Antibiotic resistance patterns of faecal indicator organisms and occurrence of *Salmonella* spp. in wild boar (*Sus scrofa scrofa*) in Italy.

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Abstract

In order to monitor antibiotic resistance in faecal indicator organisms and evaluate the occurrence of *Salmonella* spp., faeces from 110 wild boars (*Sus scrofa scrofa*), killed during a demographic control program in two different regional parks in Bologna province, were collected from September 2002 to June 2003. A single isolate of *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* from each sample was tested for antibiotic susceptibility using the agar diffusion method recommended by CLSI (formerly, NCCLS). A total of 110 *E. coli*, 48 *E. faecium* and 5 *E. faecalis* strains were isolated and submitted to antibiotic susceptibility tests. Antibiotic resistance patterns were similar in wild boar populations from both parks. Multiple antibiotic-resistance to ciprofloxacin, rifampin and erythromycin was found with high frequency in *Enterococcus* spp. strains. *E. coli* isolates showed a low antibiotic resistance level. Two *Salmonella arizonae* and one *Salmonella* spp. strains, isolated from wild boars of one park, didn’t show any resistance concerning the antibiotics tested.

Introduction

Antibiotics have become commonplace in our environment in consequence of their wide use in animal husbandry, medical therapy. Although data concerning antibiotic resistance in wild animals are scarce, some surveys have demonstrated the presence of multi-resistant indicator organisms in their faeces (Middleton and Ambrose, 2005; Poeta et al., 2005). The aim of this study was monitoring antibiotic-resistance in faecal indicator organisms isolated from wild boar (*Sus scrofa scrofa*) population living in two natural Regional Parks of Bologna province. Furthermore, occurrence and antibiotic resistance of *Salmonella* spp. were evaluated.

Materials and Methods

From September 2002 to June 2003, faeces from 55 wild boars (*Sus scrofa scrofa*) from Gessi Bolognesi Regional Park (48.15 Km²) and 55 from Monte Sole Regional Park (69.34 Km²) located in the Emilia-Romagna Region were collected. No contact between the different populations of the two parks had been recorded. The possibility of interactions and hybridisation between wild boars and domestic pigs was demonstrated in both parks, by capturing hybrid phenotype animals. Faecal samples were collected directly from the rectum, placed in BBL-Cary-Blair Transport Medium (Becton and Dickinson and Co., BD), stored at 4°C, transported to the laboratory and processed within 24 h after collection. In order to isolate *E. coli*, faeces were streaked directly onto BBL-MacConkey Agar (BD) and incubated at 37°C for 18-24 h. Strains identification was carried out by API 20E (BioMérieux). For the isolation of *E. faecalis* and *E. faecium*, 1 g of faeces was suspended in 4 ml of saline solution and serially 10-fold diluted until 10⁻⁶; 0.1 ml of each dilution was then inoculated on Difco-Bile Esculin Azide Agar (BD) and incubated at 37°C for 72 h. Isolates were identified at genus level on the basis of colony morphology, Gram staining, catalase production, esculin hydrolysis, growth in 6.5% NaCl, bile tolerance and L-pyruvolydonyl-β-naphtylamide hydrolysis (Manero e Blanch 1999; Devriese et al. 1993). Identification of *E. faecium* and *E. faecalis* isolates was performed by PCR as described by Dutka-Malen et al. (1995a;1995b).
order to isolate *Salmonella* spp. 5 g of each sample were inoculated in Difco-Muller-Kauffmann Tetrathionate Broth (BD) and Selenite Broth (Oxoid) and incubated at 42°C and 37°C for 24 h respectively. Enrichment broth's culture were then seeded onto two different media: Brilliant Green Agar (Oxoid) and XLT4 Agar (BD). *Salmonella* suspect colonies were identified by commercial system API 20E (BioMérieux). A single isolate of *E. coli*, *E. faecalis*, *E. faecium* and *Salmonella* spp. from each sample was tested for antimicrobial susceptibility using the agar diffusion method recommended by CLSI (NCCLS, 2002). The following 16 antimicrobial agents were included in the study for *E. coli* and *Salmonella* spp. using BBL Sensi-Disc (BD): amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin, cefotaxime, chloramphenicol, colistin, enrofloxacin, gentamicin, kanamycin, nalidixic acid, spectinomycin, streptomycin, sulfisoxazole, tetracycline and trimethoprim-sulfamethoxazole. The antibiotic susceptibility of *Enterococcus* spp. was assessed using the following molecules: ampicillin, chloramphenicol, ciprofloxacin, erythromycin, penicillin, rifampin, tetracycline and teicoplanin. CLSI interpretative standards (NCCLS, 2004) for *Enterobacteriaceae* and *Enterococcus* were used and the intermediate category was considered, in this study, as resistant. Multiple resistance was defined when resistance to three or more unrelated antimicrobial agents was found. Vancomycin and the high level aminoglycoside resistances of *E. faecalis* and *E. faecium* were evaluated by the screening test described by CLSI (NCCLS, 2004).

**Results**

From the 110 wild boars tested a total of 110 *E. coli*, 48 *E. faecium* and 5 *E. faecalis* strains were isolated. Differences in isolation rate from the animals living in the two Regional parks were observed for *Enterococcus* spp: *E. faecium* was isolated from 33 subjects from Gessi Bolognesi Park and 15 animals from Monte Sole Park while *E. faecalis* was isolated only from 5 wild boars in Monte Sole Park. Resistance rates of *E. coli*, *E. faecium* and *E. faecalis* strains from wild boars living in the two regional parks are showed in Table I. Multiple antibiotic resistance was observed in 83% of *E. faecium* and in all *E. faecalis* isolated. No multiple resistance was found in *E. coli*. No significant difference in antibiotic resistance rates between isolates from both parks was detected. No vancomycin and high level aminoglycoside resistance were observed in enterococci. Two *Salmonella enterica arizonae* and 1 *Salmonella* spp. strains were isolated from wild boar from Monte Sole Park; any antibiotic resistance was detected in these strains.

**Table I: Antibiotic resistance rates (%) of the faecal isolates from two regional parks obtained by agar diffusion method. Absolute numbers are presented under brackets.**

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AN: amikacin; S: streptomycin; K: kanamycin; GM: gentamicin; ENO: enrofloxacin; NA: nalidixic acid; CL: colistin; SPT: spectinomycin; SXT: trimethoprim-sulfamethoxazole; G: sulfisoxazole; CZ: cefazolin; CTX: cefotaxime; AMC: amoxicillin-clavulanic acid; AM: ampicillin; C: chloramphenicol; Te: tetracycline; CIP: ciprofloxacin; E: erythromycin; P: penicillin; RA: rifampin and TEC: teicoplanin.
Conclusion

In this study, *E. coli* and *Salmonella* strains showed a low antibiotic resistance level. Relative low levels of antibiotic resistance were also detected in enterococcal isolates when compared with those detected in enterococci of farm animals and human (Poeta et al., 2005). In particular no vancomycin or high level aminoglycoside resistances were observed in this study. Multiple antibiotic-resistance to ciprofloxacin, rifampin and erythromycin was found with high frequency in *Enterococcus* spp. strains, however further research should be carried out in order to investigate the molecular mechanisms of these resistances as well as to evaluate if the high rates are eventually due to strain donality.

References


