Molecular Characterization of Multidrug Resistant Salmonella Isolates From a Single Finisher Building for Determination of Horizontal Transmission of Resistance Genes

A.S. Leaflet R1980

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Summary and Implications

Salmonella enterica subsp. *enterica* serovar Typhimurium Phage Type DT104 contains a region where five major antibiotic genes are located, which has been named Salmonella Genomic Island 1 (SGI1) (Boyd, et al, 2002) and bound by Class I integrons which have been shown to aid in transferring genes horizontally. Antibiotic resistance profiles, Pulsed Field Gel Electrophoresis (PFGE) and Polymerase Chain Reaction (PCR) were used to determine if these genes are present in the serovars.

Introduction

Nwosu (2001) suggested that multidrug resistant (MDR) organisms were horizontally transmitting the resistance genes to other bacterial species, found in the same environments. Isolates of *Salmonella enterica ss. enterica* serovar Typhimurium Phage Type DT104 have been found to be resistant to five major antibiotics: ampicillin, chloramphenicol, tetracycline, streptomycin, and sulfonamides (Ridley and Threlfall, 1998). Recent research has found that the genes giving DT104 its resistances are grouped together Salmonella Genomic Island 1 (SGI1), and has been found not only in DT104, but also in *S.* Typhimurium and in three other serovars of *Salmonella*, Agona (Boyd, et al, 2002), Paratyphi-B (Meunier, et al, 2002), and most recently, Albany (Doublet, et al, 2003). Detailed examination of the chromosome segment showed that the resistance genes were closely linked and bounded by integrons (Boyd, et al, 2002). These integrons are regions of the chromosome, which potentially could help SGI1, release from and attach back into the chromosome (Sandvang, et al, 1997). These same integrons have been reported in 12 other serovars of *Salmonella*, including Anatum, Brandenburg, Bredeney, Derby, and Heidelberg, which are important to food safety of pork, giving rise to the concern that these *Salmonella* may become multi-drug resistant (Casin, et al, 1999). Actual evidence for transfer of SGI1 into these serovars has not yet been found or reported. This study was done to determine if transfer of SGI1 was possible in a pig finisher building.

Materials and Methods

During May through July of 2000, rectal swabs and pen fecal samples were collected from pigs housed in a finisher building in Oklahoma and cultured for the presence of *Salmonella*. The resulting isolates included *S.* Typhimurium (n=69), *S.* Worthington (n=205), and *S.* Heidelberg (n=66). Twenty isolates from each serovar were selected, for a total of sixty isolates for this study (Table 1). The DNA from these isolates was digested with Xba1, examined by PFGE, and the results were compared using BioNumerics software (Applied Maths, Kortrijk, Belgium). Antibiotic resistance profiles were conducted with the Kirby-Bauer disk diffusion method and reconfirmed by determining the Minimum Inhibitory Concentration (MIC) for the antibiotics through micro-broth dilution microtitre procedure (NARMS panel, Sensititre, Trek Diagnostics, Inc.). PCR was conducted on the sixty selected isolates. SGI1 presence was determined by finding PCR products, in the 180 to 240 bp range, produced using primers (see Table 1) for the genes *intl1* (integron gene), *aadA2* (streptomycin resistance gene), *tetG* (tetracycline resistance gene), *pse1* (ampicillin resistance gene) and *intl1* (the class I integron gene) (Boyd, et al, 2002). Isolate was extracted from an overnight culture of the isolates using a DNesay Extraction Kit (Qiagen). Sybr Green HotStar Master Mix (Qiagen), with the individual sets of primers were used to amplify and detect the genes using iCycler (BioRad) in real time PCR, followed by a melt cuvre determination of the product. For controls, isolates of *S.* Typhimurium DT104 MDR (DT104) were used to confirm the delectability of resistant genes.

Results and Discussion

MIC: The Kirby-Bauer and MIC (Sensititer) results showed that all of the *S.* Typhimurium isolates were resistant to ampicillin (A), streptomycin (S), and sulfonamides (Su) (Table 2). Sixteen of the isolates were also resistant to tetracycline (T), while four were susceptible. *S.* Heidelberg isolates had two resistant patterns with ST being the most common with fifteen isolates. *S.* Worthington had one isolate which showed the CSSuT pattern, but the rest were either resistant to tetracycline, or were totally susceptible to the antibiotics tested. This was the only multidrug resistant isolate of the *S.* Worthingtons.

PFGE: The patterns of bands isolate produced by the Xba1 restriction endonuclease on the gels (Figure 1) and analysis by BioNumerics, show that the *S.* Heidelberg isolates are closely related with all having the same banding pattern, SH01 (see Table 2), indicating the possibility of a

single origin. With *S*. Worthington, there was more pattern diversity; ten isolates had pattern SW01 and seven had SW02. The patterns SW03, SW05, and SW06 each had one isolate. The patterns SW01 and SW06 were very similar, with a difference of one band. The patterns SW02, SW03, and SW05 had a 92.5% similarity. Another difference between these two groups of patterns is that SW01 and SW06 are resistant to tetracycline, while SW02, SW03, and SW05 are susceptible to the antibiotic. *S*. Typhimurium isolates were most diverse with 11 isolates with ST01 pattern; two each for patterns ST09 and ST22, and one isolate each for ST03, ST16, ST24, ST25, and ST26.

PCR: The primers were able to detect all four genes in the DT104 controls. This showed that the resistance genes were detectable using these primers. The results, of the primers testing for the antibiotic resistance genes, have been listed below (Table 3). The integron gene, *int1*, was found in all isolates tested (Table 3). Class 1 integron genes have been reported in *S*. Typhimurium and *S*. Heidelberg (Randall, et al, 2004), but has not been reported in *S*. Worthington. Finding the *int1* gene in *S*. Worthington, would indicate that Class 1 integrons may be common among the Salmonellae. The ampicillin resistance gene, *pse1*, was not found in the MDR *S*. Heidelberg isolate or the MDR *S*. Worthington isolate. This was expected due to the lack of resistance to ampicillin in these isolates. The gene was found in isolates, which were not expressing ampicillin resistance. The *pse1* gene was not found in the *S*. Typhimurium isolate which were expressing ampicillin resistance, indicating other resistance gene(s) for ampicillin. The streptomycin resistance gene, *aadA2*, was found in all isolates tested. The was expected for the *S*. Typhimurium, *S*. Heidelberg isolates, and the MDR *S*. Worthington isolate (CSSuT), which were expressing resistance to streptomycin. The gene was also found in the *S*. Worthington isolates, which were susceptible to streptomycin. The gene for resistance to tetracycline, *tetG*, was not found in the *S*. Typhimurium isolates with the phenotypic antibiotic profile of ASSu. The gene was not found in *S*. Heidelberg isolates whose profiles were phenotypic ST; so another gene which confers tetracycline resistance may be present. The *tetG* gene was found in the *S*. Typhimurium isolates with ASSuT, *S*. Heidelberg isolates (CSSuT) and in all *S*. Worthington isolates, (CSSuT, T, and susceptible phenotypes). The results of this study did not support horizontal transmittance of resistance genes with in the isolates of this study.

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Table 1. Results showing the detection of target genes by their respective primer sets.

		AB	Genes Detected						AB	Genes Detected			
HL #	Serovar	Profile*	int1	pse	aadA2	tetG	HL #	Serovar	Profile*	int1	pse	aadA2	tetG
10961	DT104	ACSSuT	+	+	+	+	10638	Heidelberg	ST	+	-	+	-
10848	DT104	ACSSuT	+	+	+	+	10642	Heidelberg	ST	+	-	+	-
10453	Typhimurium	ASSuT	+	-	+	-	10644	Heidelberg	CSSuT	+	-	+	+
10455	Typhimurium	ASSuT	+	-	+	-	10659	Worthington	T	+	+	+	+
10458	Typhimurium	ASSuT	+	-	+	-	10662	Worthington	T	+	+	+	+
10459	Typhimurium	ASSuT	+	-	+	-	10667	Worthington	T	+	+	+	+
10462	Typhimurium	ASSuT	+	-	+	-	10672	Worthington	CSSuT	+	+	+	+
10468	Typhimurium	ASSuT	+	-	+	-	10677	Worthington	T	+	+	+	+
10473	Typhimurium	ASSuT	+	-	+	-	10679	Worthington	T	+	+	+	+
10476	Typhimurium	ASSuT	+	-	+	-	10684	Worthington	T	+	+	+	+
10477	Typhimurium	ASSu	+	-	+	-	10687	Worthington	T	+	+	+	+
10479	Typhimurium	ASSu	+	-	+	-	10691	Worthington	T	+	+	+	+
10482	Worthington	(sucept.)	+	+	+	+	10701	Worthington	T	+	+	+	+
10484	Typhimurium	ASSuT	+	-	+	-	10706	Worthington	T	+	+	+	+
10486	Typhimurium	ASSuT	+	-	+	-	10713	Heidelberg	CSSuT	+	-	+	+
10487	Worthington	(sucept.)	+	+	+	+	10718	Worthington	(sucept.)	+	+	+	+
10489	Heidelberg	ST	+	-	+	-	10721	Worthington	(sucept.)	+	+	+	+
10490	Heidelberg	ST	+	-	+	-	10724	Worthington	(sucept.)	+	+	+	+
10504	Typhimurium	ASSu	+	-	+	-	10729	Heidelberg	ST	+	-	+	-
10509	Typhimurium	ASSuT	+	-	+	-	10731	Heidelberg	CSSuT	+	-	+	+
10523	Typhimurium	ASSuT	+	-	+	-	10734	Worthington	(sucept.)	+	+	+	+
10529	Typhimurium	ASSuT	+	-	+	-	10737	Worthington	(sucept.)	+	+	+	+
10532	Typhimurium	ASSuT	+	-	+	-	10752	Worthington	(sucept.)	+	+	+	+
10584	Heidelberg	ST	+	-	+	-	10770	Worthington	(sucept.)	+	+	+	+
10586	Heidelberg	ST	+	-	+	-	10771	Typhimurium	ASSuT	+	-	+	-
10621	Heidelberg	ST	+	-	+	-	10776	Heidelberg	ST	+	-	+	-
10622	Heidelberg	ST	+	-	+	-	10777	Typhimurium	ASSu	+	-	+	-
10624	Heidelberg	ST	+	-	+	-	10786	Heidelberg	CSSuT	+	-	+	+
10631	Typhimurium	ASSuT	+	-	+	-	10787	Heidelberg	ST	+	-	+	-
10632	Heidelberg	ST	+	-	+	-	10794	Heidelberg	CSSuT	+	-	+	+
10637	Heidelberg	ST	+	-	+	-	10797	Heidelberg	ST	+	-	+	-

*Phenotypic Antibiotic Resistance Profile: A=ampicillin, C=chloramphenicol, S=streptomycin, Su=sulfonamides, T=tetracycline, and (sucept.)=not resistant to any antibiotic tested.