J. Med. Microbiol. — Vol. 49 (2000), 403–408

ISSN 0022-2615

ANTIMICROBIAL RESISTANCE

# *β*-Lactamase expression in *Yersinia enterocolitica* biovars 1A, 1B and 3

INGO STOCK, PETER HEISIG and BERND WIEDEMANN

*Pharmazeutische Mikrobiologie, University of Bonn, Bonn, Germany*

**Characteristic patterns of *β*-lactam susceptibility are associated with different biovars of *Yersinia enterocolitica*. In a previous study differences in *β*-lactam susceptibility among biovar 2, 4 and 5 strains were largely attributed to differences in expression of *β*-lactamase A (BlaA) and *β*-lactamase B (BlaB). The basis for differences in *β*-lactam susceptibility of strains of biovars 1A, 1B and 3 is now considered. All the strains examined had *blaB*; nine of 31 biovar 3 strains and two of 13 biovar 1B strains had *blaA*, but PCR did not amplify *blaA* from biovar 1A strains. Nevertheless, inhibition data indicated that the majority of uninduced biovar 1A strains expressed BlaA and BlaB in similar amounts. Strong inducibility was seen in all these strains. Biovar 1B strains (which were less inducible than strains of biovar 1A) predominantly produced BlaA without induction; ticarcillin-sensitive strains of biovar 3 produced only BlaB but were not inducible; without induction biovar 3 strains resistant to ticarcillin and amoxycillin/clavulanate produced either predominantly BlaA, predominantly BlaB or exclusively BlaB and induction was demonstrated except for strains producing BlaB alone; biovar 3 strains resistant to ticarcillin but sensitive to amoxycillin/clavulanate predominantly produced BlaA without induction and were inducible for *β*-lactamase activity. After induction, nearly all strains predominantly or exclusively produced BlaB. Although PCR amplification fragments with primers specific for *blaA* were obtained only from some strains, the induction and inhibition data suggest that all *Y. enterocolitica* strains possess enzymes related to BlaA- as well as BlaB. Nevertheless, expression of the *β*-lactamase is regulated differently in different biovars and varies within most biovars. Failure to predict *β*-lactamase expression profiles from MIC data indicates the presence of additional mechanisms contributing to differences in susceptibility.**

## Introduction

*Yersinia enterocolitica* is a frequent agent of bacterial enteritis and enterocolitis world-wide [1]. The species is divided into six biovars (1A, 1B, 2, 3, 4 and 5) [2, 3], which differ in their geographical distribution, ecological niches and pathogenic properties. This study investigated the *β*-lactamases of strains of biovars 1A, 1B and 3. Biovar 1A strains (i.e., serovars O:5; O:6, 30; O:6, 31; O:7, 8; O:10; O:18; O:46 and non-typable strains) are distributed world-wide and are predominantly isolated from the environment, water, faeces and food [1, 4–6]. They lack the virulence determinants of invasive isolates and originally were considered to be non-pathogenic [1, 7]; however, there is evidence that some strains are human-adapted and potentially pathogenic [8]. Biovar 1A strains have been isolated from clinical specimens [9–11] and a recent study pointed to virulence-associated characteristics [12]. Strains of biovar 1B (serovars O:8; O:4,32; O:13a; O:13b; O:18; O:20 and O:21) are frequently found in association with human yersiniosis, mostly in the USA and Canada ('American strains') [1], but occasionally also in Japan and Europe [13–15]. Serovar O:8, biovar 1B is the most virulent type among *Y. enterocolitica* [16], and accounts for severe yersinosis outbreaks in North America [17–19]. The natural reservoir of biovar 1B strains is the environment and pigs (serovar O:8) [1]. Biovar 3 strains (serovars O:1, 2, 3; O:5, 27) are also a common cause of human yersiniosis world-wide. Their natural reservoirs are usually restricted to pigs and chinchillas [1, 20], but strains persist enzootically in livestock, which can transmit yersiniae to healthy chinchillas [20].

Received 25 June 1999; revised version received 2 Sept. 1999; accepted 17 Sept. 1999.
Corresponding author: Dr I. Stock (e-mail: b.wiedemann@uni-bonn.de).

Previous studies aiming to correlate antibiotic susceptibility patterns and biovar in *Y. enterocolitica* have yielded conflicting results. Stolk-Engelaar *et al.* examined strains of all biovars except biovar 5 and found no association between susceptibility and biovar or serovar [11], whereas others have found relationships [9, 10, 21–23]. This group confirmed the latter view, finding a relationship between biovar and susceptibility to several $\beta$-lactam antibiotics (Table 1 and [22]). Furthermore, differences in the regulation and expression of BlaA and BlaB, the two chromosomal $\beta$-lactamases of *Y. enterocolitica* [24], were found among strains of biovars 2, 4 and 5 [25] and these data were related to biovar-specific susceptibilities to $\beta$-lactams. This report describes the extension of these studies to *Y. enterocolitica* strains of biovars 1A, 1B and 3.

## Materials and methods

### *Bacterial strains*

A total of 66 *Y. enterocolitica* strains belonging to biovar 1A (n = 22), biovar 1B (n = 13) and biovar 3 (n = 31) was examined. Twelve biovar 1A and two biovar 3 strains were isolated from surface waters and filter plant discharges around Bonn and Cologne (Germany) and were kindly provided by M. Exner (Bonn). Twenty biovar 3 strains and three strains each of biovars 1A and 1B originated from the University Hospital of Bonn, Germany or from the culture collection of Merlin-Diagnostika, Bornheim, Germany. All these strains were from clinical specimens. A further 24 isolates were kindly provided by S. Aleksic (Hamburg, Germany); these were of human and mammalian origin and were used as reference strains, having been biotyped and serotyped at the Hygiene Institute of Hamburg. They comprised strains of biovars 1A (n = 5), 1B (n = 10) and 3 (n = 9). Two additional biovar 1A strains were from raw milk and were kindly provided by H. Neubauer (Munich, Germany). All the clinical isolates were non-replicates from separate patients on different wards.

### *PCR for blaA and blaB*

All the strains were examined by PCR for the presence of *bla*A and *bla*B by a multiplex approach [25]. For some strains, the PCR procedure was modified by using either the primer pair to amplify a 439-bp fragment of *blaA* or the primer pair to amplify a 781-bp fragment of *blaB* [25]. Each PCR for *blaA* or *blaB* was performed with mixtures containing 20 pmol of each of the two primers and was run with 35 cycles of 30 s at 50°C, 60 s at 72°C and 30 s at 95°C.

### *Measurement of β-lactamase induction and inhibition*

Fourteen biovar 1A, 12 biovar 1B and 22 biovar 3 strains were tested as described previously [25]. Induction was determined after growth with imipenem at a final concentration of 0.5 mg/L.

## Results

### *Amplification of blaA fragments*

Applying a multiplex PCR approach with four primers yielded amplification products for *bla*A for two biovar 1B (phenotypic group 1B-III) and several biovar 3 strains (Table 2). Altering the PCR conditions or incorporating a chloroform-phenol extraction of the templates yielded the same results. Most strains of biovar 1A, including all those of phenotypic group 1A-I and one of four strains belonging to phenotypic group 1A-II, yielded faint bands in the 1500-bp region instead of the 479-bp products anticipated with *bla*A primers (not shown).

### *Amplification of blaB fragments*

With the *bla*A/*bla*B multiplex PCR procedure, amplification products for *bla*B were obtained from all biovar 1B and the *bla*A-positive biovar 3 strains, but not from other strains of biovar 3 and not from biovar 1A strains (Table 2). However, *bla*B amplification

**Table 1.** Susceptibility of *Y. enterocolitica* biovars 1A, 1B and 3 to antibiotics (from ref. 22)

| Biovar | Number of isolates | Phenotypic group | Number of isolates | MIC range (mg/L) | | | | Differences in ratio of MICs of AMX:AMC |
|---|---|---|---|---|---|---|---|---|
| | | | | cefixime | cefpodoxime | ticarcillin | amoxycillin/clavulanate | |
| 1A | 22 | 1A-I | 18 | 1–4* | 1–4 | 32–128† | 8–32‡ | 1–16 |
| | | 1A-II | 4 | 0.25 | 0.25 | 32–64 | 4–8 | 4–8 |
| 1B | 13 | 1B-I | 9 | ⩽0.03–0.25 | 0.06–0.25 | 16–64 | 0.5–2 | 32–64 |
| | | 1B-II | 2 | 0.06–0.13 | 0.125 | 2–4 | 1–2 | 4 |
| | | 1B-III | 2 | 4 | 4 | 128 | 32 | 4 |
| 3 | 31 | 3-I | 19 | 1–4* | 1–4§ | 1–4 | 4–16 | 2–4 |
| | | 3-II | 8 | 1–4* | 1–4§ | 64–256 | 16–32 | 4 |
| | | 3-III | 4 | 0.13–0.25 | 0.13–0.5 | 64–128 | 1–4 | 16–64 |

AMX, amoxycillin; AMC, amoxycillin/clavulanate. MIC values were also *8 mg/L to cefixime (one strain); †16 mg/L to ticarcillin (two strains); ‡4 mg/L to amoxycillin/clavulanate (one strain); §8 mg/L to cefpodoxime (one strain).

**Table 2.** Expression of β-lactamases, and presence of β-lactamase genes, in *Y. enterocolitica* strains of biovars 1A,1B and 3

| Biovar (number of strains) | Phenotypic group* | Cluster† | Number of strains | Presence of β-lactamase A | | | Presence of β-lactamase B | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | PCR | | Inhibition assay | PCR | | Inhibition assay | Induction assay |
| | | | | Multiplex | *blaA* | | Multiplex | *blaB* | | |
| | 1A-I | ND‡ | 10 | − | −§ | + | − | + | + | + |
| 1A | 1A-II | $A_1$ | 2 | − | − | + | − | + | + | + |
| (14) | | $A_2$ | 1 | − | − | + | − | + | + | + |
| | | B | 1 | − | −§ | − | − | + | + | + |
| | 1B-I | A | 1 | − | − | + | + | NT | + | − |
| | | $B_1$ | 5 | − | − | + | + | NT | + | + |
| 1B | | $B_2$ | 1 | − | − | + | + | NT | + | + |
| (12) | | $B_3$ | 1 | − | − | + | + | NT | + | + |
| | 1B-II | ND‡ | 2 | − | − | + | + | NT | + | + |
| | 1B-III | ND‡ | 2 | + | NT | + | + | NT | + | + |
| | 3-I | ND‡ | 10 | − | − | − | − | + | + | − |
| | 3-II | A | 2 | − | − | − | − | + | + | − |
| 3 | | $B_1$ | 4 | + | NT | + | + | NT | + | + |
| (22) | | $B_2$ | 2 | + | NT | + | + | NT | + | + |
| | 3-III | $A_1$ | 3 | + | NT | + | + | NT | + | + |
| | | $A_2$ | 1 | − | − | + | − | + | + | + |

*According to [22].
†No difference, according to data obtained from induction and inhibition tests.
‡No differences.
§Bands in the 1500 bp-region.
NT, not tested.

fragments were obtained from all strains in the single PCR procedure with *bla*B primers alone (Table 2 and Fig. 1).

## *β-Lactamase induction and inhibition*

A summary of induction and inhibition results is shown in Table 3. Because BlaA and BlaB display characteristic inhibition profiles [24, 26], inhibition results were interpreted as follows. Where clavulanate inhibited activity completely, BlaA (class A) was deduced to be the sole β-lactamase; where aztreonam inhibited activity completely, BlaB (class C) was deduced to be the sole enzyme. Where clavulanate inhibited most of the activity, BlaA was deduced to be the predominant enzyme, whereas BlaB was deduced to be predominant when aztreonam inhibited most activity.

β-Lactamase expression differed between and within the biovars. Biovar 1A strains had the most homogeneous patterns of β-lactamase inhibition and induction, 11 of 13 representatives expressing similar amounts of BlaA and BlaB without induction and having strong inducibility. Most biovar 1B strains showed weaker inducibility with BlaA activity predominating without induction; however, one strain was not inducible. β-Lactamase expression in biovar 3 strains was heterogeneous, but there were correlations between MIC data and the patterns of induction and inhibition. Thus, ticarcillin-sensitive strains produced only BlaB but were – surprisingly – not inducible. Without induction, biovar 3 strains resistant to ticarcillin and amoxycillin/clavulanate produced either predominantly BlaA, predominantly BlaB or exclusively BlaB. Induction was seen with imipenem except with those strains exclusively producing BlaB. Biovar 3 strains resistant to ticarcillin but sensitive to amoxycillin/clavulanate exclusively or predominantly produced BlaA without induction; they were weakly inducible.

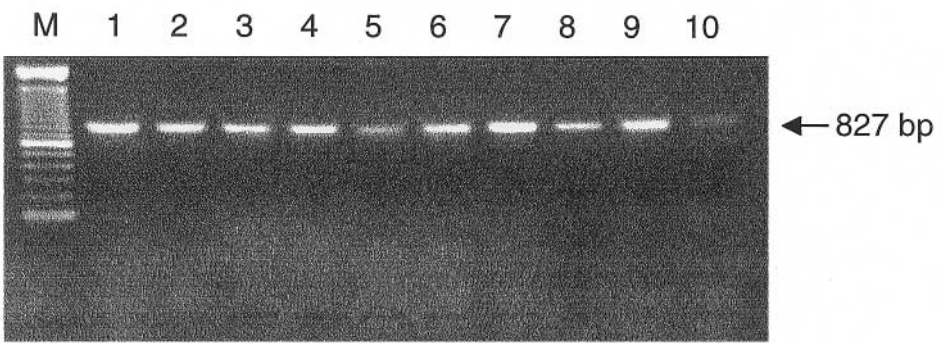


**Fig. 1.** Results from the *bla*B PCR for 10 *Y. enterocolitica* strains showing no amplification products for *bla*B with the multiplex PCR approach. The 827-bp bands indicate the presence of *bla*B. Lane M, 100-bp ladder DNA marker.

**Table 3.** $\beta$-Lactamase induction and inhibition in *Y. enterocolitica* strains of biovars 1A, 1B and 3

| | | | | $\beta$-Lactamase induction | | | $\beta$-Lactamase inhibition (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Specific activity ($\mu$mol substrate hydrolysed/min/mg) | | | Uninduced cultures | | Induced cultures | |
| | | | | | | | in presence of[‡] | | | |
| Biovar (Number of strains) | Phenotypic group[*] | Cluster[†] | Number of strains | Uninduced cultures | Induced cultures | Fold increase | AZT | CLA | AZT | CLA |
| | 1A-I | ND | 10 | 0.40–0.74 | 10–28 | 25–44 | 45–70 | 30–55 | >95–100 | 0–<5 |
| 1A | 1A-II | $A_1$ | 2 | 0.56–0.65 | 3.1–6.5 | 6–10 | 10–15 | 85–90 | 95 | 5 |
| (14) | | $A_2$ | 1 | 0.13 | 4.6 | 35 | 45 | 55 | 95 | 5 |
| | | B | 1 | 0.11 | 5.5 | 50 | 100 | 0 | 100 | 0 |
| | 1B-I | A | 1 | 0.29 | 0.36 | 1.2 | 10 | 90 | 10 | 90 |
| | | $B_1$ | 5 | 0.24–0.30 | 1.0–2.0 | 4–7 | 10–40 | 60–90 | 80–90 | 10–20 |
| 1B | | $B_2$ | 1 | 0.23 | 3.9 | 17 | 30 | 70 | >95 | <5 |
| (12) | | $B_3$ | 1 | 0.10 | 1.6 | 15 | 85 | 15 | 90 | 10 |
| | 1B-II | ND | 2 | 0.10–0.27 | 0.6–2.7 | 6–10 | 40–70 | 30–60 | >95–100 | 0–<5 |
| | 1B-III | ND | 2 | 2.1–2.8 | 18–29 | 9–10 | 45 | 55 | >95 | <5 |
| | 3-I | ND | 10 | 0.55–1.3 | 0.47–1.5 | 0.6–1.5 | 100 | 0 | 100 | 0 |
| | | A | 2 | 0.49–0.89 | 0.91–1.4 | 1.6–1.8 | 100 | 0 | 100 | 0 |
| 3 | 3-II | $B_1$ | 4 | 0.53–0.75 | 9.4–12.3 | 13–18 | 25–30 | 70–75 | >95–100 | 0–<5 |
| (22) | | $B_2$ | 2 | 1.1–2.0 | 7.2–9.9 | 5–7 | 70 | 30 | 100 | 0 |
| | 3-III | $A_1$ | 3 | 0.32–1.0 | 0.69–2.0 | 2.0–2.2 | 15–20 | 80–85 | 50–90 | 10–50 |
| | | $A_2$ | 1 | 0.60 | 1.5 | 2.5 | <5 | >95 | 80 | 20 |

ND, no differences.
[*]According to [22].
[†]According to data obtained from induction and inhibition tests.
[‡]2 mg/L.
AZT, aztreonam; CLA, clavulanate

After induction, nearly all strains of all the biovars examined predominantly or exclusively produced BlaB. Inhibition tests did not suggest the presence of other β-lactamases besides BlaA or BlaB, as complete inhibition was achieved if both clavulanic acid and aztreonam were added.

## Discussion

Amplification products for *bla*B were obtained for all the strains of biovars 1A, 1B and 3 and were previously obtained from all *Y. enterocolitica* strains of biovars 2, 4 and 5 [25]. Thus the gene for BlaB appears to be present in *Y. enterocolitica* of all biovars.

The present inhibition and induction data suggested expression of BlaA and BlaB in all biovar 1A strains, except for one with solely BlaB activity (Table 3). Pham *et al.* likewise found BlaA and a BlaB variant in biovar 1A strains [26–28]; the BlaB variant had the same inhibition as classical BlaB, but had a pI of 6.2–6.4 *versus* 5.4–5.6 [24]. A single alteration in the amino-acid sequence might give such a change, and so it is not surprising that the present study obtained amplification products for *bla*B from all biovar 1A strains. It remains to be elucidated whether the 1500-bp amplification products obtained with primers to *bla*A could be attributed to a β-lactamase gene. It may be that biovar 1A strains produce a BlaA-like enzyme, but too little DNA was amplified to allow sequencing.

β-Lactamase expression and inhibition also suggested the presence of both BlaA and BlaB enzymes in biovar 1B strains. These results are in contrast to those of Bottone [1], who predicted the presence of other β-lactamases in serogroup O:8 strains because of their sensitivity to ampicillin. Greater susceptibility to amoxycillin in biovar 1B strains was found previously in this laboratory, but only for two strains (phenotypic group 1B-II, serovars O:8 and O:20) ([22] and Table 1), which nevertheless showed induction and inhibition profiles implying expression of both BlaA and BlaB. Other serovar O:8 strains were resistant to amoxycillin and also expressed both enzymes. The results of the present study are in agreement with the data of Pham *et al*., who found BlaA and BlaB in one isolate of biovar 1B [28]. It is not known why primers specific for *bla*A allowed amplification of *bla*A fragments only from the biovar 1B strains with a high basal level of β-lactamase activity (Table 3).

Biovar 3 strains reportedly produce only the AmpC-type enzyme BlaB [26–28] and this property is reflected in their sensitivity to carboxypenicillins (carbenicillin and ticarcillin) and in the weak synergic effect of clavulanic acid with amoxycillin [4, 9]. Nevertheless, induction and inhibition results for biovar 3 revealed several phenotypes: uninduced cultures produced either solely BlaB (phenotypic groups 3-I and 3-II, cluster A), predominantly BlaB (3-II, cluster C), mainly BlaA (3-II, cluster B, 3-III, cluster $A_1$) or almost exclusively BlaA (3-III, cluster $A_2$). Sensitivity to ticarcillin was seen only for those isolates that expressed solely BlaB [26–28], but not all strains with this phenotype were sensitive to ticarcillin (3-II, cluster A). Surprisingly, biovar 3 strains expressing only the usually inducible BlaB enzyme were not inducible with imipenem. Further studies are needed to clarify the reason for this phenomenon, which might be due to alterations in the *amp*R regulator genes linked to the *bla*B structured genes.

*Y. enterocolitica* comprises a relatively heterogeneous group of micro-organisms [29–31], but only one study [32] has questioned the grouping of all the biovars into one species. Similar β-lactamases would be expected within such a species and we postulate that all *Y. enterocolitica* strains possess genes for both BlaA and BlaB, but that the expression of these enzymes depends on the biovar and the individual strain. To fully test this hypothesis, other primer pairs for *bla*A will be needed to test the presence of *bla*A homologues in all the strains irrespective of biovar. Similarly, Australian strains of biovar 4 that are known to express only BlaA [26] will have to be rigorously examined for *bla*B homologues. Failure to detect *bla*A in several strains might be due to the method and primers applied. Another explanation might be a genetic variability in *bla*A preventing binding of primers derived from known *bla*A sequences.

## References


1. Bottone EJ. *Yersinia enterocolitica*: the charisma continues. *Clin Microbiol Rev* 1997; **10**: 257–276.
2. Wauters G, Kandolo K, Janssens M. Revised biogrouping scheme of *Yersinia enterocolitica*. *Contrib Microbiol Immunol* 1987; **9**: 14–21.
3. Wauters G, Janssens M, Steigerwalt AG, Brenner DJ. *Yersinia mollaretii* sp. nov. and *Yersinia bercovieri* sp. nov., formerly called *Yersinia enterocolitica* biogroups 3A and 3B. *Int J Syst Bacteriol* 1988; **38**: 424–429.
4. Ahmedy A, Vidon DJ-M, Delmas CL, Lett M-C. Antimicrobial susceptibilities of food-isolated strains of *Yersinia enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii*. *Antimicrob Agents Chemother* 1985; **28**: 351–353.
5. Tzelepi E, Arvanitidou M, Mavroidi A, Tsakris A. Antibiotic susceptibilities of *Yersinia enterocolitica* and *Y. intermedia* isolates from aquatic environments. *J Med Microbiol* 1999; **48**: 157–160.
6. Weber G, Stanek G, Massiczek N, Klenner MF. *Yersinia enterocolitica* im Trinkwasser. [*Yersinia enterocolitica* in drinking water.] *Zentralbl Bakteriol Mikrobiol Hyg B* 1981; **173**: 209–216.
7. Cornelis G, Laroche Y, Balligand G, Sory MP, Wauters G. *Yersinia enterocolitica*, a primary model for bacterial invasiveness. *Rev Infect Dis* 1987; **9**: 64–87.
8. Burnens AP, Frey A, Nicolet J. Association between clinical presentation, biogroups and virulence attributes of *Yersinia enterocolitica* strains in human diarrhoeal disease. *Epidemiol Infect* 1996; **116**: 27–34.
9. Pham JN, Bell SM, Lanzarone JYM. Biotype and antibiotic sensitivity of 100 clinical isolates of *Yersinia enterocolitica*. *J Antimicrob Chemother* 1991; **28**: 13–18.
10. Preston MA, Brown S, Borczyk AA, Riley G, Krishnan C.

Antimicrobial susceptibility of pathogenic *Yersinia enterocolitica* isolated in Canada from 1972 to 1990. *Antimicrob Agents Chemother* 1994; **38**: 2121–2124.
11. Stolk-Engelaar VMM, Meis JFGM, Mulder JA, Loeffen FLA, Hoogkamp-Korstanje JAA. In-vitro antimicrobial susceptibility of *Yersinia enterocolitica* isolates from stools of patients in The Netherlands from 1982–1991. *J Antimicrob Chemother* 1995; **36**: 839–843.
12. Lewin A, Strauch E, Hertwig S, Hoffmann B, Nattermann H, Appel B. Comparison of plasmids of strains of *Yersinia enterocolitica* biovar 1A with the virulence plasmid of a pathogenic *Y. enterocolitica* strain. *Zentralbl Bakteriol* 1996; **285**: 52–63.
13. Ichinohe H, Yoshioka M, Fukushima H, Kaneko S, Maruyama T. First isolation of *Yersinia enterocolitica* serotype O:8 in Japan. *J Clin Microbiol* 1991; **29**: 846–847.
14. Saito M, Yamaguchi M, Toyokawa Y, Ohtomo Y, Kaneko Y, Maruyama T. *Yersinia enterocolitica* serotype O:8 infection in the Hirosaki district in Aomori prefecture from 1984 to 1991. *J Jpn Assoc Infect Dis* 1994; **68**: 960–965.
15. Chiesa C, Pacifico L, Guiyoule A, Ravagnan G, Mollaret HH. Phenotypic characterization and virulence of O:8 *Yersinia* strains isolated in Europe. *Contrib Microbiol Immunol* 1991; **12**: 182–191.
16. Hayashidani H, Ohtomo Y, Toyokawa Y *et al*. Sources of sporadic human infection with *Yersinia enterocolitica* serovar O:8 in Aomori Prefecture, Japan. *Contrib Microbiol Immunol* 1995; **13**: 29–32.
17. Black RE, Jackson RJ, Tsal T *et al*. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *N Engl J Med* 1978; **298**: 76–79.
18. Shayegani M, Menegio EJ, McGlynn DM, Gaafar HA. *Yersinia enterocolitica* in Oneida County, New York. *Contrib Microbiol Immunol* 1979; **5**: 196–205.
19. Shayegani M, Morse D, DeForge I, Root T, Parsons LM, Maupin PS. Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup O:8. *J Clin Microbiol* 1983; **17**: 35–40.
20. Wuthe HH, Aleksic S. *Yersinia enterocolitica* serovar 1, 2a, 3 biovar 3 in chinchillas. *Int J Med Mikrobiol Virol Parasitol Infect Dis* 1992; **277**: 403–405.
21. Kwaga J, Iversen JO. In vitro antimicrobial susceptibilities of *Yersinia enterocolitica* and related species isolated from slaughtered pigs and pork products. *Antimicrob Agents Chemother* 1990; **34**: 2423–2425.
22. Stock I, Wiedemann B. An in-vitro study of the antimicrobial susceptibilities of *Yersinia enterocolitica* and the definition of a database. *J Antimicrob Chemother* 1999; **43**: 37–45.
23. Toma S, Lafleur L. Survey of the incidence of *Yersinia enterocolitica* infection in Canada. *Appl Microbiol* 1974; **28**: 469–473.
24. Cornelis G, Abraham EP. $\beta$-Lactamases from *Yersinia enterocolitica*. *J Gen Microbiol* 1975; **87**: 273–284.
25. Stock I, Heisig P, Wiedemann B. Expression of $\beta$-lactamases in *Yersinia enterocolitica* strains of biovars 2, 4 and 5. *J Med Microbiol* 1999; **48**: 1023–1027.
26. Pham JN, Bell SM, Lanzarone JYM. A study of the $\beta$-lactamases of 100 clinical isolates of *Yersinia enterocolitica*. *J Antimicrob Chemother* 1991; **28**: 19–24.
27. Pham JN, Bell SM. The prevalence of inducible $\beta$-lactamase in clinical isolates of *Yersinia enterocolitica*. *Pathology* 1993; **25**: 385–387.
28. Pham JN, Bell SM. $\beta$-Lactamase induction by imipenem in *Yersinia enterocolitica*. *Pathology* 1992; **24**: 201–204.
29. Fukushima H, Gomyoda M, Aleksic S. Genetic variation of *Yersinia enterocolitica* serotype O:9 strains detected in samples from western and eastern countries. *Zentralbl Bakteriol* 1998; **288**: 167–174.
30. Lobato MJ, Landeras E, González-Hevia MA, Mendoza MC. Genetic heterogeneity of clinical strains of *Yersinia enterocolitica* traced by ribotyping and relationships between ribotypes, serotypes, and biotypes. *J Clin Microbiol* 1998; **36**: 3297–3302.
31. Ibrahim A, Goebel BM, Liesack W, Griffiths M, Stackebrandt E. The phylogeny of the genus *Yersinia* based on 16S rDNA sequences. *FEMS Microbiol Lett* 1993; **114**: 173–178.
32. Ibrahim A. Genetic diversity among *Yersinia enterocolitica* strains as revealed by sequence analysis of the 16S rRNA gene. *Contrib Microbiol Immunol* 1995; **13**: 277–280.