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# *IN VITRO* SENSITIVITY OF HUNGARIAN *ACTINOBACULUM SUIS* STRAINS TO SELECTED ANTIMICROBIALS

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*In vitro* antimicrobial sensitivity of 12 Hungarian isolates and the type strain ATCC 33144 of *Actinobaculum suis* to different antimicrobial compounds was determined both by the agar dilution and by the disc diffusion method. By agar dilution, $MIC_{50}$ values in the range of 0.05–3.125 μg/ml were determined for penicillin, ampicillin, ceftiofur, doxycycline, tylosin, pleuromutilins, chloramphenicol, florfenicol, enrofloxacin and lincomycin. The $MIC_{50}$ value of oxytetracycline and spectinomycin was 6.25 and 12.5 μg/ml, respectively. For ofloxacin, flumequine, neomycin, streptomycin, gentamicin, nalidixic acid, nitrofurantoin and sulphamethoxazole + trimethoprim $MIC_{50}$ values were in the range of 25–100 μg/ml. With the disc diffusion method, all strains were sensitive to penicillin, cephalosporins examined, chloramphenicol and florfenicol, tetracyclines examined, pleuromutilins, lincomycin and tylosin. Variable sensitivity was observed for fluoroquinolones (flumequine, enrofloxacin, ofloxacin), most of the strains were susceptible to marbofloxacin. Almost all strains were resistant to aminoglycosides but most of them were sensitive to spectinomycin. A strong correlation was determined for disc diffusion and MIC results (Spearman's rho 0.789, $p < 0001$). MIC values of the type strain and $MIC_{50}$ values of other tested strains did not differ significantly. Few strains showed a partially distinct resistance pattern for erythromycin, lincomycin and ampicillin in both methods.

**Key words:** *Actinobaculum suis*, antimicrobial sensitivity, novel antimicrobials, MIC

*Actinobaculum* (formerly *Corynebacterium, Eubacterium, Actinomyces*) *suis* is a urinary tract pathogen of swine, capable of causing acute or chronic urocystitis and pyelonephritis in breeding sows (Lawson et al., 1997; Taylor, 1999). Different antimicrobials are used for the treatment of these conditions, however, the results are usually frustrating, especially in chronic cases (Dee, 1993). The success of attempted therapy largely depends on choosing the appropriate com-

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pound, and results of *in vitro* sensitivity studies can guide clinicians in this process. Since testing antibacterial susceptibility of fastidious bacteria like *A. suis* is not routinely carried out and treatment with antibiotics is based on data in the literature, examination of the antibacterial susceptibility of *A. suis* strains including newly introduced antibiotics is of special importance.

The original description of *A. suis* by Soltys and Spratling (1957) contains data on the *in vitro* sensitivity of three strains, determined with the agar dilution method. The authors reported minimum inhibitory concentration ranges for penicillin (0.01–0.1 µg/ml), streptomycin sulphate (100 µg/ml), chloramphenicol (1–10 µg/ml), oxytetracycline-HCl (10 µg/ml), tetracycline-HCl (10 µg/ml), and chlortetracycline-HCl (100 µg/ml).

The type strain of *A. suis* (ATCC 33144) is reportedly susceptible *in vitro* to chloramphenicol (12 µg/ml), clindamycin (1.6 µg/ml), erythromycin (3 µg/ml), penicillin-G (2 units/ml), tetracycline (6 µg/ml), ampicillin (4 µg/ml) and cephalothin (6 µg/ml) (Moore and Holdeman Moore, 1986).

Ten isolates of *A. suis* tested by Jones et al. (1982) showed *in vitro* sensitivity to penicillin, ampicillin, chloramphenicol, tetracycline, erythromycin and nitrofurantoin; they were resistant to streptomycin, neomycin, nalidixic acid and trimethoprim. *A. suis* strains isolated by Høgh et al. (1984) were found to be sensitive to penicillin, a great variation in their sensitivity to polymyxin and neomycin was observed. Three *A. suis* strains isolated by Dreau and Laval (2000) were sensitive to ceftiofur, amoxicillin, penicillin and tetracycline, variably sensitive to enrofloxacin and sulphonamides, and resistant to flumequine, although testing method was not reported for these studies.

In the past decade a number of new, broad-spectrum antimicrobials, like clavulanate potentiated amoxicillin, marbofloxacin and florfenicol, were introduced to the veterinary drug market which might be effectively used to treat urinary tract disorders of swine (Wendt, 1998). *In vitro* sensitivity data were not reported yet with regard to *A. suis* for the above-mentioned compounds and for tylosin, doxycycline, tiamulin and valnemulin. There is no report on the *in vitro* sensitivity of Hungarian *A. suis* isolates either. The main aim of our study was to determine *in vitro* antimicrobial sensitivity pattern of recently isolated Hungarian *A. suis* strains to selected antimicrobials used in the swine industry. *In vitro* sensitivity of anaerobic bacteria is generally determined using the agar dilution method (Quinn et al., 1994), however, there is no standardised method. Although in most cases the guidelines of NCCLS (National Committee for Clinical Laboratory Standards, Wayne, FL, USA) are followed, results valuable for the practice were obtained even in the case of obligate anaerobic bacteria, like *Serpulina* (*Brachyspira*) *hyodysenteriae* using the disc diffusion method (Molnár, 1996). A special form of the disc diffusion method, the E-test is becoming accepted for testing antibacterial susceptibility (Nagy, 1999). Since *A. suis* is reportedly not a true obligate anaerobic bacterium (Biksi et al., 1997), and its propagation is not

as slow as that of most obligate anaerobic bacteria, we also intended to assess the feasibility of using the disc diffusion method as a possible practical alternative for the determination of sensitivity of *A. suis* isolates.

## Materials and methods

Thirteen *A. suis* strains were used in this study. Eight were isolated in a previous investigation (Biksi et al., 1997), two were isolated later from the prepuce of healthy boars, two were cultured from cases of haemorrhagic cystitis of sows, and the type strain DSM 20.639 = ATCC 33144 was previously purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). All of the isolates were identified by biochemical methods and were compared to the type strain (Moore and Holdeman Moore, 1986).

Antibiotic sensitivity testing was done by both the agar dilution and the disc diffusion method. Twenty-one antimicrobials: penicillin, ampicillin, ceftiofur, gentamicin, neomycin, streptomycin, spectinomycin, oxytetracycline, doxycycline, lincomycin, tylosin, erythromycin, tiamulin, valnemulin, chloramphenicol, florfenicol, nalidixic acid, flumequine, enrofloxacin, ofloxacin, sulphamethoxazole + trimethoprim were tested in the agar dilution method. The same antimicrobials, plus clavulanate-potentiated amoxicillin, cefotaxim and marbofloxacin were used in the disc diffusion method.

For the agar dilution method, twofold serial dilutions of each antimicrobial compound were prepared and 0.5 ml of these was mixed with 19.5 blood agar to yield twelve final test concentrations, ranging from 0.05 to 100 µg/ml. In case of each bacterial strain, 5 µl of an approximately $1.5 \times 10^4$ CFU/ml bacterial suspension (prepared using a McFarland No. 1 standard) were streaked on Westphal agar plates containing 5% defibrinated sterile sheep blood and the tested concentration of each antimicrobial. A similar plate without antimicrobials was used as a control for the presence of bacterial growth. The minimal inhibitory concentration (MIC) was determined as the lowest dilution of the antimicrobial where bacterial growth was not visible.

For the disc diffusion method, approx. 10 µl of a McFarland No. 1. suspension of each bacterial strain was streaked on a Westphal agar plate containing 5% defibrinated sterile sheep blood and four discs were placed on each agar plate at maximum. Concentration and source of sensitivity discs are presented in Table 2. Inhibitory zone diameters were determined with a caliper. Sensitivity of the strains to a given antibacterial compound was evaluated following general guidelines in the disc manufacturer's instructions.

The culture medium was selected as being the most suitable for growth of *A. suis* according to our previous experience. Plates were cultured at 37 °C for 3–4 days under anaerobic conditions using anaerobic jars (AnaeroJar, Oxoid).

A Spearman's correlation coefficient was determined for paired MIC and disc diffusion results of each strain. For this purpose, MIC values were categorised as indicating 'sensitivity' (0.05–3.125 µg/ml), 'intermediate sensitivity' (6.25–12.5 µg/ml) and 'resistance' (25–100 µg/ml). The boundaries of these categories were chosen arbitrarily. Mean MIC value of the type strain was compared to mean $MIC_{50}$ value of our own isolates by the two sample Mann-Whitney test. Statistical procedures were performed using Minitab for Windows 13.0.

## Results

Results are presented in Tables 1 and 2.

**Table 1**

Minimal inhibitory concentration (MIC) of selected antimicrobials for Hungarian *A. suis* isolates and the type strain ATCC 33144 (n = 13)

| Antimicrobial (source) | $MIC_{ATCC\ 33144}$ (µg/ml) | MIC range (µg/ml) | $MIC_{50}$ (µg/ml) | $MIC_{90}$ (µg/ml) |
|---|---|---|---|---|
| Penicillin (Sigma) | 0.2 | 0.2–12.5 | 0.2 | 0.4 |
| Ampicillin (Sigma) | 1.6 | 0.8–25 | 0.8 | 2.82 |
| Amoxicillin + clavulanic acid | ND | ND | ND | ND |
| Cefotaxim | ND | ND | ND | ND |
| Ceftiofur (Pharmacia) | 0.05 | 0.05–0.1 | 0.05 | 0.09 |
| Gentamicin (Sigma) | 25 | 25–50 | 25 | 25 |
| Neomycin (Sigma) | 100 | 100 | 100 | 100 |
| Streptomycin (Sigma) | 50 | 50–100 | 50 | 100 |
| Spectinomycin (Sigma) | 6.25 | 6.25–12.5 | 6.25 | 12.5 |
| Oxytetracycline (Sigma) | 6.25 | 6.25–12.5 | 12.5 | 12.5 |
| Doxycycline (Sigma) | 6.25 | 1.6–12.5 | 3.125 | 6.25 |
| Lincomycin (Sigma) | 0.4 | 0.2–100 | 0.4 | 80.625 |
| Tylosin (Elanco) | 0.05 | 0.05–3.125 | 0.05 | 1.44 |
| Erythromycin (Sigma) | 0.05 | 0.05–100 | 0.05 | 100 |
| Tiamulin (Novartis AH) | 0.1 | 0.1–0.4 | 0.1 | 0.4 |
| Valnemulin (Novartis AH) | 0.05 | 0.05 | 0.05 | 0.05 |
| Chloramphenicol (Sigma) | 0.8 | 0.4–0.8 | 0.4 | 0.8 |
| Florfenicol (Schering-Plough) | 0.4 | 0.4–0.8 | 0.4 | 0.8 |
| Nalidixic acid (Sigma) | 100 | 100 | 100 | 100 |
| Flumequine (Sigma) | 100 | 50–100 | 50 | 100 |
| Enrofloxacin (Sigma) | 3.125 | 0.8–3.125 | 1.6 | 2.82 |
| Marbofloxacin | ND | ND | ND | ND |
| Ofloxacin (Sigma) | 3.125 | 0.8–3.125 | 1.6 | 3.125 |
| Sulphamethoxazole + trimethoprim (Sigma) | 100 | 100 | 100 | 100 |

ND = not done

In the agar dilution study, 'low' $MIC_{50}$ values were determined for penicillin, ampicillin, ceftiofur, doxycycline, tylosin, pleuromutilins, chlorampheni-

col, florfenicol, enrofloxacin, erythromycin and lincomycin. 'Moderate' $MIC_{50}$ values were determined for oxytetracycline and spectinomycin. We obtained 'high' $MIC_{50}$ values for flumequine, neomycin, streptomycin, gentamicin, nalidixic acid and sulphamethoxazole + trimethoprim.

**Table 2**

*In vitro* sensitivity of Hungarian *A. suis* isolates and the type strain ATCC 33144 as determined by the disc diffusion method (n = 13)

| Disc (concentration, manufacturer) | Sensitive (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Penicillin (3 IU, Human) | 100 | 0 | 0 |
| Ampicillin (20 μg, Human) | 92 | 8 | 0 |
| Amoxicillin + clavulanic acid (20 + 10 μg, Unipath) | 92 | 8 | 0 |
| Cefotaxim (30 μg, Human) | 100 | 0 | 0 |
| Ceftiofur (30 μg, Rosco) | 100 | 0 | 0 |
| Gentamicin (10 μg, BioMerieux) | 0 | 8 | 92 |
| Neomycin (30 μg, Human) | 0 | 8 | 92 |
| Streptomycin (30 μg, Human) | 8 | 15 | 77 |
| Spectinomycin (100 μg, Sanofi) | 69 | 23 | 8 |
| Oxytetracycline (30 μg, Human) | 92 | 8 | 0 |
| Doxycycline (30 IU, Sanofi) | 100 | 0 | 0 |
| Lincomycin (30 μg, Rosco) | 100 | 0 | 0 |
| Tylosin (30 μg, Mast Diagnostics) | 100 | 0 | 0 |
| Erythromycin (10 μg, Human) | 85 | 0 | 15 |
| Tiamulin (30 μg, Rosco) | 100 | 0 | 0 |
| Valnemulin (30 μg, Abtek) | 100 | 0 | 0 |
| Chloramphenicol (30 μg, Human) | 100 | 0 | 0 |
| Florfenicol (30 μg, BBL) | 100 | 0 | 0 |
| Nalidixic acid (30 μg, Human) | 0 | 8 | 92 |
| Flumequine (30 μg, BioMerieux) | 15 | 46 | 39 |
| Enrofloxacin (5 μg, Unipath) | 62 | 38 | 0 |
| Marbofloxacin (5 μg, Sanofi) | 84 | 8 | 8 |
| Ofloxacin (5 μg, Human) | 46 | 46 | 8 |
| Sulphamethoxazole + trimethoprim (25 μg, Human) | 0 | 15 | 85 |

In the disc diffusion study, all of the strains proved to be sensitive to penicillin, cephalosporins tested, doxycycline, tylosin, pleuromutilins, chloramphenicol, florfenicol and lincomycin. With the exception of one, all strains were sensitive to ampicillin and clavulanate-potentiated amoxicillin. Variable sensitivity was observed for fluoroquinolones (flumequine, enrofloxacin, ofloxacin), while 84% of the strains were susceptible to marbofloxacin. Almost all strains were resistant to aminoglycosides tested but most of them were sensitive to spectinomycin. One strain showed a partially distinct resistance pattern, being moderately sensitive to amoxicillin, clavulanate-potentiated amoxicillin, oxytetracycline, and

being resistant to erythromycin. The same strain proved to be moderately sensitive to nalidixic acid, aminoglycosides tested and sulphamethoxazole + trimethoprim.

A high level of correlation was determined between the results of the two techniques (Spearman's rho: 0.789; $p < 0.0001$). However, for a few strains which seemed susceptible to ampicillin, lincomycin or erythromycin with disc diffusion, we determined high MIC values for the given antimicrobials. The determined $MIC_{50}$ values were not different from MIC values of the type strain ATCC 33144 (two-sample Mann-Whitney test, $p = 0.9$).

## Discussion

Considering the often mixed bacterial flora present in cases of urocystitis and pyelonephritis of swine (Carr and Walton, 1993), treatment of these conditions requires the use of broad-spectrum antimicrobials or antimicrobial combinations (Dee, 1993). Also, among others, the availability of the active compound at the site of infection and its activity at high pH have to be considered (Wendt, 1998). Based on these and on our *in vitro* results, where available, semisynthetic penicillins or a potentiated form of them, such as clavulanate-potentiated amoxicillin, as well as ceftiofur, florfenicol, doxycycline, and possibly marbofloxacin and enrofloxacin can potentially be useful in treating mixed urinary tract infections of swine involving *A. suis*. However, some *A. suis* strains might considerably differ in susceptibility from the above pattern. These data should be regarded as guidelines only when choosing antimicrobials for the treatment of *A. suis* infections in swine, since the pharmacokinetics of the drugs, especially the production of effective concentrations on the mucous membranes of urogenital organs, must be considered. Unfortunately, data on the concentration of different antimicrobials in the porcine urogenital tract are quite limited (Dee, 1993). As our results indicate, the disc diffusion method as performed might be a practical alternative to the agar dilution technique for determining the *in vitro* susceptibility of *A. suis* isolates.

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