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Antimicrobial susceptibility of Yersinia enterocolitica strains isolated from pigs in the north-eastern region of Poland

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Summary

The purpose of the study was to evaluate the susceptibility of Yersinia (Y.) enterocolitica strains isolated from pigs in the north-eastern region of Poland to various chemotherapeutics. In total, the study comprised 103 Y. enterocolitica strains isolated in the years 2000-2007 from pig farms located in north-eastern Poland. The in vitro susceptibility of Y. enterocolitica strains to 14 chemotherapeutics was evaluated (ciprofloxacin, enrofloxacin, norfloxacin, gentamycin, neomycin, streptomycin, oxytetracycline, tetracycline, chloramphenicol, colistin, tiamulin, sulphonamides, sulphamethoxazole/trimethoprim-SXT, nitrofurantoin). Commercially available antimicrobial disks produced by Rosco and OXOID were used. The in vitro susceptibility of Y. enterocolitica strains was evaluated by a standardized disk diffusion method using Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The study demonstrated the significant variation in the susceptibility of the isolates. The Y. enterocolitica strains were found to show the highest in vitro susceptibility to quinolones (ciprofloxacin, norfloxacin, enrofloxacin), gentamycin and colistin. A slightly lower susceptibility in case of chloramphenicol and SXT was noticed. Streptomycin and neomycin were less effective. Sulfonamides and nitrofurantoin were also found to have very limited efficacy. However, Y. enterocolitica strains were fully resistant to tiamulin. Taking into account the widespread presence of Y. enterocolitica in many animal species and their increasing risk for public health, it is necessary to continually monitor the susceptibility of Y. enterocolitica strains to chemotherapeutics.

Keywords: pig, Yersinia enterocolitica, antimicrobial susceptibility

Yersinia (*Y.*) *enterocolitica* is one of the pathogenic species of the genus *Yersinia* causing worldwide infections in humans and animals, with symptoms primarily including gastrointestinal disorders (5, 30). The bacteria is ranked third in terms of the number of clinical cases and the public health risk, after *Campylobacter spp*. and *Salmonella spp*. (17).

The source of infection in humans may be from a range of farm, domestic and wild animals and the pathogen is usually transmitted through faeces-contaminated water and food (9, 12). The main reservoir of *Y. enterocolitica* are pigs: virulent strains were usually isolated from their tonsils and offal (11, 13). However, the pathogen also occurs in other farm and domestic animals – cattle, sheep, goats, dogs, cats and poultry (5, 8, 20).

Y. enterocolitica rods are thought to be in vitro susceptible to a number of chemo-therapeutics: quinolones, III- and IV-generation cephalosporins, monobactams, carbapenems, aminoglycosides, piperacillin, tetracyclines, chloramphenicol, potentionated sulfonamides. On the other hand, the microorganism demonstrates resistance to macrolides and lincosamides, as well as resistance or intermediate sensitivity to penicillins and I- and II-generation cephalosporins (2, 4, 14, 18, 21, 23, 26).

Overuse and inappropriate application of antibiotics in animals may lead to the development of drug-resistance in the bacteria and cause losses on farms as well as human health risk (3, 31). For this reason, since 2006 antibiotic growth promoters in animal production in EU are prohibited (1, 19). Therefore, the monitoring of bacteria's susceptibility to chemotherapeutics is very important, as it is the basis for choosing appropriate therapeutic procedures and the judicious application of bactericides, also in *Y. enterocolitica* infections (6).

The purpose of the study was to evaluate the susceptibility of *Y. enterocolitica* strains isolated from pigs in the north-eastern region of Poland to various chemotherapeutics.

Material and methods

Strains. In total, 103 strains of *Y. enterocolitica* isolated in the years 2000-2007 from pigs on farms located in north-eastern Poland were selected for the study. The strains were isolated from faeces and rectal swabs of clinically healthy pigs, vaginal swabs of miscarrying sows, placentas, internal organs of aborted foetuses and from the environment. The strains belonged to biotypes 1A, 3, 4 and serotypes 0:3, 0:5, 0:6, 0:7,13, 0:8, 0:9.

Chemotherapeutics. In the study 14 chemotherapeutics from different groups were used: 1. Quinolones (ciprofloxacin, enrofloxacin, norfloxacin), 2. Aminoglycosides (gentamycin, neomycin, streptomycin), 3. Tetracyclines (oxytetracycline, tetracycline), 4. Polymyxins (colistin), 5. Amfenicoles (chloramphenicol), 6. Pleuromutylins (tiamulin), 7. Sulfonamides (three sulfonamides compound), 8. Sulfamethoxazole/trimethoprim (SXT) and 9. Nitrofurans (nitrofurantoin).

Antimicrobial disks. Commercially available antimicrobial disks were used: Rosco (tiamulin) and OXOID (other disks and all growing media). The applied chemotherapeutic concentrations in the disks are presented in tab. 1.

Antimicrobial susceptibility testing. The in vitro susceptibility of *Y. enterocolitica* strains was evaluated by a standardized disk diffusion method using Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI)) recommendations. The results of the antimicrobial susceptibility examinations were recorded by measuring the inhibition zones and scored as susceptible, intermediate, or resistant.

Results and discussion

The overall results of *in vitro* susceptibility of *Y. enterocolitica* strains isolated from pigs in Poland to chemotherapeutics are presented in tab. 1.

Over 95% of the strains proved to be susceptible to ciprofloxacin and norfloxacin. Between 80% and 95% of the isolates were susceptible to enrofloxacin, gentamycin and colistin. Between 50% and 80% of *Y. enterocolitica* strains demonstrated susceptibility to tetracycline, oxytetracycline, chloramphenicol and SXT. The least effective chemotherapeutics were streptomycin, neomycin, sulfonamides and nitrofurantoin. No activity against *Y. enterocolitica* was found in the case of tiamulin. The results of studies of *Y. enterocolitica* strains' susceptibility to β-lactam antibiotics were presented in another paper (18).

Quinolones proved to be the group with the strongest antibacterial activity. All antibiotics from this group were fully effective against 92-99% of the tested *Y. enterocolitica* isolates. The highest effectiveness was exhibited by ciprofloxacin (99%); it was the only substance among the tested chemotherapeutics without resistant strains and with only one intermediately susceptible strain.

Among the aminoglycosides, the most effective antibiotic against *Y. enterocolitica* was gentamycin with 5.8% of resistant strains. Less effective were the older aminoglycosides – streptomycin and neomycin (33% and 24.3% of susceptible strains, respectively).

Proper effectiveness was also demonstrated for colistin with 85%, tetracyclines (tetracycline – 78.6%, oxytetracycline – 76.7%) and chloramphenicol – 71.8% of the susceptible strains.

Moreover, the tested *Y. enterocolitica* strains were characterised by a high resistance rate against the sulfonamides and nitrofurantoin (13.6% and 10.7% of the susceptible strains, respectively). One must stress, however, that a combination of sulfonamides with trimethoprim (SXT) significantly increases the activity of this substance against *Y. enterocolitica* (71.8% of the susceptible strains).

The development of resistance to chemotherapeutics by bacteria is a significant problem, both in human and veterinary medicine (31). The presented study showed that strains of *Y. enterocolitica* isolated from pigs in north-eastern region of Poland varied greatly in terms of their susceptibility to different groups of chemotherapeutics.

Y. enterocolitica exhibited the highest susceptibility to quinolones, as over 92% of strains were susceptible to three representatives used

Tab. 1. Susceptibility of *Y. enterocolitica* strains isolated from pigs to chemotherapeutics

Group	Chemotherapeutics	Concentration in disks (μg)	Number (%) of *Y. enterocolitica* strains		
			susceptible	intermediate	resistant
Quinolones	ciprofloxacin	5	102 (99.03)	1 (0.97)	0 (0)
	norfloxacin	10	101 (98.06)	1 (0.97)	1 (0.97)
	enrofloxacin	5	95 (92.23)	7 (6.80)	1 (0.97)
Aminoglycosides	gentamycin	10	88 (85.44)	9 (8.74)	6 (5.83)
	streptomycin	10	34 (33.00)	28 (27.18)	41 (39.81)
	neomycin	30	25 (24.27)	64 (62.14)	14 (13.59)
Tetracyclines	tetracycline	30	81 (78.64)	12 (11.65)	10 (9.70)
	oxytetracycline	30	79 (76.70)	14 (13.59)	10 (9.70)
Others	colistin	10	88 (85.44)	4 (3.88)	11 (10.68)
	chloramphenicol	30	74 (71.84)	24 (23.30)	5 (4.85)
	tiamulin	30	0 (0)	0 (0)	103 (100)
	sulphonamides	300	14 (13.59)	1 (0.97)	88 (85.44)
	SXT	25	74 (71.84)	4 (3.88)	25 (25.27)
	nitrofurantoin	300	11 (10.68)	18 (1.48)	74 (71.84)

in this study. Stock and Wiedemann (26) classified the *Y. enterocolitica* rod as quinolones-susceptible, with ciprofloxacin as the most potent representative of this group. Our study also demonstrated that ciprofloxacin is very effective against *Y. enterocolitica*, as no strains were found to be resistant. These results are in accordance with those obtained by other scientists who found the strains to be 100% susceptible (14, 21, 22, 23, 28), and differ from the results of Kot et al. (13) showing only 44.5-66.8% strains susceptible to ciprofloxacin. A similar high susceptibility (98%) was demonstrated for norfloxacin (1 resistant strain). Studies carried out in the USA and India in the years 1990-2006 found 100% of *Y. enterocolitica* strains to be susceptible to norfloxacin (2, 14, 23). *Y. enterocolitica* rods are also susceptible to enrofloxacin, with 6.8% intermediately susceptible and only 1% of strains resistant, which is confirmed by the studies of other authors (22, 23). In the studies of Funk et al. (10), carried out in the USA, 100% of strains were noticed to be susceptible to enrofloxacin.

Y. enterocolitica is also considered to be susceptible to aminoglycosides (22, 23, 26). In our study, three aminoglycosides were evaluated and various susceptibility results were obtained. The oldest representative of this group is streptomycin. Soriano and Vega (25) found 16% of *Y. enterocolitica* strains to be resistant, the authors of the present study – 39.8%, while Singh and Virdi (23) did not find any strains to be resistant and demonstrated all isolates to be susceptible (74%) or intermediately susceptible (26%) to streptomycin. The studies of Funk et al. (10) and Singh and Virdi (23) point to neomycin as a highly active antibiotic against *Y. enterocolitica*, while in our study the majority of strains were classified as intermediately susceptible (62.1%) and only 24.3% as susceptible. Studies also confirm a high susceptibility of *Y. enterocolitica* to gentamycin, and a number of authors have demonstrated 98-100% susceptibility (2, 10, 13, 16, 21, 22). The present study found only 5.8% of *Y. enterocolitica* strains to be resistant to this antibiotic.

Tetracyclines are a group of antibiotics with a wide range of bacteriostatic activity (24). Tetracycline and oxytetracycline showed similar, high effectiveness, with 78.6% and 76.7% susceptible as well as 11.7% and 13.6% of intermediately susceptible strains, respectively. Stock and Wiedemann (27) classified *Y. enterocolitica* as susceptible or intermediately susceptible to tetracycline. Preston et al. (21) tested the susceptibility of *Y. enterocolitica* strains isolated in Canada in the years 1972-1990 and found 98-99.7%, Singh and Virdi (23) – 100%, Baumgartner et al. (4) – 99%, Kot et al. (13) – 100%, while Simonova et al. (22) only 80.3% of strains to be susceptible to tetracycline, which also in context of our results can indicate the slight decrease of sensitivity of *Y. enterocolitica* strains to this group of antibiotics.

The results of our study demonstrated a relatively high percentage of *Y. enterocolitica* strains susceptible to colistin (85.4%), while Singh and Virdi (23) observed 100% susceptibility of *Y. enterocolitica* to this antibiotic. Quite a high effectiveness of chloramphenicol was also demonstrated. The majority of *Y. enterocolitica* strains in our study were found to be susceptible (71.8%) or intermediately susceptible (23.3%), which was also confirmed by the study of Simonova et al. (22), who found 67.1% of strains to be susceptible and 29% intermediately susceptible. In the majority of publications *Y. enterocolitica* is presented as being very susceptible to chloramphenicol (2, 4, 21, 23, 26), while Funk et al. (10) found 95-100% of strains to be only intermediately susceptible.

Nitrofurans are a group of bacteriostatic chemotherapeutics. Funk et al. (10) in their study concerning *Y. enterocolitica* strains isolated in the USA in the years 1994-1995 found 100% of strains to be susceptible, while Singh and Virdi (23), who examined strains isolated in India in the years 1997-2001, found 87.5% of strains to be susceptible and 12.5% intermediately susceptible. In the present study, however, the majority of strains were found to be resistant, with only 10.7% of *Y. enterocolitica* strains susceptible, 17.5% intermediately susceptible and as high as 71.8% of strains resistant to nitrofurantoin.

In our study, 85.4% of *Y. enterocolitica* strains isolated from pigs were found to be resistant to sulfonamides. A number of scientists, on the other hand, have demonstrated that the majority of *Y. enterocolitica* isolates are susceptible to sulfonamides, in particular those potentionated with trimethoprim (co-trimoxozoles, SXT) (2, 23, 25). Singh and Virdi (23) found 100% of *Y. enterocolitica* strains to be susceptible to co-trimoxazole and 97.5% to trimethoprim. As regards individual sulfonamides, 73.8% of strains were susceptible and 26.2% intermediately susceptible to sulfafurazole; conversely, in the case of sulfadiazine, no strains were found to be susceptible, 47.5% – intermediately susceptible, and 52.5% – resistant to this sulfonamide. This is confirmed by the studies of other scientists; however, results vary significantly (7, 10, 25, 29). Lyons et al. (15) found 53% of strains to be resistant to sulfamethoxazole, while Preston et al. (21) demonstrated that *Y. enterocolitica* is highly susceptible (98.8-100%) to this sulfonamide. Soriano and Vega (25), who tested 167 *Y. enterocolitica* strains, failed to find strains resistant to trimethoprim and SXT, while 17.5% of the strains were resistant to sulfamethoxazole. The scientists also demonstrated a cross resistance of *Y. enterocolitica* strains to sulfamethoxazole and streptomycin. The present study, on the other hand, shows a significant increase in the antibacterial activity of sulfonamides potentionated with trimethoprim against *Y. enterocolitica*, while SXT proved to be active against 71.8% of the strains. Funk et al. (10) demonstrated that 95-100% of *Y. enterocolitica* strains were susceptible to SXT. Also in other studies a high percentage of strains (100% or nearly 100%) were found to be susceptible to cotrimoxazoles (2, 4, 14, 21, 25, 26).

Attempts to explane the differences in the in vitro susceptibility or resistance of *Y. enterocolitica* strains to various chemoterapeutics is difficult and complex. They are connected with and depend on various parameters – time of strain isolation, region of strain origin, animal species, characteristic of the strain (biotype, serotype, virulence markers), strain variability (point mutations, drug resistance), chemotherapeutic group, time and period of use. Inappropriate antibiotic therapy or long term use can lead to a change in the efficacy of chemoterapeutics or to develop strains that are drug--resistant, often multi-drug resistant, to the majority of antibacterial product groups (32). The identification of highly-resistant bacteria strains facilitates effective treatment and prevents the spread of such strains to other individuals (6). Therefore, more and more often the rational usage of antibiotics in animal and human therapy is postulated, and the EU prohibited usage of antibiotics as growth promoters from January 1st, 2006 (1, 3, 19, 32).

In conclusion, the study has proved that strains of *Y. enterocolitica* isolated from pigs in the north-eastern region of Poland varied greatly in terms of their *in vitro* susceptibility to different groups of chemotherapeutics. *Y. enterocolitica* strains were found to demonstrate the highest *in vitro* susceptibility to quinolones (ciprofloxacin, norfloxacin, enrofloxacin), gentamycin and colistin, while proving to be resistant to tiamulin. Chloramphenicol and SXT, but especially streptomycin and neomycin were less effective. Sulfonamides and nitrofurantoin, generally regarded as effective against *Y. enterocolitica*, were also found to have limited efficacy. The Polish strains of *Y. enterocolitica* varied greatly also in terms of the *in vitro* susceptibility to β-lactam antibiotics (18). Taking into account that *Y. enterocolitica* is wide spread in many animal species and the increasing risk it poses for public health, it is therefore deemed highly necessary to continually monitor the antimicrobial susceptibility of *Y. enterocolitica* strains to chemotherapeutics.

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