Ribotype and Pulsed Field Gel Electrophoresis Analysis of Multidrug Resistant *Salmonella* Newport

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INTRODUCTION

Salmonellosis is a leading cause of bacterial gastroenteritis in North America. There has been a recent rise in the amount of foodborne *Salmonella* infections attributed to *Salmonella enterica* serotype Newport (1). The increased use of antimicrobials in medicine and agriculture has created enormous pressure for the selection of antimicrobial resistance among bacterial pathogens, and the increase in foodborne outbreaks of *S.* Newport infection appears to be related to the recent emergence of multiple antimicrobial resistant strains of *S*. Newport (2). These strains are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline, kanamycin, potentiated sulfonamides, gentamicin, and third generation cephalosporins such as ceftriaxone, an antimicrobial agent commonly used to treat serious infections in children (3).

Rapid identification of antimicrobial resistant foodborne bacteria is imperative for preventing and treating foodborne illness, as well as for epidemiological analysis. Usually, pulsed-field gel electrophoresis (PFGE) is used to characterize foodborne bacterial isolates, along with antimicrobial susceptibility testing (4). Ribotyping has been investigated as a method to type foodborne bacteria. Although the discriminatory power of ribotyping has been reported to be less than PFGE, when used in conjunction with PFGE, the results can be more useful than if either method was used alone. A further gain in the analysis of multidrug resistant foodborne isolates may be obtained by characterizing the genetic elements responsible for the resistance. Transfer of antibiotic resistance genes between different species can be facilitated by mobile DNA elements such as promiscuous plasmids, conjugative transposons, and gene cassettes contained within integrons.

The objective of this study was to use the techniques of ribotyping, pulsed field gel electrophoresis, plasmid profiles and integron analysis to investigate the degree of DNA banding polymorphism exhibited by strains of antimicrobial resistant *S*. Newport.

METHODS

Eight multidrug resistant isolates of *S*. Newport were analyzed in this study. Antimicrobial resistance of the *S*. Newport isolates was determined against a panel of 18 antimicrobials using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, OH, USA) according to the manufacturer's instructions, and the results were interpreted according to National Committee Clinical Laboratory Standards guidelines for broth microdilution methods (5) (table 1).

Ribotyping was performed using the automated RiboPrinter Microbial Characterisation System (Qualicon Inc., Wilmington, DE, USA) according to the manufacturer's instructions.

Whole cell DNA for determination of PFGE patterns was prepared as described by the Centers for Disease Control and Prevention (6).

Plasmid profiles were obtained with the use of commercially available small and large plasmid isolation kits (Qiagen, Valencia, CA, USA) and integron analysis was performed by PCR, according to previously described methods (7). Analysis of the PFGE, ribotype and plasmid profiles was accomplished with the use of Bionumerics software, version 3 (Applied Maths, Belgium).

Table 1. *S*. Newport strains (SN 1-5, 7, 8, 21) used in this study, and their antibiotic resistance profiles. Eighteen antibiotics were used in this study. *S.* Newport isolates were screened for susceptibility to the following 18 antimicrobials: amikacin (AMI), amoxicillin - clavulanic acid (AMO), ampicillin (AMP), apramycin (APR), cefoxitin (CEF), ceftiofur (CET), ceftriaxone (CER), cephalothin (CEP), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMI), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SUL), tetracycline (TET), and trimethoprim - sulfamethoxazole (TRI). The results are reported as resistant (R), intermediate resistance (I), or susceptible (S).

	AMI	AMO	AMP	APR	CEF	CET	CER	CEP	CHL	CIP	GEN	IMI	KAN	NAL	STR	SUL	TET	TRI
SN1	S	R	R	S	R	R	I	R	R	S	R	S	R	S	R	R	R	R
SN2	S	R	R	S	R	R	I	R	R	S	S	S	S	S	R	R	R	S
SN3	S	R	R	S	R	R	I	R	R	S	S	S	S	S	R	R	R	S
SN4	S	R	R	S	R	R	S	R	R	S	I	S	R	S	R	R	R	R
SN5	S	R	R	S	R	R	S	R	R	S	R	S	S	S	R	R	R	S
SN7	S	R	R	S	R	R	I	R	R	S	S	S	S	S	R	R	R	R
SN8	S	R	R	S	R	R	S	R	R	S	R	S	R	S	R	R	R	S
SN21	S	R	R	S	R	R	S	R	R	S	S	S	S	S	R	S	R	R

RESULTS

PFGE, ribotype and plasmid profiles were used to compare the genetic relatedness of the *S*. Newport isolates. All of the methods clearly resolved the *S*. Newport isolates into distinct clusters (figure 1). Plasmid analysis revealed that most isolates contained plasmids ranging in size from 2.0 - 23.0 kb. Class 1 integrons were observed in all of the isolates. The primer pair int1AF and int1AR produced amplicons of 1.0 kb, 1.2 kb, and 2.0 kb in 7 of the 8 isolates (data not shown). One isolate (SN4) had a 1.1 kb integron and one isolate (SN1) contained more than one integron (1.0 and 2.0 kb respectively). Restriction fragment length polymorphism (RFLP) indicated that integrons of the same size were identical (data not shown).

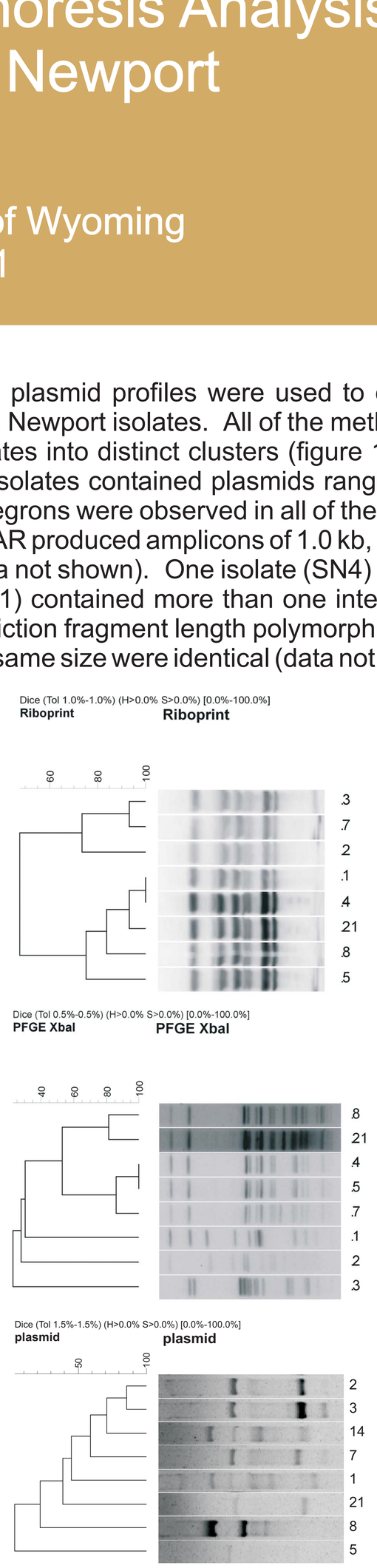

Figure 1. Cluster analysis of Riboprint, PFGE and plasmid profiles obtained for each *S*. Newport isolate. The Dice similarity coefficient (0.5 to 1.5% position tolerance) was used in the analysis. Dendrograms were produced according to the unweighted pair-group mean arithmetic method (UPGMA), and the different dendrograms were then compared using visual inspection.

CONCLUSIONS

These results demonstrate the high degree of DNA banding pattern polymorphism found in some strains of antimicrobial resistant *S.* Newport, and illustrates the presence of complex genetic structures (plasmids, integrons) contained within the isolates, which may be involved in the rapid spread of antibiotic resistance among *S*. Newport strains. While the genomic typing methods resolved the isolates into distinct clusters, the cluster results from the different typing methods did not agree with each other. Therefore, this study highlights the need to use multiple genetic analysis methods when analyzing antibiotic resistant strains of *S*. Newport.

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