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# Examination of the minimum inhibitory concentration of amoxicillin and marbofloxacin against *Streptococcus suis* using standardised methods

*Daniel Sperling[1], Hamadi Karembe[1], Monika Zouharova[2], Katerina Nedbalcova[2]**

[1]*Ceva Santé Animale, Libourne, France*
[2]*Derpartment of Immunology, Veterinary Research Institute, Brno, Czech Republic*
**Corresponding author: nedbalcova@vri.cz*

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**Abstract:** The results of the antimicrobial susceptibility testing of clinical isolates *Streptococcus suis* to amoxicillin and marbofloxacin obtained by the agar dilution method and broth microdilution method with the results obtained by the commercially available E-test were compared. Comparisons between the methods based on the determination of the minimal inhibitory concentration (MIC) of the antimicrobials were assessed based on the degree and frequency of the categorical agreement (Agar dilution method as a reference system) and the percentage of the categorical agreement and error rate. A statistical evaluation was determined using the Bland-Atman method. The presented MIC values, determined for the isolates in the E-test, were slightly different from the MIC values determined by the dilution tests, mainly due to the different defined testing concentrations. For the E-test as the test system and agar-dilution method as the reference system, no error of any class was detected (very major, major and minor error) and a complete categorical agreement was obtained between the evaluated methods for amoxicillin. For amoxicillin, the regression and correlation analysis show linear relationships between the E-test and the two dilution methods with significant coefficients of determination (0.62 and 0.75). The slopes of the equality and regression lines were not significantly different. However, the E-test tends to slightly overestimate the MIC values when compared to the microdilution. The reverse is true when compared with the agar dilution. There was good agreement between the E-test and the dilution methods with a low bias (0.001 3 and –0.005 0), all the experimental data were within the computed limits of agreement. For marbofloxacin, the same trends were observed with lower coefficients of determination (0.42 and 0.73) and a less favourable agreement. The E-test constantly underestimated the MIC values when compared to the two dilution methods. No significant difference between the microdilution and agar dilution was obtained.

**Keywords:** antimicrobial; resistance; agar dilution; microdilution; E-test

Infectious diseases caused by various species of pathogenic bacteria are very common in herds of livestock animals. Antibiotic treatment is still an important tool in order to control many bacterial infections in pigs, where effective vaccines are not available.

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The disease caused by virulent serotypes of *Streptococcus suis* is one of the most important septicaemic infections of piglets before weaning and during the nursery period. It is characterised by septicaemia with a localisation in the joints, meninges and heart valves (Taylor 2013). *Streptococcus suis* is also considered a very important potential human pathogen causing a life-threatening infection, especially in immunocompromised persons. Penam penicillins, including amoxicillin, are probably the most widely used antibiotics for the treatment and control of systemic/septicaemic infections in pigs caused by *S. suis* and are considered the drugs of choice in human medicine (Marie et al. 2002; Burch and Sperling 2018). Fluoroquinolones, including marbofloxacin, are also effective treatment choices used frequently in the field, however, they are, nowadays, considered as last resort treatments, and are included in category B, "Restrict" of the new, four-group categorisation, which corresponds to Category 2 in the first Antimicrobial Advice Ad Hoc Expert Group (AMEG) report (AMEG 2019). Amoxicillin is now classed in Category D "Prudence" for first line use, if suitable.

Resistance reported for *S. suis* isolates to the aminopenicillin group (including amoxicillin) based on the data primarily produced for ampicillin (0.6–23%) has been demonstrated to be generally very low worldwide (Varela et al. 2013).

An acute *S. suis* infection, especially septicaemia, is accompanied by serious clinical signs with a significant impact on the welfare, increased morbidity and mortality of the diseased animals, resulting in increased treatment and production costs. The early administration of an effective treatment is a key factor for the successful management.

The correct implementation of an early effective treatment based on antimicrobial susceptibility testing (AST) is guided by several principles. The proper isolation and identification of the disease-causing bacterial agent from the relevant samples taken by proper techniques are crucial for any further analysis (Jorgensen and Ferraro 2009).

The performance of AST is important for the confirmation of the susceptibility/resistance to the chosen empirical antimicrobial agents, as a treatment is usually initiated before the availability of the results from laboratory tests (Jorgensen and Ferraro 2009). The AST has to be performed in accordance with internationally accepted procedures. The methodologies for the AST of bacteria from animal sources are given and published, especially by the Clinical Laboratory Standards Institute (CLSI), which are currently considered as the major standard for veterinary AST, but there are also other national institutions, for example the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) among others (Schwarz et al. 2010).

All methodological documents are regularly updated and, since the methods and interpretive criteria can change over time, it is important to follow the latest edition. Documents from other institutions are primarily based on them.

Recently, in 2015, the Veterinary Committee on Antimicrobial Susceptibility Testing (VetCAST) was established as a subcommittee of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Its remit is to define the clinical breakpoints for antimicrobial drugs used in veterinary medicine in Europe. The main VetCAST aims are to advise on all aspects of AST for bacterial pathogens of animal origin and animal bacteria with a zoonotic potential and to permit the standardisation of AST methodology to ensure the reproducibility of the data between laboratories for estimating the resistance prevalence (Toutain et al. 2017).

Agar dilution and broth dilution are the most commonly used methods to determine the minimal inhibitory concentrations (MIC's) of antimicrobial agents and both methods are considered as the "gold standards" of AST suitable for fastidious bacterial pathogens (Ericsson and Sherris 1971). MICs for commonly used antibiotics can also be obtained by using an agar diffusion method with commercially available strips and discs (disc diffusion) providing qualitative results only, which are considered as a disadvantage. Classical diffusion tests are rapid and easy to use, but they are limited to the antibiotic range supplied by the manufacturers; another important point is the lack of suitability of the AST assessment of fastidious pathogens. An E-test is an innovative gradient technique that combines the principles of both the disk diffusion and agar dilution methods and was introduced for the first time in 1988 and assessed for several pathogens especially in human medicine and is becoming more commonly used in veterinary diagnostic laboratories (Baquero et al. 1992; Berghaus et al. 2015).

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The aim of the presented study is to compare the results of the AST of the clinical isolates of *S. suis* from diseased pigs to amoxicillin and marbofloxacin using the agar-dilution method and the broth microdilution method with the results obtained by the commercially available E-test.

## MATERIAL AND METHODS

### Isolates and preparing of bacterial cultures

Fifteen field isolates of *S. suis* were selected from cultures of bacteria, which were isolated from clinical samples of diseased pigs in 2018–2019, from farms in the Czech Republic. The individual isolates of *S. suis* represented different farms covering all geographical areas of the Czech Republic. The identification of the genus and species was performed by mass spectrometry (MALDI TOF) and all the isolates were serotyped by a co-agglutination test with co-agglutination reagents prepared from rabbit hyperimmune sera (Mittal et al. 1983; Gottschalk et al. 1993) or Polymerase chain reaction (PCR) (Wang et al. 2012). Only freshly grown cultures were used to perform the ASTs.

### Antimicrobial susceptibility testing

The agar dilution tests and microdilution tests were performed strictly according to the procedures described in the Clinical and Laboratory Standards Institute (CLSI) documents VET01 (2013) and VET08 (2018). The E-test was performed according to the recommendations of the manufacturer. The isolates were tested twice. If the same MIC values were not obtained in both examinations and the difference was 2 times higher than the test of the same strain, a third evaluation was carried out in order to select the value for the statistical comparison.

The following media and diagnostic products were used: Mueller-Hinton agar and Mueller-Hinton Broth (BD Difco, United States), VITOX-supplement (Oxoid, England), Lysed Horse Blood (LabMediaServis, Czech Republic), the test substances, amoxicillin and marbofloxacin (Discovery Fine Chemicals, P.R. China), and E-test strips (Liofilchem, Italy). The agars with various concentrations of antimicrobials for performing the agar dilution test and the microtiter plates with a antimicrobial dilution series for performing the microdilution test were prepared in the Bacteriology Laboratory of the Department of Immunology in the Veterinary Research Institute in Brno, Czech Republic, which has the status of a Veterinary Antibiotic Centre for the Czech Republic.

The *S. suis* isolates were categorised as susceptible, intermediate and resistant based on the proposed clinical breakpoints for amoxicillin and the septicaemic and respiratory bacteria: an MIC ≤ 0.5 µg/ml amoxicillin concentration was used for the susceptible ones, 1.0 µg/ml was used for the intermediate ones and ≥ 2.0 µg/ml was used for the resistant ones, according to Schwarz et al. (2008) as the CLSI (2013; 2018) have not published interpretation criteria for amoxicillin against *S. suis* isolates. The breakpoints for marbofloxacin have been established based on El Garch et al. (2017) and validated for the aerobic pathogenic Gram-positive or Gram-negative bacteria were isolated from cattle, pigs and pets, following the CLSI guidelines (CLSI 2008) – although official marbofloxacin breakpoints are not published by the CLSI. The marbofloxacin-resistant strains were determined as having a marbofloxacin MIC ≥ 4 µg/ml, strains that had a marbofloxacin MIC 2 µg/ml were considered as intermediate ones and susceptible strains had a marbofloxacin MIC ≤ 1 µg/ml.

### Statistical analysis

The data were captured in an Excel spreadsheet (Microsoft Excel 2000; Microsoft, Redmond, USA) and imported into GraphPad Prism (v8, GraphPad software Inc.) and Sigmaplot (v14, Systat software Inc.) was used for the statistical analysis.

Comparisons between the methods were performed by using the Bland-Atman method (Bland and Altman 1986). The summary statistics were calculated for each antibiotic and method. The measurement of the association between the tests was provided by calculating and comparing the slope of the trend line with the line of identity (which has a slope 1). The mean of the MIC values obtained with the two methods (x-axis) was plotted against the differences between the two methods (y-axis).

The degree and frequency of the categorical agreement (Agar dilution method as the reference system) was determined as follows (Stuckey 2007):

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Very major error (test system susceptible/reference system resistant): false susceptible result; Major error (test system resistant/reference system susceptible): false resistant result; Minor error (test system susceptible or resistant/reference system intermediate). The percentage of the categorical agreement and error rate was calculated based on Elder et al. (1997).

## RESULTS

The results of the AST for amoxicillin and marbofloxacin against *S. suis* isolates obtained by using the tested methods (E-test, microdilution method and agar dilution method) are given in Tables 1 and 2.

The $MIC_{90}$ for amoxicillin obtained by the testing methods were within the fully sensitive range, 0.064 µg/ml for the E-test and 0.03 µg/ml for the microdilution and agar dilution methods, respectively (for both classical methods the same value was used). For the E-test as the test system and the agar dilution method as the reference system, no error of any class was detected (very major, major and minor error) and a complete categorical agreement was obtained between the evaluated methods.

The $MIC_{90}$ results for marbofloxacin were 1 µg/ml, 2 µg/ml and 0.75 µg/ml for the agar dilution, broth microdilution and E-test, respectively. The minor error rate was 13.33% between the compared methods and 86.6% categorical agreement was achieved.

The mean and median MIC values for amoxicillin obtained with the E-test were slightly, but non-significantly, higher than the mean and median values obtained with the microdilution method (Table 2, Figures 1 and 3). Generally, higher values were obtained with the agar dilution. The agreements between the methods (E-test versus microdilution and E-test versus agar dilution) are shown in Figures 2 and 4. All the values are within the limits of confidence intervals and the mean biases were low, 0.001 3 and −0.005 0, for the E-test versus the microdilution method and the E-test versus the agar dilution method, respectively (Figures 2 and 4).

The mean and median MIC values for marbofloxacin obtained with the E-test were significantly lower than the mean and median values obtained with the microdilution and agar dilution methods

Table 1. *Streptococcus suis* – AST of amoxicillin

| No. | ID No. | Serotype | E-test MIC (mg/l) | Micro-dilution MIC (mg/l) | Agar dilution MIC (mg/l) |
|---|---|---|---|---|---|
| 1 | 3 | 2 / 1/2* | 0.016 | 0.008 | 0.015 |
| 2 | 5 | 23 | 0.023 | 0.008 | 0.015 |
| 3 | 7 | 21 | 0.016 | 0.008 | 0.03 |
| 4 | 32 | 7 | 0.016 | 0.015 | 0.015 |
| 5 | 38 | NT | 0.016 | 0.008 | 0.015 |
| 6 | 48 | NT | 0.032 | 0.03 | 0.03 |
| 7 | 49 | NT | 0.023 | 0.03 | 0.03 |
| 8 | 55 | 1 / 14** | 0.032 | 0.008 | 0.015 |
| 9 | 57 | NT | 0.016 | 0.015 | 0.015 |
| 10 | 73 | 9 | 0.047 | 0.03 | 0.03 |
| 11 | 83 | NT | 0.064 | 0.06 | 0.125 |
| 12 | 100 | 11 | 0.023 | 0.015 | 0.03 |
| 13 | 103 | 31 | 0.064 | 0.03 | 0.06 |
| 14 | 109 | NT | 0.016 | 0.03 | 0.015 |
| 15 | 115 | 4 | 0.023 | 0.015 | 0.03 |

NT = non-typable isolate

*cross reaction between serotypes 2 and 1/2; **cross reaction between serotypes 1 and 14

Table 2. *Streptococcus suis* – AST of marbofloxacin

| No. | ID No. | Serotype | E-test MIC (mg/l) | Micro-dilution MIC (mg/l) | Agar dilution MIC (mg/l) |
|---|---|---|---|---|---|
| 1 | 3 | 2 / 1/2* | 0.38 | 0.5 | 2 |
| 2 | 5 | 23 | 0.25 | 0.5 | 0.5 |
| 3 | 7 | 21 | 0.25 | 0.5 | 0.5 |
| 4 | 32 | 7 | 0.38 | 0.5 | 1 |
| 5 | 38 | NT | 0.5 | 0.5 | 1 |
| 6 | 48 | NT | 0.19 | 0.25 | 0.25 |
| 7 | 49 | NT | 0.125 | 0.25 | 0.5 |
| 8 | 55 | 1 / 14** | 0.25 | 0.25 | 0.5 |
| 9 | 57 | NT | 0.5 | 1 | 1 |
| 10 | 73 | 9 | 0.25 | 0.5 | 0.5 |
| 11 | 83 | NT | 0.19 | 0.5 | 0.5 |
| 12 | 100 | 11 | 0.75 | 1 | 1 |
| 13 | 103 | 31 | 0.5 | 0.5 | 1 |
| 14 | 109 | NT | 0.75 | 1 | 2 |
| 15 | 115 | 4 | 0.38 | 1 | 1 |

NT = non-typable isolate

*cross reaction between serotypes 2 and 1/2; **cross reaction between serotypes

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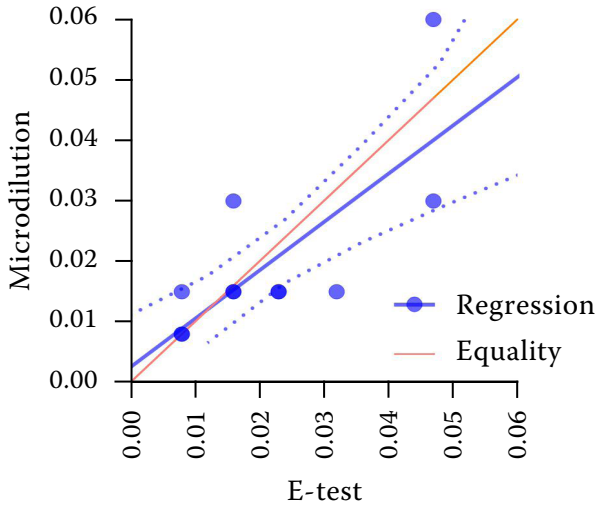


Figure 1. *Streptococcus suis*/Amoxicillin – the regression between the MIC values measured with the E-test and microdilution method, with a line of equality (red line), a regression line (blue line) and 95% confidence intervals (dotted blue lines) – The regression line has a slope of 0.79 (0.42 to 1.17) and a *y*-intercept of 0.002 5 (–0.006 to 0.011). The coefficient of determination between the two methods is R-sqr = 0.62. The slopes are not significantly different ($P$ = 0.236 1). The *y*-intercepts are also not significantly different ($P$ = 0.604 3)

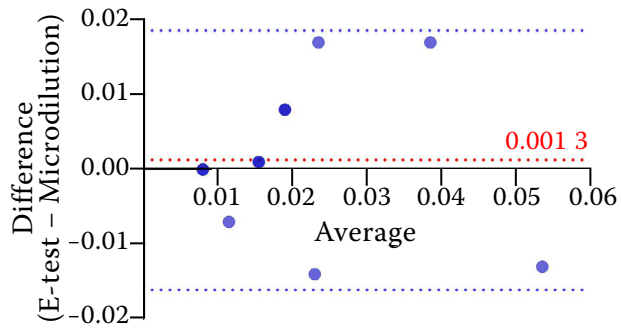


Figure 2. Agreement between the E-test and microdilution method (Bland-Altman plot). Mean bias: 0.001 267, SD of bias: 0.008 8, 95% limits of agreement: –0.016 10 to 0.018 63

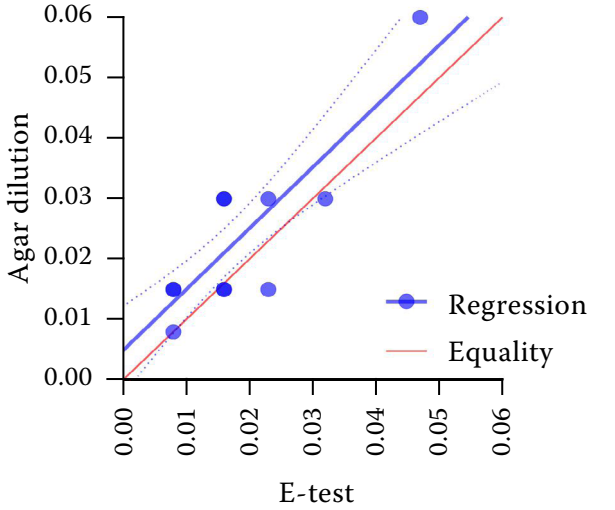


Figure 3. *Streptococcus suis*/Amoxicillin – the regression between the MIC values measured with the E-test and agar dilution method, with a line of equality (red line), a regression line (blue line) and 95% confidence intervals (dotted blue lines) – The regression line has a slope of 0.65 (0.65 to 1.4) and a *y*-intercept of 0.004 9 (–0.002 4 to 0.012). The coefficient of determination between the two methods is R-sqr = 0.75. The slopes are not significantly different ($P$ = 0.946 5). The *y*-intercepts are significantly different ($P$ = 0.007 1)

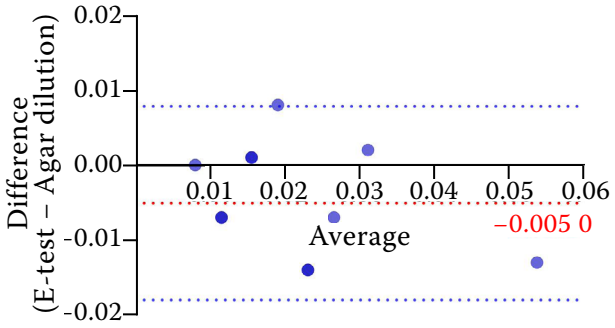


Figure 4. *Streptococcus suis*/Amoxicillin – agreement between the E-test and the agar dilution method (Bland-Altman plot). Mean bias: –0.005 071, SD of bias: 0.006 6, 95% limits of agreement: –0.018 to 0.078

Table 3. Amoxicillin – the mean MIC values and 95% confidence intervals (CI) of 15isolates of *Streptococcus suis* tested by the E-test, microdilution and agar dilution methods

| | E-test | Microdilution | Agar dilution |
|---|---|---|---|
| Median | 0.016 | 0.015 | 0.015 |
| 95% CI of median | 0.008–0.023 | 0.008–0.015 | 0.015–0.030 |
| Mean | 0.017 | 0.015 | 0.022 |
| 95% CI of mean | 0.010–0.023 | 0.010–0.018 | 0.014–0.029 |
| Geometric mean | 0.014 | 0.013 | 0.019 |
| 95% CI of geo. mean | 0.010–0.020 | 0.010–0.017 | 0.014–0.026 |

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Table 4. Marbofloxacin – the mean MIC values and 95% confidence intervals (CI) of 15isolates of *Streptococcus suis* tested by the E-test, microdilution and agar dilution methods

| | E-test | Microdilution | Agar dilution |
|---|---|---|---|
| Median | 0.250 | 0.500 | 0.500 |
| 95% CI of median | 0.125 – 0.380 | 0.500 – 0.500 | 0.500 – 1.000 |
| Mean | 0.276 | 0.541 | 0.751 |
| 95% CI of mean | 0.200 – 0.352 | 0.394 – 0.688 | 0.491 – 1.012 |
| Geometric mean | 0.240 | 0.477 | 0.565 |
| 95% CI of geo. mean | 0.175 – 0.330 | 0.351 – 0.648 | 0.325 – 0.985 |

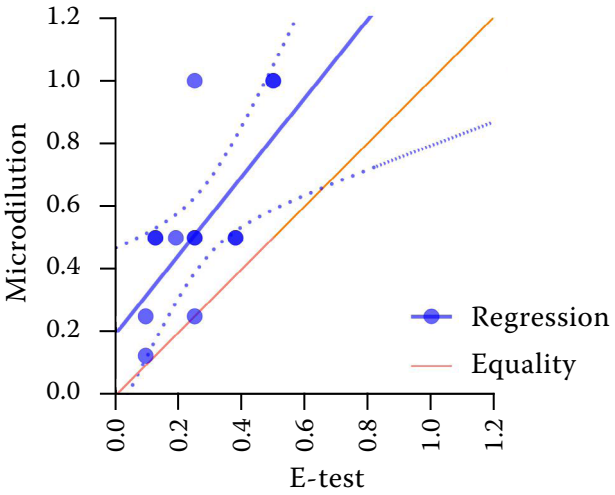


Figure 5. *Streptococcus suis*/Marbofloxacin – the regression between the MIC values measured with the E-test and microdilution method, with a line of equality (red line), a regression line (blue line) and 95% confidence intervals (dotted blue lines) – The regression line has a slope of 1.25 (95% CI: 0.36 to 2.13) and a *y*-intercept of 0.197 1 (–0.074 to 0.468 2). The coefficient of determination between the two methods is R-sqr = 0.42. The slopes are not significantly different ($P$ = 0.522 0). The *y*-intercepts are significantly different ($P$ < 0.000 1)

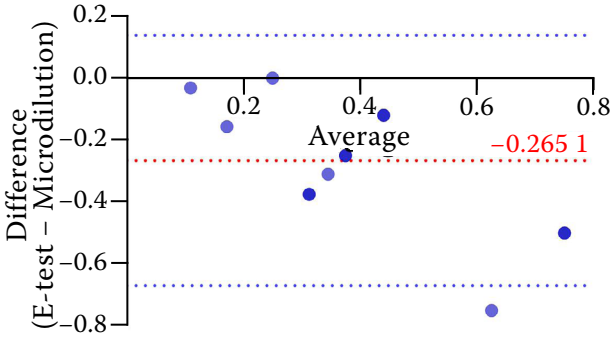


Figure 6. *Streptococcus suis*/Marbofloxacin – agreement between the E-test and the microdilution method (Bland-Altman plot). Mean bias: –0.265 1, SD of bias: 0.205 9, 95% limits of agreement: –0.668 6 to 0.138 4

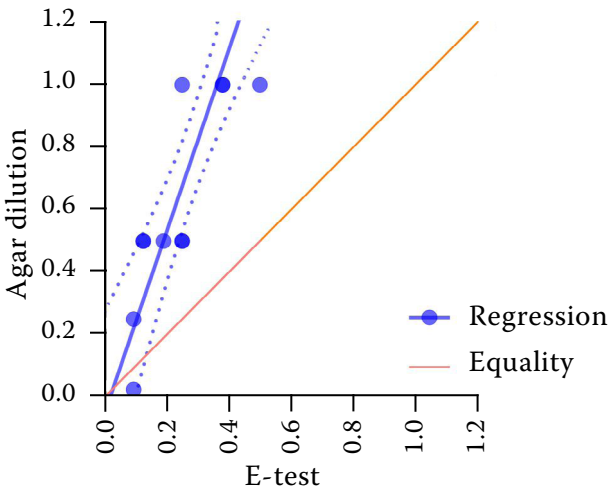


Figure 7. *Streptococcus suis*/Marbofloxacin – the regression between the MIC values measured with the E-test and agar dilution method, with a line of equality (red line), a regression line (blue line) and 95% confidence intervals (dotted blue lines) – The regression line has a slope of 2.91 (95% CI: 1.85 to 3.97) and a *y*-intercept of –0.053 20 (–0.379 9 to 0.273 5). The coefficient of determination between the two methods is R-sqr = 0.73. The slopes are significantly different ($P$ = 0.000 3). Because the slopes differ so much, it was not possible to test whether the intercepts differ significantly

(Table 4, Figures 5 and 7). Generally, equivalent or higher values were obtained with the agar dilution method. The agreements between the methods (E-test versus microdilution and E-test versus agar dilution) are shown in Figures 6 and 8. All the values, except one or two, are within the limits of confidence intervals and the mean biases were significant, –0.265 and –0.475 for the E-test versus the microdilution method and the E-test versus the agar dilution method, respectively (Figures 2 and 4). There is no significant difference between the microdilution and agar dilution methods. The E-test tends to underestimate the MIC values systematically as shown in Figures 5 and 6.

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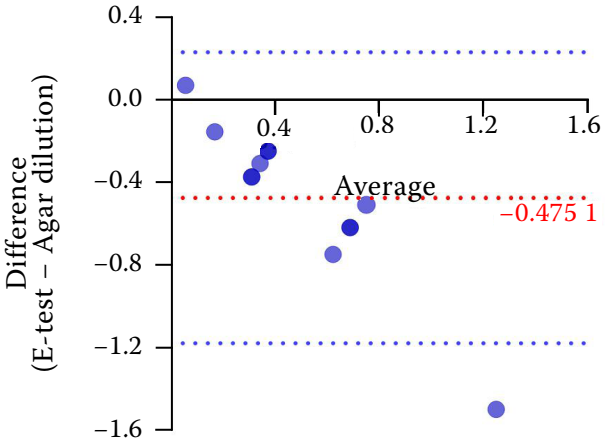


Figure 8. *Streptococcus suis*/Marbofloxacin – agreement between the E-test and the agar dilution method (Bland-Altman plot). Mean bias: −0.475 1, SD of bias: 0.359 3, 95% limits of agreement: −1.179 to 0.229 2

## DISCUSSION

Based on our knowledge, this is the first study to evaluate the validity of the E-test as an alternative method for the sensitivity testing of *S. suis* strains from clinical isolates from diseased piglets. The E-test might be preferable in clinical practice and the laboratory due to the lower cost and is less time consuming, in comparison with the gold standard dilution methods, especially when several antibiotics must be tested on a single strain (Tande et al. 1997). At the same time, the limitation of disk diffusion tests for more fastidious pathogens is well known.

*S. suis* is considered an important pathogen of pigs and potentially a zoonotic pathogen of humans, especially in Asian countries, where *S. suis* has been an important cause of adult meningitis, endocarditis, septicaemia, and arthritis (Mai et al. 2008; Callens et al. 2013) following the consumption of contaminated meat. The main control is still based on an antimicrobial treatment, especially in the case of acute outbreaks characterised by septicaemia leading frequently to meningitis, where the morbidity and mortality is usually very high. Both selected antimicrobials – amoxicillin and marbofloxacin – are frequently used in the field in order to control septicaemic and respiratory infections in pigs, caused by sensitive pathogens including *S. suis*. Amoxicillin is especially considered as the drug of choice to control *S. suis* infections and the sensitivity remains very high despite of the use of amoxicillin for a long time for treatment and metaphylactic programmes (Burch and Sperling 2018). We have confirmed the excellent sensitivity to amoxicillin for the clinical isolates of *S. suis* representing several multiple serotypes isolated from clinical cases in a recent period of 2 years in the Czech Republic. For both dilution methods, the $MIC_{90}$ of 0.03 µg/ml for amoxicillin was established and belongs to the sensitive range according to the clinical interpretive criteria established for amoxicillin, and well below the proposed epidemiological cut-off value (ECOFF) 0.5 µg/ml (Schwarz et al. 2008; Burch and Sperling 2018). The $MIC_{90}$ of 0.047 µg/ml established by the E-test was very similar to the one obtained by the dilution methods. For a direct comparison, the different scale of concentrations needs to be considered and ideally included in the gradient strip of the E-test as well as a wider dilution range.

A similar sensitivity profile was described in other studies from France and the EU VetPath monitoring, where the $MIC_{90}$ was 0.06 µg/ml for amoxicillin in 151 isolates originating from 8 different EU countries (Richez et al. 2012; El Garch et al. 2016).

The level of the categorical agreement was 100% compared to the E- test with the reference method, the agar dilution. No error was identified based on the assessment when the agar dilution method was chosen as the "gold standard method" for the comparison (Schumacher et al. 2018; Miftahussurur et al. 2020). Gold standard methods are regularly standardised by various organisations, such as the CLSI and the International Organisation for Standardisation (ISO) and the interpretation of the test results is, among others, standardised by the EUCAST.

Similarly, the suitability of the E-test as a rapid and reliable evaluation of the sensitivity of Gram-positive pathogens, like *Streptococcus pneumoniae* and the penicillin class, including amoxicillin, was reported (Tande et al. 1997).

For marbofloxacin, the $MIC_{90}$ was 1 µg/ml, 2 µg/ml and 0.75 µg/ml by the agar dilution method, broth microdilution method and the E-test, respectively. There are no CLSI susceptible/resistance breakpoints available for *S. suis* and marbofloxacin, thus, recently proposed and used breakpoints were implemented (El Garch et al. 2017). Most of the values belong in the sensitive range, with the exception of two intermediate isolates with MICs of

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2 µg/ml, obtained by the agar dilution method. The difference of one dilution in the $MIC_{90}$ parameter is generally accepted as normal within the accepted range and also reported in other studies comparing both techniques (Waites et al. 1991). The MIC results for marbofloxacin correspond with the values reported in the VetPath project, where the broth microdilution technique was used giving an $MIC_{90}$ of 1 µg/ml (El Garch et al. 2016; CLSI 2018). A minor error rate (test system susceptible/reference system intermediate) was established at a level of 13.33% and, consequently, a lower categorical agreement of 86.6% was confirmed for the E- test and marbofloxacin compared with the agar dilution method.

This discrepancy was also influenced by the fact that all the tested strains had MICs on the borderline between the susceptible and intermediate categories and one dilution difference in testing can change the interpretation. Another reason might be the limited number of *S. suis* strains tested in the study, despite the fact that, for both antibiotics tested, a very narrow distribution of MICs was obtained.

For amoxicillin, the regression and correlation analyses show linear relationships between the E-test and the two dilution methods with significant coefficients of determination (0.62 and 0.75). The slopes of the equality and regression lines were not significantly different. However, the E-test tends to slightly overestimate the MIC values when compared to the microdilution method. The reverse is true when compared with the agar dilution method.

There was a good agreement between the E-test and the dilution methods with a low bias (0.001 3 and −0.005 0), all the experimental data are within the computed limits of agreement.

For marbofloxacin, the same trends are observed with lower coefficients of determination (0.42 and 0.73) and a less favourable agreement. The E-test constantly underestimated the MIC values when compared to the two dilution methods.

The E-test combined the basic principles of both the disk diffusion and the agar dilution method using a defined gradient of concentrations in a dry format on a plastic strip providing a direct MIC value for a tested isolate. The E-test has been evaluated as a suitable method for the AST of different pathogens including fastidious pathogens important in veterinary medicine (Lobova and Cizek 2004).

From a practical point of view, the E-test already shows a faster performance, especially in comparison with the agar dilution method. The plate reading is very important, as the exact zone of inhibition is sometimes difficult to establish in the presence of microcolonies and α-haemolysis for the *S. suis* isolates. This might cause a certain level of variability in the reading of the results especially in the case of inter-laboratory comparisons.

The E-test is a suitable method for sensitivity testing especially for amoxicillin and *S. suis* isolates providing a fast alternative to the gold standard dilution-based methods in veterinary medicine.

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## Conflict of interest

The authors declare no conflict of interest.

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