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Pulsed field, ribotype and integron analysis of multidrug resistant isolates of *Salmonella enterica* serovar Newport

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Abstract

Twenty-one isolates of *Salmonella enterica* serovar Newport were evaluated for their antimicrobial resistance, pulsed field gel electrophoresis (PFGE) profiles, ribotype profiles, and their integron profiles. Antimicrobial resistance profiles indicated that 20 of the 21 isolates were resistant to the following antibiotics: amoxicillin–clavulanic acid (AMOX/CA), ampicillin (AMPC), cefoxitin (CFOX), ceftiofur (TIO), cephalothin (CRIN), chloramphenicol (CHL), streptomycin (STR), tetracycline (TET), and sulfamethoxazole (SMX). Five isolates showed resistance to gentamycin (GEN) and kanamycin (KAN). Trimethoprim–sulfamethoxazole (SMX/TMP) resistance was observed in six isolates. Eight of the twenty one isolates showed intermediate resistance to ceftriaxone (CTRX), with one isolate exhibiting complete resistance. PFGE clearly resolved the *Salmonella* Newport isolates into nine distinct clusters, and a good congruence was observed between PFGE and antibiotic resistance patterns. Automated riboprinting clearly distinguished between antibiotic resistant and sensitive strains of *Salmonella* Newport, and resolved the isolates into two ribogroups. One group consisted of the multidrug resistant isolates, and the other grouping contained the sensitive isolate. Three different integrons (1.0, 1.2, and 1.8 kb) were observed in many of the isolates, and several isolates contained more than one integron. Restriction fragment length polymorphisms (RFLP) indicated that integrons of the same size were indistinguishable.

When integron analysis and ribotype analysis were used in conjunction, four subtypes of multidrug resistant *Salmonella* Newport isolates were clearly defined. These results demonstrate the possibility of utilizing automated ribotyping and integron analysis to rapidly subtype multidrug resistant *Salmonella* Newport isolates.

Keywords: *Salmonella enterica* serovar Newport; Outbreaks; Antimicrobial resistance; Pulsed field gel electrophoresis; Automated ribotyping; Integron analysis; Restriction fragment length polymorphisms

1. Introduction

Salmonellosis is a leading cause of bacterial gastroenteritis in North America. An estimated 1.4 million cases of salmonellosis occur annually in the United States (Mead et al., 1999). Many serotypes of *Salmonella* have been identified as a cause of human and animal disease, although the majority of laboratory confirmed human salmonellosis cases are caused by four serotypes (Sanchez, Hofacre, Lee, Maurer, & Doyle, 2002).

There has been a recent rise in the amount of foodborne *Salmonella* infections attributed to *Salmonella enterica* serovar Newport. For example, In 2001, nine cases of *S.* Newport infection with a possible link to a salad item were identified in England (Sagoo, Little, Ward, Gillespie, & Mitchell, 2003). The cases were identified following the isolation of *S.* Newport from a salad item as part of a survey of retail prepared pre-packed ready to eat salad vegetables

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in the United Kingdom (Sagoo et al., 2003). During January–April 2002, a multistate outbreak of *S*. Newport was associated with the consumption of contaminated ground beef (CDC, 2002). Another outbreak of salmonellosis due to *S*. Newport was associated with the consumption of soft style cheeses in Connecticut (McCarthy et al., 2002). *S*. Newport is now recognized as the third most common *Salmonella* serotype in the US (CDC, 2002).

Salmonellosis is usually a self-limited infection and does not require antibiotic treatment. However, invasive infections, including septicemia and meningitis, occur in 5% to 10% of culture-confirmed cases, mainly among children, elderly individuals, and patients with immunocompromised systems. In the event of an invasive disease, effective antibiotic therapy can be lifesaving. The increased use of antimicrobials in human and veterinary medicine, agriculture, and as growth hormones in food animals 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. These strains are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline (CDC, 2002). Additionally, multiple antimicrobial resistant *S*. Newport strains often show resistance to other antimicrobials including kanamycin, potentiated sulfonamides, gentamicin, and third generation cephalosporins such as ceftriaxone, an antimicrobial agent commonly used to treat salmonellosis in children (CDC, 2002).

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 (Fontana, Stout, Bolstorff, & Timperi, 2003). Automated ribotyping has been investigated as a method to subtype *Salmonella* spp. (Clark et al., 2003; Fontana et al., 2003). 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 (Fontana et al., 2003). A further gain in the analysis of multidrug resistant foodborne isolates may be obtained by characterizing the genetic elements responsible for the resistance (Severino & Magalhães, 2004). Transfer of antibiotic resistance genes between different species can be facilitated by mobile DNA elements such as promiscuous plasmids, and gene cassettes contained within integrons (Severino & Magalhães, 2004). In addition, integron analysis has been demonstrated as a useful tool for studying the molecular epidemiology of antibiotic resistant Gram negative bacteria implicated in human infection, and in determining their relatedness to each other (Severino & Magalhães, 2004).

The objective of this study was to use the techniques of PFGE, automated ribotyping and integron analysis to investigate the possibility of developing a rapid, molecular identification and subtyping scheme for characterization of antimicrobial resistant *S*. Newport isolates. While the methods described here are too expensive to be employed routinely, they could be used to obtain critical data in certain situations. For example, the use of integron and ribotype analysis in a subtyping scheme may result in the development of a rapid (one day) genetic method to determine antibiotic resistance in strains of *S*. Newport, implicated in outbreaks of foodborne disease.

2. Materials and methods

2.1. Bacterial strains

Twenty one *Salmonella* Newport isolates from the foodborne bacterial culture collection at the University of Wyoming were examined in this study. One isolate (SN22) was previously shown to be susceptible to the antibiotics tested in this study, and was included as a negative control. Two isolates of *Salmonella enterica* serovar Typhimurium (SN4, multidrug resistant; SN24, antibiotic susceptible), and one isolate of *Salmonella enterica* serovar Montevideo (SN23, antibiotic susceptible) were also included in the study. The serovars of all the isolates were confirmed by serotyping using the Modified Kauffman–White Scheme as described by Brenner (1998). Stock bacterial cultures were maintained in 30% glycerol and were frozen at −70 °C. Prior to each experiment, fresh bacterial host cultures for use in experiments were produced by inoculating frozen stock cultures onto tryptic soy agar (TSA) plates and incubating the plates overnight at 37 °C.

2.2. Antimicrobial susceptibility

The antimicrobial MICs for the *S*. Newport isolates tested were determined with the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) according to the manufacturer's instructions, and the results were interpreted according to Clinical and Laboratory Standards Institute guidelines for broth microdilution methods (CLSI, 1998). *Salmonella* Newport isolates were screened for susceptibility to the following 18 antimicrobials: amikacin (AMK), amoxicillin–clavulanic acid (AMOX/CA), ampicillin (AMPC): apramycin (APR), cefoxitin, (CFOX) ceftiofur (TIO), ceftriaxone (CTRX) cephalothin (CRIN), chloramphenicol (CHL) ciprofloxacin (CIPX), gentamycin (GEN), imipenem (IMP), kanamycin (KAN), nalidixic acid (NAL) streptomycin (STR), sulphamethoxazole (SMX), tetracycline (TET), and trimethoprim–sulphamethoxazole (SMX/TMP).

2.3. Pulsed field gel electrophoresis

Whole cell DNA for determination of PFGE patterns was prepared as described by the Centers for Disease