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Antimicrobial resistance of *Yersinia enterocolitica* 0:3 isolated from tonsils and lymph nodes of slaughtered pigs in Croatia

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Introduction

Human yersiniosis presents one of the main foodborne zoonoses in European Union (1). The main carriers of pathogenic *Yersinia enterocolitica* are pigs, and meat can be contaminated during slaughter processing. Very limited research of *Y. enterocolitica* in the context of food chain are available in Croatia. First published study (2) showed low prevalence (0.08%) of pathogen in pork meat, processed meat and surface swabs of meat processing units. However, authors isolated pathogenic *Y. enterocolitica* strain resistant to main clinical antibiotics relevant at the time of study (1990ties). Recent studies in Croatia (3, 4) evaluated the prevalence of *Y. enterocolitica* in food chain, including pig tonsils and mandibular lymph nodes, pork meat and meat preparations, thermally processed and fermented meat products, raw milk and unpasteurized milk cheeses. *Y. enterocolitica* 0:3 strains were only recovered from 26 tonsils (33.33%), 8 mandibular lymph nodes (10.25%) and retailed pork meat (6.25%).

Since antimicrobial resistance (AMR) in food chain is one of a leading One Health issues, the aim of presented study was to evaluate it in *Yersinia enterocolitica* 0:3 strains collected from tonsils and mandibular lymph nodes of slaughtered pigs in Croatian abattoirs.

Materials and Methods

Pig tonsils (n=78) and mandibular lymph nodes (n=78) were sampled on slaughter-line and subjected to microbiological testing for presence of *Y. enterocolitica*, as reported elsewhere (2). Three different types of abattoirs were selected, and pigs were originated from individual households, medium-size family farms and large farms.

Presumptive colonies (n=49) were selected from CIN and CHROMagar™ *Y. enterocolitica* and subjected to MALDI-TOF MS identification (Bruker Daltonik, Bremen, Germany) and serotyping (Statens Serum Institute, Denmark). Antimicrobial susceptibility was tested by disk diffusion method toward levofloxacin, ciprofloxacin, ampicillin, cephalothin, cefotaxime, tetracycline, nalidixic acid, ceftazidime, trimethoprim/sulfamethoxazole, chloramphenicol and streptomycin. Antimicrobial susceptibility/

resistance of strains was assessed following EUCAST/CLSI guidelines.

Results

All selected colonies were identified by MALDI-TOF MS as *Yersinia enterocolitica* and belonged to 0:3 serotype. The majority of strains was resistant toward ampicillin (91.6%) and cephalothin (85.4%), followed by chloramphenicol (31.2%), nalidixic acid (31.2%), streptomycin (27.0%), tetracycline (8.3%) and trimethoprim/sulfamethoxazole (2.0%). Only one strain was susceptible to all antimicrobial agents tested. *Y. enterocolitica* strains from medium-scale farms were mostly resistant to ampicillin and cephalothin, while strains collected from large farms were additionally resistant to chloramphenicol, nalidixic acid and streptomycin. Multiresistance (resistance to three or more agents) was found in 17 strains (35.4%). Higher prevalence of multiresistant *Y. enterocolitica* was evident in pigs originated from large farms (Table 1).

Discussion and Conclusion

Y. enterocolitica strains are usually resistant to penicillin, ampicillin, and first-generation cephalosporins. First-line drugs used against the bacterium include aminoglycosides and trimethoprim-sulfamethoxazole and other effective drugs include third-generation cephalosporins, tetracyclines and fluoroquinolones (5). The presence of resistant *Y. enterocolitica* in pigs at slaughter has been studied in recent years in many European countries (6, 7), but not in Croatia. Similar to our results, Fois et al. (6) reported the most common resistance to ampicillin and cephalothin in slaughtered pigs in Sardinia, Italy. In Latvia (8), additional resistance of all tested *Y. enterocolitica* was found toward erythromycin and sulphamethoxazole. Bonardi et al. (7) in North Italy also reported high level of resistance against sulphonamides in slaughtered pigs. In contrast, the resistance level toward sulphonamides in our study was low, as reported by other authors in Switzerland or Germany (9, 10). Opposite to other studies (11, 12), our isolates showed relative high resistance (about 30%) toward chloramphenicol, nalidixic acid or streptomycin. In conclusion, AMR in *Y. enterocolitica* of slaughtered pigs in Croatia is comparable to data from other European countries. The majority of strains were susceptible to clinically relevant antimicrobial agents.

References

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Table 1: Number of (multi)resistant *Y. enterocolitica* strains and resistance profiles

	Slaughterhouse 1 (medium-size farms)	Slaughterhouse 2 (large farms)	Slaughterhouse 3 (medium-size farms)
Tested strains	15	26	8
Resistant strains	14	26	8
Multiresistant strains	2	15	0
Dominant resistance patterns	AMP-KF (n=10)	AMP-KF-NA-C-STR (n=11)	AMP-KF
Multiresistance patterns	AMP-KF-TET, AMP-KF-TET-C, AMP-KF-NA	AMP-KF-TET-NA-C-STR, AMP-KF-NA-C-STR, TET-NA- CAZ-TST, KF-NA-C-STR, NA-C-STR	-

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