Molecular Characterization of Human Clinical Isolates of
Yersinia enterocolitica Bioserotype 1B/O8 in Poland:
Emergence and Dissemination of Three
Highly Related Clones†‡

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Thirty-three clinical isolates of the highly pathogenic Yersinia enterocolitica bioserotype 1B/O8 were collected from sporadic cases in Poland from January 2004 to July 2008. The isolates carried major virulence markers and were strongly clonal. This is the first report of the emergence and dissemination of highly related clones of Y. enterocolitica 1B/O8 in Europe.

Yersinia enterocolitica is known to be an important human enteric pathogen, causing a large variety of clinical and immunological manifestations (2). Infections in humans are mostly sporadic and related to contaminated food or water. Seven biotypes (1A, 1B, 2, 3, 4, 5, and 6) and more than 60 serotypes have been described for Y. enterocolitica. However, certain serotypes (O3; O5,27; O8; and O9) are prevalent among human isolates (2, 23). This species encompasses three grades of pathogenicity: mostly nonpathogenic strains (biotype 1A), weakly pathogenic strains of biotypes 2 to 6, and highly pathogenic strains (biotype 1B). The high pathogenicity is attributed to the yersiniabactin siderophore-mediated iron uptake system. Yersiniabactin is maintained by genes located in the Yersinia enterocolitica high-pathogenicity island (3). The geographical distribution of Y. enterocolitica 1B/O8 is generally restricted to North America (2, 4); however, this pathogen has been isolated sporadically in Japan (8, 9, 11, 15). Recently a single isolate was reported in Germany (17). Other reports of Y. enterocolitica O8 occurrence in Europe were published elsewhere (for a review, see reference 17); however, only strain 893/87 from Italy (16) and the German isolate were confirmed where (for a review, see reference 17); however, only strain 893/87 from Italy (16) and the German isolate were confirmed where. The Polish 1B/O8 isolates belong to the highly pathogenic Y. enterocolitica high-pathogenicity island, which was investigated to demonstrate that the Polish 1B/O8 isolates belong to the highly pathogenic Y. enterocolitica lineage (2, 3). PCRs were carried out as described previously (7) with primers listed in Table S1 in the supplemental material. All of the tested isolates yielded PCR ampliﬁcons for the Y. enterocolitica 16S rRNA gene and the afore-mentioned virulence marker genes. There is strong evidence suggesting that the Polish 1B/O8 isolates belong to highly pathogenic Y. enterocolitica.

Congo red magnesium oxalate agar medium (14) and the PCR assay with primers for the yadA gene, which encodes Yersinia adhesin A (2, 6), were used to detect the Yersinia virulence plasmid (pYV). The virulence plasmid is considered the gold standard for Yersinia virulence determination. However, in contrast to the chromosomal virulence markers, pYV can easily be lost when bacteria are cultured at 37°C (1, 2, 17). In this study, pYV was detected in nine isolates only (Table 1). Given the high sensitivity of PCR, this result may suggest that the majority of tested isolates lost pYV during manipulations in a routine diagnostic laboratory.

In order to gain better insight into the pathogenic potential of Polish Y. enterocolitica 1B/O8 isolates, the PCR assay was carried out for the genes yts1M and ysrS, which encode components of the Yersinia chromosomal type II and III secretion systems, termed Yts1 and Ysa, respectively (12, 21). In addition, the putative virulence genes YE2407 and YE2447 of Y. enterocolitica 1B/O8 strain 8081 were investigated (10, 20). All of these genes have been reported to occur exclusively in highly

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pathogenic _Yersinia enterocolitica_. In this study, PCR amplicons of _ytsM_, _yrsS_, YE2407, and YE2447 were detected for all the tested isolates. Our data demonstrate that _Y. enterocolitica_ 1B/O8 isolates from sporadic cases in Poland carry the _Yts1_ and _Ysa_ gene clusters and share the high-pathogenicity- _Y. enterocolitica_-specific virulence traits reported elsewhere (2, 3, 23; time period, 26 h). These findings might suggest that the remarkable homogeneity of the genetic backbone shown by _Y. enterocolitica_ 1B/O8 isolates 893/87 and 900/90, collected in Italy and Japan, respectively (Table 1). In contrast, the reference 1B/O8 strain WA-314 revealed a distinct NotI pattern (genotype B). Isolates of the A1 genotype could be reproducibly distinguished from those of the predominating genotype A, even under various PFGE conditions (data not shown).

Interestingly, in the study by Saken and coworkers (16), we found NotI PFGE patterns that strongly resemble patterns of the here-described genotypes A and A1. The patterns showing resemblance were observed for the _Y. enterocolitica_ 1B/O8 isolates 893/87 and 900/90, collected in Italy and Japan, respectively. Notably, Saken and coworkers used the reference strain WA-314 and comparable PFGE conditions (pulse ramp, 8 to 23; time period, 26 h). These findings might suggest that the Polish _Y. enterocolitica_ 1B/O8 clinical isolates are related to the isolates from Italy and Japan. Further studies, however, are required to confirm this thesis.

The remarkable homogeneity of the genetic backbone shown in this study by PFGE may suggest that Polish _Y. enterocolitica_ 1B/O8 isolates are clonal. Struelens and coworkers emphasize that identification of clones must be based on monitoring of several markers of sufficient discriminatory power (18). For
this reason, we performed additional PFGE analyses using the enzyme XbaI (Fermentas, Lithuania). Even though application of an additional endonuclease has been reported to enhance the genotyping resolution of PFGE (4), all but one of the Polish 1B/O8 clinical isolates were indistinguishable (genotype II). The only exception was the isolate 180/08 (genotype III), which exhibited a single additional band in the XbaI pattern (Fig. 1B). The reference strain, WA-314, revealed a distinct XbaI pattern (genotype I).

To determine the similarity of the genotypes, PFGE patterns were analyzed using the GelCompar II software, version 5.10 (Applied Maths, Saint-Matin-Latem, Belgium). Similarity clustering analyses were performed using the single-linkage algorithm and the Dice correlation coefficient with a tolerance of 1.0%. The similarity of the PFGE genotypes distinguished in this study was high and ranged from 94.7% to 96.0% (Fig. 1) for NotI and XbaI, respectively. When NotI and XbaI PFGE profiles were analyzed simultaneously, the Polish 1B/O8 isolates were diversified into three highly similar (97.7%) types (clones), 1, 2, and 3, which comprised 27 and 5 isolates and a single isolate, respectively (Table 1). In conclusion, our results demonstrate that all of the tested Y. enterocolitica 1B/O8 isolates from Poland are closely related.

In accordance with the criteria proposed by Tenover and coworkers (19) and recommendations for genotyping data interpretation described by Struelens and colleagues (18), our results may indicate that the tested Y. enterocolitica 1B/O8 isolates collected in Poland constitute the same strain comprising three highly related PFGE clones. The genetic homogeneity of the tested isolates may result from the recent emergence of Y. enterocolitica 1B/O8 in Poland. Notably, the majority of the tested isolates were collected within the last 2 years, including all of the isolates classified as clones 2 and 3 (Table 1). This might reflect recent speciation events in the predominant clone 1. It is also noteworthy that we have recently observed in Poland a dramatic increase in the number of patients seropositive for both the Y. enterocolitica O8 lipopolysaccharide and the Yersinia outer protein antigens (13). Taken together, these findings show that the emergence and rapid dissemination of Y. enterocolitica 1B/O8 may be a challenge for the public health authorities in Poland.

To date, only a few sporadic isolates of the highly pathogenic
Y. enterocolitica bioserotype 1B/O8 have been found in Europe (16, 17). The genetic similarity of these isolates has not yet been defined. In this study, we characterized a number of clinical isolates of Y. enterocolitica 1B/O8 by PFGE. To the best of our knowledge, this is the first report of the dissemination of the closely related clones of highly pathogenic Y. enterocolitica 1B/O8 in Europe. 1B/O8 isolates indistinguishable by PFGE were reported in Japan (11, 15), where an epidemiological link between patients and contaminated food or direct contact with infected animals has been documented. In light of these reports, the strong clonality of Polish Y. enterocolitica 1B/O8 isolates may suggest a common origin. Further epidemiological investigations supported by local veterinary authorities are required to elucidate the reservoir of the pathogen in Poland and its route of transmission. Our findings also indicate the need to develop novel, high-resolution genotyping approaches devoted to Yersinia enterocolitica 1B/O8 subtyping.

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