

## RESEARCH NOTE

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### Molecular characterisation of multidrug-resistant *Salmonella enterica* serovar Typhimurium isolates from Gomel region, Belarus

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#### ABSTRACT

This study describes the characterisation by pulsed-field gel electrophoresis (PFGE), multi-locus variable number tandem repeat analysis (MLVA) typing and antimicrobial resistance profiles of 35 *Salmonella enterica* serovar Typhimurium isolates, mostly from infections in children who acquired an infection outside hospitals in the Gomel region of Belarus. Thirty-one isolates were highly similar according to PFGE and MLVA typing, were multidrug-resistant, including resistance to ceftiofur, and harboured the *bla*<sub>CTX-M-5</sub> gene. These results indicate that a common source may have been responsible for most of the infections.

**Keywords** Antimicrobial resistance, Belarus, MLVA typing, PFGE, *Salmonella* Typhimurium, typing

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*Salmonella* is one of the most common causes of human bacterial gastroenteritis worldwide, with *Salmonella enterica* serovar Typhimurium and

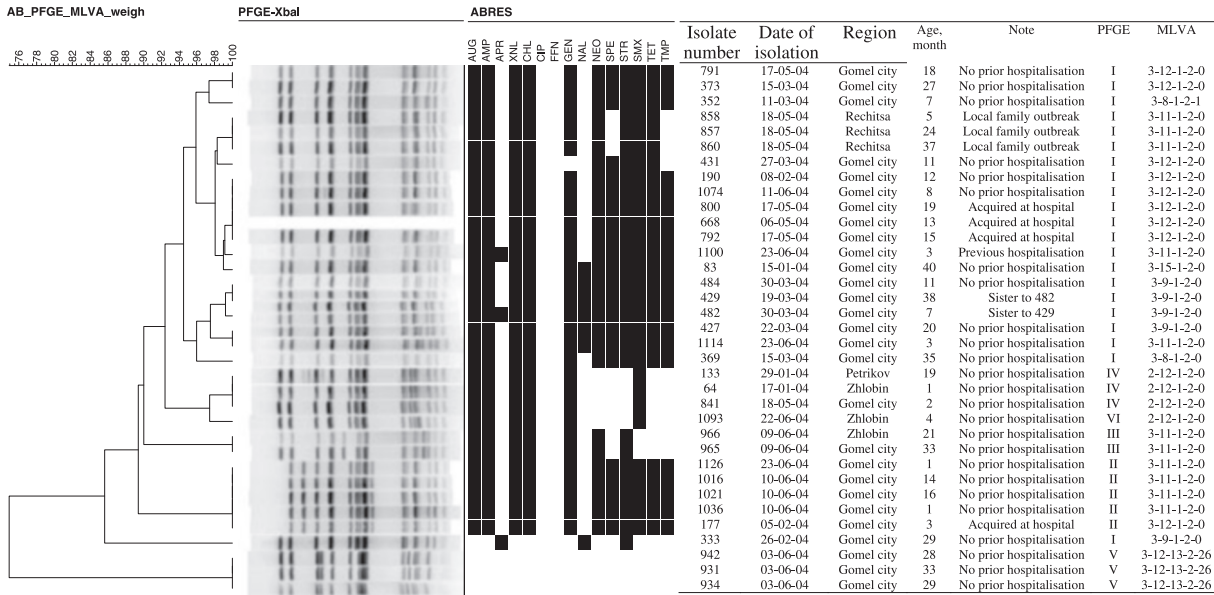
*S. enterica* serovar Enteritidis being the serovars involved most frequently [1,2]. In recent years, an increase in the occurrence of antimicrobial-resistant *Salmonella* isolates has been observed in several countries [3–5]. Not all resistance to antimicrobial agents is of equal importance. Fluoroquinolones are the drug of choice in many countries for the treatment of salmonellosis in humans, and resistance to this class of antimicrobial agent is associated with increased morbidity and mortality [6,7]. Cephalosporins are the drugs of choice for the treatment of salmonellosis in children, to whom fluoroquinolones must not be administered because of the risk of toxicity.

Multidrug-resistant *Salmonella* Typhimurium isolates have recently emerged as an important cause of nosocomial infections in children in Belarus and Russia [8]. Molecular characterisation of 34 isolates from nosocomial infections revealed that the isolates probably belonged to the same clone, and that all contained the *bla*<sub>CTX-M-5</sub> gene [8]. The purpose of the present study was to characterise isolates obtained primarily from children who had acquired an infection outside hospitals in order to determine whether these isolates might also have a common origin.

In total, 35 isolates were selected from among 457 *S. Typhimurium* isolates collected at Gomel State Medical University, Belarus, and shipped subsequently to the National Food Institute, Copenhagen, Denmark. Multidrug-resistant isolates were frequently detected in the diagnostic laboratory, and most of the isolates in the present study were selected from among these. Three fully-susceptible isolates were also included. The isolates were chosen randomly to represent isolates from Gomel city and the surrounding districts. Four isolates were from patients who developed infection during hospitalisation, and one isolate was from a patient who had been hospitalised previously. The remaining 30 isolates were from patients with no previous record of hospitalisation. Three isolates from Rechitsa district were from members of the same family, as was the case for two isolates from Gomel city. All other isolates were from different families with no known contacts. All isolates were identified as *S. enterica*, were serotyped and phage-typed, and their MICs of antimicrobial agents were determined in microtitre plates according to CLSI guidelines, as described previously [9–11]. The following antimicrobial agents were tested:

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**Fig. 1.** Cluster analysis of 35 *Salmonella enterica* serovar Typhimurium isolates from Gomel region, Belarus, based on a combination of results from pulsed-field gel electrophoresis (PFGE), multilocus variable number tandem repeat analysis (MLVA) and antibiogram typing. ABRES, antimicrobial resistance; AUG, amoxycillin–clavulanic acid; AMP, ampicillin; APR, apramycin; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; FFN, florfenicol; GEN, gentamicin; NAL, nalidixic acid; NEO, neomycin; SPE, spectinomycin; STR, streptomycin; SMX, sulphamethoxazole; TET, tetracycline; TMP, trimethoprim.

ampicillin, amoxycillin–clavulanic acid, apramycin, ceftiofur, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, streptomycin, sulphamethoxazole, tetracycline and trimethoprim. The isolates were further characterised using pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis (MLVA) as described previously [12,13]. Comparison of the PFGE and MLVA profiles was undertaken with Bionumerics v.2.5 (Applied Maths, Sint-Martens-Latem, Belgium), using a weighted comparison with the following weights: PFGE, 3; MLVA, 2; and antibiogram, 1. The similarity coefficient was based on an experimental average and the use of UPGMA. The tolerance percentage for the PFGE patterns was 3.8%. The presence of the *bla*<sub>TEM</sub>, *bla*<sub>CTX</sub>, *bla*<sub>CMY-1</sub>, *bla*<sub>CMY-2</sub>, *bla*<sub>SHV</sub> and *bla*<sub>ACC</sub> genes was investigated by PCR as described previously [14].

All 35 isolates were confirmed as *S. Typhimurium*, but were non-typeable with phages. Thirty-one of the isolates were multidrug-resistant, with the most common pattern comprising resistance to amoxycillin–clavulanic acid, ampicillin, ceftiofur, chloramphenicol, gentamicin,

neomycin, spectinomycin, streptomycin, sulphamethoxazole, tetracycline and trimethoprim. Five of the 31 isolates were also resistant to quinolones. All 31 isolates harboured the *bla*<sub>CTX-M-5</sub> gene. Six different PFGE types were found among the 35 isolates (Fig. 1). Four of these types, representing the 31 multidrug-resistant isolates and a single isolate resistant to only three antibiotics, showed high similarity (one to three bands difference from the most common type). MLVA typing revealed seven different types, and was, in some cases, able to subdivide the PFGE types. However, the opposite was also the case. Combined use of MLVA, PFGE and antimicrobial resistance profiling divided the isolates into 17 different types, with some minor clusters. Thus, isolates 857, 858 and 860 were from patients from the same family and were distinguishable from the remaining isolates.

Molecular and phenotypic characterisation revealed that the multidrug-resistant isolates were probably clonally related. Although 15 different types were found among the 31 cephalosporin-resistant isolates, the profiles had so many characteristics in common that a common origin seems likely [15,16]. However, this variation also

indicates that the clone has spread for some time in order to evolve these differences.

Outbreaks with *S. enterica* are associated most commonly with consumption of contaminated food products of animal origin. A previous report described nosocomial outbreaks involving a single clone of cephalosporin-resistant *S. enterica* Typhimurium containing the *bla*<sub>CTX-M-5</sub> gene in Belarus, although the source of these isolates was not identified [8]. In the present study, *bla*<sub>CTX-M-5</sub> was found to be associated with cephalosporin resistance. In contrast to the study conducted by Edelstein *et al.* [8], the isolates characterised in the present study were mainly from non-hospitalised patients, indicating a community reservoir for this extended-spectrum  $\beta$ -lactamase producer. The isolates from the previous study were not available for comparison, and the PFGE profiles were performed using different methodologies, but a visual comparison of the profiles indicates that the isolates from the two studies were closely related.

Enterobacteriaceae producing CTX-M enzymes are emerging worldwide, with the CTX-M-5 variant having been reported mainly in eastern Europe and Russia [8,17,18], indicating the regional spread of this gene. MLVA typing is a new and sensitive typing technique that has, to date, been used only in a limited number of studies. The present study demonstrated that MLVA can further subdivide PFGE types, as reported previously [19], although PFGE typing can also subdivide MLVA types. MLVA typing may be too sensitive to determine clonality among isolates that have spread over a prolonged period. Thus, typing techniques must be chosen according to the purpose of the investigation, and should always be used in combination and be supported by epidemiological data [15,16]. The present study indicated that multidrug-resistant *S. enterica* Typhimurium isolates infecting children in the Gomel region of Belarus belong to the same or a closely related clone, which suggests a common source of the infections caused by these organisms.

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## REFERENCES

- Herikstad H, Motarjemi Y, Tauxe RV. *Salmonella* surveillance: a global survey of public health serotyping. *Epidemiol Infect* 2002; **129**: 1–8.
- Humphrey TJ. Public-health aspects of *Salmonella* infections. In: Wray C, Wray A, eds, *Salmonella in domestic animals*. Wallingford: CABI Publishing, 2000; 245–263.
- Cailhol J, Lailler R, Bouvet P *et al.* Trends in antimicrobial resistance phenotypes in non-typhoid salmonellae from human and poultry origins in France. *Epidemiol Infect* 2006; **134**: 171–178.
- van Duijkeren E, Wannet WJ, Houwers DJ, van Pelt W. Antimicrobial susceptibilities of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. *J Clin Microbiol* 1984; **41**: 3574–3578.
- Lauderdale TL, Aarestrup FM, Chen PC *et al.* Multidrug resistance among different serotypes of clinical *Salmonella* isolates in Taiwan. *Diagn Microbiol Infect Dis* 2006; **55**: 149–155.
- Helms M, Vastrup P, Gerner-Smidt P, Mølbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium*. *Emerg Infect Dis* 2002; **8**: 490–495.
- Helms M, Simonsen J, Mølbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; **190**: 1652–1654.
- Edelstein M, Pimkin M, Dmitrachenko T *et al.* Multiple outbreaks of nosocomial salmonellosis in Russia and Belarus caused by a single clone of *Salmonella enterica* serovar Typhimurium producing an extended-spectrum beta-lactamase. *Antimicrob Agents Chemother* 2004; **48**: 2808–2815.
- Aarestrup FM, Lertworapreecha M, Evans MC *et al.* Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. *J Antimicrob Chemother* 2003; **52**: 715–718.
- Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic- and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). *APMIS* 1998; **106**: 745–770.
- Baggesen DL, Sandvang D, Aarestrup FM. Characterization of *Salmonella enterica* serovar typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. *J Clin Microbiol* 2000; **38**: 1581–1586.
- Ribot EM, Fair MA, Gautom R *et al.* Standardization of pulsed-field gel electrophoresis (PFGE) protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 2006; **3**: 59–67.
- Lindstedt BA, Vardund T, Aas L, Kapperud G. Multiple-locus variable-number tandem-repeats analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. *J Microb Methods* 2004; **59**: 163–172.
- Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM.  $\beta$ -Lactamases among extended-spectrum  $\beta$ -lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products

- and human patients in The Netherlands. *J Antimicrob Chemother* 2005; **56**: 115–121.
15. Maslow JN, Mulligan ME, Arbeit RD. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. *Clin Infect Dis* 1993; **17**: 153–162.
  16. Ørskov F, Ørskov I. From the National Institutes of Health. Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the enterobacteriaceae and other bacteria. *J Infect Dis* 1983; **148**: 346–357.
  17. Bradford PA, Yang Y, Sahn D, Grope I, Gardovska D, Storch G. CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. *Antimicrob Agents Chemother* 1998; **42**: 1980–1984.
  18. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother* 2003; **47**: 3724–3732.
  19. Torpdahl M, Sørensen G, Ethelberg S, Sandø G, Gammelgaard K, Porsbo LJ. A regional outbreak of *S. Typhimurium* in Denmark and identification of the source using MLVA typing. *Euro Surveill* 2006; **11**: 134–136.

## RESEARCH NOTE

### Survival of *Mycobacterium ulcerans* at 37°C

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#### ABSTRACT

Bone infection and metastatic spread in cases of Buruli ulcer imply that *Mycobacterium ulcerans* is able to survive and multiply at 37°C. This study investigated the survival at 37°C of *M. ulcerans* isolates from diverse geographical and clinical sources. Although the viability of all isolates decreased after a few days at 37°C, viable bacilli remained after 13 days at 37°C in most instances. African isolates of *M. ulcerans* were more thermotolerant than isolates from temperate

regions. Isolates from skin and bone lesions of the same patients showed no difference in thermotolerance.

**Keywords** Buruli ulcer, geographical origin, *Mycobacterium ulcerans*, survival, thermotolerance

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*Mycobacterium ulcerans*, the causative agent of Buruli ulcer (BU), grows optimally on mycobacteriological media at 30–32°C [1]. *M. ulcerans* classically infects skin and subcutaneous tissue, where the pathogen encounters favourable growth temperatures. However, metastatic spread to distant skin sites or bone also occurs [2–5], suggesting that *M. ulcerans* is able to survive and/or multiply at 37°C. The present study investigated the survival of *M. ulcerans* at 37°C on Löwenstein–Jensen medium. The results may have implications for: (i) a better understanding of the clinical features of the disease, with bone involvement resulting from metastatic spread; (ii) the treatment of lesions with local heat; and (iii) the epidemiology and transmission of the disease in relation to survival of *M. ulcerans* in the environment at high temperatures.

Seventeen isolates of *M. ulcerans* from ten countries were initially studied. Tubes of Löwenstein–Jensen medium were inoculated with serial dilutions of suspensions of bacteria and incubated at 37°C for 0, 3, 6, 9 or 24 h, and 2, 3, 6, 9 or 13 days, and thereafter at 32°C for 12 weeks. The number of surviving bacilli was estimated by counting the number of CFU. Inactivation curves were obtained for each isolate by plotting CFU/mL against exposure time at 37°C on a semi-logarithmic scale. The slope of the inactivation curves was expressed as decimal reduction time (D), measuring heat resistance and the time needed to inactivate 90% of the bacterial population at a given temperature. A higher D value thus indicates a greater thermotolerance [6,7].

Table 1 shows that a decrease in viability occurred for all isolates after a few days at 37°C, but with large variations for individual isolates. However, for all isolates except those from Japan, China and French Guiana, viable bacilli were still present after incubation for 13 days at 37°C. The

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