Short Communication

Antimicrobial susceptibility and genetic relatedness of *Salmonella enterica* subsp. *enterica* serovar Mbandaka strains, isolated from a swine finishing farm in Greece

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The current study investigated the antimicrobial susceptibility of *Salmonella enterica* subsp. *enterica* serovar Mbandaka (*Salmonella* Mbandaka) isolated from finishing swines in Greece. Pulsed-field gel electrophoresis (PFGE) was used to examine the genetic relatedness of the isolates. The study was carried out for 1 year as part of a project focusing on antimicrobial resistance of salmonellae recovered from asymptomatic pigs. A total of 400 finishing pigs stabled in 20 swine farms in central Greece were included in the study. Fecal samples taken directly from the rectum, one sample from each pig, were cultured for *Salmonella* spp. Five of the 400 tested finishing pigs, originating from the same herd, were asymptomatic carriers of *Salmonella* Mbandaka. All five isolates were resistant to tetracycline, four were resistant to trimethoprim/sulfamethoxazole, and three to ampicillin and amoxicillin/clavulanic acid. In contrast, all five isolates were susceptible to cefuroxime and ceftriaxone, as well as to nalidixic acid, ciprofloxacin, and levofloxacin. All five isolates had indistinguishable PFGE patterns. The present study confirms the existence of a nontyphoid *Salmonella* serotype, *Salmonella* Mbandaka in asymptomatic carrier pigs in Greece. Further, the *Salmonella* Mbandaka isolates were found to be resistant to several antimicrobials.

Key words: *Salmonella enterica* subsp. *enterica* serovar Mbandaka, antimicrobial susceptibility, swine, Pulsed-field gel electrophoresis (PFGE), zoonotic.

INTRODUCTION

*Salmonella* spp. is recognized worldwide as important pathogens in the intestinal tracts of both animals and humans. Infected animals are usually asymptomatic carriers, a fact that has a major effect on the spread of infection. The number of human cases of salmonellosis caused by the nontyphoid *Salmonella enterica* subsp. *enterica* serovar Mbandaka (*Salmonella* Mbandaka) increased during the 1990s. Related outbreaks of human infection characterized by diarrhoea have been reported in the United Kingdom (Reid et al., 1993), Italy (Fantasia et al., 1989), Australia (Scheil et al., 1998), and the United States (Gill et al., 2003). Pigs infected with *Salmonella* Mbandaka, as well as pig products, have been implicated throughout pork production and processing in cases of human infection and clinical disease (Davies, 1998). Poultry has also been considered an important source of *Salmonella* Mbandaka for humans (Hoszowski and Wasyl, 2001).

A prominent reason for concern with regard to gastro-enteritis-causing bacteria is the recognized emergence of antimicrobial resistance among key species. Over the past decade, particularly in developing countries, the increase in resistance of animal origin nontyphoid salmonellae to broad-spectrum antibiotics such as cephalosporins, tetracycline, and quinolones has been extremely worrisome (Streit et al., 2003). As a result, attempts are being made to trace salmonellosis outbreaks to contaminated sources, and numerous typing methodologies have been used. Information concerning the prevalence of *S.
enterica serotypes in Greek finishing swine herds has previously been published (Leontides et al., 2002; Wong et al., 2003). In this study, we report the antimicrobial susceptibility and the genetic relatedness of five Salmonella Mbandaka isolates originating from healthy finishing swines in a Greek herd.

MATERIALS AND METHODS

Study design

Asymptomatic carriers have a major effect on the spread of Salmonella infections to humans (Wong et al., 2003). Therefore our study, carried out from March 2003 to October 2004, was focused on antimicrobial resistance and genetic variation of salmonellae recovered from asymptomatic pigs. Samples were collected from 20 swine finishing farms, representing 10% of the swine finishing farms in central Greece. Twenty finishing pigs from each farm were sampled (almost 5% of the stabled animals), and 400 fecal samples were collected. Faecal samples were taken directly from the rectum, one sample from each pig. Samples were transported (4°C) and incubated in 37°C for 24 h. After enrichment (41.5°C for 24 h) in Modified Semi-solid Rappaport-Vassiliadis (MSRV, Merck KGaA, 64271 Darmstadt, Germany), Salmonella spp. was isolated on Xylose-Lysine-Deoxycholate (XLD, Merck KGaA, 64271 Darmstadt, Germany) and Hektoen agar (EMD, Merck KGaA, 64271 Darmstadt, Germany, according to 6579:2002 ISO. Suspect colonies were sub cultured on nutrient agar (Merck KGaA, 64271 Darmstadt, Germany) and were characterized biochemically using Triple Sugar Iron agar (TSI, Merck KGaA, 64271 Darmstadt, Germany), Lysine broth, Urea broth, Simmons Citrate agar and Tryptophan broth (Merck KGaA, 64271 Darmstadt, Germany). Serotyping was carried out by the National Veterinary Services Laboratories (Halkida, Greece) according to the Kaufmann-White scheme (Popoff and Le Minor, 1997).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of Salmonella Mbandaka isolates to nine antimicrobials (Ampicillin, Amoxicillin/clavulanic acid, Cefuroxime, Ceftriaxone, Nalidixic acid, Ciprofloxacin, Levofloxacin, Tetracycline and Trimethoprim/sulfamethoxazole) was determined by diffusion antimicrobial susceptibility tests in accordance to the M100-S16 Standards Insitute (CLSI, 2006). The MIC was determined as the lowest concentration of antimicrobial agent that inhibited visible growth. Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were used as quality control strains.

PFGE

The genetic relatedness of the isolates was investigated by pulsed-field gel electrophoresis (PFGE) as described in detail by Gautorn (1997). After cleavage with XbaI, electrophoresis was performed at 6 V/cm with 1.0% AgaroseNA agar (Amersham Biosciences, Uppsala, Sweden) by using the CHEF-DR II system (Bio-Rad Labo-
demonstrated for penicillins, cephalosporins, macrolides, tetracyclines, aminoglycosides, and several other antibacterial agents (Aiello S.E., 1998). In the case of increasing resistance to antibiotics, other methods such as identification and elimination of carrier pigs might prevent the spread of this Salmonella serotype.

The XbaI PFGE patterns of the Salmonella Mbandaka isolates are presented in Figure 1. All five isolates had indistinguishable PFGE patterns. The results are not surprising as all the isolates were from the same farm. Hosowski and Wasyl (2001) also demonstrated a high genetic relatedness among Salmonella Mbandaka strains from poultry and human origin. However, no studies concerning the genetic relatedness among swine and human isolates have been published so far.

No studies have yet been published concerning isolation of Salmonella Mbandaka in any case of human disease in Greece. Therefore, we did not have the opportunity to include data relating to human isolates in our study. However, human cases in Europe were mainly attributed to contact with asymptomatic carrier pigs. It is thus recommended that this particular Salmonella serotype may be considered in investigations of future cases of animals and humans salmonellosis.

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REFERENCES


Figure 1. PFGE patterns of 5 Salmonella Mbandaka isolates. The patterns were obtained by the PFGE protocol with XbaI digestion. Lane M, lambda ladder with a size range of 45.5 to 727.5 kb (High Range PFG Marker, New England Biolabs Inc.). Lanes 1 – 5, the five Salmonella Mbandaka isolates analyzed.