A Comparative Study between a Quantitative, Multi-class, Multi-residue Drug Analysis by LC-MS/MS and the Qualitative, Charm Kidney Inhibition Swab (KIS™) Test Supporting the Detection and Identification of Antimicrobial Drugs in Pork

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Executive Summary/Abstract

A comparative study was conducted between the Kidney Inhibition Swab (KIS ™) test and a Multiresidue Drug (MRD) LC-MS/MS method on 127 condemned pork carcasses from two Ontario processing locations. All samples were KIS ™ test negative however, 16 submissions contained non-violative residues (ie. below the Maximum Residue Limit or MRL) and 1 submission contained violative residues (sulfamethazine) (ie. above MRL) in either the kidney and/or muscle on the MRD LC-MS/MS test. Of the residues found, 47% contained lincomycin, 37% contained chlortetracycline and 16% contained sulfamethazine. Feed was the most likely source of the residues found. When looking at detection levels and MRL's alone the MRD LC-MS/MS test is far superior to the KIS ™ test, however, it must be remembered that the KIS ™ was developed to provide a simple, broad spectrum, cost effective preliminary screen, in the field whereas the MRD LC-MS/MS test is designed to be a quantitative confirmatory test in the lab.

Background

The Canadian Food Inspection Agency requires each federal processing establishment to test, at a rate prescribed by the CFIA – National Manager Chemical Residues, for sulfamethazine in market hogs using the Charm Kidney Inhibition Swab (KIS™) test. Samples from presumptive positive KIS TM™ tests are then shipped to the Centre for Veterinary Drug Residues, Saskatoon for confirmatory testing for the presence of sulfamethazine. The carcass, viscera and offal from the suspect animal must then either be held until the laboratory testing is completed or condemned and sent for rendering (1). Presently, Ontario pork producers receive only a report of presence or absence of sulfamethazine in their carcasses based on the KIS ™ test. However, the KIS ™ test is a broad spectrum microbial inhibition test. Therefore, it also detects antimicrobials other than sulfamethazine. After a positive result for inhibitors is laboratory confirmed negative for sulfamethazine, this presents the question of whether other antimicrobials were present. Without this information, the pork producers face difficulty in determining the actual risk of exposure to any antimicrobial residues to consumers. Additionally, residues could be of concern when exporting product globally. In an effort to assure food safety and to review the current use of antibiotics in the pork processing industry a comparative study was conducted between the KIS ™ test and an LC-MS/MS method.

The Charm KIS ™ (Kidney Inhibition Swab) Test is a rapid microbial inhibition screening test for antimicrobial drugs in bovine and porcine kidney tissue (2). Bacteria, cultured in agar with purple pH indicator media and kidney swab extract, generate acid that produces a yellow color. In the presence of antibiotic, the bacterial growth is inhibited and the test remains blue/purple. The Charm KIS ™ Test detects numerous antimicrobial drug families at different levels. The Multiresidue Drug (MRD) test is a quantitative confirmatory method that uses LC-MS/MS

technology to detect veterinary antibiotics, antimicrobials and anti-inflammatory drugs in animal tissues (3). It has been validated in bovine, porcine, poultry, sheep and goat muscle and kidney. See Table 1 below for a comparison of the performance of these two methods.

Objectives

The objectives of this study were:

1. Method Comparison: Charm KIS ™ Test versus LC-MS/MS detection
2. Identify the incidence of sulfamethazine versus other detected residues in condemned pork carcasses
3. Estimate the prevalence of non-sulfamethazine residues in condemned pork carcasses
4. Provide information to protect the reputation of Ontario Pork
5. Endorse Ontario Pork product excellence

Procedure

Kidney and muscle tissue samples were collected from condemned swine carcasses' at two Ontario processing locations, Conestoga Meat Packers and Fearman's Pork. Condemned carcasses were used so that there is no risk of penalty implications from the study. The condemned carcasses were sampled in a random manner at the slaughter plants from all condemned carcasses available. The tissue samples were delivered in coolers to the University of Guelph – Agriculture and Food Laboratory for testing using both the Charm KIS ™ Test and the MRD LC-MS/MS methods. Samples were collected and frozen upon receipt and tested in batches to provide the most economical processing possible.

Results

A total of 127 submissions (kidney and diaphragm/muscle) were tested in this trial, 77 from Conestoga Meat Packers and 50 from Fearman's Pork. An additional 20 submissions were tested for the client (ie. Unknown Nursery Pigs) but were not considered to be part of this report for a total of 147 submissions. All kidneys tested in the study were KIS ™ test negative, however, 17 submissions contained residues in either the kidney and/or diaphragm on the MRD LC-MS/MS test (see Table 2). This represents a positive submission rate of 13.4%. (ie. 17/127). Residues were found in 19 tissues (9 kidneys and 10 diaphragms). Some test results were unusual in that a residue was found in the diaphragm only and nothing in the corresponding kidney. Seven kidneys contained lincomycin, 1 contained sulfamethazine and 1 contained chlortetracycline whereas 6 diaphragms contained chlortetracycline, 2 contained sulfamethazine and 2 contained lincomycin. Many of the samples (ie. 8/19) were very low levels and non-quantifiable on the MRD LC-MS/MS (ie. < MQL or MDL). Only 1 sample would be considered violative or above the Maximum Residue Limit (diaphragm at 110 ppb SMZ) as set in the Food and Drugs Act.

Discussion

Antibiotics are commonly used in the swine industry for the prevention and treatment of disease and to enhance growth rate and efficiency of feed utilization (4, 5). They are administered to swine by injections and orally in the food and water. If precautions aren't taken all of these methods of administration can lead to antibiotic residues in the tissue. Many human and animal health concerns have been expressed over the years in regards to the overuse of antibiotics in agricultural production as well as the presence of residues in the food chain. Such concerns include: the potential for allergenic reactions in sensitized individuals, toxicity, the emergence of

resistant bacteria within animals and the transfer of antibiotic resistance genes to human pathogens (4, 5). While the validity of any public health threat posed by these concerns has been debated in the scientific community for many years, nevertheless, antibiotic residues in foods are illegal when above established MRL's.

The vast majority of antibiotics are administered to swine in the feed. A survey conducted in Alberta in found that the chlortetracycline/sulfamethazine/penicillin combination and tylosin were the most frequently used in-feed antibiotics in weaners and growers/finishers, respectively (6). A survey conducted in Ontario found that the most commonly used antimicrobials in feed were tylosin, carbadox and furazolidone in weaners and tylosin, lincomycin and tetracycline in finishers (7). The use of antibiotics through water was reported occasionally in all categories. The use of injectable antibiotics was reported mostly in sick pigs. Penicillin was the most common water and injectable antibiotic used. These survey results are supported by the test results of this study in which 47% of the residues found contained lincomycin, 37% contained chlortetracycline and 16% contained sulfamethazine. In addition, feed was the most likely source of the residues found.

As can be seen in Table 1 when looking at detection levels and MRL's the MRD LC-MS/MS test is far superior to the KIS ™ test. For example, since this study is interested in sulfamethazine in particular, the KIS ™ test cannot detect this residue at the MRL of 100 ppb in kidney. However, intended end use must be taken into consideration to be fair when comparing the two tests. The purpose of the KIS ™ test is to provide a simple, broad spectrum, cost effective preliminary screen, in the field, to identify a relatively low number of samples which might contain residues so that only these samples would require further confirmatory testing (8). On the other hand, the MRD LC-MS/MS test is designed to be a quantitative confirmatory test and is more expensive, laborious and not suited to testing in the field. In a residue testing program the KIS ™ test could be used for surveillance or suspect testing where turnaround time is important while the MRD LC-MS/MS test would be more suited for monitoring programs where information is gathered on the prevalence and type of residue violations in healthy populations that can be used as the basis for any future actions.

Conclusions

When looking at detection levels and MRL's alone the MRD LC-MS/MS test is far superior to the KIS ™ test in terms of performance. However, it must be remembered that the KIS ™ test has been designed as a screening test for use in the field whereas the MRD LC-MS/MS test is a quantitative confirmatory test. In this study, the most commonly found residues were lincomycin (47%), chlortetracycline (37%) and sulfamethazine (16%) and the most likely source of the residues was feed.

References

1. Canadian Food Inspection Agency. Meat Hygiene Directive 2011-30: Chapter 5-Section 5.2.9.1-5.2.9.2
2. Operator's Manual: Charm ™ Kidney Inhibition Swab (KIS) Test for Antimicrobial Drug Detection in Bovine and Porcine Kidney. 2013.
3. Martos P., Jayasundara F., Dolbeer J., Jin W., Spilsbury L., Mitchell M., Varilla C., Shurmer B. Multiclass, Multiresidue Drug Analysis, Including Aminoglycosides, in Animal Tissue Using Liquid Chromatography Coupled to Tandem Mass Spectrometry. J. Agric. Food Chem. 2010, 58, 5932–5944
4. Friendship RM. Antimicrobial drug use in swine. In: Prescott JF, Baggot JD, Walker RD,

eds. Antimicrobial Therapy in Veterinary Medicine. 3rd ed. Iowa State Univ Pr, 2000:602–611
5. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clin Infect Dis. 2002;34:S93–S106.
6. Rjic A., Reid-Smith R., Deckert AE, Dewey CE, McEwen SE. Reported antibiotic use in 90 swine farms in Alberta. Can Vet J. 2006, 47 (5): 446-52.
7. Dunlop RH, McEwen SA, Meek AH, Friendship RA, Clarke RC, Black WD. Antimicrobial drug use and related management practices among Ontario swine producers. Can Vet J. 1998, 39 (2): 87-96
8. Schneider, M.J., Lehotay, S.J. A Comparison of the FAST, Premi® and KIS(TradeMark) Tests for Screening Antibiotic Residues in Beef Kidney Juice and Serum. Analytical and Bioanalytical Chemistry. 2008, 390(7):1775-1779.

9. **Table 1 MRD and KIS TM Detection Levels in Pork (ppb)**

Drug Group	#	Compound	*MRD MDL (ppb)		KIS ™ Test	†MRL (ppm)	
			Muscle	Kidney	Kidney	Muscle	Kidney
Aminoglycosides	1	GENTAMICIN	10	124	750	100	400
	2	NEOMYCIN	43	1800	1000	500	10,000
	3	STREPTOMYCIN	38	29		500	2,000
Amphenicol	4	CHLORAMPHENICOL	3	2			
	5	FLORFENICOL	19	37		250	1,400 (liver)
	6	THIAMPHENICOL	3	2			
Beta-lactams	7	AMOXICILLIN	4	13		10	10
	8	AMPICILLIN	1	1		10	10
	9	PENICILLIN G	2	2	30	50	50
Macrolides	10	CLINDAMYCIN	2	2			
	11	ERYTHROMYCIN	4	42		100	100
	12	JOSAMYCIN	2	5			
	13	LINCOMYCIN	4	44		100	¤ 1,500
	14	PIRLIMYCIN	16	8			
	15	SPIRAMYCIN	2	1			
	16	TILMICOSIN	6	53		100	1,500 (liver)
	17	TULATHROMYCIN	67	250	1000	1500	5,000
	18	TYLOSIN	7	8	400	200	200
NSAID	19	FLUNIXIN	1	1		20	30
Quinolones	20	CIPROFLOXACIN	1	3			
	21	ENROFLOXACIN	2	1			
	22	SARAFLOXACIN	2	3			
	23	DANOFLOXACIN	4	5			
Sulfonamides	24	SULFADIMETHOXINE	4	18	250	100	100
	25	SULFADOXINE	5	14		100	100
	26	SULFADIAZINE	8	20		100	100
	27	SULFAMETHOXYPYRIDAZINE	3	7			
	28	SULFAMERAZINE	5	10		100	100
	29	SULFAMETHAZINE	13	6	500	100	100
	30	SULFANILAMIDE	110	62		100	100
	31	SULFAQUINOXALINE	4	10			
	32	SULFATHIAZOLE	4	25		100	100
Tetracyclines	33	OXYTETRACYCLINE	7	52	3000	200	1,200
	34	TETRACYCLINE	7	52		200	1,200
	35	CHLORTETRACYCLINE	6	50		200	1,200

* MDL = Method Detection Limit
† MRL = Maximum Residue Limit
¤ Proposed MRL

Table 2 - MRD Positive Samples vs KIS TM Test

Sample ID	Submitter Sample ID	Sample Type	Plant	KIS	MRD Screen				MRD Confirmation		
				KIS	MRD Screen	Linocmycin	Sulfamethazine	Chlortetracycline	Lincomycin	Sulfamethazine	Chlortetracycline
									ppb	ppb	ppb
11-114146-0006		Kidney-Porcine	CONESTOGA	NEG	POS	POS			<MDL		
11-114146-0010		Kidney-Porcine	CONESTOGA	NEG	POS	POS			<MDL		
11-114146-0018		Kidney-Porcine	CONESTOGA	NEG	POS	POS			<MDL		
11-114146-0034		Kidney-Porcine	CONESTOGA	NEG	POS			POS			<MDL
11-114146-0048		Diaphragm-Porcine	CONESTOGA		POS	POS			53		
11-114146-0068		Diaphragm-Porcine	CONESTOGA		POS			POS			<MDL
11-114146-0070		Diaphragm-Porcine	CONESTOGA		POS			POS			<MDL
11-114146-0072		Diaphragm-Porcine	CONESTOGA		POS			POS			<MDL
11-114146-0074		Diaphragm-Porcine	CONESTOGA		POS			POS		13	
11-114378-0004		Kidney-Porcine	CONESTOGA	NEG	POS		POS			79	
11-114378-0032		Diaphragm-Porcine	CONESTOGA		POS		POS			110	
11-114378-0042		Diaphragm-Porcine	CONESTOGA		POS			POS			<MDL
11-114378-0044		Diaphragm-Porcine	CONESTOGA		POS			POS			<MQL
11-114692-0010		Kidney-Porcine	CONESTOGA	NEG	POS	POS			210		
12-002007-0113		Kidney-Porcine	CONESTOGA	NEG	POS	POS			210		
12-002007-0126		Diaphragm-Porcine	CONESTOGA		POS			POS			77
12-025912-0028		Kidney-Porcine	FEARMANS PORK	NEG	POS	POS			<MDL		
12-027697-0037		Diaphragm-Porcine	FEARMANS PORK		POS	POS			<MQL		
12-027697-0038		Kidney-Porcine	FEARMANS PORK	NEG	POS	POS			200		

violative

MDL- Method Detection Limit

MQL- Method Quantitation Limt