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In-vitro antimicrobial susceptibility of *Yersinia enterocolitica* isolates from stools of patients in The Netherlands from 1982–1991

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The MICs of 24 antimicrobial agents were determined for 335 strains of *Yersinia enterocolitica* isolated from faeces in the Netherlands during 1982–1991. The isolates belonged to biotypes 1A, 1B, 2, 3, 4 and to serotypes O3, O5.27, O6.3, O7.8, O8, O9. Almost all strains were susceptible to piperacillin, piperacillin/tazobactam, imipenem, all cephalosporins except cefazolin, the aminoglycosides, quinolones, co-trimoxazole, doxycycline and chloramphenicol but resistant to amoxycillin, co-amoxiclav and macrolides. No association was observed between susceptibility patterns, biotype or serotype nor were there marked changes in the susceptibilty during the last decade. The agents traditionally used to treat human infection, including co-trimoxazole, doxycycline and chloramphenicol may remain drugs of first choice.

Introduction

Yersinia enterocolitica is an enteric pathogen associated with a wide spectrum of clinical and immunopathological manifestations. Recognition as a human pathogen was soon followed by successful isolation from clinical specimens in many countries. The availibility of selective media and comprehensive identification and biotyping schemes contributed further to the detection of *Y. enterocolitica* in stool specimens. Results of in-vitro antimicrobial susceptibilty testing of *Y. enterocolitica* from different countries have been reported in several studies (Hornstein *et al.*, 1985; Hoogkamp-Korstanje, 1987; Pham, Bell & Lanzarone, 1991*a*).

The aim of the present study was to evaluate the in-vitro activities of a wide range of antimicrobial agents, including the novel quinolone BAY y3118, against stool isolates of *Y. enterocolitica*, the relationship between antibiotic sensitivity and biotype, antibiotic sensitivity and serotype and to find out whether there has been any change in susceptibility during the last decade.

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Materials and methods

Bacteria

Three hundred and thirty-five strains of *Y. enterocolitica* isolated from stool specimens of patients with diarrhoea between January 1982 and July 1991 were used in this study (1982: 5 strains, 1983: 2, 1984: 26, 1985: 47, 1986: 75, 1987: 65, 1988: 56, 1989: 22, 1990: 22 and 1991: 15 strains). The strains were isolated in the Public Health Laboratory in Friesland (The Netherlands). One strain per patient was included in the study.

Culture methods

Stool specimens were inoculated on to Yersinia selective agar (Oxoid CM653 PCH-Diagnostica, Haarlam, The Netherlands), deoxycholate citrate agar (Oxoid CM35 PCH-Diagnostica) and into Rappaport broth. Plates were incubated at 22°C for 48 h. Broth was incubated at 22°C for 72 h and then subcultured on the above mentioned culture plates. Colonies resembling those of *Yersinia* spp. were identified according to Wauters (1973) and biotyped according to Wauters, Kandolo & Janssens (1987). Isolates were serotyped by slide agglutination using commercially available O-antisera for O3 and O9 (Sanofi Diagnostics Pasteur, Genk, Belgium) and with specific rabbit antisera for O5.27, O6.3 and O8 (Hoogkamp-Korstanje *et al.*, 1992).

Antimicrobial agents

Standard powders were obtained from the following sources: Sigma (erythromycin, amoxycillin, piperacillin, cefazolin, gentamicin, amikacin), Bayer (ciprofloxacin, Bay y3118), Hoechst (ofloxacin), Hofmann-La Roche (fleroxacin, co-trimoxazole), Pfizer (azithromycin, doxycycline), Abbott (clarithromycin), Smith Kline Beecham (co-amoxiclav), Lederle (piperacillin/tazobactam), Merck Sharp & Dohme (imipenem), Glax (cefuroxime, ceftazidime), Bristol Myers Squibb (cefepime), Roussel UCLAF (cefpirome), Lilly (tobramycin), Gist-Brocades (chloramphenicol).

Determination of the MIC

The minimum inhibitory concentrations were determined in duplicate by broth dilution method in microtitration plates using Iso-Sensitest broth (Oxoid CM 491 PCH-Diagnostica). Each well was filled with 150 μL antibiotic solution except for the last well which was used to control growth. Inocula were prepared according to the direct colony suspension method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1990), by suspending at least four colonies from overnight cultures on blood agar in 3 mL of sterile 0.85% NaCl to a McFarland turbidity standard of 0.5 (1.5×10^8 cfu/mL) inoculated into the microtiter plates with an automatic multipoint inoculator (MIC 2000, Dynatech, USA). Wells were inoculated with 1.5 μL suspension to yield 10^5 cfu/mL. The inoculum size and purity were controlled by plating 1 μL of the bacterial suspension on to blood agar. The plates were incubated at 37°C for 18 h. The MIC was defined as the lowest concentration to prevent visible growth. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control organisms. For analysis, we adopted the breakpoints recommended by the Dutch working group on antimicrobial susceptibility testing (Mouton & van Klingeren, 1990).

Results and discussion

The isolates were uniformly susceptible to piperacillin, piperacillin/tazobactam, imipenem, ceftazidime, cefepime, cefpirome, aminoglycosides, quinolones and co-trimoxazole (Table I). Almost all were susceptible to cefuroxime, cefdinir, doxycycline and chloramphenicol. All isolates were resistant to amoxycillin and most resistant to co-amoxiclav, cefazolin and the macrolides. The MIC_{90}s of BAY y3118 and ciprofloxacin were lower than those of ofloxacin and fleroxacin. It was also noteworthy that more strains were susceptible to azithromycin than to the other macrolides and that a high MIC of azithromycin corresponded with a high MIC of the other macrolides and vice versa.

The ranges of MICs of the different antimicrobial agents are in agreement with the data reported from other parts of the world (Hornstein *et al.*, 1985; Pham *et al.*, 1991*a*).

Of 335 isolates, 139 strains belonged to serotype O3, 76 strains to serotype O9, 16 strains to serotype 5.27, 13 strains to serotype O6.3, 20 strains to serotype O7.8 and 22 strains to serotype O8. The remaining strains were not typeable with the antisera used. These findings are not different from the rest of Europe (Cover & Aber, 1989).

The sensitivities of strains to β-lactam antibiotics *in vitro* have been shown to correspond to the major serotypes (Hornstein *et al.*, 1985) but we found no difference in the range of MICs within the serotypes for any of the antibiotics tested.

Table I. MICs (mg/L) of 24 antibiotics for 335 *Y. enterocolitica* isolates

Antibiotic	Breakpoint concentration (mg/L)[a]	MIC_{50}	MIC_{90}	Range	% Susceptible
Amoxycillin	≤2	≥32	≥32	4–≥32	0
Co-amoxiclav	≤2/1	16	≥32	≤1–≥32	5
Piperacillin	≤16	2	8	≤1–16	100
Piperacillin/tazobactam	≤16/4	1	4	≤0.5–8	100
Imipenem	≤4	0.25	0.25	≤0.031–0.5	100
Cefazolin	≤4	32	64	≤2–≥64	10
Cefuroxime	≤4	2	4	≤0.5–≥16	90
Ceftazidime	≤4	0.5	1	≤0.062–4	100
Cefepime	≤4	0.032	0.032	≤0.008–0.128	100
Cefdinir	≤4	4	8	≤0.5–⩾16	86
Cefpirome	≤4	≤0.5	≤0.5	≤0.5–2	100
Gentamicin	≤1	0.5	1	≤0.125–2	99
Tobramycin	≤1	0.5	1	≤0.125–2	99
Amikacin	≤4	2	2	0.25–4	100
Ciprofloxacin	≤0.5	0.016	0.016	≤0.008–0.032	100
Ofloxacin	≤0.5	0.064	0.064	≤0.008–0.128	100
Fleroxacin	≤0.5	0.064	0.064	0.016–0.128	100
BAY y3118	≤0.5	≤0.008	≤0.008	≤0.008–0.016	100
Erythromycin	≤2	32	64	≤2–⩾64	2
Azithromycin	≤2	2	4	0.5–8	50
Clarithromycin	≤2	32	32	≤2–128	3
Co-trimoxazole	≤2/38	≤0.064	0.125	≤0.064–0.25	100
Doxycycline	≤1	1	2	≤0.125–8	90
Chloramphenicol	≤4	2	4	≤0.5–8	94

[a]Recommended by Mouton & van Klingeren (1990) (except for azithromycin, clarithromycin, cefepime, cefdinir and cefpirome).

Table II. Susceptibility of different *Y. enterocolitica* biotypes to various antibiotics (MIC_{50} in mg/L)

Antibiotic	Biotype 1A ($n=71$)	Biotype 1B ($n=43$)	Biotype 2 ($n=34$)	Biotype 3 ($n=21$)	Biotype 4 ($n=134$)
Amoxycillin	≥32	≥32	≥32	≥32	≥32
Co-amoxiclav	≥32	≥32	≥32	≥32	8
Piperacillin	4	4	4	4	2
Piperacillin/tazobactam	2	2	2	2	≤0.5
Imipenem	0.25	0.25	0.25	0.25	0.25
Cefazolin	16	16	16	8	16
Cefuroxime	2	4	4	4	2
Ceftazidime	0.5	0.5	0.5	0.5	≤0.062

A biotype for 303 isolates could be determined. Pham *et al.* (1991*a*,*b*) found a specific relationship between biotype and sensitivity pattern to β-lactam agents and suggested that differential sensitivity to the members of the β-lactam antibiotics was due to the production of different β-lactamases by the members of each biotype. In the present study we could not demonstrate any obvious difference in the range of MICs of the β-lactams for the various biotypes. The MIC_{50} for the biotypes are shown in Table II. The MIC_{50} for biotype 4 to the combination of a β-lactam with clavulanic acid or tazobactam was clearly lower than that of the other biotypes but there were only four strains of biotype 4 with a MIC ≤ 1 mg/L for co-amoxiclav.

Finally, there was no significant change in the susceptibility patterns of the different isolates over the years and no multiply resistant isolates were found. In conclusion, our data show that the agents traditionally used to treat human infection, including co-trimoxazole, doxycycline and chloramphenicol may remain drugs of first choice.

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