those infected through injecting drug use, a characteristic similar to that of those infected through sexual intercourse. Also similar to cases infected through sexual transmission, people in this group are more likely to be late presenters, possibly because they were not aware of being at risk or because they did not seek medical care for different reasons. The observed age shift towards older ages represents either people infected at older ages or people who were diagnosed with HIV many years after being infected. The latter hypothesis is supported by a rapidly growing number of late presenters. On the other hand, data on HIV testing patterns in different age groups are not available and the observed age increase may result from increased testing in older age groups.

In the era of HAART, the number of AIDS cases continues to increase in Poland. Many developed countries experienced a distinct decrease in AIDS incidence when HAART became generally available [8]. Assuming the wide availability of HAART, stable or even increasing AIDS incidence may represent persons who were unaware of their HIV status due to low risk perception or limited access to HIV testing and appropriate medical consulting or care [8, 9]. Poland has, at present, one of the lowest HIV testing rates in Europe [10]. Approximately 36% of incident AIDS cases are diagnosed simultaneously with the HIV diagnosis. The increasing rate of these cases and the fact that a large proportion were infected through sexual contact (60% of cases with reported transmission route) indicate that the HIV epidemic in Poland may be underestimated and not limited to specific population compartments such as injecting drug users. Furthermore, despite the availability of the mother-to-child transmission prophylaxis since 1994, incidence of vertically transmitted AIDS in Poland continues to rise. The transmission mainly occurs in women who did not know about their serostatus during the pregnancy [11]. Based on a study of over 25 000 newborns tested in 2001 – 2002 in the Mazowieckie region, between 100 and 200 seropositive women give birth each year in Poland [12]. Pregnant women are still not routinely being offered testing for HIV.

To conclude, in order to generate more accurate data, HIV surveillance must be enhanced by collecting detailed risk information. Even though further studies to guide prevention strategies are warranted, it is clear that implementation of a comprehensive programme of vertical transmission prophylaxis including voluntary testing of all pregnant women should be a priority. Moreover, there exists a need to increase access to and use of HIV testing by offering it more widely in accessible settings, or even by approving self-testing kits. Considering that the majority of late presenters were infected through sexual transmission, an effort is also needed to enhance collaboration between the HIV and STI surveillance and prevention programs.

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ORIGINAL ARTICLES

Surveillance report

SURVEILLANCE OF HUMAN SALMONELLOSIS IN BULGARIA, 1999-2004: TRENDS, SHIFTS AND RESISTANCE TO ANTIMICROBIAL AGENTS

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This article analyses the distribution of resistant salmonella and resistance mechanisms among the most frequently encountered serotypes in Bulgaria. Culture, biochemical tests and serotyping were used for identification. Screening for resistance to 14 antimicrobial agents with the standard Bauer-Kirbi disk-diffusion method. The double disk synergy method was used to determine production of extended-spectrum ß-lactamases (ESBL). Transfer of genes coding for ESBLs with experimental conjugation. Specific primers were used for PCR detection of *bla*-CTX-M, *bla*-SHV and *bla*-TEM. 245 resistant salmonella strains were determined in our study; the majority originated from sporadic cases of human illness or asymptomatic infection and the remaining 23 were isolated from outbreaks. 79 producers of ESBL were detected: 5 *S.* Enteritidis, 1 *S.* Typhimurium, 9 *S.* Isangi and 62 *S.* Corvallis with types of enzymes: CTX-M3, TEM and SHV. Gene coding for extended-spectrum ß-lactamases were successfully transferred into a recipient *Escherichia coli* C1A strain simultaneously with genes coding for resistance to aminoglycosides and sulphonamides (for *bla*-CTX-M3) and gene coding for resistance to aminoglycosides and chloramphenicol (for *bla*-SHVand *bla*-TEM). PCR amplification revealed *bla*-CTX-M3 genes in *S.* Enteritidis, and *bla*-SHV and *bla*-TEM in *S.* Corvallis. Salmonellae have revealed increasing resistance to all clinically important groups of antimicrobial agents. Bulgaria is the first country in the world where ESBL in serotype Corvallis has been reported. A wide diversity of resistance genes is found among the leading serotypes of salmonella causing human disease in Bulgaria.

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Euro Surveill 2006;11(5): 97-100 Published online May 2006

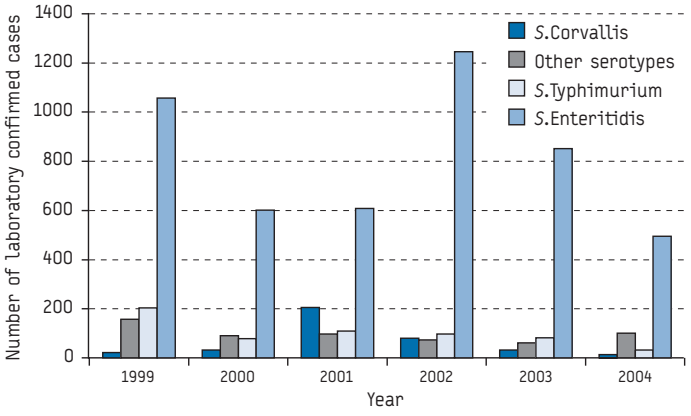
Keywords: ESBL-producing salmonellae, multidrug-resistant

Introduction

The Bulgarian Ministry of Health has named salmonellosis one of the country's priority communicable diseases, and healthcare providers are legally required to record and report cases of illness discovered in their regions. Surveillance of salmonellosis in Bulgaria is laboratory based. The network of microbiological laboratories functioning in the country performs the primary diagnosis and forwards both outbreak and sporadic strains to the National Reference Laboratory for Enteric Pathogens for confirmation, serotyping and antimicrobial susceptibility testing. Since the second half of the 1990s Bulgaria participated in two international networks targeting surveillance of salmonellosis: Global Salm-Surv and Enter-net. National surveillance data on laboratory confirmed cases of human salmonelloses, including the total salmonella count and the total number of serotyped salmonellae, is reported annually to Global Salm-Surv. The databases enable us to follow the trends of these important infections at national and international level. Resistance to antimicrobial agents in enterobacteriaceae, including salmonella, is now an issue of international concern. The purpose of this paper is to analyse the distribution of resistant salmonella in Bulgaria and the resistance mechanisms among strains causing different forms of human illness: outbreaks, sporadic cases of disease and asymptomatic infection. In Bulgaria, as in many European countries, *S.* Enteritidis and *S.* Typhimurium are the first and second most common causative agents of human salmonellosis, respectively [1]. A shift in the position of *S.* Corvallis has been observed in our country since 1997 when it did not belong to the leading serotypes causing human salmonellosis. During the period under study, *S.* Corvallis was the third most common cause of salmonellosis in Bulgaria [FIGURE 1] [2].

FIGURE 1

Distribution of the top three *Salmonella* serotypes by number of laboratory confirmed cases, Bulgaria, 1999-2004



Methods

A total of 6707 salmonella strains were isolated and reported by 28 Regional Inspectorates for Prevention and Control of Public Health in Bulgaria between 1999 and 2004. These data were sent to Global Salm-Surv so that a country database could be established. 2123 (32 %) of all salmonella isolates were sent to the National Reference Laboratory and were included in this study; 55 of them had caused outbreaks, and the remaining 2068 were from sporadic cases of salmonellosis or carrier state. Conventional microbiological methods: culture, biochemical tests, serotyping (BIO-RAD) have been performed for identification of strains. Screening for resistance to 14 antimicrobial agents (cefotaxime, Cefoxitin, carbenicillin, ceftazidime, cefuroxime, cephalothin, ampicillin, amoxicillin/ clavulanic acid, gentamicin, tetracycline, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim/ sulfamethoxazole (Biomerieux)) was done using standard Bauer-Kirby disk- diffusion method and screening for ESBL-production with the double disk synergy method [3]. All resistant strains were divided into two groups depending on their phenotypes:

1) resistant to < 4 antimicrobial agents
2) resistant to ≥ 4 antimicrobial agents.

The transfer of *bla*-CTX-M, *bla*-TEM and *bla*-SHV genes was studied with experimental conjugation using 14 salmonella strains as donors and an Escherichia coli C1A strain as a recipient. Transconjugates were selected on McConkey agar containing cefotaxime 10µg/ml and nalidixic acid 40 µg/ml. For PCR detection of *bla* genes the following primers were applied:

ALA2/P2D for *bla*-CTX-M 5' ATGGTTAAAAAATCACTGCG 3'/ 5' CAGCGCTTTTGCCGTCTAAG 3' [11], OS5/OS6 for *bla*-SHV 5' TTATCTCCCTGTTAGCCACC 3'/ 5' GATTTGCTGATTTCGCTCGG 3' [12] and 5'-3' ATGAGTATTCAACATTTCCG; ACCAATGCTTAATCAGTGAG for *bla*-TEM.

Results

A total of 245 resistant salmonella strains were found in our study. 222 (91%) originated from sporadic cases of salmonelloses or human carriers; 23 were obtained from outbreaks. Table 1 shows the distribution of resistant strains among the leading three salmonella serotypes causing human disease in Bulgaria for the period 1999-2004.

Characteristically, resistance increases in dynamics for *S.* Enteritidis, *S.* Typhimurium and *S.* Corvallis. Table 2 represents the distribution of resistant strains among the less frequently detected serotypes in Bulgaria for the period 1999-2004. Seventy nine of 245 strains (32%) produced extended-spectrum ß-lactamases (ESBL): 5 *S.* Enteritidis, 1 *S.* Typhimurium, 9 *S.* Isangi and 62 *S.* Corvallis. The remaining 166 (68%) revealed resistance to ampicillin, carbenicillin, first and second generation cephalosporins, aminoglycosides, tetracycline, chloramphenicol, nalidixic acid, trimethoprim sulfamethoxazole alone and in combinations. ESBL- producing salmonellae have demonstrated multidrug-resistance to more than seven antimicrobial agents, and were therefore classified into the group of microorganisms resistant to ≥ 4 antibiotics [TABLES 1 and 2]. This mechanism of resistance has been proved in strains originating from 6 regions situated in central and western Bulgaria. *Bla* genes coding for ESBL were successfully transferred into a recipient *E. coli* C1A strain simultaneously with

TABLE 1

Resistant *Salmonella* among the top three serotypes detected in Bulgaria between 1999-2004

Serotype	Year	Total number of tested strains/ number of resistant strains (%)	Resistant to < 4 antibiotics (%)	Resistant to ≥ 4 antibiotics (%)	Number of ESBL-producing strains
S. Enteritidis	1999	230/ 31 (13.4)	25 (10.8)	6 (2.6)	3
	2000	184/ 52 (28.2)	50 (27.1)	2 (1.1)	1
	2001	72/ 24 (33.3)	23 (31.9)	1 (1.4)	0
	2002	15/ 5 (33.3)	5 (33.3)	0 (0)	0
	2003	47/ 17 (36.2)	11 (23.4)	6 (12.8)	1
	2004	24/ 10 (41.6)	9 (37.5)	1 (4.2)	0
	TOTAL	**572/ 139 (24.3)**	**123 (21.5)**	**16 (2.7)**	**5**
S. Typhimurium	1999	40/ 7(17)	1 (2.5)	6 (15)	1
	2000	23/ 0 (0)	0 (0)	0 (0)	0
	2001	18/ 5 (27.7)	1(5.5)	4 (22.2)	0
	2002	3/ 1 (33.3)	1 (33.3)	0 (0)	0
	2003	6/ 2 (33.3)	1 (16.6)	1 (16.6)	0
	2004	3/ 0 (0)	0 (0)	0 (0)	0
	TOTAL	**93/ 15 (16.1)**	**4 (4.3)**	**11 (11.8)**	**1**
S. Corvallis	1999	Not tested	Not tested	Not tested	Not tested
	2000	Not tested	Not tested	Not tested	Not tested
	2001	23/ 9 (39.1)	1 (4.3)	8 (34.7)	8
	2002	38/ 38 (100)	0 (0)	38 (100)	38
	2003	16/ 16 (100)	1 (6.3)	15 (93.7)	15
	2004	6/ 3 (50)	2 (33.3)	1 (16.6)	1
	TOTAL	**83/ 66 (79.5)**	**4 (4.8)**	**62 (74.6)**	**62**

Table 2

Distribution of resistant *Salmonella* among the less frequently detected serotypes in Bulgaria for the period 1999-2004

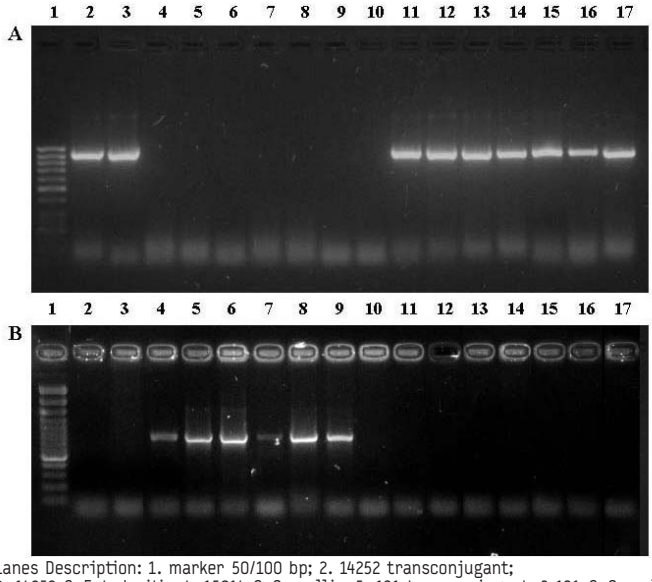
Serotype	Number of tested strains /number of resistant strains (%)	Resistant to < 4 antibiotics (%)	Resistant to ≥ 4 antibiotics (%)	Number of ESBL-producing strains
S. Isangi	19/ 15 (78.9)	1 (5.3)	14 (73.4)	9
S. Tshiongue	1/ 1 (100)	0 (0)	1 (100)	0
S. Gallinarum	3/ 3 (100)	3 (100)	0 (0)	0
S. Gloucester	2/ 1 (50)	0 (0)	1 (50)	0
S. Hadar	3/ 2 (66.7)	1 (33.3)	1 (33.3)	0
S. Breda	1/1 (100)	0 (0)	1 (100)	0
S. Blockley	3/ 2 (66.7)	2 (66.7)	0 (0)	0

genes coding for resistance to aminoglycosides and sulphonamides (for *bla*-CTX- M3) and genes coding for resistance to aminoglycosides, and chloramphenicol (for *bla*- TEM and *bla*-SHV). PCR amplification with primers ALA2 and P2D revealed *bla*- CTX- M3 genes in 5 *S.* Enteritidis and transconjugants derived from them [FIGURE 2A]. Bulgaria is the first country in the world where such multidrug -resistant and ESBL in serotype Corvallis has been reported. The first ESBL-positive strain *S.* Corvallis to be identified emerged in a community in 2001 and was isolated from a sick child. All salmonellae belonging to this serotype are subject to systematic screening of ESBL-production. In Bulgaria it is very rare to find *S.* Corvalis sensitive to all antimicrobials, or resistant to only one antimicrobial such as trimethoprim/ sulfamethoxazole. Figure 2B demonstrates *bla*-SHV detected in strains of *S.* Corvallis and transconjugants derived from them.

Figure 2

A. *Bla*-CTX-M3 in *S.* Enteritidis and transconjugants

B. *Bla*-SHV in *S.* Corvallis and transconjugants



Lanes Description: 1. marker 50/100 bp; 2. 14252 transconjugant; 3. 14252 *S.* Entederitis; 4. 15014 *S.* Corvallis; 5. 191 transconjugant; 6 191 *S.* Corvalis; 7. 111 transconjugant; 8. *S.* Corvallis; 9. 15015 *S.* Corvalis; 10. 14096 *S.* Typhimuriium; 11. 14355 transconjugant; 12. 14355 *S.* Enteritidis; 13. 14309 transconjugant; 14. 14306 *S.* Enteritidis; 15. 14292 transconjugant; 16. 14292 *S.* Enteritidis; 17. 432 *S.* Enteritidis

Discussion

In Bulgaria, the leading serotypes of non-typhoid salmonella have become resistant to most of clinically important antimicrobials. This major change had occurred during the last few years. Many countries consider resistance to nalidixic acid combined with retained susceptibility to ciprofloxacin to be the most typical mechanism for salmonella. Bacteria expressing such phenotypes have mutation in their chromosomal *gyr*A. In Bulgaria, this mechanism of resistance has been seen mainly in strains of *S.* Enteritidis. Despite the wide occurrence of ESBLs in *Klebsiella pneumoniae, E.coli, Citrobacter, Proteus* and other members of the family *Enterobacteriaceae*, they remained rare in salmonella until the second half of the 1990s. The number of salmonella serotypes expressing ESBL continues to increase [4-7]. The first ESBL- producing strains were detected in 1999 and belonged to serotypes *S.* Enteritidis and *S.* Typhimurium. Our study has revealed CTX-M3 ESBL in *S.* Enteritidis. To date, 36 types of CTX-M ß-lactamases are known. They are grouped into four clusters and CTX-M3 enzymes have been classified into the first cluster of cefotaximases [8]. Few reports of *bla*CTX-M 3 harbouring *S.* Enteritidis are available in literature, for example in Poland in 2000 [9].

In comparison to other non-typhoid salmonellae, *S.* Typhimurium are regarded as more resistant [10]. In Bulgaria strains from this serotype expressed mainly resistance to ampicillin, carbenicillin, tetracycline and chloramphenicol, that could be explained with widely distributed plasmids in European countries. Only one of the *S.* Typhimurium strains in our collection expressed ESBL, but failed to hybridise with any of the primer pairs used in the study, though its phenotype was suggestive for TEM or SHV types of ESBLs [FIGURE 2B, lane 10]. This strain did not transfer resistance genes to the recipient *E.coli* C1A during the experimental conjugation possibly because of their chromosomal location. Selection and dissemination of multidrug- resistant *S.* Corvallis is a characteristic finding for our country and the majority of strains belonging to this serotype are ESBL- producers. *S.* Corvallis is a rare serotype in Europe, but in Bulgaria, it has been the third most commonly reported causative agent of human infection after *S.* Enteritidis and *S.* Typhimurium since 1997. Our study has shown for the first time that the SHV type of ESBL are present in *S.* Corvallis. Salmonellae from serotype Isangi are characteristically resistant to multiple antimicrobial agents. Since their first isolation in Bulgaria in 1956, there has been little work on understanding the mechanisms of resistance among salmonellae from this serotype. Our study has revealed 9 CTX- M producing *S.* Isangi.

Conclusions

Diversity of resistance genes are widely distributed among the leading serotypes of salmonellae causing human disease in Bulgaria. Resistance to important for treatment groups of antimicrobial agents: ß-lactams, sulphonamides, nalidixic acid has been increasing among *S.* Enteritidis, *S.* Typhimurium, *S.* Corvallis. No resistance to ciprofloxacin has been found. CTX-M3 type of ESBL has been proved in *S.* Enteritidis. Bulgaria is the first country in the world reporting ESBL in serotype Corvallis.

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ORIGINAL ARTICLES

Surveillance report

SURVNET@RKI – A MULTISTATE ELECTRONIC REPORTING SYSTEM FOR COMMUNICABLE DISEASES

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In 2001 Germany implemented a new electronic reporting system for surveillance of notifiable infectious diseases (Surv*N*et@RKI). The system is currently being used in all 431 local health departments (LHD), the 16 state health departments (SHD) and the Robert Koch-Institut (RKI), the national agency for infectious disease epidemiology. The Surv*N*et@RKI software is written in MS Access 97 and Visual Basic and it supports MS Access as well as MS SQL Server database management systems as a back-end. The database is designed as a distributed, dynamic database for 73 reporting categories with more than 600 fields and about 7000 predefined entry values. An integrated version management system documents deletion, undeletion, completion and correction of cases at any time and entry level and allows reproduction of previously conducted queries. Integrated algorithms and help functions support data quality and the application of case definitions. RKI makes the system available to all LHDs and SHDs free of charge. RKI receives an average of 300 000 case reports and 6240 outbreak reports per year through this system. A public web-based query interface, Surv*S*tat@RKI, assures extensive and timely publication of the data. During the 5 years that Surv*N*et@RKI has been running in all LHDs and SHDs in Germany it has coped well with a complex federal structure which makes this system particularly attractive to multinational surveillance networks. The system is currently being migrated to Microsoft C#/.NET and transport formats in XML. Based on our experiences, we provide recommendations for the design and implementation of national or international electronic surveillance systems.

Euro Surveill 2006;11(4): 100-3 Published online April 2006
Keywords: disease notification, electronic reporting, infectious diseases, surveillance

Introduction

In January 2001 a new law for the prevention and control of infectious diseases (*Infektionsschutzgesetz*, IfSG) was enacted in Germany. This has resulted in a modernisation of the national surveillance system for notifiable infectious diseases. In order to assure information flow between local, state and federal institutions we developed a new electronic reporting system (Surv*N*et@RKI) as the technical backbone of the new surveillance system. While various evaluations of the German surveillance system have already been published elsewhere [1-4], this report intends to present and critically discuss the technical aspects of the software and database architecture for electronic data transfer within the surveillance system.

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The objective of this paper is to present technical solutions developed in Germany which could be applicable in surveillance systems of other countries or international networks.

Methods

Background and requirements

Germany is a federal republic with 16 states (*Bundesländer*) and 439 counties (*Stadt-/Landkreise*). Typically, there is one local health department (LHD) per county, responsible for managing single cases and outbreaks of infectious diseases and carrying out necessary prevention and control activities. The IfSG defines 47 pathogens and 14 diseases that laboratories and clinicians, respectively, have to notify to the local health department. LHD complete and verify the case information based on national case definitions. These cases are then transmitted on a single case basis to the state health departments (SHD) and from there to the Robert Koch-Institut (RKI), the central national agency for infectious disease epidemiology. A requirement analysis revealed the need for an electronic reporting system with the following functional and non-functional features:

The system capacity needed to be sufficient for over 300 000 reported cases per year with 25 to 60 variables per case entered by 431 LHDs throughout the country. The system needed to take issues of data security of privacy-related patient data as well as specific additional requirements of individual states into account. For economic reasons the software had to run on common hardware without the need for additional software licenses and expensive back-end systems. As permanent internet connection was not available in all LHDs, the system needed to be operable offline as well. The system should incorporate reporting of complex outbreaks and be flexible enough to adapt quickly to unexpected changes caused by new emerging diseases (e.g., SARS).

In July 2000 the two legislative houses of representatives in Germany (*Bundestag* and *Bundesrat*) ratified the IfSG to be enacted by 1 January 2001. Within 6 months the RKI developed the electronic reporting system for the national surveillance system.

Software design

The architecture of the system was designed inhouse at the RKI. However, a major part of the programming was done by an external IT company. The newly developed system was called Surv*N*et@RKI.

The data flow is depicted in the figure. The front-end of Surv*N*et@RKI is written in MS Access 97 and Visual Basic. Depending on the data volume it supports MS Access as well as MS SQL Server database management systems as a back-end. Adding or removing reporting categories, fields or allowed values do not require changes to the programme structure, which is based on the fractal© concept of