Antimicrobial resistance and virulence factors of *Streptococcus suis* strains isolated from diseased pigs in southern Italy (Sardinia)

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Summary

Streptococcus suis is a major swine pathogen responsible for important economic losses to the porcine industry worldwide. The objective of this study was to characterize strains of *S suis* isolated from dead piglets from farms located in southern Italy (Sardinia) between 2012 and 2014, by determining their genotype profiles, antimicrobial resistance profiles, and presence of associated virulence factors in order to evaluate a potential association between antimicrobial resistance serotypes and virulence factors. A total of 39 *S suis* isolates were examined for possession of virulence-associated factors using multiplex polymerase chain reaction assays. All isolates were tested for susceptibility to antimicrobial agents. Fisher's exact test was performed in order to study the correlation between antimicrobial resistance and virulence factors *epf+/epf-*. Genotypes *cps2+/epf+/sly+/mrp+/arcA+, cps2+/epf-/sly+/mrp+/arcA+,* and *cps2-/epf-/sly+/mrp+/arcA+* were identified, representing 18.0%, 74.6%, and 7.4% of the isolates, respectively. A high frequency of resistance was observed for tetracycline (88.9%) and erythromycin (38.9%). No correlation between the virulence factor *epf* and resistance to multiple antibiotics was found.

Keywords: swine, *Streptococcus suis,* multiplex polymerase chain reaction, virulence factor, antimicrobial susceptibility

Received: July 28, 2015
Accepted: February 18, 2016

Resumen - Resistencia antimicrobiana y factores de virulencia de las cepas aisladas del *Estreptococo suis* de cerdos muertos en el sur de Italia (Sardinia)

El *Estreptococo suis* es un importante patógeno porcino responsable de relevantes pérdidas económicas a la industria porcina mundialmente. El objetivo de este estudio fue clasificar cepas del *E suis* aisladas de lechones muertos de granjas localizadas en el sur de Italia (Sardinia) entre 2012 y 2014, por medio de la determinación de los perfiles de su genotipo, perfiles de su resistencia antimicrobiana, y la presencia de factores de virulencia relacionados para evaluar la asociación potencial entre estereotipos de resistencia antimicrobiana y factores de virulencia. Se examinaron un total de 39 *E suis* aislados en busca de la posesión de factores de virulencia asociados utilizando múltiples pruebas de reacción en cadena de polimerasa. Se analizaron todos los aislados en busca de susceptibilidad a agentes antimicrobianos. Se realizó la prueba exacta de Fisher para estudiar la correlación entre la resistencia antibacteriana y los factores de virulencia *epf+/epf-*. Se identificaron los genotipos *cps2+/epf+/sly+/mrp+/arcA+*, *cps2+/epf-/sly+/mrp+/arcA+*, y *cps2-/epf-/sly+/mrp+/arcA+*, representando 18.0%, 74.6%, y 7.4% de los aislados, respectivamente. Se observó una alta frecuencia de resistencia a la tetraciclina (88.9%) y eritromicina (38.9%). No se encontró correlación entre el factor de virulencia *epf* y la resistencia a múltiples antibióticos.

Résumé - Antibiorésistance et facteurs de résistance de souches de *Streptococcus suis* isolées de porcs malades en Italie du sud (Sardaigne)

Streptococcus suis est un agent pathogène majeur du porc responsable d'importantes pertes économiques dans l'industrie porcine à l'échelle mondiale. L'objectif de la présente étude était de caractériser des souches de *S suis* isolées de porcelets morts provenant de fermes localisées en Italie du sud (Sardaigne) entre 2012 et 2014, en déterminant les profils génotypiques, les profils d'antibiorésistance, et la présence de facteurs de virulence afin d'évaluer l'association potentielle entre l'antibiorésistance et les facteurs de virulence. Trente-neuf isolats de *S suis* ont été examinés pour la présence de facteurs de virulence par épreuves d'amplification en chaine par la polymérase multiplex. Tous les isolats ont été testés pour leur sensibilité à différents agents antimicrobiens. Le test exact de Fisher a été utilisé afin d'étudier la corrélation entre l'antibiorésistance et les facteurs de virulence *epf+/epf-*. Les génotypes *cps2+/epf+/sly+/mrp+/arcA+*, *cps2+/epf-/sly+/mrp+/arcA+*,

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This article is available online at http://www.aasv.org/shap.html.

Tedde MT, Pilo C, Frongia M, et al. Antimicrobial resistance and virulence factors of *Streptococcus suis* strains isolated from diseased pigs in southern Italy (Sardinia). *J Swine Health Prod.* 2016;24(5):253–258.

https://doi.org/10.54846/jshap/942

et *cps2-/epf-/sly+/mrp+/arcA+* ont été identifiés, représentant respectivement 18,0%, 74,6%, et 7,4% des isolats. On nota une fréquence élevée de résistance à la tétracycline (88,9%) et à l'érythromycine (38,9%). Aucune corrélation entre le facteur de virulence *epf* et une antibiorésistance multiple n'a été notée.

Streptococcus suis infection is considered a major problem worldwide in the swine industry, resulting in great economic losses.[1] Pigs may acquire *S suis* via both vertical and horizontal transmission. Colonized animals typically harbor the organism in their tonsils and may never develop disease (carrier animals). On the contrary, some carrier piglets eventually develop bacteremia, septicemia, meningitis, or all three, due to dissemination of *S suis* from tonsils or other mucosal surfaces or both, usually when maternal antibodies decline.[2] Of the various manifestations of the disease, septicemia and meningitis are by far the most striking, but endocarditis, pneumonia, and arthritis may also be observed. Nevertheless, in hyperacute cases of infection, pigs are often found dead with no premonitory signs of disease.[3] Moreover, *S suis* has zoonotic potential, causing septicemia with or without septic shock, meningitis, and other less common infections in humans.[4] Human infection with *S suis* has become a serious zoonosis and has been observed in many countries where intensive swine production is practised.[2,5] Overall, three human cases of *S suis* meningitis have been reported in Italy, one in the 1990s and two in 2007. One of the two cases reported in 2007 was identified in the geographical area covered by this study and was characterized as *S suis* serotype 2.[6,7] A total of 35 serotypes of *S suis* have been described and defined on the basis of the antigenicity of their capsular polysaccharide (CPS).[8] *Streptococcus suis* strains differ in virulence, and strains of the same serotype can be differentiated by the expression of virulence-associated factors, including muramidase-released protein (MRP, encoded by *mrp*); a peptidoglycan-associated protein probably acting as an adhesin; an extracellular protein factor (EF, encoded by *epf*); and suilysin (SLY, encoded by *sly*), which is a thiol-activated hemolysin with a cytotoxic effect that might allow penetration into deeper tissues.[9] Muramidase-released protein, EF, and SLY have been considered the major virulence-associated markers of *S suis* 2,[10] and the arginine deiminase enzyme (ADS, encoded by *arcA*) has been recently described[11] and seems to play an important role in survival of the bacterium by increasing resistance to acidity. The gene *cps2* is specific for *S suis* serotypes 2 and 1/2 and is considered a fifth virulence factor.[12] The virulence factors MRP, EF, and SLY are associated with *S suis* serotype 2 strains in Europe and Asia, but not with the less virulent North American strains.[12] The efficacy of many commercially available *S suis* killed whole-cell vaccines is poor because protection is limited to homologous strains.[13] Previous studies revealed a wide diversity of antimicrobial resistance and varied distribution of virulence-associated factors in different serotypes of *S suis,* but few studies have focused on the relationship between antimicrobial resistance and virulence factors.[14-17] To our knowledge, this study represents the first characterization of clinical strains of *S suis* isolated from dead piglets from farms located in southern Italy (Sardinia) between 2012 and 2014. Hence, the objective of this work was to determine the antimicrobial resistance, serotypes, and virulence factors of these clinical isolates in order to estimate a correlation among these three characteristics.

Materials and methods

This study did not require ethical review because the activities comprised a part of a periodic, routine diagnostic monitoring program and did not involve animal experimentation.

Bacterial strains and culture conditions

In this study, 39 strains of *S suis* were recovered from pigs at necropsy at the Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi" (Public Veterinary Diagnostic Laboratory, Sardinia, Italy) between 2012 and 2014 from samples submitted for disease diagnosis in piglets from farms located in southern Italy (Sardinia). The samples were collected from a variety of tissues from dead pigs with lesions such as pneumonia or pleurisy or both, meningitis, endocarditis, and septicemia. *Streptococcus suis* strains were isolated frequently from pigs between 5 and 10 weeks of age. The bacterial strains were identified as *S suis* by cultural methods. Specimens were inoculated onto Columbia agar plates (Oxoid Ltd, Basingstoke, United Kingdom) supplemented with 5% sheep blood and incubated in aerobic conditions at 37°C for 24 to 48 hours. Two to three colonies that did not exceed 1 mm in diameter and exhibited α-hemolysis were picked from each plate to subculture on Columbia 5% blood agar plates and were incubated in the same manner. Suspicious colonies, ie, gram-positive cocci negative on the catalase test, were confirmed by the API-20STREP system (BioMérieux, Marcy l'Etoile, France).

DNA extraction and PCR conditions

Genomic DNA was isolated and purified with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) using the method for gram-positive microorganisms indicated in the manufacturer's instructions. Genomic DNA was stored at -20°C until further processing. Multiplex PCR (mPCR)[18] was used to determine the presence of *cps2*, *epf, sly, mrp,* and *arcA* genes. In order to evaluate the sensitivity of the mPCR assays, *S suis* type strain DSMZ 9682 was used as a reference strain. The sequences of oligonucleotidic primers are listed in Table 1. The mPCR mixture contained PCR buffer 1X (Invitrogen, Cergy Pontoise, France), 2 mM $MgCl_2$ (Invitrogen); a 300-µM concentration of each of deoxynucleoside triphosphate (Invitrogen), 0.1 µM of each primer for *epf*, 0.06 µM of each primer for *cps2*, 0.03 µM of each primer for *sly*, 0.05 µM of each primer for *mrp,* and 0.06 µM of each primer for *arcA*; 0.04 U of *Taq* DNA polymerase (Invitrogen); and 50 ng of DNA template. UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific, Waltham, Massachusetts) was used as negative control. The reaction procedure consisted of an initial denaturation at 94°C for 2 minutes, 40 cycles at 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 90 seconds, with a final extension at 72°C for 5 minutes. The amplified products were separated in a 2% agarose gel in Tris-acetate-EDTA (TAE) buffer (Thermo Fisher Scientific) for 1 hour at a constant voltage of 125 V. Amplified products were stained with Syber Safe (Thermo Fisher Scientific) and detected by ultraviolet transillumination. The 100 bp Smart Ladder (Invitrogen) was used as a molecular size standard.

Antimicrobial susceptibility testing

The 36 strains determined to be *S suis cps2+* were tested for susceptibility to nine antimicrobials according to the Clinical and Laboratory Standards Institute (CLSI)

Table 1: Polymerase chain reaction primers used to amplify virulence genes of *Streptococcus suis* strains*

Gene	GenBank accession number	Primer sequence (5^–3^)	Position in coding sequence
epf	X71881	5′-CGC AGA CAA CGA AAG ATT GA-3′ 5′-AAG AAT GTC TTT GGC GAT GG-3′	744 bp
cps2	AF118389	5′-TTT GTC GGG AGG GTT ACT TG-3′ 5′-TTT GTC GGG AGG GTT ACT TG-3′	498 bp
mrp	X64450	5′-ATT GCT CCA CAA GAG GAT GG-3′ 5′-TGA GCT TTA CCT GAA GCG GT-3′	188 bp
sly	Z36907	5′-GCT TGA CTT ACG AGC CAC AA-3′ 5′-CCG CGC AAT ACT GAT AAG C-3′	248 bp
arcA	AF546864	5′-TGA TAT GGT TGC TGC TGG TC-3′ 5′-GGA CTC GAG GAT AGC ATT GG-3′	118 bp

* Between 2012 and 2014, thirty-nine *S suis* strains were isolated from dead piglets from farms located in southern Italy (Sardinia). Strains were examined for possession of virulence-associated factors using multiplex polymerase chain reaction.

2013 guidelines.[19] Three to four colonies from an overnight culture on Columbia agar supplemented with 5% sheep blood were suspended in Mueller Hinton (MH) broth (Becton Dickinson, Pont de Claix, France). The suspension was adjusted to a 0.5 McFarland standard and diluted to obtain an inoculum of 10^6 colony-forming units (CFU) per mL of *S suis*. For each isolate, two plates were inoculated by flooding MH agar supplemented with 5% sheep blood with the diluted suspension (4 mm depth) (Becton Dickinson). The antibiotic discs were placed with a disc dispenser (Bio-Rad, Hercules, California), and plates were incubated at 37°C for 18 hours. Antimicrobial agents tested were as follows: amoxicillin (10 µg per disc), amoxicillin-clavulanic acid (30 µg per disc), penicillin G (10 units per disc), ampicillin (10 µg per disc), ceftiofur (30 µg per disc), enrofloxacin (5 µg per disc), tetracycline (30 µg per disc), trimethoprim-sulfamethoxazole (25 µg per disc) (Oxoid Ltd), and erythromycin (15 µg per disc) (Cefar Diagnóstica Ltda, São Paulo, Brazil). *Escherichia coli* ATCC 25922, *E coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212 were used as control strains. The zone of growth inhibition was interpreted as sensitive, intermediate, or resistant. The inhibition zone diameters of *S suis* strains and the reference strains were measured on the same day using a sliding caliper. For amoxicillin, amoxicillin-clavulanic acid, ceftiofur, erythromycin, penicillin G, and trimethoprim-sulfamethoxazole, disc diffusion susceptibility was tested according to the Clinical and Laboratory Standards Institute (Approved Standard VET01-A4; CLSI, 2013) guidelines specific for *S suis* strains.[19] For ampicillin, enrofloxacin, and tetracycline, specific breakpoints for *S suis* are not available in CLSI guidelines. For those antimicrobials, interpretation of disc diffusion susceptibility was evaluated according to breakpoints indicated by the manufacturer as specific for *Streptococcus* species (Oxoid Ltd).

Statistical analysis

The correlation between antimicrobial resistance and virulence factors was studied. Microbial resistance values were expressed as percentages and compared using a Fisher's exact test. Statistical analysis was performed with SPSS software (version 15.0; SPSS Inc, Chicago, Illinois), and statistical significance was defined at $P < .05$.

Results

All isolates were determined to be *S suis* serotype 2 by biochemical characteristics (API -20 strep, BioMerieux SA France) and further confirmed by positive PCR for the genes coding for the 16S rRNA of *S suis* and for the capsule of *S suis* serotype 2 (*cps2*+).

In this study, 39 *S suis* strains were isolated, and 36 of them (92.3%) belonged to genotype 2 (*cps2*+). Among the 39 cases, 38.5% were categorized as lung infections, 18.0% as meningitis, 18.0% as endocarditis, and 25.5% as septicemia (cases where *S suis* was isolated from multiple organs or from mediastinal lymph nodes, spleen, liver, and kidney were classified as septicemia) (Table 2). Numbers of isolates from various organs are shown in Table 3. Virulence factor gene *epf* was detected in 18.0% of the isolates, whereas virulence factor genes *sly, mrp,* and *arcA* were detected in 100% of the isolates. Three genotypes, *cps2+/epf+/sly+/mrp+/arcA+, cps2+/epf-/sly+/mrp+/arcA+*, and *cps2-/epf-/sly+/mrp+/arcA+* were identified, representing 18.0%, 74.6%, and 7.4%, of the isolates respectively (Table 3).

The collection of 36 *S suis cps2+* strains was tested for susceptibility to nine antimicrobials (Table 4). A high frequency of resistance was observed for tetracycline (88.9%), erythromycin (38.9%), trimethoprim-sulfamethoxazole (16.7%), and enrofloxacin (11.1%). Multiple antimicrobial resistance (two or more antimicrobials) was observed in 58.3% of the *S suis* isolates. Sensitivity testing showed that ampicillin had the greatest antimicrobial effect on the 36 isolates (100% of strains were susceptible), followed by amoxicillin, amoxicillin-clavulanic acid, penicillin G, and ceftiofur (Table 4).

The correlation between antimicrobial resistance and virulence factors *epf+/epf-* was evaluated (Table 5). The virulence factors *cps2+/cps2-* were not analyzed for any correlation with antimicrobial resistance because of the small number of *cps2-* strains. (Table 2). Since no strains resistant to ampicillin were found, no correlation data were collected for this antibiotic. The virulence factor *epf* was not correlated with resistance to multiple antibiotics in this study.

Table 2: Distribution of *Streptococcus suis* genotypes among diseased pigs*

Disease	No. of isolates (%)	*Streptococcus suis* genotypes		
		cps2+ /epf+/sly+/mrp+/arcA+	***cps2+ /epf-/sly+/mrp+/arcA+***	***cps2- /epf-/sly+/mrp+/arcA+***
Pneumonia/ pleuritis	15 (38.5)	5	10	0
Meningitis	7 (18.0)	2	5	0
Septicemia	10 (25.5)	0	9	1
Endocarditis	7 (18.0)	0	5	2
Total	39 (100.0)	7	29	3

* Study described in Table 1.

Table 3: Number of *Streptococcus suis* genotypes isolated from organs of dead piglets*

Genotypes	Brain	Spleen	Heart	Lung	Liver	Mediastinal LN	Kidney	Total isolates (%)
cps2+/epf+/sly+/mrp+/arcA+	2	0	0	5	0	0	0	7 (18.0)
cps2+/epf-/sly+/mrp+/arcA+	5	2	5	10	2	4	1	29 (74.6)
cps2-/epf-/sly+/mrp+/arcA+	0	0	2	0	0	1	0	3 (7.4)
Total (%)	**7 (18.0)**	**2 (5.1)**	**7 (18.0)**	**15 (38.5)**	**2 (5.1)**	**5 (12.8)**	**1 (2.5)**	**39 (100.0)**

* Study described in Table 1.
LN = lymph node.

Discussion

Streptococcus suis is a major swine pathogen, responsible for important economic losses to the porcine industry worldwide. In western countries, *S suis* infections in humans have mostly been restricted to workers in close contact with pigs or swine by-products. However, in Southeast and East Asia, this bacterium also affects the general population and thus represents a significant public-health concern.[2] During the 3 years of monitoring, the organs of all piglets dead within the geographical district of interest were screened for *S suis* infection. *Streptococcus suis* strains isolated in this study represent the entire set of clinical strains whose infection led to piglet deaths. The number of isolates was small because this study focused attention on strains that cause piglet mortality. Strains were isolated from different farms; nevertheless, clonality among isolates cannot be excluded. The small number of studied strains and the potential for clonality can add bias to the evaluation of correlation between antimicrobial resistance and virulence factors.

An accurate isolation strategy was carried out in order to avoid selective isolation of clonal strains from the same farm. Different capsular serotypes display various clinical manifestations and differ vastly among countries. The most prevalent capsular gene in the isolates in this study was *cps2*; *cps2*+ strains are known to be highly virulent. In this study, *cps2*- strains caused endocarditis and septicemia. This is not surprising, because other *cps2*- serotypes in Europe, particularly with the profile *cps2-/epf-/sly+/mrp+/arcA+*, have proved to be highly virulent and can cause septicemia and meningitis in pigs.[20] We found that *mrp+/sly+/epf+* and *mrp+/sly+/epf-* genotypes were predominant among the tested isolates. The *arcA* gene was identified in all strains, confirming previous studies.[21] From European epidemiological studies[10] and experimental infections in pigs,[22] strains of the *sly+/mrp+/epf+* genotype are known to be highly virulent. This type was significantly associated with systemic infection in pigs and was highly pathogenic to mice.[17] This genotype has also been identified in many isolates from human clinical cases.[20] Though *epf* is not an essential virulence factor for *S suis* serotype 2 strains, it is probably associated with other factors that play a more crucial role in determining virulence and host specificity in *S suis* strains.[23]

Moreover, in this study, resistance of *S suis* to antibiotics commonly used in pig farms in the area was examined. A high rate of resistance was observed for tetracycline (88.9%) and erythromycin (38.9%). In this study, *S suis* had the greatest susceptibility to beta-lactam antibiotics and the greatest resistance to erythromycin and tetracycline. High rates of resistance to macrolides and tetracyclines suggest widespread resistance to these antibiotics in Italy.[21] In Europe, rising rates of resistance have been attributed to the intensive use by swine breeders of the macrolide-class antibiotics, tylosin as a growth promoter and tetracycline as a therapeutic agent.[20] Co-resistance to macrolides and tetracyclines can be explained by the fact that tetracycline and erythromycin resistance determinants are often linked to mobile genetic elements.[24] The trend of *S suis* resistance to macrolides and tetracyclines has been reported worldwide.[25] Studies of genetic resistance traits have demonstrated that *erm*(B) and *mef*(A) are involved in macrolide resistance, whereas *tet*(M) and *tet*(O) are involved in tetracycline resistance.[21] Resistance to erythromycin is a concern for public health, as macrolide drugs are important for therapeutic treatment of severe streptococcal cases in humans.[17] Resistance of the isolates in the present study to penicillin and ceftiofur was lower than resistance to other tested antibiotics, but was, however,

Table 4: Antibiotic resistance phenotype of *Streptococcus suis cps2*+ isolates*

Antibiotic	S strains (%)	R strains (%)
Amoxicillin	35 (97.2)	1 (2.8)
Amoxicillin-clavulanic acid	35 (97.2)	1 (2.8)
Ampicillin	36 (100.0)	0 (0.0)
Ceftiofur	34 (94.4)	2 (5.6)
Enrofloxacin	32 (88.9)	4 (11.1)
Erythromycin	22 (61.1)	14 (38.9)
Penicillin G	35 (97.2)	1 (2.8)
Tetracycline	4 (11.1)	32 (88.9)
Trimethoprim-sulfamethoxazole	30 (83.3)	6 (16.7)

* Study described in Table 1. *Streptococcus suis* isolates (n = 36) were tested for susceptibility to antimicrobial agents. No isolates demonstrated intermediate resistance.
S = susceptible; R = resistant.

clinically significant. Thus, development of resistance to these antibiotics would reduce the efficacy of antibiotic treatment. To the best our knowledge, this study represents the first epidemiological investigation of lethal cases of *S suis* infection in piglets in southern Italy (Sardinia). Moreover , this study represents a first attempt to correlate antimicrobial resistance and virulence factors in *S suis* isolated in southern Italy (Sardinia). The results reveal that the majority of *S suis* isolates from dead pigs carry multiple virulence factors and that *cps2*+ strains display resistance to multiple antimicrobials. Infection with invasive *S suis* requires antibiotic treatment. Possession of both antimicrobial resistance and virulence makes these pathogenic strains potentially highly dangerous to both animal production and public health. Worldwide, *S suis* serotype 2 is the most frequently isolated serotype.

Implications

- *Streptococcus suis* strains from piglets from participating farms located in southern Italy (Sardinia) were resistant to tetracycline and erythromycin between 2012 and 2014.
- Under the conditions of this study, antimicrobial resistance and genomic virulence factors in *S suis* isolated from swine are not correlated.

Acknowledgements

We thank Dr Timo G. Lutz for his revision of the language of this manuscript.

Conflict of interest

None reported.

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Table 5: Correlation between antimicrobial resistance and virulence factors in *Streptococcus suis* strains*

Antibiotic	Genotype	No. of susceptible strains (%)	No. of resistant strains (%)	Total (n)	*P*†
Amoxicillin	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Amoxicillin-clavulanic acid	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Penicillin G	*epf*-	29 (100.0)	0 (0.0)	29	.19
	epf+	6 (85.7)	1 (14.3)	7	
	Total	35 (97.2)	1 (2.8)	36	
Ampicillin	*epf*-	29 (100.0)	0 (0.0)	29	NA
	epf+	7 (100.0)	0 (0.0)	7	
	Total	36 (100.0)	0 (0.0)	36	
Ceftiofur	*epf*-	27 (93.1)	2 (6.9)	29	1.00
	epf+	7 (100.0)	0 (0.0)	7	
	Total	34 (94.4)	2 (5.6)	36	
Enrofloxacin	*epf*-	26 (89.7)	3 (10.3)	29	1.00
	epf+	6 (85.7)	1 (14.3)	7	
	Total	32 (88.9)	4 (11.1)	36	
Tetracycline	*epf*-	3 (10.3)	26 (89.7)	29	1.00
	epf+	1 (14.3)	6 (85.7)	7	
	Total	4 (11.1)	32 (88.9)	36	
Trimethoprim-sulfamethoxazole	*epf*-	23 (79.3)	6 (20.7)	29	.24
	epf+	7 (100.0)	0 (0.0)	7	
	Total	30 (83.3)	6 (16.7)	36	
Erythromycin	*epf*-	19 (65.5)	10 (34.5)	29	.25
	epf+	3 (42.9)	4 (57.1)	7	
	Total	22 (61.1)	14 (38.9)	36	

* Study described in Table 1.
† Fisher's exact test. $P < .05$ considered statistically significant.
NA = not applicable. No statistical analyses were performed because ampicillin is a constant.

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