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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 gastroenteritis-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.*

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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 sus-ceptibility 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^{o}C) within 4 h to the laboratory.

Sampling and cultivation

25 g of each faecal sample was diluted in sterile plastic bags with 225 ml of buffered peptone water (BPW, Merck KGaA, 64271 Darmstadt, Germany) and incubated in 37^{o}C for 24 h. After enrich-ment (41.5^{o} 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 dilution antimicrobial susceptibility tests in accordance to the M100-S16 method and the interpretive criteria of the Clinical and Laboratory Standards Insitute (CLSI, 2006). The MIC was determined as the lowest concentration of antimicrobial agent that inhibited visible growth. *Escherichia coli* ATTC 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 Gautom (1997). After cleavage with *Xba*I, 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-ratories, Richmond, California, USA). Running conditions for *XbaI*-digested DNA were 2.2 to 64 s for 20 h. Lambda ladder with a size range of 0.13 to 194 kb (Low Range PFG Marker, New England Biolabs Inc.), were used as molecular weight standard. The DNA bands were visualized on a UV transilluminator and Polaroid photographs of the gels were visually examined.

RESULTS AND DISCUSSION

Epidemiological data show that salmonellosis is a serious problem worldwide in pigs, cattles, horses, poultry, and even exotic animals. Nontyphoid salmonellae are among the leading causes of food-borne disease in humans and are therefore of increased importance in public health. In the present study, five (1.25%) of the 400 tested finishing pigs were asymptomatic carriers of salmonellae, which were further serotyped as *Salmonella* Mbandaka. All five pigs originated from the same herd; the herd included 180 sows all of the same breed. The prevalence was lower than previously reported in other European countries (Wong et al., 2003). No other *Salmonella* serotypes were identified in these animals. Therefore the present findings confirm the existence of the particular nontyphoid *Salmonella* serotype in asymptomatic carrier swines in Greece and therefore indicate a potential risk for trans-mission of the bacterium from swine to humans, directly or in meat products.

Concerning the susceptibility to antimicrobial agents all five isolates were found to be resistant to tetracycline (MIC90 8 g/ml) four to trimethoprim/sulfamethoxazole (MIC90 0.5 g/ml), and three to ampicillin and amoxi-cillin/clavulanic acid (MIC90 16 g/ml). In contrast, all five isolates were susceptible to cefuroxime and ceftria-xone, as well as to nalidixic acid, ciprofloxacin, and levo-floxacin. Given the zoonotic potential of *Salmonella* Mbandaka, research is needed to determine which antimicrobials might be efficacious against this pathogen. In the present study, susceptibility to some third-genera-tion cephalosporins (cefuroxime, ceftriaxone) and quino-lones (nalidixic acid, ciprofloxacin, levofloxacin) was detected in all five isolates. This susceptibility can be partially explained by the limited use of these antibiotics in Greek farms (data not shown). However, an increasing resistance in *Salmonella* Mbandaka strains to some cephalosporin -lactams (cefotaxime, ceftazidime and aztreonam) has been observed by other researchers (Szych et al., 2001). The resistance of *Salmonella* Mbandaka isolates to tetracycline, ampicillin, amoxicil-lin/clavulanic acid, and trimethoprim/sulfamethoxazole has previously been reported (Streit et al., 2003) and reflects the use of these antimicrobials as growing factors in swine finishing farms.

Salmonella Mbandaka sensitivity to antibiotics was exa-mined *in vitro* in this study. *In vivo* confirmation of these results is necessary because persistent antibacterial effects at subinhibitory concentrations (postantibiotic effects), which facilitate removal of affected bacteria by host defense mechanisms, have been

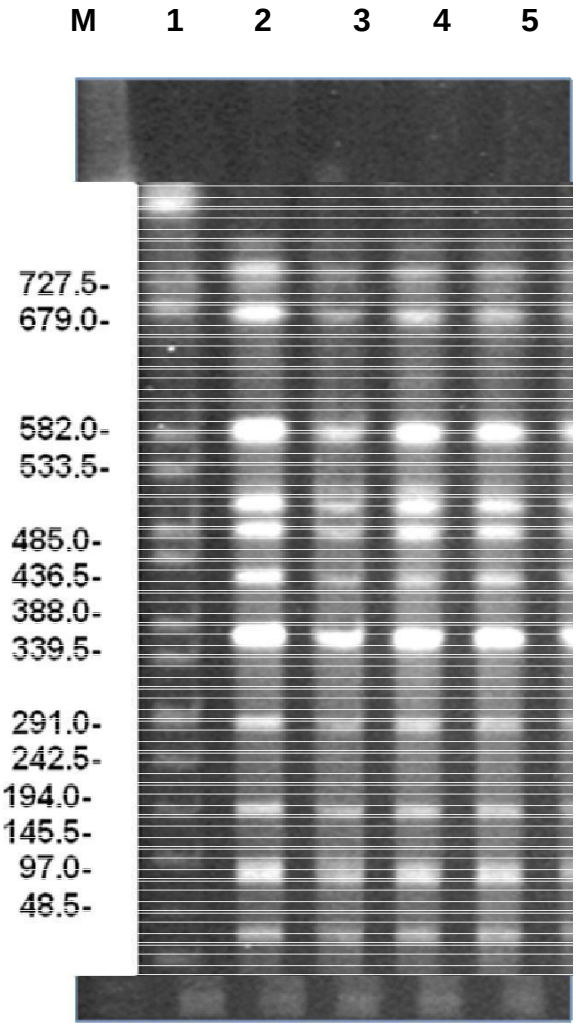

Figure 1. PFGE patterns of 5 *Salmonella Mbandaka* isolates. The patterns were obtained by the PFGE protocol with *XbaI* digestion. Lane M, lambda ladder with a size range of 45.5 to 727.5 kb (High Range PFG Marker, New England Biolabs Inc.). Lanes 1 – 5, the five *Salmonella* Mbandaka isolates analyzed.

demonstrated for penicillins, cephalosporins, macrolides, tetracyclines, aminoglycosides, and several other antibac-terial agents (Aiello S.E, 1998). In the case of increasing resistance to antibiotics, other methods such as identi-fication 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. Hoszowski 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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