SUSCEPTIBILITY OF POLISH YERSINIA ENTEROCOLITICA STRAINS ISOLATED FROM PIGS TO 12 β-LACTAM ANTIBIOTICS

KAROLINA PERKOWSKA, ALEKSANDRA PLATT-SAMORAJ, AGATA BANCERZ-KISIEL, AND WOJCIECH SZWEDA

Department of Epizootiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-718 Olsztyn, Poland

Received: December 17, 2011 Accepted: February 16, 2011

Abstract

The aim of the study was to assess the susceptibility of 103 Yersinia enterocolitica strains isolated from pigs in Poland in the years 2000–2007 to 12 β-lactam antibiotics. The in vitro susceptibility of the bacteria to seven selected cephalosporins of all generations and to five penicillins was tested by means of a disk diffusion method following the criteria and recommendations provided by the National Committee for Clinical Laboratory Standards. The strains varied greatly in regard to their in vitro susceptibility to β-lactam antibiotics. The strains were found to be relatively highly susceptible to the third and fourth generation cephalosporins, while being generally resistant to the first generation cephalosporins and most penicillins. Taking into account the wide spread of Y. enterocolitica, in particular in the pig population, but also among other animal species, which creates an increasing risk to the public health, it is deemed necessary to systematically monitor the susceptibility of Y. enterocolitica strains to antibiotics.

Key words: pig, Yersinia enterocolitica, antibiotic susceptibility, β-lactams.

Yersinia enterocolitica is a microorganism with increasing significance for public health worldwide (5). In Europe, the number of cases of intestinal yersiniosis is the second only to salmonellosis and intestinal campylobacteriosis (5). Y. enterocolitica is widely spread in land and aquatic environments and in various animal populations, particularly in pigs, which constitute the main reservoir and source of infection for humans, but it was also found in wild boars, cattle, sheep, goats, dogs, cats, poultry, fish, and wild animals (5, 7, 8, 12, 21, 25). Virulent strains of Y. enterocolitica are transmitted to humans mainly through faeces contaminating water, milk, and food, usually of animal origin, as a result of hygienic negligence during processing and storage.

Y. enterocolitica is characterised by varied biochemical properties, antigenicity, and pathogenicity. At the moment, six biotypes are distinguished, including 1B, 2, 3, 4, and 5 regarded as pathogenic, and 70 serotypes, including serotypes 0:3, 0:8, 0:9, and 0:5,27, which are the most significant for public health (5). A microorganism’s virulence is related to pathogenicity factors encoded by plasmids and chromosomes; the main role seems to play the enterotoxins Yst and Ail protein (11, 20).

Y. enterocolitica infections in animals are usually symptomless, while in humans predominant symptoms include gastro-intestinal disorders with diarrhoea. However, other forms of the disease such as septic, pseudoappendicular, lympho-nodular, cutaneous (erythema nodosum or Reiter’s syndrome), as well as reactive polyarthritis, pharyngitis, panophthalmitis, meningoencephalitis, osteomyelitis, and microabscesses in the liver, spleen, kidneys, lungs, and colon are also observed (5, 19).

The need to use antibiotics in the treatment of humans and animals, preventive use thereof, errors in application and sometimes overuse, may all lead to the development of antibiotic resistance in the bacteria, causing a growing risk to human and animal health. For this reason, from January 1, 2006, the use of antibiotic growth promoters in animal production is prohibited in EU Member States (1, 24). This requirement has caused an increased demand for medicated feed that may contain tylosin, lincomycin, chlorotetracycline, doxycycline, amoxicillin, tiamulin, and sulphaguanidine. Systematic monitoring of the susceptibility of bacterial strains, including Y. enterocolitica, must therefore be regarded as highly justified to ensure appropriate treatment of humans and to limit the spread of microorganisms’ drug resistance in animals.

The aim of this study was to assess the susceptibility of Y. enterocolitica strains isolated from...
pigs in Poland in the years 2000–2007 to β-lactam antibiotics.

**Material and Methods**

**Strains.** *Yersinia enterocolitica* strains were isolated from the faeces of clinically healthy pigs, rectal and vaginal swabs of miscarrying sows, placentas and internal organs of aborted foetuses (lymph nodes, tonsils, liver, spleen, lungs, small and large intestines) and from the environment. In total, 103 strains of *Y. enterocolitica* isolated in 2000-2007 from pigs in farms located in the North-East Poland were selected for the study.

Biotyping carried out in accordance with the scheme set out in annex D PN-EN ISO 10273 (27), including determination of the ability to ferment trehalose, xylose, and aesculin, and detection of pyrazinamidase, Tween esterase, and indole, proved that the strains belonged to biotypes 1A, 3, and 4.

Serotyping was carried out by means of a slide agglutination test using as antigen 24 h *Y. enterocolitica* blood agar culture (Graso Biotech, Poland) and specific sera for somatic antigens. The examined strains belonged to the following serotypes: O:3, O:5, O:6, O:7, 13, O:8, 19, O:20, and O:22.

The examinations for the presence of virulence markers were carried out using a multiplex PCR with primers amplifying fragments of the *ail*, *ystA*, and *ystB* genes synthesised in the DNA Sequencing Laboratory, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences – Oligo, Warsaw. Products with respective masses: 531 bp (14), 134 bp (26), and 180 bp (26) were obtained, which proved that the *ail* and *ystA* genes are present in biotype 4 strains, while in biotype 1A strains only the *ystB* gene (26) was found to be present.

**Antibiotic discs.** Commercially available antibiotic discs (BioLab, Hungary; OXOID, UK) were used. Twenty selected β-lactam antibiotics were applied (antibiotic concentrations in the discs in brackets), including seven cephalosporins of all generations: I – cephalothin (30 μg), cefazolin (30 μg), II – cefamandole (30 μg), cefuroxime (30 μg), III – cefotaxime (30 μg), ceftriaxone (30 μg), and IV – cefepime (30 μg) and five penicillins – amoxyccillin/克拉维酸 (30 μg), ampicillin (10 μg), ampicillin/sulbactam (20 μg), carbenicillin (100 μg), and piperacillin (100 μg).

**Antibiotic susceptibility testing.** The susceptibility of *Y. enterocolitica* strains was tested with a standardized disc diffusion method using Müiller-Hinton agar and antibiotic discs, following the criteria and recommendations provided by the National Committee for Clinical Laboratory Standards. The results of antibiotic susceptibility were recorded by measuring the inhibition zones and scored as susceptible (S), intermediate susceptible (I), or resistant (R).

**Results**

The results of the *in vitro* susceptibility assessment of Polish *Y. enterocolitica* strains isolated from pigs to β-lactam antibiotics are presented in Table 1 and Fig. 1. β-lactam antibiotics were characterised by a widely varied effectiveness against the 103 *Y. enterocolitica* strains. Among the cephalosporins, the most effective was cefepime, a representative of the fourth generation cephalosporins, with 101 (98.06%) strains susceptible and only two (1.94%) strains resistant. Susceptibility to cefotaxime and ceftriaxone, third generation cephalosporins, was found in 86.41% and 84.47% and resistance in 6.8% and 9.7% of strains, respectively. Susceptibility to the second generation cephalosporins: cefuroxime and cefamandole, was found in 63.1% and 58.25% of strains, respectively, and resistance was the same for the two antibiotics: 34.95%.

The first generation cephalosporins showed little or no activity against *Y. enterocolitica*. Only three (2.91%) strains were susceptible to cephalzin with seven (6.8%) intermediate susceptible and 93 (90.29%) resistant. All the strains were resistant to cephalothin.

Among penicillins, the best results were obtained for piperacillin with 53 strains (51.54%) susceptible, 34 (33.01%) intermediate susceptible, and 16 (15.53%) resistant. The vast majority of *Y. enterocolitica* strains proved to be resistant to other antibiotics from this group, with 72.82% of strains resistant to ampicillin/sulbactam and over 90% of strains resistant to carbenicillin (93.2%), amoxicillin/clavulanate (94.17%), and ampicillin (98.06%). None of the *Y. enterocolitica* strains was susceptible to ampicillin and only two strains were susceptible to amoxyccillin/clavulanate and carbenicillin (1.94%).

**Discussion**

Inappropriate antibiotic treatment and prophylaxis, as well as overuse of antibiotics, in both human and veterinary medicine, may lead to the development of bacterial strains resistant to one or several groups of anti-bacterial drugs, including antibiotics (32). This is a growing problem, and therefore more and more attention is paid to reasonable, wise, and prudent usage of antimicrobials. This is reflected by e.g. the prohibition of using antibiotics as growth promoters, implemented in 2006 in EU Member States (1, 24).

Bacteria have a wide variety of ways and mechanisms to develop antibiotic resistance, and therefore it is important to systematically assess their susceptibility to individual antibiotics, thus enabling selection an optimal treatment and preventing the spread of drug resistance among bacteria (6, 8). The presented studies showed that Polish strains of *Y. enterocolitica* isolated from pigs varied greatly in terms of their *in vitro* susceptibility to β-lactam antibiotics.
### Table 1
Results of susceptibility assessment of Polish *Y. enterocolitica* strains isolated from pigs to β-lactam antibiotics

<table>
<thead>
<tr>
<th>Group</th>
<th>Antibiotics</th>
<th>Number (%) of <em>Y. enterocolitica</em> strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>susceptible</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>cefepime</td>
<td>101 (98.06)</td>
</tr>
<tr>
<td></td>
<td>cefotaxime</td>
<td>89 (86.41)</td>
</tr>
<tr>
<td></td>
<td>ceftriaxone</td>
<td>87 (84.47)</td>
</tr>
<tr>
<td></td>
<td>cefuroxime</td>
<td>65 (63.10)</td>
</tr>
<tr>
<td></td>
<td>cefamandole</td>
<td>60 (58.25)</td>
</tr>
<tr>
<td></td>
<td>cephazolin</td>
<td>3 (2.91)</td>
</tr>
<tr>
<td></td>
<td>cephalothin</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Penicillins</td>
<td>piperacillin</td>
<td>53 (51.54)</td>
</tr>
<tr>
<td></td>
<td>ampicillin/sulbactam</td>
<td>17 (16.50)</td>
</tr>
<tr>
<td></td>
<td>amoxycillin/clavulanate</td>
<td>2 (1.94)</td>
</tr>
<tr>
<td></td>
<td>carbenicillin</td>
<td>2 (1.94)</td>
</tr>
<tr>
<td></td>
<td>ampicillin</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Percentage of susceptible *Y. enterocolitica* strains to selected cephalosporins and penicillins.

The first generation cephalosporins, including cephazolin and cephalotin, are active against Gram-positive bacteria, while showing little or no activity against bacteria of the *Enterobacteriaceae* family. The authors’ studies proved the resistance of 100% of *Y. enterocolitica* strains to cephalotin and of 90.29% of strains to cephazolin; these results are in accordance with the results of other researchers (9, 16, 17, 28, 29).

The studies of Hammerberg et al. (13) did not find any *Y. enterocolitica* strains to be susceptible to cephalotin, while the studies of Kwaga and Iversen (18) found only 3% of strains to be susceptible to cephalotin and 36% to cephalozin. In the recent studies of Abdel-Haq et al. (2), 2% of *Y. enterocolitica* strains were found to be susceptible and 8% intermediate susceptible to cephalozin, while Kot et al. (17) demonstrated that 88.9% of biotype 1A and 60% of biotype 4 strains were susceptible to cephalozin.

The second generation of cephalosporins with a stronger activity against Gram-negative than Gram-positive bacteria includes e.g. cefuroxime and cefamandole. These antibiotics are effective in the treatment of *Streptococcus* sp., *Staphylococcus* sp., *Neisseria* sp., *Moraxella* sp., *Haemophilus influenza*, *Escherichia coli* and other anaerobic rod infections. The authors’ studies have demonstrated that 63.1% of *Y. enterocolitica* strains are susceptible to cefuroxime...
and 58.25% to cefamandole, while Singh and Virdi (30) found 41.3% and 37.5%, of strains to be susceptible and 52.5% and 50% respectively, respectively. The study by Hoogkamp-Korstanje (15), involving 189 Y. enterocolitica isolates from patients with clinical yersiniosis, showed that 87% of the isolates were susceptible to cefuroxime. On the other hand, the studies of Kwaga and Iversen (18), carried out in the years 1972-1990, demonstrated 100% susceptibility of Y. enterocolitica to cefuroxime and cefamandole. Lack of resistance to these antibiotics was also found by Rastawicki et al. (29), who studied Polish serotype 0:3 strains in 1996-1998. The majority of strains were also found to be susceptible to the aforementioned antibiotics in the course of a study carried out in Spain by Gaspar and Soriano (10). This proves that Y. enterocolitica strains have developed a significant resistance to the second generation cephalosporins over the last twenty years.

The third generation of cephalosporins, of which cefotaxime and ceftriaxone were included in the studies, demonstrates bactericidal activity mainly against Staphylococcus sp. and Streptococcus sp., but also against Enterobacteriaceae, Haemophilus influenzae, Borrelia sp., and Pasteurella sp. Y. enterocolitica rods are categorised as microorganisms susceptible to the two cephalosporins. This was confirmed in own studies; only 6.8% of Y. enterocolitica strains were found to be resistant to cefotaxime and 9.7% to ceftriaxone, which is in accordance with the results obtained by Abdel-Haq et al. (2), who observed ca. 8% of strains to be resistant. Singh and Virdi (30) did not find these strains to be resistant, while 11% and 10% of Y. enterocolitica strains were already susceptible, respectively. In other comparative studies, resistance to antibiotics of this generation was not found (4, 15-18, 22, 31). The studies of the Polish clinical strains of Y. enterocolitica, serotype O:3, have demonstrated full susceptibility of the strains to the third generation cephalosporins (29).

The fourth generation cephalosporins exhibit the strongest activity against both Gram-positive and Gram-negative bacteria. Cefepime, used in our study, is applied in the treatment of Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, and Enterobacteriaceae infections. Stock and Wiedermann (31) categorise Y. enterocolitica strains as susceptible, which was confirmed by Abdel-Haq et al. (2). However, our study found only two (1.94%) strains to be resistant, which may indicate that a certain percentage of strains are susceptible to cephalosporins occurs within the studied reservoir, e.g. pigs and their environment.

Treatment with penicillins in veterinary medicine is frequently used. The following antibiotics from this group were selected for the study: amoxycillin/clavulanate, ampicillin, ampicillin/ sulbactam, carbencillin, and piperacillin. The literature cites assessments of Y. enterocolitica susceptibility to amoxicillin, as well as amoxycillin/clavulanate. In own study, only 1.94% of Y. enterocolitica strains was found to be susceptible and 3.98% intermediately susceptible to amoxycillin/clavulanate. The studies of Singh and Virdi (30) demonstrated that only 2.5% of strains were susceptible, while remaining strains were only susceptible to amoxicillin/clavulanate. On the other hand, Funk et al. (9) found only 5% of Y. enterocolitica strains to be resistant, while as much as 94% were already susceptible to amoxicillin/clavulanate. Studies of other authors (17, 31) demonstrated full Y. enterocolitica resistance to amoxicillin. Similar results were obtained for carbencillin with 1.94% of susceptible strains, while Singh and Virdi (30) found 24% susceptible and 64% susceptible strains. The studies of Preston et al. (28) in Canada demonstrated nearly identical (2%) percentage of susceptible strains as in our study. Studies of Hammerberg et al. (13) showed 100% Y. enterocolitica resistance to carbencillin, while Ahmed et al. (3) found 13% of strains to be susceptible.

Ampicillin shows strong bactericidal activity towards a wide range of microorganisms; however, Y. enterocolitica rods are in general resistant to this antibiotic. The authors’ study found no susceptible strains and only 1.94% of strains to be susceptible. Singh and Virdi (30) found 100% of strains to be resistant, which is also confirmed by the results of other authors (13, 16, 18, 29). On the other hand, Abdel-Haq et al. (2) found 2% of strains to be susceptible and 11% to be intermediate susceptible. A susceptibility of 1%-2% of strains was also confirmed by Funk et al. (9). The combination of ampicillin with sulbactam significantly broadens the scope of activity and increases the percentage of susceptible strains from 0% to 16.5% and the intermediate susceptible strains from 1.94% to 10.68%. A significant increase in the percentage of susceptible and intermediate susceptible strains, from 0% to 24% and 34%, respectively, was reported by Singh and Virdi (30), while the study results of Abdel-Haq et al. (2) found 73% of Y. enterocolitica strains to be susceptible to ampicillin/sulbactam.

Piperacillin, a representative of ureidopenicillins with a broad scope of antibacterial activity, is frequently used e.g. in the treatment of Pseudomonas aeruginosa infections. Stock and Wiedermann (31) categorise Y. enterocolitica strains as susceptible or intermediate susceptible, which was confirmed by the authors’ study, who found the vast majority of strains to be susceptible (51.54%) or immediately susceptible (33.01%). In the studies of Kot et al. (17), 77.8% of susceptible strains were found to belong to biotype 1A and 60% to biotype 4. Kwaga and Iversen (18) in their studies carried out in the years 1972-1990, found all Y. enterocolitica strains to be susceptible to piperacillin, while Abdel-Haq et al. (2) observed that only 78% of strains were susceptible. Singh and Virdi (30) obtained varied results of piperacillin susceptibility assessment with 27.5% of strains susceptible, 47.5% immediately susceptible, and 25% resistant. Pham et al. (23) explain the significant diversity in the susceptibility to β-lactam antibiotics as due to the different capabilities of Y. enterocolitica strains to produce β-lactamases.
One may conclude that in the course of the study, the Polish strains of *Y. enterocolitica* isolated from pigs varied greatly in terms of their *in vitro* susceptibility to β-lactam antibiotics. *Y. enterocolitica* strains were found to be relatively highly susceptible to the third and fourth generation cephalosporins, whilst being generally resistant to the first generation cephalosporins and most penicillins. Taking into account the wide spread of *Y. enterocolitica*, particularly in the pig population but also among other animal species, which has an increasing risk to the public health, it is deemed necessary to systematically monitor the susceptibility of *Y. enterocolitica* strains to antibiotics.

**Acknowledgments:** The authors would like to thank Mrs Mrs Danuta Pieludź, Eliza Lipińska, and Bogumiła Pietruszka for the excellent technical assistance they provided during the realization of this study.

**References**


