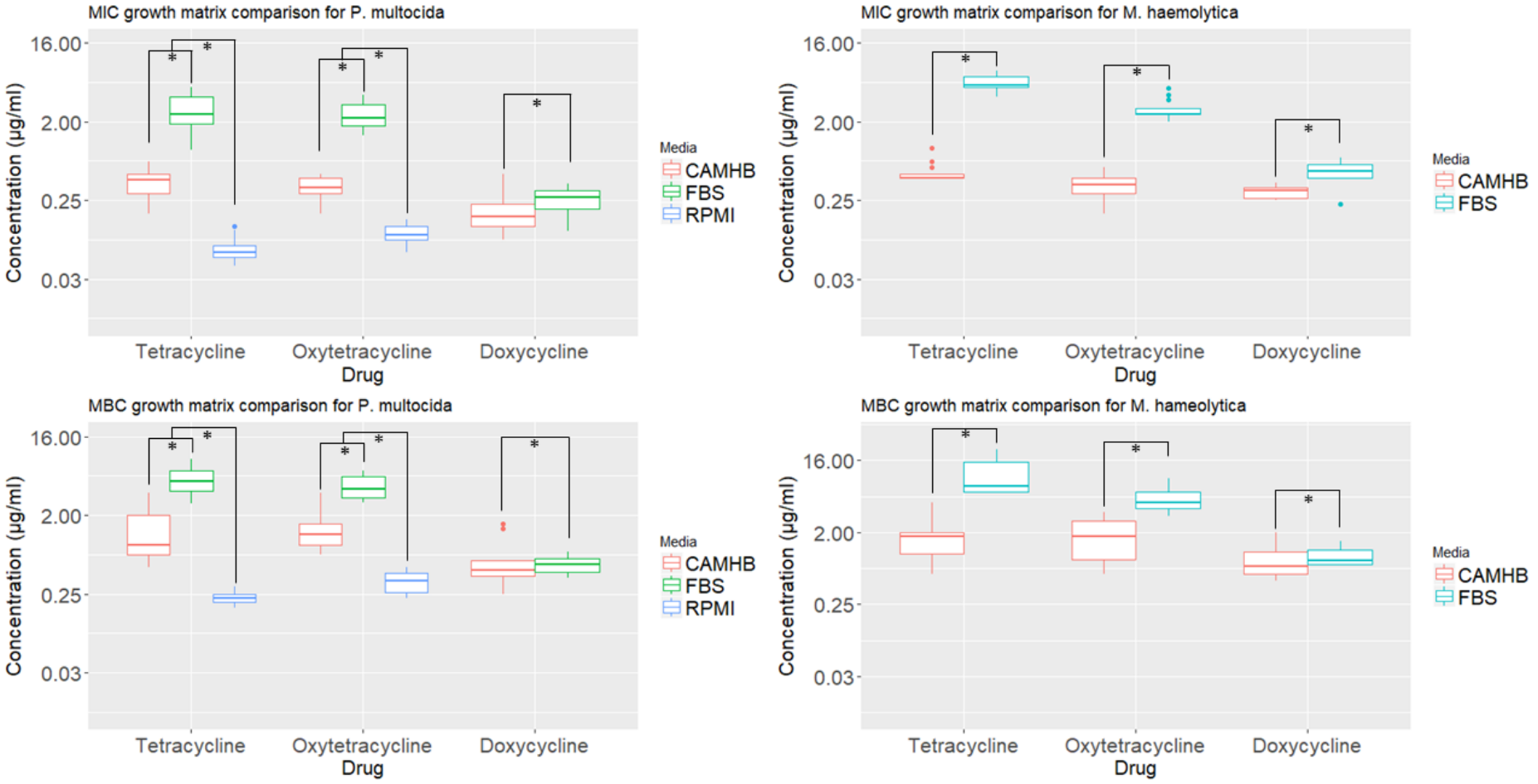


Figure 2. Comparative growth curves in CAMHB, FBS, and RPMI (supplemented with 0.1M phosphate, pH 6.8).

Viable cell counts (CFU/mL) for each of six clinical isolates of *P. multocida* in the growth media CAMHB, FBS and RPMI (supplemented with 0.1M phosphate, pH 6.8).

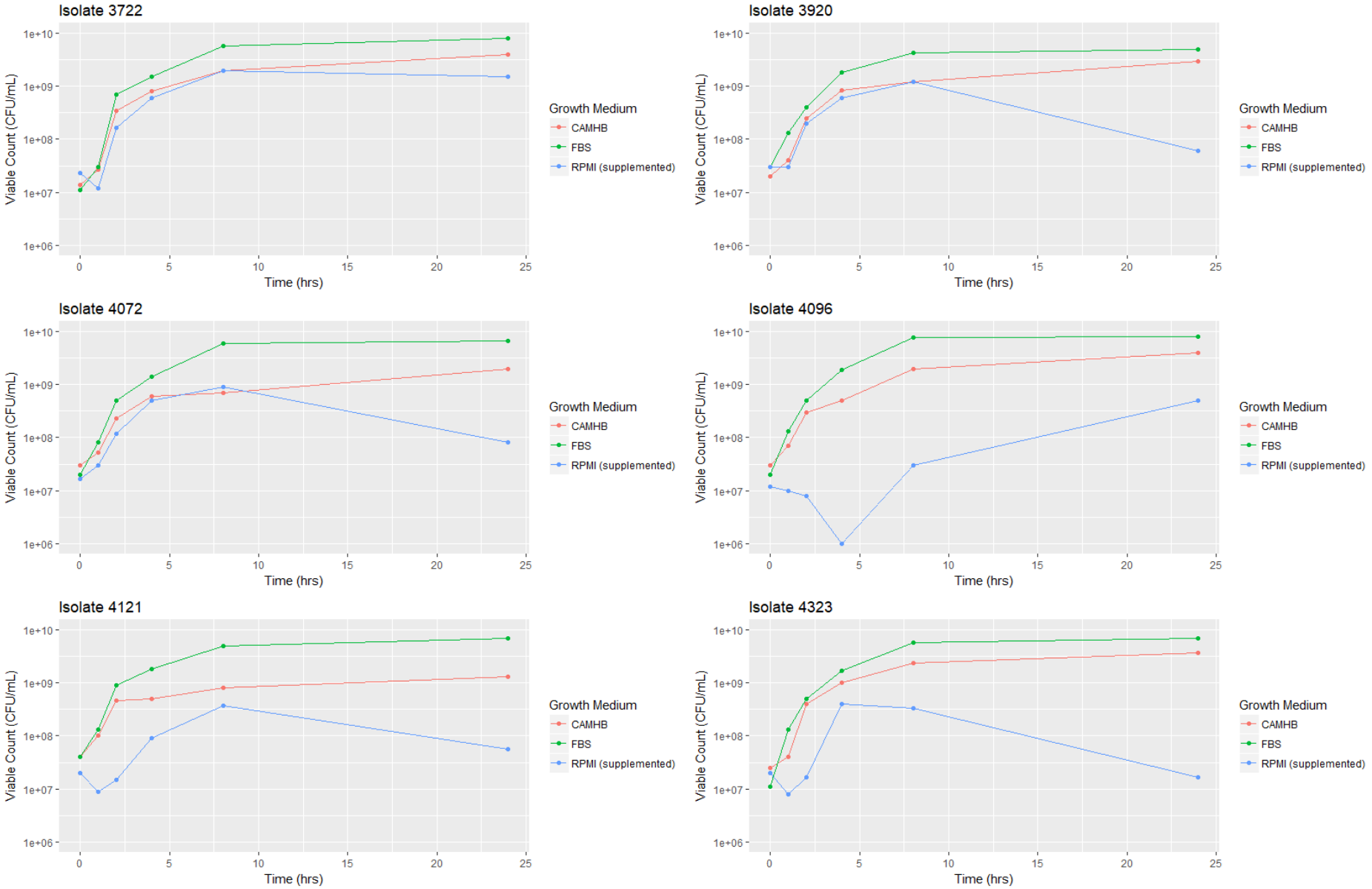
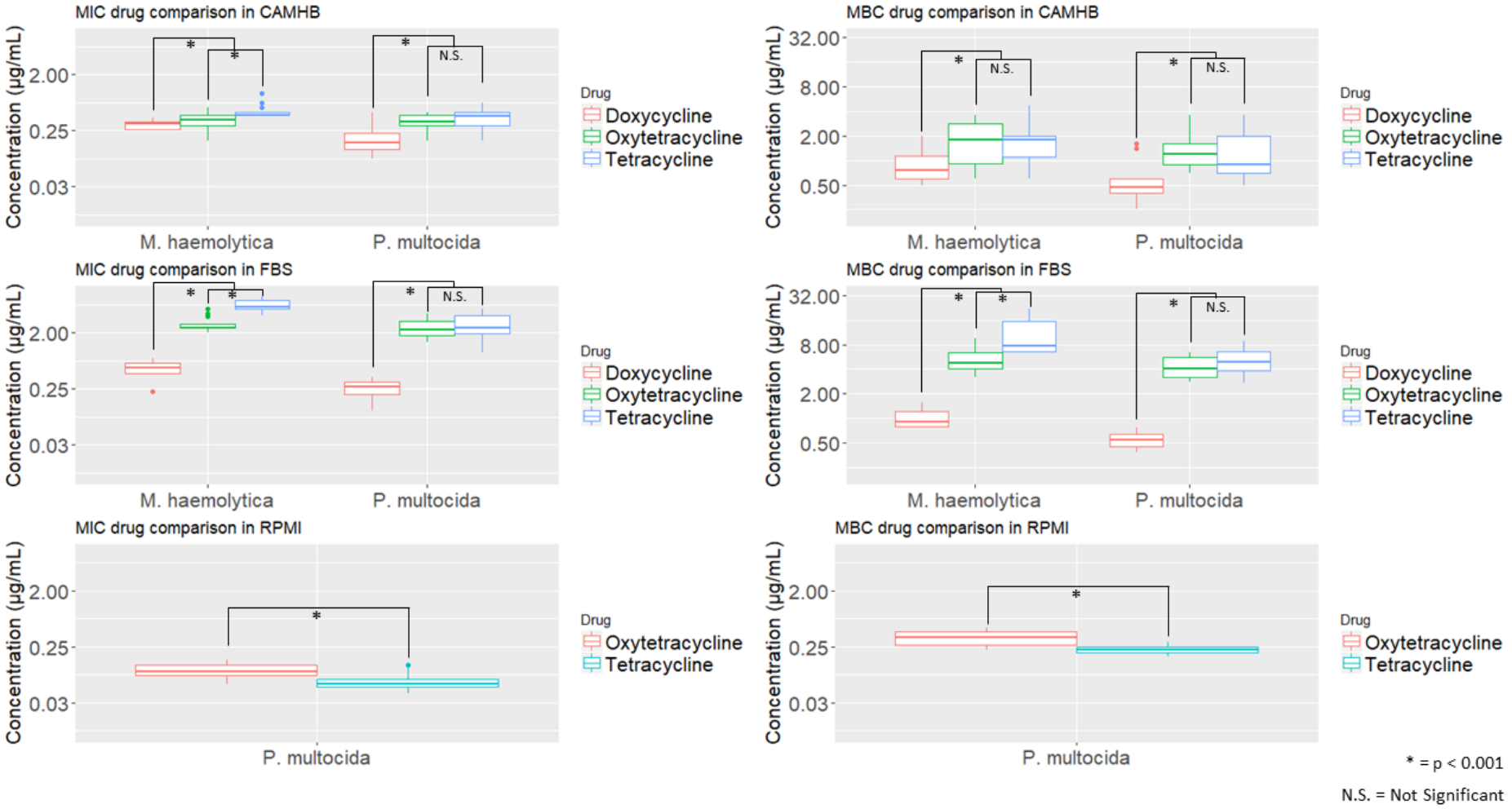


Figure 3. MIC and MBC comparisons for three tetracyclines in FBS, CAMHB, and RPMI.

Mean MIC and MBC (µg/mL) for three tetracyclines (doxycycline, oxytetracycline and tetracycline) measured in CAMHB, FBS and RPMI for *M. haemolytica* and *P. multocida* after protein-binding correction. *P < 0.001 (analysis of variance with Tukey *post-hoc* analysis). N.S: No significant difference. MIC and MBC determinations were based on 5-overlapping sets of doubling dilutions to increase accuracy.

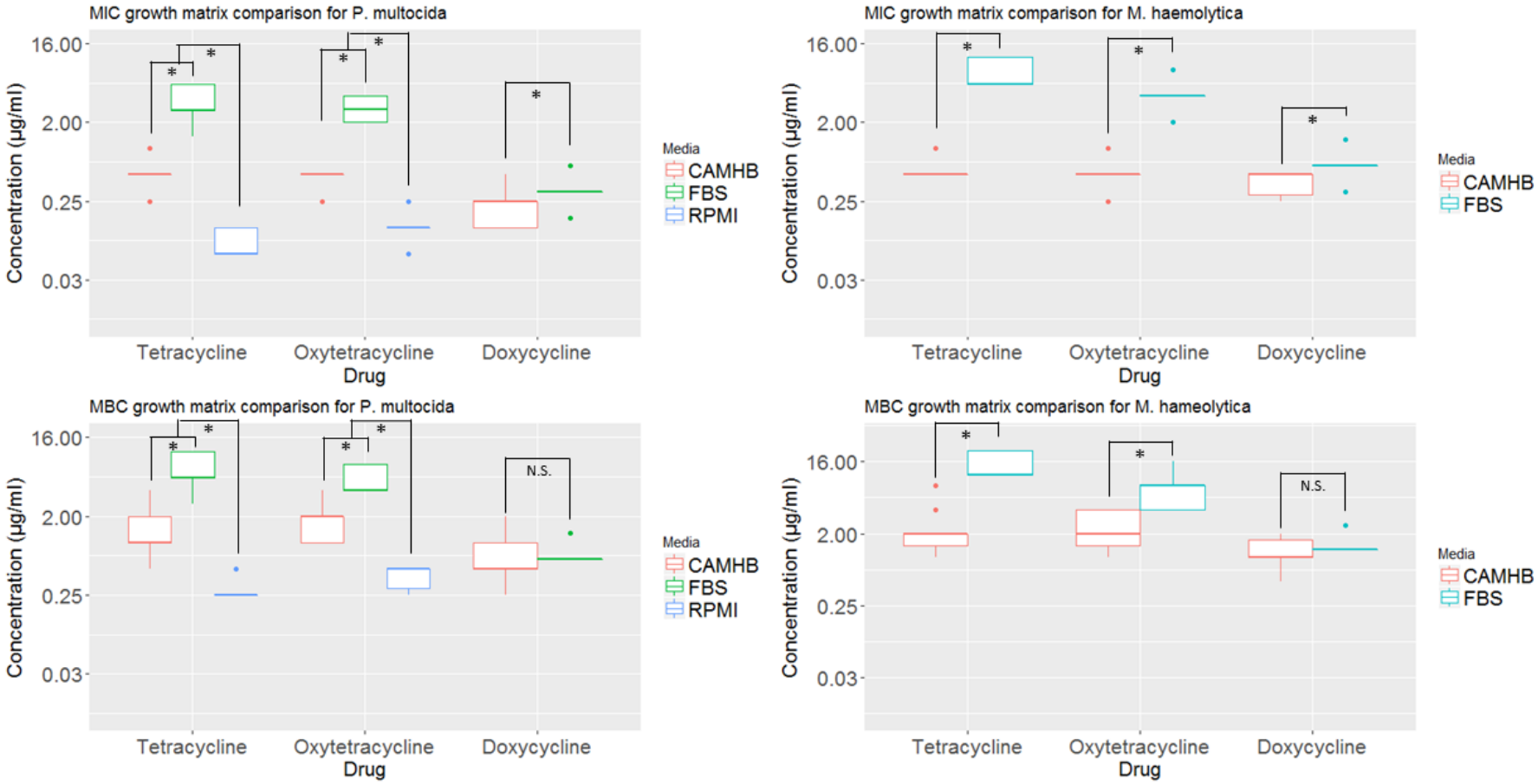


Supplementary Data Table 1:
Geometric mean free drug concentration (µg/mL) MIC, MBC and standard deviation (SD, n=6) for tetracycline, oxytetracycline and doxycycline, measured in CAMHB, FBS and RPMI for *P. multocida* and *M. haemolytica* using standard 2-fold dilution series.
N/A= not applicable

P. multocida	**Tetracycline**		**Oxytetracycline**		**Doxycycline**	
Medium	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CAMHB	0.48 (0.2)	1.33 (1.22)	0.45 (0.10)	1.63 (1.11)	0.22 (0.13)	0.68 (0.59)
FBS	3.35 (1.69)	6.50 (3.09)	2.83 (1.03)	5.42 (2.05)	0.32 (0.17)	0.69 (0.21)
RPMI	0.08 (0.03)	0.26 (0.06)	0.14 (0.06)	0.41 (0.12)	N/A	N/A
M. haemolytica	**Tetracycline**		**Oxytetracycline**		**Doxycycline**	
Medium	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
CAMHB	0.58 (0.21)	2.00 (1.80)	0.46 (0.21)	2.30 (1.35)	0.41 (0.12)	1.12 (0.52)
FBS	6.95 (2.68)	13.54 (5.19)	3.85 (1.19)	6.60 (2.93)	0.67 (0.23)	1.44 (0.49)

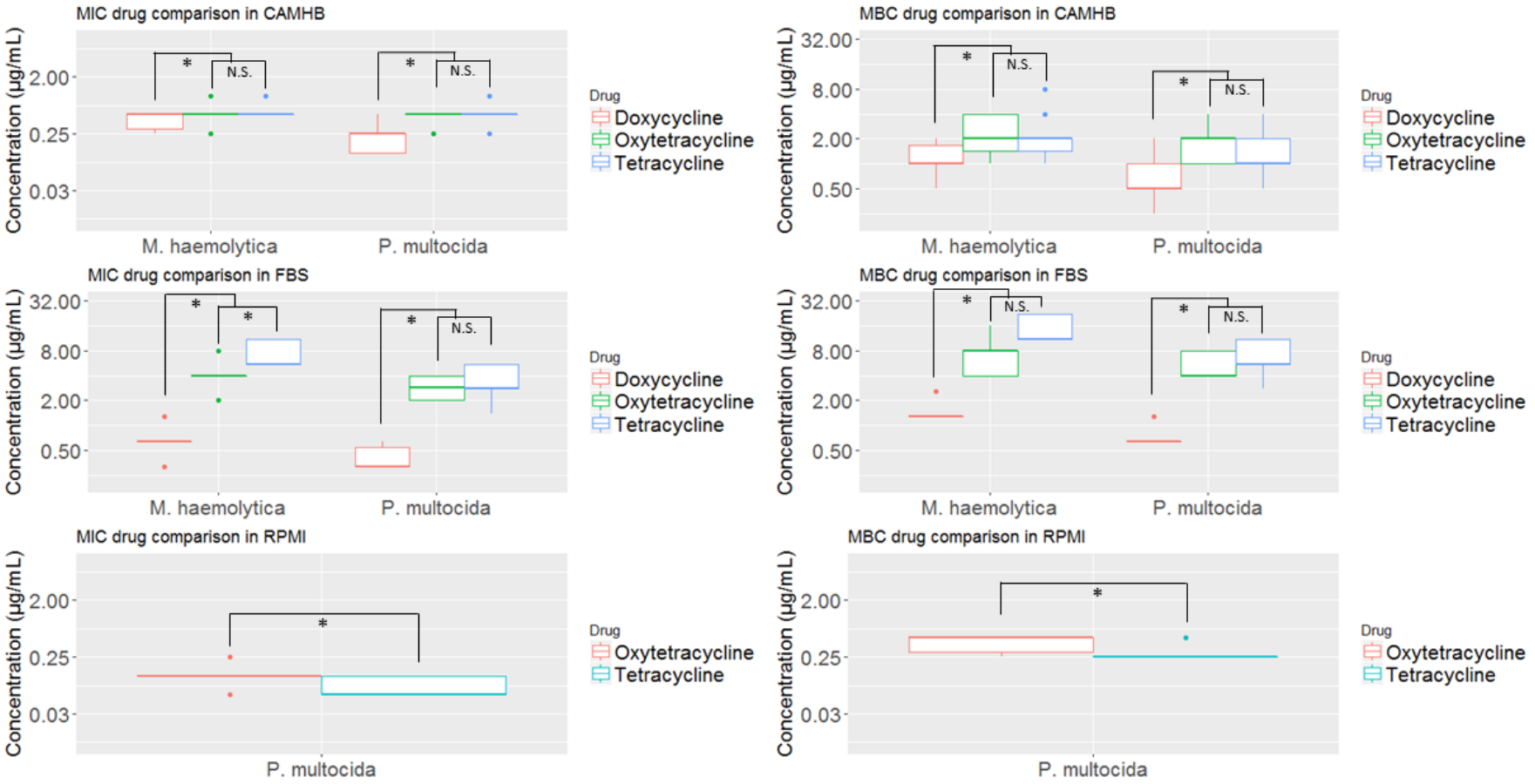
Supplementary Figure S1. MIC and MBC comparisons between CAMHB, FBS and RPMI for tetracycline, oxytetracycline and doxycycline

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for tetracycline, oxytetracycline and doxycycline, measured in CAMHB, FBS and RPMI for *M. haemolytica* and *P. multocida* using standard 2-fold dilution series after protein-binding correction. *P < 0.001 (analysis of variance with Tukey *post-hoc* analysis).



Supplementary Figure S2. MIC and MBC comparisons for three tetracyclines in FBS, CAMHB, and RPMI.

Mean MIC and MBC for three tetracyclines (doxycycline, oxytetracycline and tetracycline) measured in CAMHB, FBS and RPMI for *M. haemolytica* and *P. multocida* using standard 2-fold dilutions series after protein-binding correction. *P < 0.001 (analysis of variance with Tukey *post-hoc* analysis). N.S: No significant difference.



Supplementary Table 2:
Raw data: MIC and MBC measurements in CAMHB and FBS measured as total and free concentrations.

Media	Test	Drug	Strain	Concentration (µg/ml)	Free concentration (µg/ml)
CAMHB	MIC	Tetracycline	P.mult_3722	0.45	0.45
CAMHB	MIC	Tetracycline	P.mult_3722	0.45	0.45
CAMHB	MIC	Tetracycline	P.mult_3722	0.5	0.5
CAMHB	MIC	Oxytetracycline	P.mult_3722	0.45	0.45
CAMHB	MIC	Oxytetracycline	P.mult_3722	0.45	0.45
CAMHB	MIC	Oxytetracycline	P.mult_3722	0.5	0.5
CAMHB	MIC	Doxycycline	P.mult_3722	0.45	0.45
CAMHB	MIC	Doxycycline	P.mult_3722	0.5	0.5
CAMHB	MIC	Doxycycline	P.mult_3722	0.45	0.45
CAMHB	MBC	Tetracycline	P.mult_3722	0.8	0.8
CAMHB	MBC	Tetracycline	P.mult_3722	0.8	0.8
CAMHB	MBC	Tetracycline	P.mult_3722	0.9	0.9
CAMHB	MBC	Oxytetracycline	P.mult_3722	0.9	0.9
CAMHB	MBC	Oxytetracycline	P.mult_3722	0.9	0.9
CAMHB	MBC	Oxytetracycline	P.mult_3722	0.9	0.9
CAMHB	MBC	Doxycycline	P.mult_3722	0.45	0.45
CAMHB	MBC	Doxycycline	P.mult_3722	0.6	0.6
CAMHB	MBC	Doxycycline	P.mult_3722	0.6	0.6
CAMHB	MIC	Tetracycline	P.mult_3920	0.45	0.45
CAMHB	MIC	Tetracycline	P.mult_3920	0.5	0.5
CAMHB	MIC	Tetracycline	P.mult_3920	0.5	0.5
CAMHB	MIC	Oxytetracycline	P.mult_3920	0.45	0.45
CAMHB	MIC	Oxytetracycline	P.mult_3920	0.45	0.45
CAMHB	MIC	Oxytetracycline	P.mult_3920	0.5	0.5
CAMHB	MIC	Doxycycline	P.mult_3920	0.25	0.25
CAMHB	MIC	Doxycycline	P.mult_3920	0.225	0.225
CAMHB	MIC	Doxycycline	P.mult_3920	0.2	0.2
CAMHB	MBC	Tetracycline	P.mult_3920	3.2	3.2
CAMHB	MBC	Tetracycline	P.mult_3920	3.2	3.2
CAMHB	MBC	Tetracycline	P.mult_3920	3.6	3.6
CAMHB	MBC	Oxytetracycline	P.mult_3920	2.8	2.8
CAMHB	MBC	Oxytetracycline	P.mult_3920	2.8	2.8
CAMHB	MBC	Oxytetracycline	P.mult_3920	3.6	3.6
CAMHB	MBC	Doxycycline	P.mult_3920	1.6	1.6
CAMHB	MBC	Doxycycline	P.mult_3920	1.6	1.6
CAMHB	MBC	Doxycycline	P.mult_3920	1.4	1.4
CAMHB	MIC	Tetracycline	P.mult_4072	0.175	0.175
CAMHB	MIC	Tetracycline	P.mult_4072	0.2	0.2
CAMHB	MIC	Tetracycline	P.mult_4072	0.175	0.175

CAMHB	MIC	Oxytetracycline	P.mult_4072	0.175	0.175
CAMHB	MIC	Oxytetracycline	P.mult_4072	0.2	0.2
CAMHB	MIC	Oxytetracycline	P.mult_4072	0.2	0.2
CAMHB	MIC	Doxycycline	P.mult_4072	0.0875	0.0875
CAMHB	MIC	Doxycycline	P.mult_4072	0.0875	0.0875
CAMHB	MIC	Doxycycline	P.mult_4072	0.1	0.1
CAMHB	MBC	Tetracycline	P.mult_4072	0.7	0.7
CAMHB	MBC	Tetracycline	P.mult_4072	0.7	0.7
CAMHB	MBC	Tetracycline	P.mult_4072	0.8	0.8
CAMHB	MBC	Oxytetracycline	P.mult_4072	0.9	0.9
CAMHB	MBC	Oxytetracycline	P.mult_4072	0.9	0.9
CAMHB	MBC	Oxytetracycline	P.mult_4072		
CAMHB	MBC	Doxycycline	P.mult_4072	0.4	0.4
CAMHB	MBC	Doxycycline	P.mult_4072	0.4	0.4
CAMHB	MBC	Doxycycline	P.mult_4072	0.4	0.4
CAMHB	MIC	Tetracycline	P.mult_4096	0.35	0.35
CAMHB	MIC	Tetracycline	P.mult_4096	0.3	0.3
CAMHB	MIC	Tetracycline	P.mult_4096	0.3	0.3
CAMHB	MIC	Oxytetracycline	P.mult_4096	0.35	0.35
CAMHB	MIC	Oxytetracycline	P.mult_4096	0.35	0.35
CAMHB	MIC	Oxytetracycline	P.mult_4096	0.35	0.35
CAMHB	MIC	Doxycycline	P.mult_4096	0.15	0.15
CAMHB	MIC	Doxycycline	P.mult_4096	0.15	0.15
CAMHB	MIC	Doxycycline	P.mult_4096	0.15	0.15
CAMHB	MBC	Tetracycline	P.mult_4096	0.5	0.5
CAMHB	MBC	Tetracycline	P.mult_4096	0.5	0.5
CAMHB	MBC	Tetracycline	P.mult_4096	0.5	0.5
CAMHB	MBC	Oxytetracycline	P.mult_4096	0.7	0.7
CAMHB	MBC	Oxytetracycline	P.mult_4096	0.7	0.7
CAMHB	MBC	Oxytetracycline	P.mult_4096	1	1
CAMHB	MBC	Doxycycline	P.mult_4096	0.3	0.3
CAMHB	MBC	Doxycycline	P.mult_4096	0.25	0.25
CAMHB	MBC	Doxycycline	P.mult_4096	0.25	0.25
CAMHB	MIC	Tetracycline	P.mult_4121	0.4	0.4
CAMHB	MIC	Tetracycline	P.mult_4121	0.4	0.4
CAMHB	MIC	Tetracycline	P.mult_4121	0.3	0.3
CAMHB	MIC	Oxytetracycline	P.mult_4121	0.3	0.3
CAMHB	MIC	Oxytetracycline	P.mult_4121	0.3	0.3
CAMHB	MIC	Oxytetracycline	P.mult_4121	0.3	0.3
CAMHB	MIC	Doxycycline	P.mult_4121	0.125	0.125
CAMHB	MIC	Doxycycline	P.mult_4121	0.125	0.125
CAMHB	MIC	Doxycycline	P.mult_4121	0.125	0.125
CAMHB	MBC	Tetracycline	P.mult_4121	2	2
CAMHB	MBC	Tetracycline	P.mult_4121	2	2

CAMHB	MBC	Tetracycline	P.mult_4121	2	2
CAMHB	MBC	Oxytetracycline	P.mult_4121	1.6	1.6
CAMHB	MBC	Oxytetracycline	P.mult_4121	1.6	1.6
CAMHB	MBC	Oxytetracycline	P.mult_4121	1.6	1.6
CAMHB	MBC	Doxycycline	P.mult_4121	0.6	0.6
CAMHB	MBC	Doxycycline	P.mult_4121	0.6	0.6
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CAMHB	MIC	Tetracycline	P.mult_4323	0.7	0.7
CAMHB	MIC	Tetracycline	P.mult_4323	0.7	0.7
CAMHB	MIC	Oxytetracycline	P.mult_4323	0.35	0.35
CAMHB	MIC	Oxytetracycline	P.mult_4323	0.4	0.4
CAMHB	MIC	Oxytetracycline	P.mult_4323	0.4	0.4
CAMHB	MIC	Doxycycline	P.mult_4323	0.225	0.225
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CAMHB	MBC	Tetracycline	P.mult_4323		
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CAMHB	MBC	Tetracycline	P.mult_4323	1	1
CAMHB	MBC	Oxytetracycline	P.mult_4323	1.2	1.2
CAMHB	MBC	Oxytetracycline	P.mult_4323	1.2	1.2
CAMHB	MBC	Oxytetracycline	P.mult_4323	1.6	1.6
CAMHB	MBC	Doxycycline	P.mult_4323	0.5	0.5
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CAMHB	MIC	Oxytetracycline	M.haem_1056	0.5	0.5
CAMHB	MIC	Oxytetracycline	M.haem_1056	0.6	0.6
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CAMHB	MIC	Doxycycline	M.haem_1056	0.3	0.3
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CAMHB	MBC	Doxycycline	M.haem_1056	0.7	0.7
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CAMHB	MIC	Tetracycline	M.haem_1250	0.45	0.45
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CAMHB	MIC	Oxytetracycline	M.haem_1250	0.4	0.4
CAMHB	MIC	Oxytetracycline	M.haem_1250	0.4	0.4
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CAMHB	MIC	Doxycycline	M.haem_1250	0.35	0.35
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CAMHB	MIC	Tetracycline	M.haem_1978	1	1
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CAMHB	MIC	Doxycycline	M.haem_1978	0.4	0.4
CAMHB	MIC	Doxycycline	M.haem_1978	0.4	0.4
CAMHB	MBC	Tetracycline	M.haem_1978	2	2
CAMHB	MBC	Tetracycline	M.haem_1978	4.7	4.7
CAMHB	MBC	Tetracycline	M.haem_1978	2	2
CAMHB	MBC	Oxytetracycline	M.haem_1978	2.4	2.4
CAMHB	MBC	Oxytetracycline	M.haem_1978	2.8	2.8
CAMHB	MBC	Oxytetracycline	M.haem_1978	2.8	2.8
CAMHB	MBC	Doxycycline	M.haem_1978	1.6	1.6
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CAMHB	MIC	Oxytetracycline	M.haem_2008	0.2	0.2
CAMHB	MIC	Oxytetracycline	M.haem_2008	0.175	0.175
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CAMHB	MIC	Doxycycline	M.haem_2008	0.35	0.35
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CAMHB	MIC	Doxycycline	M.haem_2008	0.35	0.35

CAMHB	MBC	Tetracycline	M.haem_2008	0.6	0.6
CAMHB	MBC	Tetracycline	M.haem_2008		
CAMHB	MBC	Tetracycline	M.haem_2008	1	1
CAMHB	MBC	Oxytetracycline	M.haem_2008	0.7	0.7
CAMHB	MBC	Oxytetracycline	M.haem_2008		
CAMHB	MBC	Oxytetracycline	M.haem_2008	1.2	1.2
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CAMHB	MBC	Tetracycline	M.haem_2059		
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CAMHB	MBC	Oxytetracycline	M.haem_2059	1.6	1.6
CAMHB	MBC	Oxytetracycline	M.haem_2059		
CAMHB	MBC	Oxytetracycline	M.haem_2059	1.6	1.6
CAMHB	MBC	Doxycycline	M.haem_2059	0.5	0.5
CAMHB	MBC	Doxycycline	M.haem_2059	0.6	0.6
CAMHB	MBC	Doxycycline	M.haem_2059	0.7	0.7
CAMHB	MIC	Tetracycline	M.haem_2563	0.45	0.45
CAMHB	MIC	Tetracycline	M.haem_2563	0.45	0.45
CAMHB	MIC	Tetracycline	M.haem_2563	0.45	0.45
CAMHB	MIC	Oxytetracycline	M.haem_2563	0.3	0.3
CAMHB	MIC	Oxytetracycline	M.haem_2563	0.3	0.3
CAMHB	MIC	Oxytetracycline	M.haem_2563	0.35	0.35
CAMHB	MIC	Doxycycline	M.haem_2563	0.25	0.25
CAMHB	MIC	Doxycycline	M.haem_2563	0.25	0.25
CAMHB	MIC	Doxycycline	M.haem_2563	0.25	0.25
CAMHB	MBC	Tetracycline	M.haem_2563	2.4	2.4
CAMHB	MBC	Tetracycline	M.haem_2563	1.8	1.8
CAMHB	MBC	Tetracycline	M.haem_2563	1.8	1.8
CAMHB	MBC	Oxytetracycline	M.haem_2563	1.8	1.8
CAMHB	MBC	Oxytetracycline	M.haem_2563		
CAMHB	MBC	Oxytetracycline	M.haem_2563	2.4	2.4
CAMHB	MBC	Doxycycline	M.haem_2563	1.2	1.2
CAMHB	MBC	Doxycycline	M.haem_2563	1.2	1.2

CAMHB	MBC	Doxycycline	M.haem_2563	1	1
FBS	MIC	Doxycycline	P.mult_3722	3.6	0.288
FBS	MIC	Doxycycline	P.mult_3722	3.6	0.288
FBS	MIC	Doxycycline	P.mult_3722	4	0.32
FBS	MBC	Doxycycline	P.mult_3722	5.6	0.448
FBS	MBC	Doxycycline	P.mult_3722	5.6	0.448
FBS	MBC	Doxycycline	P.mult_3722	5.6	0.448
FBS	MIC	Doxycycline	P.mult_3920	2	0.16
FBS	MIC	Doxycycline	P.mult_3920	2.4	0.192
FBS	MIC	Doxycycline	P.mult_3920	2.8	0.224
FBS	MBC	Doxycycline	P.mult_3920	4.8	0.384
FBS	MBC	Doxycycline	P.mult_3920	5.6	0.448
FBS	MBC	Doxycycline	P.mult_3920	5.6	0.448
FBS	MIC	Doxycycline	P.mult_4072	1.4	0.112
FBS	MIC	Doxycycline	P.mult_4072	1.4	0.112
FBS	MIC	Doxycycline	P.mult_4072	1.6	0.128
FBS	MBC	Doxycycline	P.mult_4072	6.4	0.512
FBS	MBC	Doxycycline	P.mult_4072	5.6	0.448
FBS	MBC	Doxycycline	P.mult_4072	5.6	0.448
FBS	MIC	Doxycycline	P.mult_4096	4	0.32
FBS	MIC	Doxycycline	P.mult_4096	4.8	0.384
FBS	MIC	Doxycycline	P.mult_4096	4.8	0.384
FBS	MBC	Doxycycline	P.mult_4096	8	0.64
FBS	MBC	Doxycycline	P.mult_4096	8	0.64
FBS	MBC	Doxycycline	P.mult_4096	9.6	0.768
FBS	MIC	Doxycycline	P.mult_4121	4.8	0.384
FBS	MIC	Doxycycline	P.mult_4121	4.8	0.384
FBS	MIC	Doxycycline	P.mult_4121	3.6	0.288
FBS	MBC	Doxycycline	P.mult_4121	7.2	0.576
FBS	MBC	Doxycycline	P.mult_4121	7.2	0.576
FBS	MBC	Doxycycline	P.mult_4121	7.2	0.576
FBS	MIC	Doxycycline	P.mult_4323	3.2	0.256
FBS	MIC	Doxycycline	P.mult_4323	3.2	0.256
FBS	MIC	Doxycycline	P.mult_4323	2.8	0.224
FBS	MBC	Doxycycline	P.mult_4323	9.6	0.768
FBS	MBC	Doxycycline	P.mult_4323	8	0.64
FBS	MBC	Doxycycline	P.mult_4323	8	0.64
FBS	MIC	Doxycycline	M.haem_1056	6.4	0.512
FBS	MIC	Doxycycline	M.haem_1056	5.6	0.448
FBS	MIC	Doxycycline	M.haem_1056	5.6	0.448
FBS	MBC	Doxycycline	M.haem_1056	12.8	1.024
FBS	MBC	Doxycycline	M.haem_1056	12.8	1.024
FBS	MBC	Doxycycline	M.haem_1056	11.2	0.896
FBS	MIC	Doxycycline	M.haem_1250	9.6	0.768

FBS	MIC	Doxycycline	M.haem_1250	8	0.64
FBS	MIC	Doxycycline	M.haem_1250	8	0.64
FBS	MBC	Doxycycline	M.haem_1250	9.6	0.768
FBS	MBC	Doxycycline	M.haem_1250	9.6	0.768
FBS	MBC	Doxycycline	M.haem_1250	9.6	0.768
FBS	MIC	Doxycycline	M.haem_1978	8	0.64
FBS	MIC	Doxycycline	M.haem_1978	9.6	0.768
FBS	MIC	Doxycycline	M.haem_1978	8	0.64
FBS	MBC	Doxycycline	M.haem_1978	11.2	0.896
FBS	MBC	Doxycycline	M.haem_1978	9.6	0.768
FBS	MBC	Doxycycline	M.haem_1978	9.6	0.768
FBS	MIC	Doxycycline	M.haem_2008	5.6	0.448
FBS	MIC	Doxycycline	M.haem_2008	6.4	0.512
FBS	MIC	Doxycycline	M.haem_2008	5.6	0.448
FBS	MBC	Doxycycline	M.haem_2008	19.2	1.536
FBS	MBC	Doxycycline	M.haem_2008	19.2	1.536
FBS	MBC	Doxycycline	M.haem_2008	19.2	1.536
FBS	MIC	Doxycycline	M.haem_2059	7.2	0.576
FBS	MIC	Doxycycline	M.haem_2059	8	0.64
FBS	MIC	Doxycycline	M.haem_2059	8	0.64
FBS	MBC	Doxycycline	M.haem_2059	11.2	0.896
FBS	MBC	Doxycycline	M.haem_2059	11.2	0.896
FBS	MBC	Doxycycline	M.haem_2059	11.2	0.896
FBS	MIC	Doxycycline	M.haem_2563	6.4	0.512
FBS	MIC	Doxycycline	M.haem_2563	5.6	0.448
FBS	MIC	Doxycycline	M.haem_2563	2.8	0.224
FBS	MBC	Doxycycline	M.haem_2563	16	1.28
FBS	MBC	Doxycycline	M.haem_2563	16	1.28
FBS	MBC	Doxycycline	M.haem_2563	11.2	0.896
FBS	MIC	Oxytetracycline	P.mult_3722	4	2
FBS	MIC	Oxytetracycline	P.mult_3722	3.6	1.8
FBS	MIC	Oxytetracycline	P.mult_3722	3.6	1.8
FBS	MBC	Oxytetracycline	P.mult_3722	6.4	3.2
FBS	MBC	Oxytetracycline	P.mult_3722	7.2	3.6
FBS	MBC	Oxytetracycline	P.mult_3722	6.4	3.2
FBS	MIC	Oxytetracycline	P.mult_3920	4	2
FBS	MIC	Oxytetracycline	P.mult_3920	3.6	1.8
FBS	MIC	Oxytetracycline	P.mult_3920	2.8	1.4
FBS	MBC	Oxytetracycline	P.mult_3920	8	4
FBS	MBC	Oxytetracycline	P.mult_3920	8	4
FBS	MBC	Oxytetracycline	P.mult_3920		0
FBS	MIC	Oxytetracycline	P.mult_4072	4	2
FBS	MIC	Oxytetracycline	P.mult_4072	3.6	1.8
FBS	MIC	Oxytetracycline	P.mult_4072	3.6	1.8

FBS	MBC	Oxytetracycline	P.mult_4072	6.4	3.2
FBS	MBC	Oxytetracycline	P.mult_4072	5.6	2.8
FBS	MBC	Oxytetracycline	P.mult_4072	6.4	3.2
FBS	MIC	Oxytetracycline	P.mult_4096	4.8	2.4
FBS	MIC	Oxytetracycline	P.mult_4096	4.8	2.4
FBS	MIC	Oxytetracycline	P.mult_4096	5.6	2.8
FBS	MBC	Oxytetracycline	P.mult_4096	8	4
FBS	MBC	Oxytetracycline	P.mult_4096	9.6	4.8
FBS	MBC	Oxytetracycline	P.mult_4096		0
FBS	MIC	Oxytetracycline	P.mult_4121	8	4
FBS	MIC	Oxytetracycline	P.mult_4121	7.2	3.6
FBS	MIC	Oxytetracycline	P.mult_4121	8	4
FBS	MBC	Oxytetracycline	P.mult_4121	11.2	5.6
FBS	MBC	Oxytetracycline	P.mult_4121	11.2	5.6
FBS	MBC	Oxytetracycline	P.mult_4121	11.2	5.6
FBS	MIC	Oxytetracycline	P.mult_4323	7.2	3.6
FBS	MIC	Oxytetracycline	P.mult_4323	6.4	3.2
FBS	MIC	Oxytetracycline	P.mult_4323	5.6	2.8
FBS	MBC	Oxytetracycline	P.mult_4323	11.2	5.6
FBS	MBC	Oxytetracycline	P.mult_4323	12.8	6.4
FBS	MBC	Oxytetracycline	P.mult_4323	12.8	6.4
FBS	MIC	Oxytetracycline	M.haem_1056	8	4
FBS	MIC	Oxytetracycline	M.haem_1056	9.6	4.8
FBS	MIC	Oxytetracycline	M.haem_1056	7.2	3.6
FBS	MBC	Oxytetracycline	M.haem_1056	9.6	4.8
FBS	MBC	Oxytetracycline	M.haem_1056	9.6	4.8
FBS	MBC	Oxytetracycline	M.haem_1056	9.6	4.8
FBS	MIC	Oxytetracycline	M.haem_1250	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_1250	5.6	2.8
FBS	MIC	Oxytetracycline	M.haem_1250	5.6	2.8
FBS	MBC	Oxytetracycline	M.haem_1250	8	4
FBS	MBC	Oxytetracycline	M.haem_1250	9.6	4.8
FBS	MBC	Oxytetracycline	M.haem_1250	8	4
FBS	MIC	Oxytetracycline	M.haem_1978	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_1978	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_1978	5.6	2.8
FBS	MBC	Oxytetracycline	M.haem_1978	9.6	4.8
FBS	MBC	Oxytetracycline	M.haem_1978	9.6	4.8
FBS	MBC	Oxytetracycline	M.haem_1978	6.4	3.2
FBS	MIC	Oxytetracycline	M.haem_2008	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_2008	4	2
FBS	MIC	Oxytetracycline	M.haem_2008	4.8	2.4
FBS	MBC	Oxytetracycline	M.haem_2008	12.8	6.4
FBS	MBC	Oxytetracycline	M.haem_2008	12.8	6.4

FBS	MBC	Oxytetracycline	M.haem_2008	12.8	6.4
FBS	MIC	Oxytetracycline	M.haem_2059	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_2059	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_2059	5.6	2.8
FBS	MBC	Oxytetracycline	M.haem_2059	8	4
FBS	MBC	Oxytetracycline	M.haem_2059	7.2	3.6
FBS	MBC	Oxytetracycline	M.haem_2059	8	4
FBS	MIC	Oxytetracycline	M.haem_2563	4.8	2.4
FBS	MIC	Oxytetracycline	M.haem_2563	5.6	2.8
FBS	MIC	Oxytetracycline	M.haem_2563	4	2
FBS	MBC	Oxytetracycline	M.haem_2563	19.2	9.6
FBS	MBC	Oxytetracycline	M.haem_2563	14.4	7.2
FBS	MBC	Oxytetracycline	M.haem_2563	12.8	6.4
FBS	MIC	Tetracycline	P.mult_3722	2.8	1.904
FBS	MIC	Tetracycline	P.mult_3722	2.8	1.904
FBS	MIC	Tetracycline	P.mult_3722	2.8	1.904
FBS	MBC	Tetracycline	P.mult_3722	4.8	3.264
FBS	MBC	Tetracycline	P.mult_3722	4	2.72
FBS	MBC	Tetracycline	P.mult_3722	4	2.72
FBS	MIC	Tetracycline	P.mult_3920	2.8	1.904
FBS	MIC	Tetracycline	P.mult_3920	2.8	1.904
FBS	MIC	Tetracycline	P.mult_3920	3.2	2.176
FBS	MBC	Tetracycline	P.mult_3920	5.6	3.808
FBS	MBC	Tetracycline	P.mult_3920	5.6	3.808
FBS	MBC	Tetracycline	P.mult_3920		0
FBS	MIC	Tetracycline	P.mult_4072	1.4	0.952
FBS	MIC	Tetracycline	P.mult_4072	1.4	0.952
FBS	MIC	Tetracycline	P.mult_4072	1.8	1.224
FBS	MBC	Tetracycline	P.mult_4072	8	5.44
FBS	MBC	Tetracycline	P.mult_4072	7.2	4.896
FBS	MBC	Tetracycline	P.mult_4072	7.2	4.896
FBS	MIC	Tetracycline	P.mult_4096	4	2.72
FBS	MIC	Tetracycline	P.mult_4096	4.8	3.264
FBS	MIC	Tetracycline	P.mult_4096	5.6	3.808
FBS	MBC	Tetracycline	P.mult_4096	5.6	3.808
FBS	MBC	Tetracycline	P.mult_4096	7.2	4.896
FBS	MBC	Tetracycline	P.mult_4096	9.6	6.528
FBS	MIC	Tetracycline	P.mult_4121	7.2	4.896
FBS	MIC	Tetracycline	P.mult_4121	6.4	4.352
FBS	MIC	Tetracycline	P.mult_4121	7.2	4.896
FBS	MBC	Tetracycline	P.mult_4121	9.6	6.528
FBS	MBC	Tetracycline	P.mult_4121	8	5.44
FBS	MBC	Tetracycline	P.mult_4121	9.6	6.528
FBS	MIC	Tetracycline	P.mult_4323	5.6	3.808

FBS	MIC	Tetracycline	P.mult_4323	6.4	4.352
FBS	MIC	Tetracycline	P.mult_4323	5.6	3.808
FBS	MBC	Tetracycline	P.mult_4323	11.2	7.616
FBS	MBC	Tetracycline	P.mult_4323	12.8	8.704
FBS	MBC	Tetracycline	P.mult_4323	11.2	7.616
FBS	MIC	Tetracycline	M.haem_1056	9.6	6.528
FBS	MIC	Tetracycline	M.haem_1056	11.2	7.616
FBS	MIC	Tetracycline	M.haem_1056	9.6	6.528
FBS	MBC	Tetracycline	M.haem_1056	11.2	7.616
FBS	MBC	Tetracycline	M.haem_1056	11.2	7.616
FBS	MBC	Tetracycline	M.haem_1056	12.8	8.704
FBS	MIC	Tetracycline	M.haem_1250	7.2	4.896
FBS	MIC	Tetracycline	M.haem_1250	7.2	4.896
FBS	MIC	Tetracycline	M.haem_1250	5.6	3.808
FBS	MBC	Tetracycline	M.haem_1250	9.6	6.528
FBS	MBC	Tetracycline	M.haem_1250	9.6	6.528
FBS	MBC	Tetracycline	M.haem_1250	9.6	6.528
FBS	MIC	Tetracycline	M.haem_1978	9.6	6.528
FBS	MIC	Tetracycline	M.haem_1978	9.6	6.528
FBS	MIC	Tetracycline	M.haem_1978	7.2	4.896
FBS	MBC	Tetracycline	M.haem_1978		0
FBS	MBC	Tetracycline	M.haem_1978	9.6	6.528
FBS	MBC	Tetracycline	M.haem_1978	9.6	6.528
FBS	MIC	Tetracycline	M.haem_2008	7.2	4.896
FBS	MIC	Tetracycline	M.haem_2008	7.2	4.896
FBS	MIC	Tetracycline	M.haem_2008	7.2	4.896
FBS	MBC	Tetracycline	M.haem_2008	28.8	19.584
FBS	MBC	Tetracycline	M.haem_2008	22.4	15.232
FBS	MBC	Tetracycline	M.haem_2008	22.4	15.232
FBS	MIC	Tetracycline	M.haem_2059	8	5.44
FBS	MIC	Tetracycline	M.haem_2059	9.6	6.528
FBS	MIC	Tetracycline	M.haem_2059	8	5.44
FBS	MBC	Tetracycline	M.haem_2059	9.6	6.528
FBS	MBC	Tetracycline	M.haem_2059	11.2	7.616
FBS	MBC	Tetracycline	M.haem_2059	11.2	7.616
FBS	MIC	Tetracycline	M.haem_2563	7.2	4.896
FBS	MIC	Tetracycline	M.haem_2563	8	5.44
FBS	MIC	Tetracycline	M.haem_2563	5.6	3.808
FBS	MBC	Tetracycline	M.haem_2563	32	21.76
FBS	MBC	Tetracycline	M.haem_2563	22.4	15.232
FBS	MBC	Tetracycline	M.haem_2563	16	10.88