Antimicrobial susceptibility of porcine *Pasteurella multocida*, *Streptococcus suis,* and *Actinobacillus pleuropneumoniae* from the United States and Canada, 2001 to 2010

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Summary

Objective: To provide data on the in vitro antimicrobial susceptibility of three bacterial respiratory disease pathogens isolated from swine across the United States and Canada over the period 2001 to 2010.

Materials and methods: A total of 1097 *Actinobacillus pleuropneumoniae,* 2389 *Pasteurella multocida,* and 2617 *Streptococcus suis* isolates recovered from diseased or dead swine from North America over a 10-year period were tested for in vitro susceptibility to antimicrobial agents approved for treatment of swine respiratory disease (SRD). Clinical and Laboratory Standards Institute standardized methods were used to determine the minimum inhibitory concentrations (MICs) of ceftiofur, enrofloxacin, florfenicol, penicillin, tetracycline, tilmicosin, and tulathromycin.

Results: Over the years 2001to 2010, *A pleuropneumoniae* and *P multocida* remained susceptible to ceftiofur, enrofloxacin, florfenicol, tilmicosin, and tulathromycin, and *S suis* remained susceptible to ceftiofur, enrofloxacin, and florfenicol. Low penicillin MIC values for *P multocida* and *S suis* and higher MIC values for *A pleuropneumoniae* were also seen. Most isolates of all three organisms were resistant to tetracycline over the 10 years of the survey.

Implications: Monitoring antimicrobial susceptibility among swine pathogens over time provides valuable information about changes which may be occurring in the antimicrobial susceptibility of these organisms and is an important tool in effective antimicrobial therapy. Surveillance of the in vitro susceptibility of these SRD pathogens continues to be an important component in antimicrobial stewardship.

Keywords: swine, antimicrobial susceptibility, *Actinobacillus, Pasteurella, Streptococcus*

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Resumen - Susceptibilidad antimicrobiana de *Pasteurella multocida*, *Streptococcus suis*, y *Actinobacillus pleuropneumoniae* en porcinos en los Estados Unidos de Norteamérica y Canadá, 2001 a 2010

Objetivo: Proveer información sobre la susceptibilidad antimicrobiana in vitro de tres patógenos de enfermedad respiratoria bacteriana aislados de cerdos a lo largo de Estados Unidos de Norteamérica y Canadá en el periodo 2001 a 2010.

Materiales y métodos: Un total de 1097 aislados de *Actinobacillus pleuropneumoniae*, 2389 de *Pasteurella multocida*, y 2617 de *Streptococcus suis* recuperados de cerdos enfermos ó muertos de Norteamérica en un periodo de 10 años, fueron puestos a prueba en busca de su susceptibilidad in vitro a agentes antimicrobianos aprobados para el tratamiento de la enfermedad respiratoria porcina (SRD por sus siglas en inglés). Se utilizaron los métodos estandarizados del Instituto de Estándares de Laboratorio y Clínicos para determinar las concentraciones inhibitorias mínimas (MICs por sus siglas en inglés) de ceftiofur, enrofloxacina, florfenicol, penicilina, tetraciclina, tilmicosina, y tulathromicina.

Resultados: Durante los años 2001 a 2010, *A pleuropneumoniae* y *P multocida* permanecieron susceptibles al ceftiofur, enrofloxacina, florfenicol, tilmicosina, y tulathromicina, y *S suis* permaneció susceptible al ceftiofur, enrofloxacina, y florfenicol. También se observaron valores bajos de MIC para penicilina para *P multocida* y *S suis* y valores más altos de MIC para *A pleuropneumoniae*. La mayoría de los aislados de los tres organismos fueron resistentes a la tetraciclina durante los 10 años del estudio.

Implicaciones: El monitoreo de la susceptibilidad antimicrobiana entre los patógenos porcinos a lo largo del tiempo provee información valiosa sobre los cambios que pueden estar ocurriendo en la susceptibilidad microbiana de estos organismos y es una herramienta importante en la terapia antimicrobiana efectiva. La vigilancia de la susceptibilidad in vitro de estos patógenos en la SRD continua siendo un componente importante en el manejo antimicrobiano de este complejo respiratorio.

Résumé - Sensibilité antimicrobienne d'isolats porcins de *Pasteurella multocida*, *Streptococcus suis*, et *Actinobacillus pleuropneumoniae* provenant des États-Unis et du Canada, 2001 à 2010

Objectif: Fournir des données sur la sensibilité antimicrobienne de trois espèces bactériennes pathogènes responsables de maladies respiratoires chez le porc provenant des États-Unis et du Canada pour la période allant de 2001 à 2010.

Matériels et méthodes: Un total de 1097 isolats d'*Actinobacillus pleuropneumoniae*,

EP,CL,LJ: Pfizer Animal Health, Kalamazoo, Michigan.

GS: Waterwood Consulting, Kalamazoo, Michigan.

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2389 isolats de *Pasteurella multocida,* et 2617 isolats de *Streptococcus suis* obtenus de porcs malades ou morts en Amérique du Nord pendant une période de 10 ans ont été testés in vitro pour leur sensibilité à des agents antimicrobiens approuvés pour le traitement de maladies respiratoires porcines (SRD). Les méthodes standardisées du Clinical Laboratory Standardss Institute ont été utilisées pour déterminer les concentrations minimales inhibitrices (MIC) des antibiotiques suivants: ceftiofur, enrofloxacine, florfénicol, pénicilline, tétracycline, tilmicosin, et tulathromycine.

Résultats: Au cours des années 2001 à 2010, *A pleuropneumoniae* et *P multocida* sont demeurées sensibles au ceftiofur, à l'enrofloxacine, au florfénicol, au tilmicosin, et à la tulathromycine, et *S suis* est demeuré sensible au ceftiofur, à l'enrofloxacine, et au florfénicol. De faibles valeurs de MIC pour la pénicilline, ont été notées pour *P multocida* et *S suis* et des valeurs plus élevées de MIC pour *A pleuropneumoniae* ont également été observées. La majorité des isolats des trois espèces bactériennes amassés durant la période de l'étude étaient résistants à la tétracycline.

Implications: La surveillance de la sensibilité antimicrobienne d'agents pathogènes porcins dans le temps fournie des informations utiles sur les changements qui peuvent survenir dans la sensibilité antimicrobienne de ces microorganismes et est un outil important pour une thérapie antimicrobienne efficace. La surveillance de la sensibilité in vitro de ces agents de SRD continue d'être un élément important de l'intendance des antimicrobiens.

Livestock veterinarians in the United States and Canada use antimicrobial drugs to treat sick animals and to control the spread of pathogenic bacteria to their healthy pen mates.[1,2] Reduction of stress and suffering of animals is an important component of humane husbandry. Antimicrobial drugs are also used to promote growth in many production animals, including swine.[1,3] Any use of antimicrobial drugs, however, does carry a risk that resistant bacteria will emerge,[4,5] reducing the effectiveness of the drugs and resulting in prolonged illness and suffering, as well as increased numbers of sick animals. Monitoring antimicrobial susceptibility among significant pathogens is therefore an important activity in maintaining effective antimicrobial therapy.[6,7] Swine respiratory disease is among the most frequently encountered bacterial infections in swine and can be caused by a number of bacteria, including *Pasteurella multocida*, *Streptococcus suis,* and *Actinobacillus pleuropneumoniae.*[8] In this survey, the activities of ceftiofur, penicillin, enrofloxacin, florfenicol, tetracycline, tilmicosin, and tulathromycin against respiratory pathogens recovered from pigs across the United States and Canada between 2001 and 2010 were investigated as part of an on-going, long-term veterinary antimicrobial susceptibility surveillance program.

Materials and methods

Participating laboratories and characterization of isolates

Twenty-four veterinary diagnostic laboratories from the major pork-producing areas of the United States and Canada participated in this surveillance program. The regions from which isolates were obtained are shown in Table 1.

All *A pleuropneumoniae, P multocida,* and *S suis* were recovered from diseased or deceased pigs. The diagnostic laboratories selected the isolates on the basis of their own protocols, but were requested not to use susceptibility as a criterion for selection. In order to limit over-representation from any one geographic area, the participating laboratories were asked to submit no more than a maximum number of isolates each year. While this maximum number changed slightly during the 10-year period, the number was always ≤ 40 isolates of each bacterial species per laboratory per year. Starting in 2003, Pfizer Animal Health requested that the participating laboratories send no more than one isolate of each bacterial species from a herd each quarter-year to reduce the risk of over-representation of clones from local outbreaks. The total number of isolates recovered each year by each of the laboratories was not provided to Pfizer Animal Health.

Isolates were identified to the genus and species level by the submitting laboratory before shipment to the Pfizer Animal Health laboratory in Kalamazoo, Michigan. Standard biochemical tests and commercially available identification systems (API Microbial Identification Kits; bioMérieux, Durham, North Carolina, and Biolog Microbial Identification System; Biolog Systems, Hayward, California) were used to confirm or further characterize the isolates when necessary. All isolates were stored in 1.0 mL trypticase soy broth (BD Biosciences/Diagnostics, Sparks, Maryland) supplemented with 10% glycerol and were held at approximately -70°C until tested.

Minimal inhibitory concentration determinations

Over the 10 years of the survey, all minimal inhibitory concentration (MIC) determinations were conducted by two laboratories (Pfizer Animal Health, Kalamazoo, Michigan, and Microbial Research Inc, Fort Collins, Colorado) to minimize potential testing bias.[9,10] Both laboratories adhered to Clinical and Laboratory Standards Institute (CLSI) standardized methods and quality control during susceptibility testing (Table 2). Minimal inhibitory concentrations (MICs) for all isolates were determined using a dehydrated broth microdilution system (Sensititre Division, Trek Diagnostic Systems, Inc, Cleveland, Ohio). This method conforms to the standards of the CLSI for testing veterinary pathogens.[11] Direct colony suspensions were used when testing all organisms, and suspensions were prepared to yield a final bacterial concentration of approximately 5×10^5 colony forming units (CFU) per mL. The custom 96-well microtiter panels initially included serial doubling dilutions of the following antimicrobial agents: ceftiofur, enrofloxacin, florfenicol, penicillin, tetracycline, and tilmicosin. Enrofloxacin was not tested between 2004 and 2007, and when it was included in the panel again in 2008, the lowest concentration range was decreased from 0.03 μg per mL to 0.004 μg per mL to better accommodate quality-control ranges for this drug. Tulathromycin was added to the panel in 2004, prior to its approval by the US Food and Drug Administration (FDA) in 2005 for treatment of infections due to swine respiratory disease- (SRD-) associated pathogens, including *A pleuropneumoniae* and *P multocida*. Concentration ranges for each antimicrobial agent were chosen to encompass appropriate quality-control ranges and applicable clinical break points when available. In 2008, the range of tetracycline concentrations was altered to accommodate additional antimicrobial agents in the 96-well microtiter plates.

In 2008, the CLSI formally issued a clarification on the methodology for susceptibility testing of veterinary streptococci.[11] They recommended that the inoculation medium for MIC testing of streptococci be a cation-adjusted Mueller-Hinton broth (CAMHB)

Table 1: Origin and number of bacterial isolates per year by region for a 10-year study of antimicrobial susceptibility of three respiratory disease pathogens from pigs in the United States and Canada*

Region	Year										
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Actinobacillus pleuropneumoniae											
Canada	0	0	0	4	14	6	31	15	4	6	80
East	24	12	12	2	5	14	4	15	11	6	105
Mid-central	59	90	113	105	87	109	56	47	47	50	763
Plains	6	30	9	9	7	11	11	10	6	9	108
West	0	0	20	17	0	2	1	0	0	1	41
Total	**89**	**132**	**154**	**137**	**113**	**142**	**103**	**87**	**68**	**72**	**1097**
Pasteurella multocida											
Canada	0	18	15	35	35	30	108	59	40	37	377
East	28	22	18	10	30	47	45	28	32	12	272
Mid-central	111	97	155	146	156	182	161	114	103	106	1331
Plains	41	23	1	21	35	53	52	34	26	32	318
West	6	8	11	20	13	5	8	6	8	6	91
Total	**186**	**168**	**200**	**232**	**269**	**317**	**374**	**241**	**209**	**193**	**2389**
Streptococcus suis											
Canada	22	19	11	40	40	32	107	69	49	57	446
East	22	25	30	10	26	47	38	35	37	16	286
Mid-central	80	98	144	143	178	193	180	147	127	121	1411
Plains	40	37	4	32	59	53	50	45	41	56	417
West	3	4	3	6	9	10	5	8	5	4	57
Total	**167**	**183**	**192**	**231**	**312**	**335**	**380**	**304**	**259**	**254**	**2617**

* Provinces and states or territories that submitted isolates originating from within the regions included Canada: Alberta, British Columbia, Manitoba, Ontario, Quebec, Saskatchewan; East: Alabama, Arkansas, Kentucky, Maryland, Mississippi, New York, North Carolina, Pennsylvania, Puerto Rico, South Carolina, Virginia; Mid-central: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, Wisconsin; Plains: Kansas, Nebraska, North Dakota, Oklahoma, South Dakota, Texas; and West: California, Colorado, Hawaii, Mexico, Montana, Utah, Washington, Wyoming.

that contained 2.5% to 5% lysed horse blood. Previous testing of streptococci in our laboratory included use of CAMHB without lysed horse blood. To determine the effect of this change, the *S suis* strains isolated in 2007 were retested in 2008 using both types of broth, and the results were compared. The MIC_{50} and MIC_{90} values for florfenicol, penicillin, tetracycline, tilmicosin, and tulathromycin against *S suis* were the same using either method. However, with the addition of lysed horse blood in the medium, the ceftiofur MIC_{50} increased from ≤ 0.03 to 0.06 µg per mL, and the MIC_{90} increased from 0.12 to 1.0 µg per mL. The MIC results reported here for the *S suis* isolated from 2007 to 2010 were tested under the updated (2008) recommendations.

Results

Actinobacillus pleuropneumoniae

Table 3 shows the MIC distributions for the seven antimicrobial drugs tested against *A pleuropneumoniae,* along with the MIC_{50} and MIC_{90} values. All isolates tested each year showed MIC values that were less than the CLSI break points for susceptibility to ceftiofur (MIC ≤ 2 µg per mL) and to florfenicol (MIC ≤ 2 µg per mL). There was an increase in the ceftiofur MIC_{90} for *A pleuropneumoniae* in 2005, but this was not seen in subsequent years. Penicillin susceptibility did not change substantially over the 10 years. The penicillin MIC_{50} values were either 0.5 or 1 µg per mL for each year of the study, and the MIC_{90} values were all ≥ 32 µg per mL, except for 2010, when the MIC_{90} was 8 µg per mL. In 2003 and 2008, 0.6% and 3.5% of *A pleuropneumoniae* isolates, respectively, had enrofloxacin MICs that were higher than the CLSI-approved (but not yet published) susceptible break point (MIC ≤ 0.25 µg per mL), but no isolates with an MIC greater than the susceptible break point were detected in the other 4 years in which enrofloxacin was included in the testing panel. The susceptible break point for tetracycline is ≤ 0.5 µg per mL, and ≥ 92% of the *A pleuropneumoniae* isolates were above this, with MIC_{50} values > 8 µg per mL in each year of the survey. The number of *A pleuropneumoniae* isolates with tilmicosin MIC values greater than the susceptible break point (MIC ≤ 16 µg per mL) varied during the 10-year surveillance period, with all isolates having MIC

Table 2: Summary of methods used for determining minimal inhibitory concentrations for seven antimicrobial agents against three swine respiratory pathogens*

Organism	Medium	Incubation	Quality-control organism(s)
Actinobacillus pleuropneumoniae	VFM	20-24 hours; 5% ± 2% CO_2; 35°C ± 2°C	*A pleuropneumoniae* ATCC 27090
Pasteurella multocida, *Streptococcus suis* (2001 to 2006)	CAMHB	18-24 hours; ambient air; 35°C ± 2°C	*Staphylococcus aureus* ATCC 29213 *Enterococcus faecalis* ATCC 29212 *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853
S suis (2007 to 2010)	CAMHB + 2.5%-5% LHB	20-24 hours; ambient air; 35°C ± 2°C	*Streptococcus pneumoniae ATCC 49619*

* Sources of pathogens reported in Table 1. Minimal inhibitory concentrations were determined using CLSI published methods (CLSI M31-A3).[11]

VFM = veterinary fastidious medium; CAMHB = cation-adjusted Mueller-Hinton broth; LHB = lysed horse blood.

values below the susceptible break point in 2001, and 10% to 15% testing above the susceptible break point the last 2 years of testing. All isolates had tulathromycin MIC values ≤ 64 µg per mL, the CLSI-approved (but not yet published) susceptible break point. The MIC_{50} and MIC_{90} values for both tilmicosin and tulathromycin increased over the surveillance period.

Pasteurella multocida

Table 4 shows the MIC distribution frequencies of *P multocida* isolates collected between 2001 and 2010 from across the United States and Canada. All *P multocida* isolates tested each year remained susceptible to ceftiofur. Penicillin was active against *P multocida*: > 95% of the isolates tested between 2001 and 2010 had MICs ≤ 0.25 µg per mL. The enrofloxacin MIC_{50} value changed from ≤ 0.03 µg per mL to 0.015 µg per mL between 2001 and 2008, but this might reflect the lower concentrations tested from 2008 to 2010. The enrofloxacin MIC_{90} values remained ≤ 0.03 µg per mL between the two testing periods, and the proportion of isolates that had MICs that were equal to or less than the CLSI break point for enrofloxacin susceptibility (MIC ≤ 0.25 µg per mL) also remained consistent, at or near 100%.

With the exception of the first year of the survey (2001), all florfenicol MIC_{50} and MIC_{90} values remained at 0.5 µg per mL over the years of this study. The proportion of *P multocida* isolates that were susceptible to florfenicol by CLSI standards remained at or near 100% across all years. Less than 47% of the *P multocida* isolates were susceptible to tetracycline in each year of the survey (MIC ≤ 0.5 µg per mL). *Pasteurella multocida* showed high levels of susceptibility to the macrolides tilmicosin and tulathromycin, although against both drugs, MIC_{50} and MIC_{90} values increased with time. The number of *P multocida* isolates with tilmicosin MIC values greater than the susceptible break point (MIC ≤ 16 µg per mL) was very low from 2001 to 2010, ranging from 0% in 2003 to approximately 6% above the susceptible break point the last 2 years of testing. The proportion of isolates that were susceptible to tulathromycin, according to CLSI break points, did not change substantially over time: 100% of isolates in most years were categorized as susceptible.

Streptococcus suis

As a consequence of the change in MIC testing methodology for *S suis* in 2007, an increase in ceftiofur MIC_{50} and MIC_{90} values between 2006 and 2007 is evident (Table 5). For each method of testing, ceftiofur MIC_{50} values remained consistent and MIC_{90} values indicate small fluctuations. The penicillin MIC_{50} values did not change over the 10 years of the survey, although the MIC_{90} values increased from 0.25 to 1 µg per mL after the change in testing method. The proportion of enrofloxacin MIC values among the *S suis* that were susceptible (MIC ≤ 0.5 µg per mL) during 2008 to 2010 declined slightly from the 2001 to 2003 testing period. Susceptibility to florfenicol remained high, with > 97% of isolates each year susceptible to this drug. Conversely, < 4% of *S suis* isolates each year were susceptible to tetracycline. Tilmicosin and tulathromycin are not indicated for *S suis,* and therefore there are no CLSI-approved break points for these two macrolides against *S suis.* The data indicate that there was very little antimicrobial activity of these drugs against this organism, with MIC_{50} and MIC_{90} values > 64 µg per mL during all years of this study.

Discussion

The relatively high prevalence of the three potentially serious pathogens in swine herds in the United States and Canada[8,12-14] and the need to treat and control further infection in litter and pen mates indicates the importance of high levels of susceptibility to the antimicrobial drugs that are available to veterinarians. However, as the FDA and the American Veterinary Medical Association have noted, there is no publicly funded nationwide monitoring of antimicrobial susceptibility among swine pathogens in the United States.[15] Only a few countries conduct nationwide surveys of swine pathogens, and most of these surveys focus upon zoonotic bacteria such as *Salmonella* and *Escherichia coli*. Systematic surveillance of porcine respiratory pathogens conducted annually or at regular time intervals are even scarcer. Germany has a national program, GERM-Vet,[7,16] which examines the antimicrobial susceptibility of *A pleuropneumoniae, P multocida,* and *S suis* isolated from swine in Germany, and results of recent testing have been published.[17]

Table 3: Minimal inhibitory concentration (MIC) summary values and frequency distributions for seven antimicrobial agents tested against *Actinobacillus pleuropneumoniae* isolated from swine and submitted to Pfizer Animal Health by veterinary diagnostic laboratories located in the United States and Canada from 2001 to 2010*

Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Ceftiofur MIC frequency distribution (% of isolates)†										
					0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	> 16
2001	89	≤ 0.03	≤ 0.03	100.0	97.8	2.2	0	0	0	0	0	0	0	0	0
2002	132	≤ 0.03	≤ 0.03	100.0	94.7	3.8	1.5	0	0	0	0	0	0	0	0
2003	154	≤ 0.03	≤ 0.03	100.0	94.8	5.2	0	0	0	0	0	0	0	0	0
2004	137	≤ 0.03	≤ 0.03	100.0	95.6	4.4	0	0	0	0	0	0	0	0	0
2005	113	≤ 0.03	0.06	100.0	64.6	29.2	6.2	0	0	0	0	0	0	0	0
2006	142	≤ 0.03	≤ 0.03	100.0	93.0	6.3	0.7	0	0	0	0	0	0	0	0
2007	103	≤ 0.03	≤ 0.03	100.0	91.3	8.7	0	0	0	0	0	0	0	0	0
2008	87	≤ 0.03	≤ 0.03	100.0	97.7	1.1	1.1	0	0	0	0	0	0	0	0
2009	68	≤ 0.03	≤ 0.03	100.0	97.1	2.9	0	0	0	0	0	0	0	0	0
2010	72	≤ 0.03	≤ 0.03	100.0	100.0	0	0	0	0	0	0	0	0	0	0
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Penicillin MIC frequency distribution (% of isolates)†**										
					0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
2001	89	0.5	32	NA	9.0	36.0	28.1	6.7	0	0	1.1	3.4	12.4	3.4	0
2002	132	0.5	> 64	NA	2.3	26.5	43.9	3.0	0	0	1.5	1.5	0	7.6	13.6
2003	154	1	> 64	NA	0.0	11.0	35.7	14.9	0.6	0.6	1.3	2.6	2.6	3.9	26.6
2004	137	0.5	> 64	NA	1.5	9.5	42.3	19.7	0	0	0.7	0	0.7	0	25.5
2005	113	1	> 64	NA	1.8	4.4	26.5	26.5	2.7	0	0.9	3.5	0	0	33.6
2006	142	0.5	> 64	NA	7.7	19.7	34.5	7.7	0.7	0.7	0.7	1.4	2.1	3.5	21.1
2007	103	0.5	> 64	NA	13.6	33.0	23.3	4.9	1.0	0	1.9	1.9	1.0	1.9	17.5
2008	87	1	> 64	NA	1.1	10.3	36.8	18.4	2.3	1.1	0	0	11.5	6.9	11.5
2009	68	1	64	NA	2.9	8.8	29.4	36.8	0	1.5	0	0	8.8	2.9	8.8
2010	72	0.5	8	NA	4.2	4.2	62.5	18.1	0	0	1.4	0	0	8.3	1.4
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Enrofloxacin MIC frequency distribution (% of isolates)†**										
					0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	> 2
2001	89	≤ 0.03	≤ 0.03	100.0	NT	NT	NT	96.6	3.4	0	0	0	0	0	0
2002	132	≤ 0.03	≤ 0.03	100.0	NT	NT	NT	99.2	0.8	0	0	0	0	0	0
2003	154	≤ 0.03	0.06	99.4	NT	NT	NT	53.9	40.9	4.5	0	0.6	0	0	0
2004-2007	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
2008	87	0.06	0.06	96.5	0	1.1	2.3	25.3	63.2	4.6	0	0	2.3	1.1	0
2009	68	0.06	0.06	100.0	0	0	0	33.8	57.4	8.8	0	0	0	0	0
2010	72	0.06	0.06	100.0	0	0	0	18.1	73.6	8.3	0	0	0	0	0
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Florfenicol MIC frequency distribution (% of isolates)†**										
					0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
2001	89	0.25	0.5	100.0	1.1	0	70.8	28.1	0	0	0	0	0	0	0
2002	132	0.5	0.5	100.0	0	0	45.5	54.5	0	0	0	0	0	0	0
2003	154	0.25	0.5	100.0	0.6	0.6	50.0	47.4	1.3	0	0	0	0	0	0
2004	137	0.25	0.5	100.0	0	0.7	61.3	38.0	0	0	0	0	0	0	0
2005	113	0.25	0.5	100.0	0	0	69.0	29.2	0	1.8	0	0	0	0	0
2006	142	0.25	0.5	100.0	0	0.7	63.4	35.2	0.7	0	0	0	0	0	0
2007	103	0.25	0.5	100.0	0	1.0	58.3	40.8	0	0	0	0	0	0	0
2008	87	0.25	0.5	100.0	1.1	1.1	49.4	47.1	1.1	0	0	0	0	0	0

Table 3 continued

2009	68	0.5	0.5	100.0	0	0	33.8	64.7	1.5	0	0	0	0	0	0
2010	72	0.5	0.5	100.0	0	0	33.3	66.7	0	0	0	0	0	0	0
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tetracycline MIC frequency distribution (% of isolates)†‡**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**> highest conc‡**	
2001	89	16	32	5.6	NT	1.1	4.5	1.1	0	13.5	15.7	32.6	28.1	3.4	
2002	132	16	> 32	3.0	NT	0	3.0	0.8	0.8	9.1	21.2	28.8	25.0	11.4	
2003	154	16	> 32	3.2	NT	0	3.2	1.9	1.9	3.2	26.6	22.1	28.6	12.3	
2004	137	16	> 32	2.2	NT	0	2.2	6.6	1.5	0.7	30.7	16.1	30.7	11.7	
2005	113	16	32	1.8	NT	0	1.8	11.5	0	0.9	23.0	13.3	45.1	4.4	
2006	142	16	32	0.7	NT	0.7	0	11.3	0	0.7	24.6	15.5	39.4	7.7	
2007	103	16	32	1.0	NT	0	1.0	10.7	1.0	0	26.2	18.4	35.0	7.8	
2008	87	> 8	> 8	3.4	0	1.1	2.3	9.2	2.3	1.1	20.7	63.2			
2009	68	> 8	> 8	7.4	0	0	7.4	5.9	0	0	23.5	63.2			
2010	72	> 8	> 8	4.2	0	0	4.2	20.8	0	0	18.1	56.9			
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tilmicosin MIC frequency distribution (% of isolates)†**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2001	89	2	4	100.0	1.1	0	0	3.4	76.4	19.1	0	0	0	0	0
2002	132	2	4	99.2	0	0	0	1.5	62.1	34.1	0.8	0.8	0	0	0.8
2003	154	8	16	99.4	0	0.6	0	2.6	24.0	4.5	37.0	30.5	0	0	0.6
2004	137	8	8	98.6	0	0	0.7	0	0.7	19.0	70.1	8.0	0.7	0.7	0
2005	113	16	16	91.1	0	0	0	0.9	0	0.9	23.9	65.5	8.0	0.9	0
2006	142	16	16	98.6	0.7	0	0	0	0	0.7	43.0	54.2	0.7	0	0.7
2007	103	16	16	96.1	0	0	0	0	0	1.0	31.1	64.1	3.9	0	0
2008	87	16	16	93.2	0	0	0	2.3	0	2.3	5.7	82.8	5.7	1.1	0
2009	68	16	32	83.8	0	0	0	0	0	0	11.8	72.1	14.7	0	1.5
2010	72	16	16	90.3	0	0	0	0	0	0	4.2	86.1	9.7	0	0
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tulathromycin MIC frequency distribution (% of isolates)†§**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2004	137	8	8	100.0	0	0	0.7	0	0.7	22.6	67.2	8.8	0	0	0
2005	113	32	64	100.0	0	0	0	0.9	0	0	0	1.8	50.4	46.9	0
2006	142	16	16	100.0	0	0	0.7	0	0	0	8.5	81.0	9.9	0	0
2007	103	32	32	100.0	0	0	0	0	0	0	5.8	39.8	51.5	2.9	0
2008	87	32	64	100.0	0	0	0	0	1.1	0	0	2.3	66.7	29.9	0
2009	68	32	64	100.0	0	0	0	0	0	0	0	2.9	47.1	50.0	0
2010	72	64	64	100.0	0	0	0	0	0	0	0	0	19.4	80.6	0

* Sources of pathogens reported in Table 1. Minimal inhibitory concentrations were determined using CLSI published methods (CLSI M31-A3).[11] Bold vertical lines indicate the CLSI approved final or tentative break points for susceptibility and resistance in SRD pathogens. Unshaded areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC values ≤ the lowest concentration in the range.

† MIC frequency may not add up to 100% due to rounding.

‡ Highest concentration of tetracycline tested was 32 µg/mL from 2001 to 2007 and 8 µg/mL from 2008 to 2010.

§ Tulathromycin was not included in the testing panels between 2001 and 2003.

MIC_{50} = lowest MIC at which 50% of the isolates are inhibited; MIC_{90} = lowest MIC at which 90% of the isolates are inhibited; %S = percentage of isolates interpreted as susceptible; CLSI = Clinical and Laboratory Standards Institute; SRD = swine respiratory disease; NT = not tested at this antimicrobial concentration; NA = not applicable (no veterinary-specific break points approved by CLSI).

Table 4: Minimal inhibitory concentration (MIC) summary values and frequency distributions for seven antimicrobial agents tested against *Pasteurella multocida* isolated from swine and submitted to Pfizer Animal Health by veterinary diagnostic laboratories located in the United States and Canada from 2001 to 2010*

Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Ceftiofur MIC frequency distribution (% of isolates)†										
					0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	> 16
2001	186	≤ 0.03	≤ 0.03	100.0	97.8	1.1	1.1	0	0	0	0	0	0	0	0
2002	168	≤ 0.03	≤ 0.03	100.0	98.8	0.6	0.6	0	0	0	0	0	0	0	0
2003	200	≤ 0.03	≤ 0.03	100.0	99.5	0	0.5	0	0	0	0	0	0	0	0
2004	232	≤ 0.03	≤ 0.03	100.0	98.3	1.7	0	0	0	0	0	0	0	0	0
2005	269	≤ 0.03	≤ 0.03	100.0	99.3	0.7	0	0	0	0	0	0	0	0	0
2006	317	≤ 0.03	≤ 0.03	100.0	99.7	0.3	0	0	0	0	0	0	0	0	0
2007	374	≤ 0.03	≤ 0.03	100.0	98.7	1.1	0.3	0	0	0	0	0	0	0	0
2008	241	≤ 0.03	≤ 0.03	100.0	92.1	5.0	2.9	0	0	0	0	0	0	0	0
2009	209	≤ 0.03	≤ 0.03	100.0	97.6	1.9	0.5	0	0	0	0	0	0	0	0
2010	193	≤ 0.03	≤ 0.03	100.0	99.5	0.5	0	0	0	0	0	0	0	0	0
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Penicillin MIC frequency distribution (% of isolates)†										
					0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
2001	186	≤ 0.12	≤ 0.12	NA	91.4	3.8	1.6	0	0.5	0	1.6	0	0	0.5	0.5
2002	168	≤ 0.12	0.25	NA	89.3	8.9	0.6	0	0	0	0	0	1.2	0	0
2003	200	≤ 0.12	0.25	NA	63.0	36.0	1.0	0	0	0	0	0	0	0	0
2004	232	≤ 0.12	0.25	NA	87.9	9.5	0.4	0	0	0	0	0	0.9	0	1.3
2005	269	≤ 0.12	≤ 0.12	NA	92.9	4.8	0	0	0	0	0.4	0	0.7	1.1	0
2006	317	≤ 0.12	≤ 0.12	NA	94.3	4.4	0	0	0	0.3	0.6	0	0	0	0.3
2007	374	≤ 0.12	≤ 0.12	NA	93.6	2.7	0.5	0	1.3	0.3	1.1	0.5	0	0	0
2008	241	≤ 0.12	≤ 0.12	NA	92.5	5.0	0.4	0	0	0.4	0	0	0.8	0.4	0.4
2009	209	≤ 0.12	≤ 0.12	NA	94.7	3.3	0	0	0	0	0	0	0	0	1.9
2010	193	≤ 0.12	≤ 0.12	NA	96.9	1.6	0	0	0	0	0	0	0.5	0	1.0
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Enrofloxacin MIC frequency distribution (% of isolates)†										
					0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	> .2
2001	186	≤ 0.03	≤ 0.03	99.5	NT	NT	NT	97.3	1.1	0	1.1	0.5	0	0	0
2002	168	≤ 0.03	≤ 0.03	98.8	NT	NT	NT	97.6	1.2	0	0	1.2	0	0	0
2003	200	≤ 0.03	≤ 0.03	100.0	NT	NT	NT	99.0	0.5	0.5	0	0	0	0	0
2004-2007	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
2008	241	0.015	0.03	100.0	1.2	19.9	55.2	18.7	5.0	0	0	0	0	0	0
2009	209	0.015	0.03	100.0	0	3.3	64.1	25.8	5.7	0.5	0.5	0	0	0	0
2010	193	0.015	0.03	100.0	0	8.3	67.4	21.8	2.1	0	0.5	0	0	0	0
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Florfenicol MIC frequency distribution (% of isolates)†										
					0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
2001	186	0.25	0.5	100.0	1.1	0	53.8	41.9	2.7	0	0.5	0	0	0	0
2002	168	0.5	0.5	100.0	0	1.2	45.8	51.2	1.8	0	0	0	0	0	0
2003	200	0.5	0.5	100.0	0	0	26.5	73.5	0	0	0	0	0	0	0
2004	232	0.5	0.5	100.0	0	0	21.1	77.6	0.9	0.4	0	0	0	0	0
2005	269	0.5	0.5	99.6	0	0	44.2	53.9	1.5	0	0	0	0	0.4	0
2006	317	0.5	0.5	100.0	0.3	0	21.8	77.3	0.6	0	0	0	0	0	0
2007	374	0.5	0.5	99.7	0	0	20.3	78.1	0.8	0.5	0.3	0	0	0	0
2008	241	0.5	0.5	99.2	0	0	5.4	90.5	3.3	0	0	0	0.8	0	0

Table 4 continued

2009	209	0.5	0.5	99.5	0	0	1.4	91.4	6.7	0	0.5	0	0	0	0
2010	193	0.5	0.5	100.0	0	0	2.1	96.9	1.0	0	0	0	0	0	0
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tetracycline MIC frequency distribution (% of isolates)†‡**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**> highest conc‡**	
2001	186	2	32	27.4	NT	11.3	16.1	16.7	26.3	4.3	5.4	7.5	10.2	2.2	
2002	168	2	16	34.0	NT	6.0	28.0	11.3	32.1	2.4	8.9	4.2	6.5	0.6	
2003	200	2	16	32.5	NT	5.5	27.0	11.5	29.5	2.5	8.5	7.5	5.5	2.5	
2004	232	2	16	42.3	NT	6.5	35.8	5.2	28.4	3.9	4.7	6.9	6.5	2.2	
2005	269	1	32	36.4	NT	11.9	24.5	17.8	17.1	2.2	7.8	5.2	10.8	2.6	
2006	317	2	16	33.1	NT	6.3	26.8	10.7	28.7	3.5	8.2	6.9	6.6	2.2	
2007	374	1	16	46.5	NT	3.5	43.0	4.0	28.3	1.6	5.9	6.4	5.9	1.3	
2008	241	2	>8	29.8	0.8	0.8	28.2	14.1	30.7	7.1	1.7	16.6			
2009	209	2	>8	13.4	0	0	13.4	30.6	25.8	12.4	1.9	15.8			
2010	193	2	>8	28.5	0	0	28.5	15.5	30.1	6.2	3.1	16.6			
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tilmicosin MIC frequency distribution (% of isolates)†**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2001	186	4	8	97.3	0.5	0	0	2.2	21.5	42.5	26.3	4.3	0.5	1.1	1.1
2002	168	4	16	99.4	0	0	0.6	4.8	17.3	36.3	27.4	13.1	0	0.6	0
2003	200	4	8	100.0	0	0	0	1.5	19.0	43.0	31.0	5.5	0	0	0
2004	232	4	8	99.6	1.3	0	0.4	4.7	20.7	37.9	29.7	4.7	0	0.4	0
2005	269	4	8	98.6	0	0	0.4	3.7	13.4	33.1	43.9	4.1	0	0.7	0.7
2006	317	8	8	100.0	0	0	0.3	0.9	14.2	33.8	42.3	8.5	0	0	0
2007	374	8	16	98.1	0	0	0	0.5	10.4	30.5	43.6	13.1	0.5	1.1	0.3
2008	241	8	16	98.4	0	0	0	1.2	10.4	30.7	35.7	20.3	0.8	0.4	0.4
2009	209	8	16	93.7	0	0	0	1.0	3.8	20.6	27.3	41.1	5.3	1.0	0
2010	193	8	16	94.4	0	0	0.5	0.5	5.7	32.6	28.5	26.4	4.1	1.0	0.5
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tulathromycin MIC frequency distribution (% of isolates)†§**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2004	232	1	0.5	100.0	0	23.3	36.6	35.8	3.4	0.9	0	0	0	0	0
2005	269	2	1	99.6	0	3.3	26.4	48.7	20.1	0	1.1	0	0	0	0.4
2006	317	2	1	100.0	0	4.7	29.3	49.5	15.1	1.3	0	0	0	0	0
2007	374	2	2	99.7	0	0.3	7.5	41.2	43.3	7.0	0.3	0.3	0	0	0.3
2008	241	4	1	100.0	0	0.8	10.4	43.6	34.0	10.4	0.8	0	0	0	0
2009	209	4	2	100.0	0	0	2.4	17.2	38.8	37.3	3.3	1.0	0	0	0
2010	193	8	2	100.0	0	0	0	11.9	39.4	35.2	13.5	0	0	0	0

* Sources of pathogens reported in Table 1. Minimal inhibitory concentrations were determined using CLSI published methods (CLSI M31-A3).[11] Bold vertical lines indicate the CLSI approved final or tentative break points for susceptibility and resistance in SRD pathogens. Unshaded areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC values ≤ the lowest concentration in the range.

† MIC frequency may not add up to 100% due to rounding.

‡ Highest concentration of tetracycline tested was 32 µg/mL from 2001 to 2007 and 8 µg/mL from 2008 to 2010.

§ Tulathromycin was not included in the testing panels between 2001 and 2003.

MIC_{50} = lowest MIC at which 50% of the isolates are inhibited; MIC_{90} = lowest MIC at which 90% of the isolates are inhibited; %S = percentage of isolates interpreted as susceptible; CLSI = Clinical and Laboratory Standards Institute; SRD = swine respiratory disease; NT = not tested at this antimicrobial concentration; NA = not applicable (no veterinary-specific break points approved by CLSI).

Table 5: Minimal inhibitory concentration (MIC) summary values and frequency distributions for seven antimicrobial agents tested against *Streptococcus suis* from swine submitted to Pfizer Animal Health by veterinary diagnostic laboratories located in the United States and Canada from 2001 to 2010*

Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Ceftiofur MIC frequency distribution (% of isolates)†‡										
					0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	> 16
2001	167	≤ 0.03	0.06	99.4	85.0	9.6	2.4	0.6	0.6	0.6	0.6	0.6	0	0	0
2002	183	≤ 0.03	0.06	100	86.9	6.6	3.8	2.7	0	0	0	0	0	0	0
2003	192	≤ 0.03	0.06	100	84.9	6.8	4.2	2.6	1.0	0	0.5	0	0	0	0
2004	231	≤ 0.03	0.12	100	81.8	7.8	7.4	0.4	1.3	0.4	0.9	0	0	0	0
2005	312	≤ 0.03	0.06	99.7	83.7	7.1	4.2	2.9	1.3	0.3	0.3	0.3	0	0	0
2006	335	≤ 0.03	≤ 0.03	100	91.9	3.0	3.3	0.6	0.6	0	0.6	0	0	0	0
2007	380	0.06	1	98.1	8.7	49.7	16.6	6.3	5.0	8.2	3.7	1.6	0.3	0	0
2008	304	0.06	1	96.7	3.3	49.0	19.1	9.2	5.9	6.6	3.6	2.0	1.0	0.3	0
2009	259	0.06	1	95.7	9.3	42.5	22.4	6.6	7.3	5.8	1.9	2.7	0.8	0.8	0
2010	254	0.06	1	98.0	8.7	48.4	18.9	4.7	5.9	5.1	6.3	0	1.2	0.4	0.4
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Penicillin MIC frequency distribution (% of isolates)†‡										
					0.12	0.25	0.5	1	2	4	8	16	32	64	> 64
2001	167	≤ 0.12	0.25	NA	86.8	3.6	3.0	2.4	1.8	1.2	0	0.6	0.6	0	0
2002	183	≤ 0.12	0.25	NA	88.5	1.6	3.8	0.5	2.2	1.6	1.6	0	0	0	0
2003	192	≤ 0.12	0.5	NA	83.9	3.1	4.2	2.1	3.1	1.6	0.5	0.5	0	0.5	0.5
2004	231	≤ 0.12	0.5	NA	83.5	3.5	5.6	3.0	0.4	2.2	0.9	0.4	0.4	0	0
2005	312	≤ 0.12	0.5	NA	83.7	4.5	3.2	4.2	1.9	1.0	1.3	0.3	0	0	0
2006	335	≤ 0.12	0.25	NA	88.1	4.2	2.1	2.7	1.8	0.9	0.3	0	0	0	0
2007	380	≤ 0.12	1	NA	81.8	3.4	3.4	4.5	5.0	1.3	0.5	0	0	0	0
2008	304	≤ 0.12	1	NA	72.4	8.2	7.2	4.9	3.9	2.0	1.3	0	0	0	0
2009	259	≤ 0.12	1	NA	80.7	4.2	3.9	5.4	4.6	0.8	0.4	0	0	0	0
2010	254	≤ 0.12	1	NA	83.9	2.4	3.5	3.5	4.3	1.2	1.2	0	0	0	0
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Enrofloxacin MIC frequency distribution (% of isolates)†‡										
					0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	> 2
2001	167	0.25	0.5	98.2	NT	NT	NT	3.0	3.0	9.6	49.1	33.5	1.8	0	0
2002	183	0.25	0.5	99.0	NT	NT	NT	4.4	3.3	15.3	56.8	19.1	0.5	0.5	0
2003	192	0.25	0.5	99.5	NT	NT	NT	0.5	0.5	13.5	58.9	26.0	0.5	0	0
2004-2007	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
2008	304	0.25	0.5	96.7	0.3	0	0	0	1.3	6.9	58.9	29.3	2.6	0.7	0
2009	259	0.5	1	78.4	0	0	0	0	0	0.8	12.0	64.9	20.1	1.5	0.8
2010	254	0.5	0.5	93.7	0	0	0	0	0	3.1	29.5	61.0	5.5	0.8	0
Year	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	%S	Florfenicol MIC frequency distribution (% of isolates)†‡										
					0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
2001	167	1	2	98.8	0	1.8	1.8	8.4	52.1	34.7	1.2	0	0	0	0
2002	183	1	2	98.9	1.1	0	2.2	12.6	61.2	21.9	1.1	0	0	0	0
2003	192	1	2	99.5	0.5	0	0	5.7	55.7	37.5	0.5	0	0	0	0
2004	231	1	2	100	0	0.4	3.0	4.8	45.0	46.8	0	0	0	0	0
2005	312	1	2	99.7	0.3	0.3	1.3	5.4	70.2	22.1	0.3	0	0	0	0
2006	335	1	2	99.4	2.1	1.2	2.1	10.7	48.4	34.9	0.3	0.3	0	0	0
2007	380	2	2	98.2	0	0	0	0	18.4	79.7	1.8	0	0	0	0
2008	304	2	2	99.7	0	0	0	4.3	43.8	51.6	0.3	0	0	0	0

Table 5 continued

2009	259	2	2	97.3	0	0	0.4	0.4	28.6	68.0	2.7	0	0	0	0
2010	254	2	2	98.0	0	0	0	1.2	31.9	65.0	1.6	0	0	0	0.4
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tetracycline MIC frequency distribution (% of isolates)†‡**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**> highest conc§**	
2001	167	>32	>32	0.6	NT	0.6	0	0	0	0.6	1.2	1.8	28.7	67.1	
2002	183	>32	>32	2.2	NT	1.1	1.1	1.6	1.6	1.6	1.1	3.8	25.1	62.8	
2003	192	>32	>32	0.5	NT	0	0.5	1.0	2.1	0.5	0.5	2.1	26.6	66.7	
2004	231	>32	>32	1.3	NT	0.4	0.9	1.7	1.7	0.9	0.4	6.1	26.4	61.5	
2005	312	>32	>32	1.6	NT	0.3	1.3	1.0	0.6	0.6	0.3	2.6	34.6	58.7	
2006	335	>32	>32	3.3	NT	2.7	0.6	0.9	0.9	0.9	0.9	1.5	28.7	63.0	
2007	380	>32	>32	1.1	NT	0.8	0.3	0	2.4	1.1	0.3	0	6.6	88.7	
2008	304	>8	>8	0.6	0.3	0.3	0	1.0	1.3	1.3	0.7	95.1			
2009	259	>8	>8	0.4	0	0	0.4	0.4	0.4	1.5	2.3	95.0			
2010	254	>8	>8	0.8	0	0	0.8	0.8	0.4	4.3	2.0	91.7			
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tilmicosin MIC frequency distribution (% of isolates)†‡**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2001	167	> 64	> 64	NA	1.2	0.6	0.6	1.8	7.8	5.4	0.6	0	1.8	1.2	79.0
2002	183	> 64	> 64	NA	3.3	3.3	1.6	4.9	3.8	3.8	1.1	1.6	0	1.1	75.4
2003	192	> 64	> 64	NA	0	0.5	1.0	3.1	9.9	5.2	0.5	0	0	0.5	79.2
2004	231	> 64	> 64	NA	2.2	0.9	0.4	1.7	13.0	5.2	0.4	0	1.3	0	74.9
2005	312	> 64	> 64	NA	0.6	0.3	1.6	2.2	7.7	7.1	0	0.3	0.6	0.6	78.8
2006	335	> 64	> 64	NA	1.8	0.9	3.3	3.3	7.8	7.2	1.5	0.3	0.6	0.3	73.1
2007	380	> 64	> 64	NA	0.8	0.3	0	0	0.3	11.8	7.1	0.3	0	0	79.5
2008	304	> 64	> 64	NA	0.7	0.3	0.3	0.3	0.7	9.5	6.3	1.0	0	0.3	80.6
2009	259	> 64	> 64	NA	0.4	0.8	0	5.0	16.2	1.5	0.8	0	0	0.4	74.9
2010	254	> 64	> 64	NA	0.8	0	0	0.4	3.5	13.8	0.4	0	0	0.4	80.7
Year	**n**	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**%S**	**Tulathromycin MIC frequency distribution (% of isolates)†‡¶**										
					0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**> 64**
2004	231	> 64	> 64	NA	2.2	2.2	2.2	8.7	8.7	0	2.2	3.5	3.0	2.6	64.9
2005	312	> 64	> 64	NA	0.6	1.0	2.2	3.2	6.7	4.8	1.9	3.5	1.6	4.8	69.6
2006	335	> 64	> 64	NA	1.2	1.2	4.2	5.4	9.3	3.6	2.7	1.8	3.9	6.6	60.3
2007	380	> 64	> 64	NA	0	0	0.3	3.2	8.2	7.6	0.5	0.8	0.5	3.4	75.5
2008	304	> 64	> 64	NA	0	0.3	2.0	1.3	4.9	9.9	0.7	0	2.3	4.6	74.0
2009	259	> 64	> 64	NA	2.3	5.8	10.4	4.6	0.4	1.5	1.9	3.1	4.2	8.1	57.5
2010	254	> 64	> 64	NA	0	0.8	2.4	6.3	8.7	0	0.8	0.4	2.0	1.6	77.2

* Sources of pathogens reported in Table 1. Minimal inhibitory concentrations were determined using CLSI published methods (CLSI M31-A3).[11] Bold vertical lines indicate the CLSI approved final or tentative break points for susceptibility and resistance in SRD pathogens. Unshaded areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC values ≤ the lowest concentration in the range.

† MIC frequency may not add up to 100% due to rounding.

‡ Method change described in Table 2.

§ Highest concentration of tetracycline tested was 32 µg/mL from 2001 to 2007 and 8 µg/mL from 2008 to 2010.

¶ Tulathromycin was not included in the testing panels between 2001 and 2003.

MIC = minimal inhibitory concentration; MIC_{50} = lowest MIC at which 50% of the isolates are inhibited; MIC_{90} = lowest MIC at which 90% of the isolates are inhibited; %S = percentage of isolates interpreted as susceptible; NT = not tested at this antimicrobial concentration; NA = not applicable (no veterinary-specific breakpoints approved by CLSI).

This report seeks to provide a picture of the antimicrobial susceptibility of a convenience sample of SRD pathogens isolated from swine across the United States and Canada over the period 2001 to 2010. This program was not designed to estimate the prevalence of resistant porcine pathogens and indeed, denominator data, which would be required for estimating the size of the population being sampled, was not available. Instead, the program monitors changes in in vitro susceptibility among representative samples of identified pathogens and provides a warning system for the emergence of resistance, a feature of other antimicrobial susceptibility surveillance programs.[7] The program is ongoing and continues to collect SRD bacteria from across North America and test them for antimicrobial susceptibility.

As Schwarz et al[18] have stated, there are many important reasons for presenting MIC frequencies in surveillance data in the format that is used in this report. These include permitting comparisons between MIC datasets in which different break points or epidemiological cut-off points are used, or even where no break points have been established. Publishing this data also allows for observation of MIC shifts that are not reflected in calculated values such as MIC_{90} or percentages susceptible and resistant. While it added substantially to the length of this report, we believe that there is value in including the MIC frequency distributions and that these provide more details of the dynamics of antimicrobial susceptibility changes among swine respiratory pathogens than would be available if just the summarized values were included.

Antimicrobial resistance surveillance programs are subject to a number of limitations, including sampling bias.[9,10,19] In a study of published antimicrobial resistance surveillance studies,[20] it was concluded that sampling bias and failure to address the potential bias introduced by isolates from a common outbreak are frequent, but that case definition and laboratory practices and procedures may also influence the validity of the results. In the current study, the sampling strategy changed in 2003, when the number of isolates of a target species from any herd was restricted to one isolate during any quarter. The impact of this change has not been determined, but the data from 2003 and all years following were from isolates collected using this restriction. The number of isolates submitted by each laboratory was different each year, and not all of the participating laboratories submitted isolates every year. Additionally, testing methods have changed for streptococci.

Bias due to laboratory testing practices were minimized by using only two laboratories to conduct MIC testing, and both adhered to standard microbiological methods for susceptibility testing and quality-control standards. The data in this program came from over 6000 clinical swine isolates, and while this is a substantial number, it is only a small representative sample of the SRD pathogen population in the United States and Canada. To increase the likelihood that our sample was representative, veterinary diagnostic laboratories from across the major pork-producing areas of the United States and Canada provided isolates for this program. The isolates may have been collected and sent to state or provincial laboratories only after treatment with antimicrobial drugs had failed, and so the isolates in this study may reflect a more resistant bacterial population compared to isolates collected from animals without previous antimicrobial treatment or where treatments were successful, resulting in additional selection bias.

This report provides the first extensive survey of the antimicrobial susceptibility of major SRD pathogens isolated from swine across the United States and Canada during the years 2001 to 2010. The data show that, over those 10 years, *A pleuropneumoniae* and *P multocida* remained susceptible to ceftiofur, enrofloxacin, florfenicol, tilmicosin, and tulathromycin. *Streptococcus suis* remained susceptible to ceftiofur, enrofloxacin, and florfenicol. While the data show consistently low penicillin MIC values for *P multocida* and *S suis,* along with higher MIC values for *A pleuropneumoniae*, the CLSI has not approved interpretive criteria for penicillin against swine pathogens. The inoculating broth used for susceptibility testing of *S suis* was modified in 2007, during this study, and it is unknown if the increases in penicillin MIC_{90} values between 2008 and 2010 are related to this change. Most isolates of the three organisms were resistant to tetracycline, with little change in the MIC distributions over the 10 years of the survey.

The data presented in this report, especially those data that show that there has been some increase in MICs of important antimicrobial agents, should serve to underscore the importance of prudent use of these drugs when treating SRD (and other infections). Careful stewardship may allow for effective use of these drugs for many years. On-going surveillance of the in vitro susceptibility of these SRD pathogens will continue to be an important component in antimicrobial stewardship.

Implications

- Monitoring antimicrobial susceptibility among swine pathogens over time provides valuable information about changes which may be occurring in the antimicrobial susceptibility of these organisms. Having current susceptibility information is an important function in the maintenance of effective antimicrobial therapy.
- Surveillance of the in vitro susceptibility of SRD pathogens should continue as an important component in antimicrobial stewardship.

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References

1. Cromwell GL. Why and how antibiotics are used in swine production. *Anim Biotechnol.* 2002;13:7–27.

2. Mackinnon JD. The proper use and benefits of veterinary antimicrobial agents in swine practice. *Vet Microbiol.* 1993;35:357–367.

3. Hughes P, Heritage J. Antibiotic Growth-Promoters in Food Animals. 2002. Available at **http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/555_EN.HTM**. Accessed 15 October 2012.

4. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. *Clin Infect Dis.* 2002;34(suppl 3):S93-S106.

5. Prescott JF. Antimicrobial use in food and companion animals. *Animal Health Research Reviews / Conference of Research Workers in Animal Diseases.* 2008;92:127–133.

6. Aarestrup FM. Monitoring of antimicrobial resistance among food animals: principles and limitations. *J Vet Med B Infect Dis Vet Public Health.* 2004;51:380–388.

7. Wallmann J. Monitoring of antimicrobial resistance in pathogenic bacteria from livestock animals. *Int J Med Microbiol.* 2006;296(suppl 2):81–86.

8. MacInnes JI, Gottschalk M, Lone AG, Metcalf DS, Ojha S, Rosendal T, Watson SB, Friendship RM. Prevalence of *Actinobacillus pleuropneumoniae, Actinobacillus suis, Haemophilus parasuis, Pasteurella multocida* and *Streptococcus suis* in representative Ontario swine herds. *Can J Vet Res.* 2008;72:242–248.

9. Kahlmeter G, Brown DF. Resistance surveillance studies—comparability of results and quality assurance of methods. *J Antimicrob Chemother.* 2002;50:775–777.

10. Bax R, Bywater R, Cornaglia G, Goossens H, Hunter P, Isham V, Jarlier V, Jones R, Phillips I, Sahm D, Senn S, Struelens M, Taylor D, White A. Surveillance of antimicrobial resistance–what, how and whither? *Clin Microbiol Infect.* 2001;7:316–325.

11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard – Third Edition. M31-A3. Volume 28, Number 8. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2008. Available at: **http://www.clsi.org/source/orders/free/m31-a3.pdf**. Accessed 18 October 2012.

12. Marsteller TA, Fenwick B. *Actinobacillus pleuropneumoniae* disease and serology. *Swine Health Prod.* 1999;7:161–165.

13. USDA/APHIS. Swine 2006. Part II: Reference of swine health and health management practices in the United States, 2006. 2007. Available at: **http://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_dr_PartII.pdf**. Accessed 15 October 2012.

14. MacInnes JI, Desrosiers R. Agents of the "suiside diseases" of swine: *Actinobacillus suis, Haemophilus parasuis,* and *Streptococcus suis*. *Can J Vet Res.* 1999;63:83–89.

15. US FDA/CVM and AVMA. Judicious use of antimicrobials for swine veterinarians. 2002. Available at: **http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/UCM095578.pdf**. Accessed 15 October 2012.

16. Wallmann J, Schroter K, Wieler LH, Kroker R. National antibiotic resistance monitoring in veterinary pathogens from sick food-producing animals: the German programme and results from the 2001 pilot study. *Int J Antimicrob Agents.* 2003;22:420–428.

17. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. GERMAP 2008. Antibiotika-Resistenz und -Verbrauch [Antibiotic resistance and consumption]. 2008. Available at: **http://www.bvl.bund.de/SharedDocs/Downloads/08_PresseInfothek/Germap_2008.pdf?__blob=publicationFile&v=2**. Accessed 15 October 2012.

18. Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, Gaastra W. Assessing the antimicrobial susceptibility of bacteria obtained from animals. *Vet Microbiol.* 2010;141:1–4.

19. Rempel OR, Laupland KB. Surveillance for antimicrobial resistant organisms: potential sources and magnitude of bias. *Epidemiol Infect.* 2009;137:1–9.

20. Rempel O, Pitout JDD, Laupland KB. Antimicrobial resistance surveillance systems: Are potential biases taken into account? *Can J Infect Dis Med Microbiol.* 2011;22:e24–e28.