BRIEF COMMUNICATION PEER REVIEWED

Susceptibility of 45 recent French field isolates of *Streptoccocus suis* to florfenicol

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Summary

To investigate occurrence of acquired resistance, minimum inhibitory concentrations of florfenicol for *Streptococcus suis* isolated in France between 2011 and 2014 were determined. No acquired resistance to florefenicol was observed among recent field isolates of *S suis* after more than 10 years of use of this antibiotic.

Keywords: swine, swine respiratory disease, *Streptococcus suis*, florfenicol

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Resumen - Susceptibilidad al florfenicol de 45 aislamientos franceses de campo recientes de *Streptoccocus suis*

Para investigar la ocurrencia de la resistencia adquirida, las concentraciones mínimas inhibitorias de florfenicol para aislamientos de *Streptoccocus suis* en Francia entre 2011 y 2014 fueron determinados. No se observó resistencia adquirida al florfenicol entre aislamientos de campo recientes de *S suis* después de más de 10 años de uso de este antibiótico.

Résumé - Sensibilité au florfénicol de 45 isolats récents de *Streptococcus suis* provenant de France

Afin d'étudier l'occurrence de résistance acquise envers le florfénicol, on détermina les concentrations minimales inhibitrices d'isolats de *Streptococcus suis* obtenus entre 2011 et 2014 d'élevages en France. Aucune résistance acquise au florfénicol ne fut observée parmi les isolats récents de *S suis* après plus de 10 ans d'utilisation de cet antibiotique.

Streptococcus suis is a major pathogen in swine production, causing meningitis, arthritis, septicemia, bronchopneumonia, polyserositis, and endocarditis.[1] It is also recognized as an important zoonotic agent.[2]

A florfenicol concentrate solution is labelled in the United States for treatment of swine respiratory disease (SRD) associated with several bacterial pathogens, including *S suis*. Treatment is administered by the oral route through drinking water. In France, florfenicol is approved for treatment and control of respiratory disease caused by the major pathogens *Actinobacillus pleuropneumoniae* and *Pasteurella multocida,* but *S suis* is not included in the claim. However, *S suis* may occur simultaneously or sequentially with such bacteria in SRD and is also found in the upper respiratory tracts of healthy animals.[3] Thus *S suis* may be exposed to florfenicol during treatment of animals suffering from SRD caused by bacterial pathogens.

The objective of this study was to determine susceptibility to florfenicol of recent *S suis* field isolates from pig herds in western France to determine whether acquired resistance has emerged. Minimum inhibitory concentrations (MICs) were determined to provide epidemiological survey data.

Materials and methods

Bacterial isolates

Bacteria were isolated at the Institut en Santé Agro-Environnement laboratory (Public Veterinary Diagnostic Laboratory, Fougeres, France) between 2011 and 2014 from samples submitted for disease diagnosis in piglets from herds located in the west of France (mainly Ille-et-Vilaine, but also in Brittany, Pays de la Loire, and Normandy). Isolation, identification, and serotyping of isolates were conducted by conventional bacteriological methods: culture, Gram staining, biochemical tests (Api 20 Strep strip; BioMérieux, Marcy l'Etoile, France) and serotyping (*S suis* antisera; LDA 22, Ploufragan, France).

All isolates were stored at < -60°C in brain-heart broth with 15% glycerol until MIC determination. Determination of MICs was performed on independent *S suis* isolates from an epidemiological point of view (ie, no more than one isolate per year per herd).

MIC determination

Minimal inhibitory concentrations were determined using a broth microdilution method. Testing was performed according to Clinical and Laboratory Standards Institute guidelines (CLSI VET01-A4[4] and VET01-S2[5]). Briefly, after isolates were incubated overnight on agar plates and purity of the cultures was confirmed, the direct colony suspension method was used. Bacterial suspensions were adjusted in cation-adjusted Mueller-Hinton broth supplemented with 2.5% lysed horse blood. The final concentration in the 50 µL of bacterial suspension added per microtiter plate well was approximately 5×10^5 colony forming units (CFU) per mL. Minimum inhibitory concentrations were determined using 96-well microtiter plates containing dehydrated antibiotic (CMP1ASPV, florfenicol custom veterinary susceptibility plate format; Trek Diagnostic Systems Inc, Cleveland, Ohio). After incubation for 24 hours at 35°C (standard deviation 2°C) in ambient air, the MIC was recorded as the lowest concentration of florfenicol that completely

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inhibited growth of the organism in wells, as detected by the unaided eye.

Quality controls

As directed in CLSI guidelines,[4,5] *Enterococcus faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619 were used as reference strains for MIC quality controls each day of testing, with acceptable quality control ranges 2 to 8 µg per mL and 1 to 4 µg per mL, respectively. A colony count of the inoculum for each plate was performed to ensure that the final inoculum in wells approximated 5×10^5 CFU per mL.

Results

The 45 *S suis* isolates used in the study were from routine submissions to the Public Veterinary Diagnostic Laboratory, from piglets 4 to 9 weeks old suffering from respiratory disease (19 isolates; 42%), endocarditis (9 isolates; 20%), septicemia (8 isolates; 18%), meningitis (5 isolates; 11%), and arthritis (4 isolates; 9%). Isolates belonged to different serotypes: type 2 (38% of isolates), non-typeable (24%), 7 (13%), 1,2 (7%), 3 (7%), 1 (4%), 8 (4%), and 9 (2%). Minimum inhibitory concentrations of florfenicol ranged from 0.5 to 2 µg per mL; MIC_{50} and MIC_{90} were 1 and 2 µg per mL, respectively, confirming the previous data, as shown in Table 1.

Discussion

All isolates included in this study were considered to be susceptible to florfenicol, according to CLSI VET01-S2-approved breakpoints for *S suis,* which are ≤ 2 µg per mL (susceptible), 4 µg per mL (intermediate), and ≥ 8 µg per mL (resistant).[5] Data provided by this study are consistent with results of previous studies in which resistance was not detected among isolates collected in France until 2002[12] and in Germany between 2000 and 2005.[8-10]

Streptococcus suis isolates classified as intermediate to florfenicol have seldom been isolated in Europe[11] or North America.[7] According to the studies of Callens et al[6] and Portis et al,[7] resistance to florfenicol is seldom if ever reported (one isolate among 331 European and approximatively 2000 American isolates tested), whereas the susceptibilities of *S suis* isolates to several antibiotics (erythromycin, lincomycin, penicillin, tiamulin, tetracycline, tilmicosin, and tylosin) have dramatically decreased in Europe over the past few years.[6]

Distribution of isolates among serotypes is in accordance with previous French data, but with a higher percentage of non-typeable isolates and a lower percentage of serotype 9.[13] Wisselink et al[11] could not show an association between the serotype of an isolate and its susceptibility pattern. The number of isolates in this study was too small to confirm this result.

Although no resistance to florfenicol was found among the *S suis* isolates tested in this study, the authors do not recommend extra-label use of florfenicol.

Implication

Under the conditions of this study, *S suis* isolates collected from piglets in the west of France between 2011 and 2014 were not resistant to florfenicol.

Conflict of interest

None reported.

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Table 1: Florfenicol MIC_{50}, MIC_{90}, and MIC ranges reported for *Streptococcus suis* isolates*

		Susceptibility of *S suis* isolates to florfenicol†			Concentrations of florfenicol (µg/mL)‡
	S suis isolation	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC ranges (µg/mL)	
Present study	2011-2014	1	2	0.5-2	0.125-128
Callens et al[6]	2010	ND	ND	0.5-8	0.03-128
Portis et al[7]	2007-2010	2	2	0.06->32	0.06-32
	2001-2006	1	2	0.06->32	0.06-32
Schwarz and Kehrenberg[8]	2000-2005	1	2	0.25-2	0.125-128
Kehrenberg et al[9]	2002-2003	1	2	0.25-2	0.125-128
Priebe and Schwarz[10]	2000-2001	1	2	0.25-2	0.125-128
Wisselink et al[11]	1987-1997	ND	ND	0.5-4	0.06-32

* Areas of isolation: West of France (present study); Belgium (Callens); North America: United States and Canada (Portis); Germany (Schwarz, Kehrenberg, Priebe); Belgium, UK, France, Italy, Spain, Germany, and The Netherlands (Wisselink).

† MIC determination methods: Broth microdilution for the present study and all references except Callens[6] (agar dilution).

‡ Range of concentrations of florfenicol tested.

MIC = Minimum inhibitory concentration; ND = not determined

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* Non-refereed references.