Rapid communication
Surveillance of vancomycin-resistant enterococci reveals shift in dominating clones and national spread of a vancomycin-variable vanA Enterococcus faecium ST1421-CT1134 clone, Denmark, 2015 to March 2019

Anette M Hammerum, Ulrik S Justesen, Mette Pinholt, Louise Roer, Hülya Kaya, Peder Worning, Sanne Nygaard, Michael Kemp, Marianne Engell Clausen, Karen Leth Nielsen, Jurgita Samulionienė, Mona Kjærgaard, Claus Østergaard, John Cola, Turid Snekleth Søndergaard, Shahin Gaini, Kristian Schanning, Henrik Westh, Henrik Hasman and Barbara Juliane Holzknecht

Outbreaks
Linked seasonal outbreaks of Salmonella Typhimurium among passerine birds, domestic cats and humans, Sweden, 2009 to 2016

Robert Söderlund, Cecilia Jernberg, Linda Trönnberg, Anna Pääjärvi, Erik Ägren and Elina Lahti

Increasing incubation periods during a prolonged monophasic Salmonella Typhimurium outbreak with environmental contamination of a commercial kitchen at Oslo Airport, Norway, 2017

Lotta Siira, Emily MacDonald, Gry Marianne Holmbakken, Tom Sundar, Lars Meyer-Myklestad, Heidi Lange, Lin T Brandal, Umaer Naseer, Gro S Johannessen, Bjarne Bergsøe, Laura Espenhain, Line Vold and Karin Nygård

Surveillance
Antimicrobial use trends, Israel, 2012 to 2017

Yaakov Dickstein, Elizabeth Temkin, Debby Ben-David, Yehuda Carmeli and Mitchell J Schwaber
We describe clonal shifts in vanA Enterococcus faecium isolates from clinical samples obtained from patients in Denmark from 2015 to the first quarter (Q1) of 2019. During Q1 2019, the vancomycin-variable enterococci (VVE) ST1421-CT1134 clone and has spread to all five regions in Denmark. Among 174 E. faecium isolates with vanA, vanB or vanA/vanB genes in Q1 2019, 44% belonged to this type.

We describe the clonal shift for vanA Enterococcus faecium during the last 4 years and the national spread of a vancomycin-variable vanA E. faecium clone and has spread to all five regions in Denmark. The aim is to highlight the importance of using molecular methods for detecting vancomycin-variable enterococci (VVE), and to alert other countries about this emerging nosocomial clone.

Vancomycin-variable enterococci
Vancomycin-variable enterococci (VVE) are E. faecium harboring the vanA gene complex, but being phenotypically vancomycin susceptible [1,2]. VVE can only be detected by molecular methods and cannot be cultured on selective vancomycin-containing media. Different clones of VVE have caused nosocomial outbreaks and development of vancomycin-resistant revertant mutants in vitro and in vivo has been described [1,3-5]. This makes the detection of VVE highly important in clinical samples in order to assure relevant antibiotic treatment and in screening samples to avoid nosocomial spread. In 2015 and 2016, sporadic VVE with different genetic background were detected in the Capital Region of Denmark, in connection with vancomycin-resistant enterococci (VRE) outbreaks (data not shown). In 2016, a VVE clone belonging to ST1421-CT1134, which displays variable vancomycin susceptibility (minimum inhibitory concentration (MIC) 1 to ≥ 256 mg/ml) was detected in screening samples from a hospital in the Capital Region [5]. One strain, Efm-V1511, belonging to this clone was characterised by Hansen et al. [5]. Efm-V1511 had a 49.6 Kp plasmid, which carried the Tn1546 (vanA transposon). Tn1546 was truncated in vanX by a 252 bp 3’ deletion explaining the vancomycin susceptibility of Efm-V1511. In ST1421-CT1134 isolates resistant to vancomycin, resistance could be attributed to changes in ddl disrupting phenotypically vancomycin susceptible [1,2].
Modified from DANMAP 2017 [7].

DCM: Department of Clinical Microbiology; ND: not detected.

Figure 1
The five healthcare regions and the 10 Departments of Clinical Microbiology, Denmark, 2019

Gene function sometimes accompanied by changes in vanS, increased pHVH-V1511 copy number or the existence of an additional vanA-containing plasmid encoding a functional vanX [5].

National surveillance of vancomycin-resistant and vancomycin-variable enterococci
We have previously described the surveillance of vancomycin-resistant enterococci (VRE) in clinical isolates in Denmark from 2005 to 2015 [6]. In the present study, we follow up and describe the data from isolates obtained from 2016 through the first quarter (Q1) of 2019. Since 2005, VRE isolates from clinical samples, e.g. urine, blood and tissue, as opposed to screening (faecal) isolates have been voluntarily submitted to Statens Serum Institut (SSI) from Danish Departments of Clinical Microbiology (DCM) for species identification, genotyping and surveillance (Figure 1) [7]. Only one isolate per patient per 12 months was included. All VRE isolates (699 E. faecium and 30 E. faecalis) were tested for the presence of vancomycin resistance genes vanA and vanB by PCR from 2005 through 2014. From 2015 through Q1 2019, all clinical VRE/VVE isolates (n=1,935) underwent whole-genome sequencing (WGS) as previously described [6]. From the WGS data, multilocus sequence type (MLST), and van genes were extracted in silico. The isolates were further subtyped in SeqSphere+(Ridom GmbH, Münster, Germany (http://www.ridom.de/seqsphere/)) using the cgMLST scheme by de Been et al. [8] for E. faecium.

VRE diagnostic algorithms have differed substantially over time and between the five Danish regions. In 2017, testing of phenotypically vancomycin-susceptible E. faecium isolates from blood cultures for the presence of vanA/vanB genes by PCR was introduced in the DCMs in the Capital Region. During 2018, this was expanded to testing of all clinical E. faecium isolates. During 2018, molecular testing by PCR of E. faecium from all clinical samples was also implemented in one of the four DCMs in the Region of Southern Denmark. Furthermore, E. faecium isolates from blood cultures were tested by PCR for vanA/vanB genes in another DCM in the Region of Southern Denmark and in the DCM in the Central Denmark Region in 2018. In Q1 2019, diagnostic algorithms to detect VVE have expanded. Most of the DCMs across Denmark test at least all blood culture E. faecium isolates for the presence of vanA genes using PCR.

Enterococcus faecium and Enterococcus faecalis isolates from clinical samples carrying vanA and vanB genes
From 2005 to Q1 2019, 2,503 vanA E. faecium, 74 vanB E. faecium, 32 vanA/vanB E. faecium, 12 vanA E. faecalis, and 43 vanB E. faecalis from clinical samples were submitted to SSI (Figure 2).

Emergence and disappearance of major Enterococcus faecium clones
Of the 1,935 VRE/VVE isolates obtained from 2015 through Q1 2019, 1,910 were E. faecium and 25 E. faecalis (Figure 2).

The E. faecium isolates belonged to 29 sequence types (STs). ST80 (22%), ST203 (65%) and ST1421 (9%) were most prevalent. Typing by cgMLST revealed 156 different complex types (CTs).

The 13 most common types of vanA, vanB and vanA/vanB E. faecium from 2015 to Q1 2019 are shown in Table 1. From 2015 to 2019, three types were dominating: ST80-CT14 vanA E. faecium, ST203-CT859 vanA E. faecium and ST1421-CT1134 vanA E. faecium (Table 1). In 2015, 22% of the E. faecium isolates belonged to ST80-CT14 vanA E. faecium. The type decreased during 2016.

ST203-CT859 vanA E. faecium isolates were first detected during the end of 2014 [6]. It emerged very fast and was the most prevalent vanA E. faecium type (together with its subtypes CT1051 and CT1507) during 2015 to 2017, but decreased in 2018 (Table1). In Q1 2019 only 12% of the VRE/VVE E. faecium isolates belonged to ST203-CT859.

In 2017, 3% of the E. faecium isolates belonged to the VVE clone, ST1421-CT1134 vanA E. faecium. This type
Figure 2
Vancomycin-resistant and vancomycin-variable Enterococcus faecium and vancomycin-resistant E. faecalis isolates from clinical samples carrying van genes, Denmark, 2005–Q1 2019 (n = 2,664)

Discussion and conclusion
During 2005 to Q1 2019, most of the Danish clinical VRE isolates have been vanA E. faecium isolates. This study shows that predominating clones shifted over time and, importantly, the emergence of a vancomycin-variable clone, ST1421-CT1134 vanA E. faecium, that has spread to all the five Danish regions in 2019.

Although the E. faecium isolates belonged to 156 CTs, three types (ST80-CT14 vanA E. faecium, ST203-CT859 vanA E. faecium, ST1421-CT1134 vanA E. faecium) have dominated during the last 4 years.

ST80-CT14 vanA E. faecium was highly prevalent in the Capital Region during 2012 to 2015 [9]. The vanA E. faecium constituting Group2_ST80 in the paper by Pinholt et al. [9] belonged to ST80-CT14 (data not shown). On a national level, the numbers of ST80-CT14 vanA E. faecium decreased during 2016 to 2018, and this clone was not detected during Q1 2019.

ST203-CT859 vanA E. faecium emerged during 2015 through 2017 and nearly disappeared 2019. This clone has spread to Sweden, the Faroe Islands and Greenland [6,7].

Because of differences in diagnostic algorithms, there is a detection bias of VVE. It seems very likely that ST1421-CT1134 vanA E. faecium have been underreported in some regions at least during some periods. Thus, the rising incidence could partly be explained by...
increasing molecular testing of vancomycin susceptible isolates. However, a sharply increasing incidence has also been seen in DCM with extensive testing for VVE.

The origin of ST80-CT14 vanA E. faecium and ST80-CT860 vanA E. faecium are still unknown. vanA E. faecium isolates belonging to ST1421-CT1134 have also been reported from Australia, but these isolates have not been VVE [10]. Why these three clones were so successful is unknown.

The spread of the VVE clone, ST1421-CT1134 vanA E. faecium, in Denmark is of concern, especially since VVE diagnostic is challenging. Because of this, the clone is likely to be underdiagnosed, which facilitates further spread. Since cross-border spread has been described for VRE, countries with patients transferred from Denmark should be aware of the vancomycin-variable ST1421-CT1134 vanA E. faecium clone.

Table 1

<table>
<thead>
<tr>
<th>Types</th>
<th>2015 (n = 369)</th>
<th>2016 (n = 427)</th>
<th>2017 (n = 425)</th>
<th>2018 (n = 535)</th>
<th>Q1 2019 (n = 174)</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<tr>
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<tr>
<td>ST203-CT859 (subtypes CT1051 and CT1507) vanA</td>
<td>188</td>
<td>51</td>
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<td>ST421-CT1134 vanA</td>
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<td>Other types</td>
<td>51</td>
<td>14</td>
<td>51</td>
<td>12</td>
<td>81</td>
</tr>
</tbody>
</table>

CT: cluster type (cgMLST); MLST: multilocus sequence typing; ND: not detected; ST: sequence type (MLST); Q1: first quarter.

Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>2016 (n = 2)</th>
<th>2017 (n = 13)</th>
<th>2018 (n = 176)</th>
<th>Q1 2019 (n = 77)</th>
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<td>9</td>
<td>23</td>
</tr>
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<td>Central Denmark Region</td>
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<td>ND</td>
<td>ND</td>
<td>2</td>
</tr>
<tr>
<td>North Denmark Region</td>
<td>ND</td>
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<td>ND</td>
<td>1</td>
</tr>
</tbody>
</table>

Q1: first quarter.

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Conflict of interest

None declared.

Authors’ contributions

Mette Pinholt, Louise Roer, Hülya Kaya, Peder Worning, Sanne Nygaard, Marianne Engell Clausen, Karen Leth Nielsen, Jurgita Samulioniene, Mona Kjærsgaard, Claus Østergaard, Ulrik S Justesen, John Coia, Turid Snękloth Søndergaard, Shahin Gaini, Kristian Schanning, Henrik Westh, Henrik Hasman and Barbara Holzknecht contributed to the revision of the manuscript and approved the final version. Louise Roer, Hülya Kaya, Anette M Hammerum and Henrik Hasman did the molecular analysis. Mette Pinholt, Peder Worning, Kristian Schanning and Henrik Westh shared WGS data for many of the VRE/VVE isolates from DCM.
Hvidovre. Ulrik S Justesen, Mette Pinholt, Marianne Engell Clausen, Karen Leth Nielsen, Sanne Nygaard, Michael Kemp, Jurgita Samulioniené, Mona Kjersgaard, Claus Østergaard, John Coia, Turid Snekloth Søndergaard, Kristian Schønning, Henrik Westh and Barbara Holzknecht detected VRE/VVE at the DCMs in Denmark. Shahin Gaini shared isolates and data on the VRE/VVE from the Faroe Islands.

Anette M Hammerum and Barbara Holzknecht drafted the manuscript. Anette M Hammerum incorporated comments, additions and feedback throughout the revision.

References


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In 2016, an outbreak of *Salmonella* Typhimurium (STm) with multilocus variable-number tandem repeat analysis (MLVA) profiles historically associated with passerine birds (2-[11-15]-[3-4]-NA-212) occurred among passerines, cats and humans in Sweden. Our retrospective observational study investigated the outbreak and revisited historical data from 2009–16 to identify seasonality, phylogeography and other characteristics of this STm variant. Outbreak isolates were analysed by whole-genome single nucleotide polymorphism (SNP) typing. The number of notified cases of passerine-associated STm among passerines, cats and humans per month and county, and their MLVA profiles, were compared to birdwatchers’ counts of passerines. Seasonal trend decomposition and correlation analysis was performed. Outbreak isolates did not cluster by host on SNP level. Passerine-associated STm was seasonal for birds, cats and humans, with a peak in March. Cases and counts of passerines at bird feeders varied between years. The incidence of passerine-associated STm infections in humans was higher in the boreal north compared with the southern and capital regions, consistent with passerine population densities. Seasonal mass migration of passerines appears to cause STm outbreaks among cats certain years in Sweden, most likely via predation on weakened birds. Outbreaks among humans can follow, presumably caused by contact with cats or environmental contamination.

**Background**

*Salmonella enterica* is a zoonotic and food-borne pathogen that is estimated to cause hundreds of millions of cases of disease worldwide every year [1]. In infected humans, *S. enterica* can cause diarrhoea, enteric fever or bacteraemia, depending on which subspecies and serovar causes the infection, as well as the susceptibility of the infected host [1]. Serovars occur in a spectrum ranging from host generalists, which can infect many animal species, to host specialists, which preferentially or only infect a single species [2]. Host adaptation can involve the acquisition of mobile genetic elements encoding new genetic traits [3,4], but also the loss of functions not needed in the preferred host or niche, e.g. metabolic capabilities [5]. Serovars that are specialists preferring particular animal hosts tend to cause infection among vulnerable human hosts like immunosuppressed people, young children and elderly people [2,6]. *S. enterica* subspp. *enterica* serovar Typhimurium (STm) is considered a predominantly generalist serovar, infecting a broad range of wild and domestic animal species [7], while also being the second most common source of human gastroenteritis caused by *Salmonella* in Europe [8]. However, certain variants within the serovar appear to be host specialists that are mostly found, for example, in pigeons [9], ducks [10] or hedgehogs [11].

Notable examples of host-specialist STm are the phage type DT 40/U277/NST variants, which have been reported as the cause of outbreaks of septicemia and mortality in wild birds in several countries including Canada, Switzerland, Sweden, Norway and the United Kingdom (UK) [12-16]. Outbreaks tend to occur in late winter or early spring, most commonly among certain passerine species such as Eurasian siskins (*Carduelis spinus*), Eurasian bullfinches (*Pyrrhula pyrrhula*), common redpolls (*Carduelis flammea*) or greenfinches (*Carduelis chloris*). Emaciation and necrotic lesions in the oesophagus, crop, liver and spleen are frequently seen at necropsy [14,16]. The bacteria are generally present in blood, lung and liver samples, but absent from the intestines [14]. However, STm has also been found in cloacal swabs taken from apparently healthy passerines of the same species [14]. This suggests that the birds may serve as year-round hosts, carrying low levels of STm in their gastrointestinal tracts, with outbreaks occurring when birds are in poor condition.
and crowded at feeding locations in late winter or early spring. Infection among cats has also been reported in some of these outbreaks [15,16], and a study found STm phage type DT 40 in dead birds, in faecal samples from cats that had eaten birds and on the ground under a bird feeder in the same domestic garden area [17]. Infected cats can display inappetence, fever, vomiting and diarrhoea [15], but cats are also known to shed Salmonella while showing mild or no clinical signs of disease [18]. Given the exposure via cats and environmental contamination, these outbreaks pose a risk of causing human cases of salmonellosis. During an outbreak of STm DT 40/NST among passerines and cats in Värmland county, Sweden in 1999, four human cases of STm NST also occurred; two of these had sick cats in the household, while the other two had been feeding wild birds [15]. A recent study found that the same genotypes of STm occured among wild birds, cats and humans in the UK [19], further demonstrating the zoonotic potential of passerine-associated STm variants.

Outbreak detection
In early 2016, several findings of dead passerine birds were reported by the Swedish public. At the same time, an uncommonly high number of STm-positive samples from domestic cats were analysed: 448 between January and March. Simultaneously, data from the national microbiological surveillance programme indicated an increase of STm with the multilocus variable-number tandem repeats analysis (MLVA) profile pattern 2-[11-15]-[3-4]-NA-212 among humans, with a total of 18 cases in the same period.

The overall number of domestic salmonellosis cases in Sweden is low; from 2009–16, there were 552–840 cases reported annually [20]. Salmonellosis is a notifiable disease in Sweden and all domestic isolates from humans are sent to the Public Health Agency (PHA)’s microbiological laboratory in Solna, Sweden for typing. The low number of cases and real-time typing of all isolates at the agency allow for a very sensitive surveillance system for outbreak detection. Results from MLVA typing showed matching profiles from passerines, cats and human cases during the same period.

The Swedish National Veterinary Institute (SVA) and the PHA initiated a collaborative project to investigate the outbreak and to review historical data on the occurrence of this passerine-associated variant of STm among passerines, cats and humans in Sweden.

Methods
A retrospective, observational study was carried out to investigate the occurrence of a specific passerine-associated variant of STm among Swedish passerine birds, domestic cats and humans from 2009–16. The analysed data included observations from annual birdwatcher surveys; nationwide passive surveillance data regarding the occurrence of salmonellosis among Eurasian bullfinches, Eurasian siskins, common redpolls, cats and humans; molecular typing (MLVA) data from all sampled sources in Sweden (human, food, feed, domestic and wild animals); and whole genome sequencing typing data from passerine, cat and human isolates collected during the outbreak in 2016. Additional data from Statistics Sweden regarding regional rates of cat ownership and housing types was included to aid interpretation of the observations made.

Birdwatcher surveys
Birdwatchers count and report the number of wild birds visiting private birdfeeders in Sweden the last weekend of January every year, an event organised by the Swedish Ornithological Society. Nationwide data on the numbers of common redpolls, Eurasian bullfinches and Eurasian siskins reported during this monitoring in the period 2009–16 were downloaded [21]. To compensate for the varying number of survey participants from year to year, these counts were normalised against the total number of birds counted from the top 30 species the same year.

Samples and bacterial isolates
Routine MLVA analysis of all STm isolates from animal, food, feed and human sources was introduced in Sweden in 2009; therefore, the study period was set to 2009–16. Swedish authorities encourage the public to submit dead wild animals to the SVA for necropsy and ancillary laboratory analysis as part of the national wildlife disease surveillance programme. Passerine birds such as Eurasian bullfinches, Eurasian siskins and common redpolls that are found dead by the public are submitted on a volunteer basis. The study was limited to these three species, as they are the most commonly observed salmonellosis cases among wild birds in the SVA’s records. In instances where multiple birds were submitted from a single location and time, they were counted as a single observation.

A high proportion of Swedish cats have health insurance (estimated at 36% in 2014 [22]), and a veterinarian suspecting salmonellosis in an animal is obliged
to perform an investigation. Therefore, many cats with signs of salmonellosis are investigated and sampled at veterinary clinics. Cat faecal samples from cats with suspected salmonellosis are submitted to the SVA’s laboratory for *Salmonella* analysis. Presumptive *Salmonella* isolates are also submitted to the SVA from other Swedish laboratories for mandatory confirmation of possible cases of salmonellosis in cats. A subset of the isolates investigated in this study was not fully serotyped, with an O4+ status considered sufficient to assume the isolate to be STm. Not all isolates from cats and wild birds were typed by MLVA because of volume caps that are implemented for practical and economic reasons in response to the high number of very similar profiles that are generated in years when many cases occur.

Human *Salmonella* spp. isolates from domestic cases are routinely submitted to the PHA by clinical microbiological laboratories for typing. All human STm isolates from 2009–16 were analysed by MLVA. The human case definition for the present study was domestic infection by STm with a MLVA profile matching the passerine-associated pattern 2-11-15-3-NA-212.

**Molecular characterisation**

Phage typing has been replaced by MLVA for typing of STm isolates in Sweden. MLVA was performed according to the European Centre for Disease Prevention and Control’s standard protocol [23]. Whole genome sequencing was performed on isolates from 15 passerines (2 Eurasian siskins, 4 common redpolls and 9 Eurasian bullfinches), 15 cats and 10 humans during the 2016 outbreak. A convenience sample of isolates from different parts of the country was included for each host category to reveal any major regional differences. DNA was extracted using the Blood & Tissue Kit protocol on a BioRobot E21 (Qiagen, Hilden, Germany), with libraries subsequently prepared using the Nextera XT kit (Illumina, San Diego, California, United States). Sequencing was performed on a MiSeq instrument (Illumina) as paired-end 2 x 250 bp reads, with all isolates sequenced to >25 x depth. Single nucleotide polymorphism typing was performed by mapping reads corresponding to ca 25 x–100 x coverage for each isolate to the complete STm LT2 genome sequence [24] using Bowtie 2 2.2.7 [25], and calling SNPs with SAMtools 1.3.1 [26]. SNPs were filtered, requiring a single variant allele for each included variable site, an overall quality >100 for each variable site, and for each variable site to be present and have a quality >25 in all isolates. Sites with a conflicting genotype call with a quality of>10% of the primary call quality were excluded. The relationship between isolates was visualised using the neighbor-net algorithm in SplitsTree 4.14.4 [27]. All sequence data were uploaded to the European nucleotide archive (ENA) and are available under project accession number PRJEB27180.

**Population and geographical data**

Counties were classified into three zones based on biogeography [28] to relate the occurrence of passerine-associated STm cases with the ecology of the investigated bird species: (i) the boreal zone, characterised by coniferous and birch forests (counties BD, AC, Z, Y, X, W, S and T in northern and central Sweden); (ii) the boreo-nemoral zone, characterised by mixed deciduous and coniferous forests and a generally lower overall forest cover (counties U, C, D, O, E, F, G, H, I and K in central and southern Sweden) and

### Table 1

MLVA profiles of *Salmonella* Typhimurium from cats, passerines and matching isolates from domestic human salmonellosis cases, Sweden, 2009–2016

<table>
<thead>
<tr>
<th>MLVA profile</th>
<th>Number of passerines</th>
<th>Number of cats</th>
<th>Number of humans</th>
</tr>
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<tbody>
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<td>20</td>
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<td>3-20-15-9-309</td>
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<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>3-18-NA-NA-211</td>
<td>0</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>54</strong></td>
<td><strong>116</strong></td>
<td><strong>86</strong></td>
</tr>
</tbody>
</table>

MLVA: multilocus variable-number tandem repeat analysis; ND: not done.
Figure 2
Age distribution of domestic cases of Salmonella Typhimurium with (n = 86) and without (n = 1,281) MLVA profiles consistent with passerines as the ultimate source of infection, Sweden, 2009–2016

MLVA: multilocus variable-number tandem repeat analysis; STm: Salmonella Typhimurium.

‘Passerine-associated’ STm refers to variants with the MLVA pattern 2-[11-15]-[3-4]-NA-212, which can indicate passerines as a probable source.

(iii) the nemoral zone, characterised by deciduous forests and lower overall forest cover (counties N and M in the southernmost part of Sweden). The capital region of Stockholm county (AB), located in the bore-nemoral zone, was analysed as a separate category (See Supplementary Table S1 for county codes). The official county-level human population for 2016 was retrieved from Statistics Sweden [29]. Supplementary data for 2016 regarding households living in apartment buildings vs detached or semi-detached houses, at the county level, were available from the Statistics Sweden online database [29] and were used as a proxy to identify possible differences in exposure to STm from wild birds via access to a garden or feeding birds. Data on household cat ownership were available by Nomenclature of Territorial Units for Statistics (NUTS) 2 county groups from a study performed in 2012, also from Statistics Sweden [30]. Age, sex and county of residence for human cases was retrieved from SmiNet, the Swedish electronic notification system for reportable communicable diseases. No data on the severity of symptoms or underlying conditions were available.

Data analysis
Associations between year-to-year microbiological and birdwatcher survey data were investigated using Spearman’s rank correlation test in R version 3.3.1 (R Foundation, Vienna, Austria), with the alternative hypothesis that the true rho (ρ) was not equal to 0. Any p value < 0.05 was considered significant; 95% confidence intervals (CIs) for incidence measurements were calculated using the Agresti-Coull method [31] implemented in the binom package in R version 3.3.1. Differences were considered significant if CIs did not overlap. Seasonal trend decomposition of the occurrence of STm among cats, birds and humans was performed using the stl function in R version 3.3.1, dividing the observed variation into seasonal, long-term trend and random components.

Ethical statement
Data on human cases were collected as part of the national surveillance of salmonellosis under the Swedish Communicable Diseases Act (SFS: 2004:168). All data were anonymised and cannot be inferred directly or indirectly to a person. Guidelines on animal ethics and welfare were followed. According to national legislation, ethical permission is not needed when animals are sampled at veterinary clinics for diagnostic purposes. Persons submitting samples to the SVA give their consent on the referral form regarding the use of the submitted material for research purposes. Wild birds were sampled within the general disease surveillance of found dead birds, where approval from an ethical review committee is not required.

Results
Birdwatcher counts of selected passerine species at bird feeders
Birdwatcher observation data analysed in the present study showed a marked year-to-year variability in the number of Eurasian siskins and common redpolls (Figure 1). Notably, 4,117 common redpolls and 9,456 Eurasian siskins were counted in 2015, increasing to 23,041 and 27,242, respectively, in the STm outbreak year of 2016. A surge of common redpolls also occurred in 2009 and of Eurasian siskins in 2012. The number of Eurasian bullfinches counted was less variable. A total of 874,483 visits from individual birds of the species of interest were counted, while the total count in the normalisation dataset was 9,299,153 birds.

Salmonella Typhimurium among passerines and cats
STm isolates from 72 Eurasian bullfinches, Eurasian siskins and common redpolls, submitted from 2009–16, were included in the study (Supplementary Table S2). The outbreak year of 2016 coincided with the highest number of birds diagnosed with STm. The number of found dead birds submitted per year varied between none and 27, with an average of nine per year.

A total of 1,165 index isolates of STm from cats, submitted by veterinarians from 2009–16 for Salmonella analysis or for confirmation of presumptive isolates, were included in the study. The number of isolates collected per year varied between seven and 487, with an average of 146 per year. Of the 1,165 isolates, 805 (69%) were not fully serotyped, as their O4+ status was considered sufficient to assume the isolate to be a likely STm.

Of the passerine and cat isolates, a subset of 54 (75%) and 116 (10%) isolates, respectively, were analysed by MLVA.
MLVA profiles were homogenous for the 54 passerine isolates, all of which matched the pattern 2-[11-15]-[3-4]-NA-212 (Table 1), referred to herein as ‘passerine-associated’. Of the 116 cat isolates, 106 had profiles matching those of the passerines, six were single-locus variants of multiple passerine profiles and four differed at three loci or more from all passerine profiles. In contrast, isolates from domestic cases of STm among humans had a rich variety of MLVA profiles and were only included in the study if they matched the passerine-associated MLVA pattern 2-[11-15]-[3-4]-NA-212, which was selected based on the variants that were observed among passerines. This pattern was found in 86 human isolates from patients with a mean age of 40 years and a median age of 49 years, of which 52% (n = 45) were women. For humans, as for the passerines and cats, 2-13-3-NA-212 was the most common passerine-associated MLVA profile, followed by 2-12-3-NA-212 (Table 1). A database search for the same profile among STm from other sources in Sweden—including food, animal feed, and wild and domestic animals—produced very few hits, mostly isolates from other passerine birds like great tits and greenfinches or from predators like red foxes and birds of prey (data not shown). All findings of STm in food, feed, domestic animals and other wildlife were typed by MLVA during the study period.

Passerine-associated *Salmonella Typhimurium* as a cause of human salmonellosis

Using the passerine-associated MLVA profiles as selection criteria, 86 cases of human salmonellosis for which passerines or cats are probable sources were identified in Sweden in 2009–16. This corresponds to 6% of the human cases of STm infection contracted within the country. The number of passerine-associated cases varied between three and 25 per year, with an average of 11 per year; young children (< 5 years) and elderly people (≥ 60 years) were overrepresented among these cases, compared with all domestic STm cases (Figure 2). The largest number of cases occurred in 2016, when 25 isolates were identified as passerine associated. Specifically, the 2-13-3-NA-212 profile dominated, with 12 cases reported in January–March 2016. Of these 12 cases, five were children < 5 years of age and five were ≥ 60 years of age. Local reports indicated that a number of cases had contact with cats (data not shown).

A comparison of the incidence of passerine-associated STm between different biogeographical zones in Sweden in 2009–16 revealed differences. The incidence was significantly higher in the northern boreal zone (0.22/100,000 inhabitants) compared with the nemoral zone (0.05/100,000) and the capital region.
of Stockholm county (0.02/100,000) (Figure 3). No significant differences between the zones were observed when comparing the incidence of domestic STm cases caused by all other types, except for Stockholm county, which has a significantly lower overall incidence (Figure 3).

Whole genome sequencing investigation of the 2016 outbreak
The 2016 outbreak among passerines, cats and humans in Sweden was caused by a genetically homogenous group of strains, as indicated by the MLVA analysis, but there was substantial variation between outbreak isolates on the whole-genome SNP level (Figure 4a). The outbreak isolates did not cluster by host, with most of the main clades of outbreak isolates containing representatives from passerines, cats and humans. A single human isolate, the earliest in the year among the 10 analysed, differed more substantially from the other outbreak isolates in terms of SNP variation; however, it was consistent with the outbreak in terms of MLVA profile (cluster H, not shown in the network in Figure 4a). The 40 outbreak isolates investigated were from 13 counties (Figure 4b). There were several instances of the same genotype occurring in multiple host types in the same county, but otherwise a limited regionality of genotypes.

Year-to-year variation and seasonality
Comparing data on the number of cases of passerine-associated STm during the 2009–16 period revealed variation between years for passerines, birds and
Figure 5

(A) Year-to-year variation and (B) seasonality of presumed passerine-associated *Salmonella* Typhimurium among passerines (n = 72), cats (n = 1,165) and humans (n = 86), Sweden, 2009–2016

Outbreak control measures

The Swedish national authorities launched a public information campaign at the time of the 2016 outbreak. Press releases and web notices highlighted the importance of hygiene when handling bird feeders and cat litter boxes, cleaning the area under bird feeders and keeping cats showing signs of disease away from children. This information was picked up by several national and regional media outlets and raised public awareness. While the human cases were comparatively few, the same precautions can also be beneficial for preventing the spread of, for example, psittacosis and avian trichomoniasis.

Discussion

Most salmonellosis cases in Sweden are related to travel abroad or contaminated food, but the occurrence of cases associated with cats in the early months of the year has been anecdotally known for many years [15]. We have used nationwide data from multiple sources, covering the period 2009–16, to show that multispecies outbreaks of passerine-associated STm occur among passerine birds, domestic cats and humans in the early months of certain years, possibly triggered by fluctuations in the passerine population and mass migration events. Birdwatcher data confirm that passerines in Sweden seek out human habitations to feed in large numbers in certain years, resulting in varying levels of exposure to passerine-associated STm from year to year for both domestic cats and humans. The
underlying drivers of this phenomenon are likely complex. Many tree species produce large seed crops intermittently, with low or no production during the interim years; these cycles can be synchronous over large geographical areas, thereby affecting the ecology of the animal species that feed on the tree seeds [32]. For example, spruce and birch seed production is known to influence the population size and winter movements of common redpolls and Eurasian siskins, respectively, with years of low seeding triggering irruptions and increased mortality [33-35]. Other factors, such as the seed crop the previous year [36], the weather [37] and the availability of alternative food sources (like the seeds of annual plants [15]) have also been thought to trigger irruptive migration among passerines. Predation on weakened birds with possible septicæmia, e.g. around bird feeders, is the most likely route of infection for cats [15,17], presumably exposing the cat to a high infectious dose.

The human incidence of passerine-associated STm was significantly higher in the boreal northern and central parts of Sweden. While this could be an artefact of the limited number of observations and years of sampling, as well as other uncertainties related to under-reporting of salmonellosis in humans, it is biologically plausible, as middle to northern Sweden is richer in spruce and birch forest habitats and therefore supports larger populations of the relevant passerine species [35]. We also note a low incidence in the capital region of Stockholm county. Fewer households in Stockholm county than nationwide have one or more cats (10% vs 17%), and more households live in apartment buildings (59% vs 41%). Thus, it is likely that a lower proportion of the population in Stockholm county interacts with a garden bird feeder or an outdoor cat, compared with other counties in the boreo-nemoral zone, presumably leading to less exposure to passerine-associated STm.

In Sweden, passerine-associated STm among humans seems to more commonly afflict young children and elderly people. It is possible that these groups have a higher exposure to outdoor cats, bird feeders and garden environments. In addition, passerine-associated STm appears to be host biased and lacks the large virulence plasmid found in many other strains of STm [19], traits associated with a lower risk of severe infection in humans [2,4]. Healthy adults may therefore hypothetically be less vulnerable to infection with this variant of STm, compared with other variants.

We observed that passerine-associated STm was strongly seasonal in passerines, cats and humans. All three host types experienced a peak in the early months of the year, particularly in March, with human cases continuing to occur during early summer, when cases among cats and birds declined. It is conceivable that asymptomatic birds or persistent environmental contamination continue to cause these human infections later in the season. The peak of this variant of STm is in contrast with other domestic STm infections and salmonellosis in general in Sweden, which peak in late summer. Both MLVA and whole genome sequencing confirmed the link between the different hosts of the pathogen, although, as expected, sequencing data were found to be more informative. The 2016 outbreak was not clonal, consistent with an outbreak caused by environmental triggers acting on multiple sources of infection simultaneously—in this case, populations of passerines in different areas, as opposed to the highly clonal outbreak strains frequently found in single-source, e.g. food-borne, outbreaks.

The presented data should be interpreted with caution, as it is largely based on passive clinical surveillance. Continued observation over longer periods is therefore warranted. However, although we are far from understanding this complex phenomenon, we propose that the observation of high numbers of passerines like Eurasian siskins and common redpolls in winter can be used as an early warning of an increased risk of outbreaks of salmonellosis among cats and humans. The common redpoll and Eurasian siskin are resident in much of northern Eurasia as well as further south, e.g. in central Europe and the Alps [38], and occur seasonally in most of mainland Europe [38]. The Eurasian bullfinch is resident in most of Europe [38]. As previously mentioned, outbreaks of salmonellosis among these birds have been reported from several European countries [13-17]. In North America, the common redpoll co-occurs with the pine siskin (Spinus pinus), a close relative of the Eurasian siskin [38], with both species

<table>
<thead>
<tr>
<th>Correlation</th>
<th>PA STm in passerines</th>
<th>PA STm in cats</th>
<th>PA STm in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA STm in passerines</td>
<td>NA</td>
<td>ρ = 0.7, p = 0.05</td>
<td>ρ = 0.5, p = 0.2</td>
</tr>
<tr>
<td>PA STm in cats</td>
<td>ρ = 0.7, p = 0.05</td>
<td>NA</td>
<td>ρ = 0.9, p = 0.005</td>
</tr>
<tr>
<td>Passerine count</td>
<td>ρ = 0.7, p = 0.06</td>
<td>ρ = 0.7, p = 0.06</td>
<td>ρ = 0.4, p = 0.4</td>
</tr>
</tbody>
</table>

NA: not applicable; PA: passerine-associated; STm: *Salmonella Typhimurium.*

* Spearman’s rank correlation test.

**TABLE 2**

Correlations in year-to-year variation in the number of cases of presumed passerine-associated *Salmonella Typhimurium* among passerines, cats and humans, and the number of selected passerine species counted by birdwatchers, Sweden, 2009–2016.
experiencing periodic outbreaks of salmonellosis [12]. Our results are therefore likely to have implications for public and animal health outside our study area of Sweden. Typing methodologies like MLVA and whole genome sequencing have facilitated data exchange between veterinarian and public health sectors, as well as the discovery of discrete lineages of pathogens, thereby improving the capacity to trace zoonotic spread of bacteria. Continued use of such methods, as well as retrospective analysis of historical isolates and international data sharing, is likely to reveal more host-adapted lineages of STm and epidemiological links between animals and humans in the years to come.

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Conflict of interest

None declared.

Authors’ contributions

RS, CJ and EL conceived the study. RS, CJ, EL, LT, AP and EÅ contributed to the collection, analysis and interpretation of data. RS drafted the manuscript. RS, CJ, EL, LT, AP and EÅ contributed to revision of the draft manuscript and approved the final version.

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26. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical


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Any supplementary material referenced in the article can be found in the online version.

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In September 2017, a cluster of monophasic *Salmonella* Typhimurium isolates was identified at the National Reference Laboratory for Enteropathogenic Bacteria in Norway. We investigated the cluster to identify the source and implement control measures. We defined a case as a person with laboratory-confirmed salmonellosis with the outbreak strain multiple locus variable-number tandem repeat analysis type. We conducted descriptive epidemiological and environmental investigations and performed whole genome sequencing (WGS) with core and accessory genome multilocus sequence typing of all isolates from cases or the environment connected with this outbreak. We identified 21 cases, residing in 10 geographically dispersed counties, all of whom had consumed food or drinks from a café at Oslo Airport. Case distribution by date of symptom onset suggested that a point source was introduced in mid-August followed by continued environmental contamination. The incubation periods ranged 0–16 days and increased as the outbreak progressed, likely due to increasingly low-dose exposure as control measures were implemented. WGS confirmed an identical cluster type-944 in all cases and six environmental specimens from the café. Control measures, including temporary closure and kitchen refurbishment, failed to eliminate the environmental source. We recommend strengthened hygiene measures for established environmental contamination during an outbreak.

**Background**

Non-typhoidal *Salmonella* infection is the second most commonly reported gastrointestinal infection in the European Union/European Economic Area (EU/EEA) [1]. In Norway, *Salmonella* infections have been notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS; http://www.msis.no/) since 1975. Since 2005, between 866 and 1,942 cases of salmonellosis have been reported annually. Typically, 60–80% of notified cases are acquired abroad, as Norway has few established domestic *Salmonella* reservoirs. *S.* Typhimurium is one of the most commonly encountered *Salmonella* serovars in Norway accounting for 33% of domestically acquired salmonellosis cases in 2000-15 [2]. In the past 20 years, monophasic *S.* Typhimurium with antigenic formula 4,[5],12:i.- has been identified in several countries, both in production animals and human cases [2-4]; monophasic *S.* Typhimurium is especially associated with pig production [5,6]. In Europe, the first described monophasic *S.* Typhimurium outbreak was caused by a ‘Spanish clone’, which emerged in 1997 [7]. In Norway, monophasic *S.* Typhimurium was first identified in 2007 and in 2016, it accounted for 12% of all salmonellosis cases and 17% of all domestic salmonellosis cases reported to the Norwegian Institute for Public Health (NIPH) [8].

The incubation period of salmonellosis is typically between 6 and 72 hours, but incubation periods of up
to 16 days have been documented following low-dose exposure [9-11]. The infectious dose for salmonellosis varies by serovar but is usually $\times 10^4$ bacteria [12]. Symptoms of non-typhoidal salmonellosis are diarrhoea, nausea, headache and abdominal cramps. Fever may also be present [13].

**Outbreak detection**

On Friday 15 September 2017, the National Reference Laboratory (NRL) for Enteropathogenic Bacteria (NRL) for human matrices at NIPH reported a cluster of six monophasic S. Typhimurium isolates sharing a rare multiple locus variable-number tandem repeat analysis (MLVA) type (3-13-12-NA-210). The cases resided in five geographically dispersed municipalities in Norway and no travel abroad was reported in the week before symptom onset. NIPH initiated an outbreak investigation in collaboration with the Norwegian Food Safety Authority (NFSA) and the municipal medical officers in the affected municipalities to identify the source of the outbreak in order to implement control measures and prevent further spread.

**Methods**

**Case definition**

For this outbreak, a case was defined as a person residing in Norway with a laboratory-confirmed infection with monophasic S. Typhimurium MLVA type 3-13-12-NA-210 sampled after 15 August 2017.

**Case finding**

In Norway, all Salmonella isolates are submitted by the medical microbiology laboratories to NRL at NIPH for confirmation and characterisation.

On 15 October 2017, the NIPH requested, through the Epidemic Intelligence Information System (EPIS) coordinated by the European Centre for Disease Prevention and Control (ECDC), information on whether other countries had identified cases of monophasic S. Typhimurium isolates and environmental exposures in the affected municipalities to identify the source of the outbreak in order to implement control measures and prevent further spread.

**Epidemiological investigation**

Four initial cases were interviewed using a standardised 19-page Salmonella-specific trawling questionnaire [14]. The questionnaire included detailed questions about food consumption and purchases, animal contact and environmental exposures in the week before the onset of symptoms, as well as clinical and demographic information. Following analysis of information from these interviews, the questionnaire was shortened to focus on categories of most interest, which included domestic travel before symptom onset and food items consumed at the cafés at Oslo Airport. The interviews were carried out by municipal medical officers in the municipality of the cases or by NFSA and NIPH staff.

A descriptive analysis of the cases and results of the interviews was conducted using Excel 2013 and STATA v15 (StataCorp, College Station, Texas, United States). The mean incubation periods of cases in the periods of August 2017 and September 2017 onwards were compared by t-test.

**Microbiological investigation**

**Human specimens**

In Norway, all faecal specimens from patients with gastroenteritis are routinely analysed for at least Salmonella, Campylobacter, Shigella and Yersinia at the medical microbiology laboratories. Salmonella isolates are submitted to the NRL at NIPH, where they are serotyped by agglutination tests with antisera (SIFIN, Berlin, Germany and Statens Serum Institut (SSI), Hillerød, Denmark) according to the White-Kauffmann scheme [15] and MLVA typed as described previously [16]. The isolates were tested for susceptibility to ampicillin, azithromycin, cefotaxime, gentamicin, meropenem, pefloxacin, tetracycline, and trimethoprim-sulphamethoxazole according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 7.1 guidelines (http://www.eucast.org). Where EUCAST breakpoints were not available, epidemiological cut-off values were used based on national zone distributions (tetracycline: R$<17$ mm) [17].

**Environmental and food specimens**

Environmental swabs and food specimens were collected by NFSA during site inspection on 22 September and environmental swabs on 21 November 2017. These were analysed at the Norwegian Veterinary Institute, NRL-Salmonella from non-human matrices, for the presence of Salmonella and Escherichia coli. Monophasic S. Typhimurium variants were identified according to the European Food Safety Authority (EFSA) recommendations [18] and sent to the NRL at NIPH for further typing as described above.

In the period 13–23 October, environmental samples collected by the company operating the café were analysed at a private laboratory and sent to the Norwegian Veterinary Institute for verification and then submitted to the NRL at NIPH for typing as described above.

**Comparison of isolates**

All human and environmental monophasic S. Typhimurium isolates connected with the outbreak were analysed at NIPH by whole genome sequencing (WGS). The raw reads were submitted to the European Nt Archive (ENA) under the accession numbers ERS3466858-ERS3466884. Classical multilocus sequence typing (MLST, 7 loci) core genome (cgMLST, 3,002 loci) and accessory genome MLST (aMLST, 1,324 loci) containing 4,333 loci was performed on WGS data. The cgMLST was performed as previously described [19]; for the in-house aMLST S. Typhimurium TW-Stm6 (CP019649.1) was used as a reference genome.

The online tool ResFinder version 3.0, available at the Center for Genomic Epidemiology (http://www.
genome epidemiology.org/) was used for sequence-based identification of acquired resistance genes using assembled genomes obtained through SPAdes Genome Assembler version 3.0 (Algorithmic Biology Laboratory, St. Petersburg University, St. Petersburg, Russia) [20]. Default threshold values were used.

Environmental investigation
The NFSA inspected the café kitchen on 20 and 22 September and 21 November 2017, swabs from kitchen surfaces were collected during the two latter inspections. NFSA also reviewed documentation for suspected food products delivered to the implicated café. During the period 13 October–15 November, the company operating the café collected environmental specimens on nine occasions.

Ethical statement
Ethical approval was not required as outbreak investigations are covered under national legislation. Cases were asked for consent to participate at the start of the interviews.

Results
Epidemiological investigation
Description of the cases
As at 1 February 2018, 21 confirmed cases were reported to the NIPH. Thirteen (13/21) were women and the median age was 27 years (range 17–60) (Table). The cases resided in 10 geographically dispersed counties in Norway. International requests returned no reports of cases in other European countries.

Date of symptom onset was available for 16 cases and ranged from 23 August to 18 November 2017, with the majority occurring in the first week of the outbreak (Figure 1). For the 15 cases with available date of symptom onset and date of exposure, the incubation period ranged between 0 and 16 days and increased as the outbreak progressed (p = 0.029) (Figure 2). The median incubation period in August was 4.5 days (range 0–5) and 9 days (range 2–16) from September onwards.

Four cases were interviewed with the *Salmonella*-specific trawling questionnaire and the remaining cases with a more focused questionnaire. All cases interviewed with the trawling questionnaire had visited Oslo Airport and had consumed different food or drink items at the same café. In total, all 21 cases reported having consumed food or drink items at the café between 18 August and 13 October 2017. The cases reported consuming at least three different types of sandwiches and seven different fruit or vegetable juices; no single common food or drink item was reported by all interviewed cases. No other common exposures at Oslo Airport were identified. The cases included members of staff at the implicated café.

Microbiological investigation
Twenty-one human monophasic *S. Typhimurium* isolates with the MLVA type 3-13-12-NA-210 were identified by the NRL at NIPH.

Six environmental specimens collected from a kitchen drain, water tap and a wall mounted steel shelf were positive for the outbreak strain. All 10 collected food specimens tested negative for *Salmonella*.

All isolates of human and environmental origin were sequence type (ST) 34 and cluster type (CT) 944. The isolates clustered together and cgMLST and aMLST showed that there were three or fewer allelic differences between the isolates (Figure 3).
The outbreak strain was resistant to ampicillin and tetracycline and carried the \textit{bla}_{TEM-1B} and \textit{tet}B genes that confer resistance to β-lactams and tetracycline, respectively.

Environmental and trace-back investigation

The company operating the café provided documentation on wholesalers where they had sourced food items; some of them were located outside Norway. NFSA received documentation on foods obtained from wholesalers in the period 9–25 August, as well as on 21 September and 26 September 2017. The review of documentation connected to food batches delivered to the café was inconclusive.

The inspections identified several weaknesses in the hygiene routines at the café, including separation between clean and unclean kitchen areas, use of appropriate detergents for different cleaning purposes and which basins that should be used to wash hands, food items and dishes, respectively. Furthermore, the washing routines of workwear was not optimal and there was lacking assessment and management of potential risks connected with the work processes and ingredients.

Outbreak control measures

The timeline for outbreak control measures can be seen in Figure 4.

On 22 September, the café closed temporarily to wash down the facilities and replace some of the kitchen equipment, while awaiting results from environmental specimens collected the same day. The café reopened on 13 October and simultaneously engaged a private company to collect environmental specimens. The next day the café was informed that preliminary results from some specimens showed the presence of \textit{Salmonella} and it closed again for an additional wash down. New specimens were collected on five occasions 15–23 October and the facilities were cleaned intensively several times during this period. On 25 October, the café reopened. According to the company operating the café, the staff that reported for work had tested negative for \textit{Salmonella}, five rounds of negative environmental specimens had been analysed and all inventory and kitchen equipment had been replaced. The company contracted cleaning staff to perform daily cleaning of all contact points and surfaces between 31 October and 7 November and had environmental specimens collected 6 November, while the café was open for business. On 7 November, one specimen with suspected \textit{Salmonella} was reported among those collected the previous day and the café was closed again. The suspected specimen had been collected from a shelf in the kitchen that had previously tested negative and had been installed after the inspection by NFSA on 22 September. Following the suspected finding, decontamination was undertaken and new specimens were collected on 8 and 15 November. These specimens tested negative. Environmental specimens collected by NFSA on 21 November were also negative.

Discussion

The dispersion of monophasic \textit{S. Typhimurium} MLVA type 3-13-12-NA-210 cases around Norway initially

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\textbf{Figure 2}

Outbreak timeline with incubation periods for cases of monophasic \textit{Salmonella Typhimurium} MLVA type 3-13-12-NA-210 infection by date of symptom onset\(^a\) or specimen collection date\(^b\), café at Oslo Airport, Norway, 2017 (n = 21)

\(^a\) Week of symptom onset (n = 16).

\(^b\) Week of specimen collection (n = 5).

\(^c\) Same day of exposure and symptom onset.

\(^d\) Uncertain day of onset.
suggested an outbreak linked to a nationally distributed product. Interviews of cases and descriptive epidemiology revealed that all cases had travelled by air or visited Oslo Airport in the days before illness onset, linking the outbreak to a local source. The results from the microbiological investigation of human and environmental specimens showed that the monophasic *S. Typhimurium* isolates were of the same MLVA type and clustered together closely in WGS analysis, which pointed to a café at Oslo Airport as the common source of the outbreak. This outbreak was likely associated with consumption of food or drink items from this café, which was located outside the security restricted area in the terminal and therefore accessible to anyone visiting the airport. Trawling interviews and descriptive epidemiology, in conjunction with environmental and microbiological investigation, were sufficient to identify the location of the source. This outbreak illustrates how WGS can be used to link patient and environmental specimens in an outbreak investigation context. Any suspected outbreak strains were analysed by WGS based cgMLST and aMLST, as they were received at the NRL at NIPH, which allowed for timely and ongoing confirmation of cases and isolates to be linked to the outbreak with high resolution.

The dates of onset of the first cases between 22 and 28 August suggested that the outbreak was caused by a point source, likely introduced at the café in mid-August. During the next several weeks the café was open for business, which resulted in ongoing exposure for customers and staff. The duration of this outbreak from August until November and the increasing incubation period over this time suggested that the source was environmental i.e. customers were continuously exposed but the dose of *Salmonella* decreased over time. Environmental contamination has previously been implicated in a protracted *S. Typhimurium* outbreak in the United Kingdom; cases were identified over a period of more than 13 months and aerosolised drain contamination from a restaurant kitchen drainage system was suspected as the source of transmission [21]. Previous studies have also indicated that low-dose exposure is associated with a prolonged incubation period [9-11], which would fit with the hypothesis of a lower dose obtained through contamination of food items through an environmental reservoir. As salmonellosis cases are normally interviewed about their exposures in the week before symptom onset for hypothesis generation, investigations of low-dose exposure outbreaks may miss exposures outside the standard incubation period.

### Control measures

The company operating the Oslo Airport café undertook extensive control measures, including several periods of voluntary closure, refurbishment of the kitchen and deep cleaning, however, eliminating the source of the environmental contamination proved difficult. This was likely due to the environmental contamination with specimens taken from several sites e.g. a kitchen drain, a tap and a steel shelf, all testing positive for the outbreak strain. The environmental inspection revealed several weaknesses in kitchen hygiene and food handling routines, possibly facilitating the cross-contamination of food items and protraction of the outbreak. This outbreak highlights the importance of ensuring food handlers are provided with adequate training to adhere to strict hygiene measures and routines throughout food handling in order to avoid environmental contamination. In addition to these food handling and hygiene practices, intensified hygiene audits and regular environmental specimen collection could be considered, especially in a context where large quantities of raw fruits and vegetables are processed in a commercial kitchen. Follow up with cleaning companies is also important to ensure wash downs are conducted as planned.

### Source of infection

We are unable to conclude how the source of infection was introduced at the café. We foresee two possible hypotheses; the source of infection was either...
Some evidence supports the hypothesis of infected staff as the source of introduction. First, the outbreak strain was present in specimens obtained from some staff members, which indicates that the strain circulated among the staff. However, staff members reported that they had consumed food items from the café, so they may have been exposed at work. Second, after being notified of the outbreak, the company operating the café required all staff members to provide a negative stool specimen before they could report to work; some asymptomatic staff members were identified this way. Finally, information obtained from the company operating the café shows staff members may have been ill in the run-up to the start of the outbreak. However, it is unknown whether any previous illness was caused by the outbreak strain.

Conclusions and recommendations

This common source monophasic *S. Typhimurium* MLVA type 3-13-12-NA-210 outbreak at Oslo Airport has implications beyond the local setting, as cases resided in several dispersed counties across the country. Although our EPIS request returned no reports of international cases, an outbreak at an international airport could easily have geographically wider implications. Furthermore, the café, where large quantities of fresh ingredients are processed, is part of an international chain with the company operating cafés in at least seven European countries, in addition to countries outside Europe.

The epidemiological and microbiological results from cases and environmental specimens obtained at the café support the hypothesis of a common source monophasic *S. Typhimurium* MLVA type 3-13-12-NA-210 outbreak. No further cases or isolates of the outbreak strain were identified at the NRL at NIPH after 18 December 2017, which indicates that the control measures that were implemented, including the voluntary closure of the café, successfully ended the outbreak. We recommend molecular surveillance for outbreak detection and investigation, strengthened hygiene measures in the case of established environmental contamination and awareness of long incubation periods where low dose contamination may be a driving factor for transmission.
Acknowledgements

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Conflict of interest

LS is a co-investigator in an unrelated study, for which the National Institute for Health and Welfare, Finland, has received research funding from GlaxoSmithKline Biologicals SA. The other authors report no potential conflicts of interest.

Authors’ contributions

LS drafted the manuscript. LS, EM, HL, LE, LV and KN carried out patient interviews, compiled the descriptive analysis and contributed to the epidemiological investigation. TS and LMM managed the outbreak response locally. GH coordinated the environmental investigations and trace-back investigations. LTB and UN conducted laboratory investigations on human specimens. GS and BB conducted laboratory investigations on food samples. All authors contributed to the writing of this manuscript and approved the final version.

References


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**Background:** In 2012, Israel’s National Center for Infection Control initiated a national stewardship programme that included mandatory annual reporting of antimicrobial use. Here we present nationwide Israeli data for the period 2012 to 2017. Aim: The goal of this study was to detect trends in antimicrobial use in Israel following the introduction of the stewardship programme, as part of an assessment of the programme’s impact. Methods: In this retrospective observational study, data were collected from Israel’s health maintenance organisations (HMOs), acute care hospitals and post-acute care hospitals (PACHs). Acute care hospital data were collected for general medical and surgical wards, and medical/surgical intensive care units (ICUs). Data were converted into defined daily doses (DDD), with use rates presented as DDD per 1,000 insured per day and DDD per 100 patient-days in hospitals and PACHs. Trends were analysed using linear regression. Results: Antimicrobial use decreased across sectors between 2012 and 2017. In the community, the decrease was modest, from 22.8 to 21.8 DDD per 1,000 insured per day (4.4%, p = 0.004). In acute care hospitals, antibiotic DDDs per 100 patient-days decreased from 100.0 to 84.0 (16.0%, p = 0.002) in medical wards, from 112.8 to 94.2 (16.5%, p = 0.004) in surgical wards and from 154.4 to 137.2 (11.1%, p = 0.04) in ICUs. Antimicrobial use decreased most markedly in PACHs, from 29.1 to 18.1 DDD per 100 patient-days (37.8%, p = 0.005). Conclusion: Between 2012 and 2017, antimicrobial use decreased significantly in all types of healthcare institutions in Israel, following the introduction of the nationwide antimicrobial stewardship programme.

Antimicrobial use trends, Israel, 2012 to 2017

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To date, relatively little data have been published on antimicrobial use in Israel. An article from the country’s largest health maintenance organisation (HMO), which insures slightly more than 50% of the population, summarised antimicrobial use data in the community [13]. The authors found that between 2000 and 2010, the rate of consumption remained steady at 23.2 defined daily doses (DDD) per 1,000 insured. Four previous articles examined antimicrobial use within Israeli hospitals, three for the period 1998 to 2004 [14-17]. The largest of the studies summarised data from 26 medical wards in six hospitals between 2003 and 2004 and found an overall rate of antimicrobial use of 79.9 DDD per 100 patient-days [17], with considerable variation between wards. An article summarising data from a single Israeli hospital in 1998 found rates, expressed in DDD per 100 bed-days, of 232, 173 and 149 for intensive care units (ICUs), medical and surgical wards, respectively [16].

Restrictive antimicrobial use and infectious disease (ID) approval for certain broad-spectrum agents were common practices in most acute care hospitals for many years before the formation of the Israeli National Center for Infection Control (NCIC), a branch of the Ministry of Health (MOH) [14]. However, after the NCIC was formed, it identified nationally monitored antimicrobial stewardship as an important component of the strategy to confront antimicrobial resistance.
In 2012, the NCIC instituted a nationwide programme for judicious use of antimicrobials [18]. As part of the programme, a circular was released requiring all healthcare institutions—including HMOs, acute care hospitals and post-acute care hospitals (PACHs)—to establish an antimicrobial stewardship committee that advises the institution's director on measures recommended for the judicious use of antimicrobials. While the creation of institutional antimicrobial use guidelines is mandatory, the nature and implementation of specific interventions to reduce antimicrobial use are at the institutions' discretion. Institutions must submit annual reports to the MOH on antimicrobial use. These are analysed, and annual comparative reports on antimicrobial use are prepared by the MOH and subsequently distributed to the institutions to support antimicrobial stewardship efforts.

The aim of this report is to present antimicrobial use trends in Israel for the period 2012 to 2017, following the launch of the programme, and to compare our data with similar data from Europe.

**Methods**

**Study design**
This report is an observational, retrospective analysis of aggregate data on antimicrobial use. Antimicrobial dispensing data were collected in the community from all clinics operating under the auspices of the four nationwide HMOs, which cover 100% of the population, and from acute care hospitals and PACHs. The HMOs represent all insurers within the national healthcare programme. From acute care hospitals, data were gathered for medical wards, general surgical wards and medical/surgical intensive care units (ICUs). The PACHs include patient populations requiring sub-acute medical care, inpatient rehabilitation, chronic mechanical ventilation and those fully dependent on nursing care for their activities of daily living.

**Data sources**
HMOs reported antimicrobials prescribed to individuals. Hospitals and PACHs used pharmacy databases to report antimicrobials dispensed to wards. Each institution used its pharmacy-operated system to generate the data, which were extracted to Microsoft Excel spreadsheets and submitted for analysis. Data on verified patient consumption of antimicrobials, such as from electronic medical records documenting drug administration, were not accessible. For the sake of simplicity, we refer to antimicrobial dispensing as antimicrobial use or consumption. Data were converted into DDD and grouped into categories using the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification method (2016 definitions) [19,20]. Use rates from HMO prescriptions were presented as DDD per 1,000 insured per day. Rates for hospitals and PACHs were presented as DDD per 100 patient-days, rather than DDD per 1,000 inhabitants, because we did not analyse hospital-wide antimicrobial use, but use in selected wards only.

Data for comparison of community antimicrobial use in Israel versus that in European countries, as determined by the European pooled-mean in 2012 and 2017, were taken from the 2018 summary published by the European Surveillance of Antimicrobial Consumption Network (ESAC-Net), a network managed and coordinated by the European Centre for Disease Prevention and Control [1]. To facilitate comparison with Israeli data, which includes individual ATC categories reported by ESAC-Net as ‘other’, data were compiled both as categorised by ESAC-Net and as categorised by the NCIC.

**Statistics**
Linear regression was performed to analyse time trends in antimicrobial use with p ≤ 0.05 defined as statistically significant. All calculations were performed with VassarStats (Vassar College, Poughkeepsie, New York, United States).

**Ethical statement**
Approval by an ethical committee was unnecessary, as this was a non-interventional study evaluating anonymised data that were collected for public health purposes and are publically available on the Israeli MOH website.
### Table

Antimicrobial use by patient population and antimicrobial category, Israel, 2012–2017

<table>
<thead>
<tr>
<th>Antimicrobial category</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Community (DDD/1,000 insured/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J01A</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>0.441</td>
</tr>
<tr>
<td>J01C</td>
<td>12.9</td>
<td>12.7</td>
<td>12.6</td>
<td>12.3</td>
<td>12.1</td>
<td>12.4</td>
<td>0.033</td>
</tr>
<tr>
<td>J01D</td>
<td>3.8</td>
<td>3.8</td>
<td>3.7</td>
<td>3.8</td>
<td>3.8</td>
<td>3.7</td>
<td>0.414</td>
</tr>
<tr>
<td>J01E</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.158</td>
</tr>
<tr>
<td>J01F</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
<td>2.3</td>
<td>2.2</td>
<td>2.1</td>
<td>0.924</td>
</tr>
<tr>
<td>J01M</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>0.003</td>
</tr>
<tr>
<td>All other J01 classes</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.116</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22.8</td>
<td>22.6</td>
<td>22.3</td>
<td>22.0</td>
<td>21.6</td>
<td>21.8</td>
<td>0.004</td>
</tr>
</tbody>
</table>

| **Medical/surgical intensive care units (DDD/100 patient-days)** |      |      |      |      |      |      |         |
| J01A                  | 3.6  | 3.0  | 2.7  | 3.3  | 2.9  | 3.3  | 0.709   |
| J01C                  | 39.1 | 35.8 | 36.6 | 34.2 | 35.0 | 31.8 | 0.014   |
| J01D                  | 41.6 | 41.3 | 38.8 | 39.7 | 41.8 | 39.8 | 0.582   |
| J01E                  | 3.0  | 2.4  | 4.1  | 3.8  | 3.7  | 2.7  | 0.754   |
| J01F                  | 12.3 | 11.1 | 9.9  | 10.1 | 10.8 | 10.8 | 0.297   |
| J01M                  | 15.0 | 13.7 | 13.7 | 14.0 | 14.8 | 11.5 | 0.213   |
| All other J01 classes | 39.8 | 45.2 | 43.0 | 38.9 | 41.1 | 37.3 | 0.270   |
| **Total**             | 154.4| 152.5| 148.8| 144.0| 150.1| 137.2| 0.041   |

| **General surgical wards (DDD/100 patient-days)** |      |      |      |      |      |      |         |
| J01A                  | 1.0  | 0.7  | 0.6  | 0.5  | 0.5  | 0.5  | 0.024   |
| J01C                  | 41.9 | 37.9 | 35.6 | 34.6 | 34.7 | 32.6 | 0.007   |
| J01D                  | 22.1 | 24.1 | 24.3 | 25.1 | 24.8 | 26.1 | 0.010   |
| J01E                  | 0.4  | 0.7  | 0.4  | 0.6  | 0.5  | 0.5  | 0.932   |
| J01F                  | 4.5  | 3.8  | 3.8  | 3.8  | 3.6  | 3.4  | 0.021   |
| J01M                  | 20.2 | 15.2 | 16.7 | 15.6 | 15.2 | 12.1 | 0.036   |
| All other J01 classes | 22.7 | 22.1 | 20.5 | 20.8 | 20.0 | 18.9 | 0.002   |
| **Total**             | 112.8| 104.5| 102.3| 101.3| 99.6 | 94.2 | 0.004   |

| **General medical wards (DDD/100 patient-days)** |      |      |      |      |      |      |         |
| J01A                  | 5.4  | 5.2  | 4.9  | 5.1  | 5.0  | 4.8  | 0.036   |
| J01C                  | 29.1 | 26.4 | 26.1 | 26.0 | 26.0 | 23.6 | 0.021   |
| J01D                  | 30.4 | 29.0 | 28.3 | 28.5 | 28.0 | 27.4 | 0.008   |
| J01E                  | 1.3  | 1.3  | 1.4  | 1.3  | 1.1  | 1.2  | 0.188   |
| J01F                  | 11.2 | 10.3 | 10.5 | 10.8 | 10.1 | 9.8  | 0.068   |
| J01M                  | 14.8 | 13.6 | 12.2 | 11.5 | 10.3 | 9.0  | <0.001  |
| All other J01 classes | 7.8  | 8.3  | 8.2  | 8.5  | 8.2  | 8.3  | 0.231   |
| **Total**             | 100.0| 94.1 | 91.7 | 91.7 | 88.7 | 84.0 | 0.002   |

| **Post-acute care hospitals (DDD/100 patient-days)** |      |      |      |      |      |      |         |
| J01A                  | 0.5  | 0.2  | 0.3  | 0.4  | 0.3  | 0.4  | 0.924   |
| J01C                  | 9.3  | 8.9  | 6.3  | 5.7  | 5.9  | 5.7  | 0.020   |
| J01D                  | 8.4  | 6.5  | 5.4  | 5.4  | 4.9  | 4.9  | 0.020   |
| J01E                  | 0.8  | 1.0  | 0.7  | 0.8  | 0.7  | 0.7  | 0.213   |
| J01F                  | 1.3  | 1.1  | 1.0  | 0.9  | 0.8  | 0.8  | 0.002   |
| J01M                  | 5.1  | 4.6  | 3.8  | 3.5  | 3.0  | 2.8  | <0.001  |
| All other J01 classes | 3.5  | 5.1  | 3.8  | 3.4  | 3.0  | 2.8  | 0.148   |
| **Total**             | 29.1 | 27.4 | 21.3 | 20.1 | 18.8 | 18.1 | 0.005   |

DDD: defined daily doses.
Results
Data were available from all HMOs for the entire period. Data from acute care hospitals were available for only a subset of hospitals in the first 2 years (15/28 in 2012 and 22/28 in 2013), but all 28 hospitals in Israel reported data thereafter. In 2017, one hospital reported data as antimicrobials consumed by patients and not as pharmacy dispensing and because of this discrepancy, data from that hospital were not included in the analysis for that year. Antimicrobial use data from the PACHs were initially not available for all institutions (6/15 reported in 2012 and 13/15 reported in 2014), but as of 2016, all 15 PACHs reported.

Between 2012 and 2017 there was a statistically significant decrease in antimicrobial use in each type of healthcare institution studied (Figure 1).

Community
In the community, the total DDD per 1,000 insured per day declined 4.4%, from 22.8 in 2012 to 21.8 in 2017 (p = 0.004) (Table and Figure 2). Most of the decrease was because of significant declines in the use of penicillins (p = 0.033) and fluoroquinolones (p = 0.003). While three of the HMOs reported similar usage data during all years studied, the fourth reported consumption rates ca 20% higher than the others in 2012. It was this fourth HMO that saw the greatest decrease in antimicrobial use, accounting for most of the overall decrease in the community. Two of the HMOs did not report any difference in consumption during the period analysed. Compared with countries reporting to ESAC-Net, the rate of antimicrobial use in the community in Israel declined from 105.1% of the European pooled-mean in 2012 to 100.0% in 2017. In 2017, the rate of antimicrobial use in the community in Israel fell within the middle third of countries reporting to ESAC-Net (Figure 3).

Figure 2
Antimicrobial use in the community by antibiotic category, Israel, 2012–2017
Among acute care hospitals, antimicrobial use decreased between 2012 and 2017 (Table and Figure 4). This decrease was significant in all three ward types. Within medical wards, the combined DDD per 100 patient days was 100.0 in 2012, declining 16.0% to 84.0 by 2017 (p = 0.002). Significant declines in antimicrobial use were seen for fluoroquinolones (14.8 to 9.0 DDD/100 patient-days, p < 0.001) and beta-lactams, specifically beta-lactam-beta-lactamase inhibitors (16.9 to 11.7 DDD/100 patient-days, p < 0.001) and cephalosporins (27.8 to 24.9 DDD/100 patient days, p = 0.012). A significant increase was observed in the consumption of chloramphenicol (approved for use in Israel), from 0.8 to 1.6 DDD per 100 patient-days (p = 0.003).

In surgical wards, the rate dropped 16.5%, from 112.8 DDD per 100 patient-days in 2012 to 94.2 in 2017 (p = 0.004). Significant declines were seen for most antimicrobial groups, with the greatest absolute decreases observed for fluoroquinolones (20.2 to 12.1 DDD/100 patient-days, p = 0.036), penicillins (20.2 to 15.9 DDD/100 patient-days, p = 0.022) and beta-lactam/beta-lactamase inhibitors (21.7 to 16.7 DDD/100 patient-days, p = 0.003). A significant increase was observed in the consumption of cephalosporins, from 20.5 to 24.1 DDD per 100 patient days (p = 0.01).

In medical/surgical ICUs, antimicrobial use dropped 11.1%, from 154.4 DDD per 100 patient days in 2012 to 137.2 in 2017 (p = 0.041). While consumption in most antimicrobial categories decreased, no decline reached statistical significance.

Discussion

Following the 2012 launch by the Israeli NCIC of a nationwide antimicrobial stewardship intervention [18], a decrease in antimicrobial use was observed in Israel in the community, in medical and surgical wards as well as medical/surgical ICUs within acute care hospitals, and in PACHs. Most of this observed decrease was attributable to declines in use of fluoroquinolones and penicillins, including beta-lactam/beta-lactamase inhibitors.

Although the decrease in antimicrobial use was seen almost universally across the healthcare spectrum in Israel, considerable variation existed between different institutions. In the community, differences were observed between HMOs, with the bulk of the overall decrease in antimicrobial use occurring in the HMO that had the greatest initial rate of consumption. In the acute care hospital setting, although significant decreases were seen in consumption rates of all ward types, inter-hospital variability was significant. There
**Figure 4**
Antimicrobial use in acute care hospitals by antimicrobial category and ward, Israel, 2012–2017

<table>
<thead>
<tr>
<th>Year</th>
<th>Medical/surgical ICUs</th>
<th>Surgical wards</th>
<th>Medical wards</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>(n = 15)</td>
<td>(n = 13)</td>
<td>(n = 15)</td>
</tr>
<tr>
<td>2013</td>
<td>(n = 21)</td>
<td>(n = 22)</td>
<td>(n = 22)</td>
</tr>
<tr>
<td>2014</td>
<td>(n = 26)</td>
<td>(n = 28)</td>
<td>(n = 28)</td>
</tr>
<tr>
<td>2015</td>
<td>(n = 26)</td>
<td>(n = 28)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>2016</td>
<td>(n = 26)</td>
<td>(n = 28)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>2017</td>
<td>(n = 25)</td>
<td>(n = 27)</td>
<td>(n = 25)</td>
</tr>
</tbody>
</table>

DDD per 100 patient-days

BLI: Beta-lactamase inhibitors; DDD: defined daily doses; ICUs: intensive care units.

The number of acute care hospital wards reporting that year is shown under the year.

**Figure 5**
Antimicrobial use in post-acute care hospitals by antimicrobial category, Israel, 2012–2017

<table>
<thead>
<tr>
<th>Year</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>2013</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>2014</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>2015</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>2016</td>
<td>(n = 15)</td>
</tr>
<tr>
<td>2017</td>
<td>(n = 15)</td>
</tr>
</tbody>
</table>

DDD per 100 patient-days

BLI: Beta-lactamase inhibitors; DDD: defined daily doses.

The number of post-acute care hospitals reporting that year is shown under the year.
were numerous wards and medical/surgical ICUs where no change was seen over time and others in which antimicrobial use increased. In contrast, a trend towards decreased antimicrobial use was nearly uniform among PACHs, with only a few reporting increased or static rates of consumption.

Israel is a country with relatively high rates of AMR, comparable to some southern Europe countries [21]. Previous nationwide antimicrobial stewardship programs that have been successful, e.g. in Sweden, France and Scotland [22-24], were implemented in countries where the baseline rates of AMR and antimicrobial use were lower than those in Israel. It is likely that as overall antimicrobial use decreases, it becomes ever more challenging to reduce it further and this may account for the relatively static rates observed in many countries with below-average antimicrobial use [1]. As noted above, the significant decreases in antimicrobial use that we observed are in contrast to a lack of change in antimicrobial use in these settings across the majority of European countries reporting to ESAC-Net within the same time period, particularly in countries with rates of consumption greater than the population-weighted mean [1]. However, the degree of reduction within Israel was uneven, with considerably greater drops in use in inpatient settings than in the community, findings that will inform future stewardship interventions.

This report has a number of limitations. As noted, our data relied on antimicrobial dispensing records. Discrepancies can arise between quantities dispensed and consumed, for example when a patient fails to complete a course of therapy or when the decision is made to change antimicrobials mid-way through treatment. In both cases, the quantity of antimicrobial consumed will be less than that dispensed. Thus, our data may overestimate rates of antimicrobial use; however, it is not anticipated that this problem affects the analysis of temporal trends. As data reported to ESAC-Net may suffer from a similar issue given that rates of antimicrobial use are based on national sales or reimbursement data [25], the issue should also not affect the comparison with European data. A second limitation is the lack of a comparator for our data from acute care hospitals and PACHs because of the use of a different denominator. Data on antimicrobial use in hospitals are reported in ESAC-Net as DDD per 1,000 inhabitants per day rather than per 100 patient-days. Data from the Global Point Prevalence Survey reflect only the percentage of patients receiving antimicrobials on the survey date [26]. The decision to gather ward-level rather than hospital-level data in acute care hospitals in Israel was pragmatic, permitting timely feedback to units where changes in antimicrobial use can be relatively easily implemented. Furthermore, it enabled the identification of departments whose antimicrobial use deviates significantly from the mean, assuring antimicrobial stewardship efforts.

In conclusion, following the introduction of a nationwide antimicrobial stewardship intervention, there was an observed decrease in the rates of antimicrobial use in all types of studied healthcare institutions in Israel between the years 2012 and 2017.

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We thank all of the healthcare institutions and personnel who contributed to the antimicrobial use data summarised in this report.

Conflict of interest

None declared.

Authors’ contributions

YD: Performed analyses and wrote the report.
ET: Data collection and performed analyses.
DBD: Data collection.
YC: Conceived the report and data collection.
MJS: Conceived the report and data collection.

All authors approved the final version to be published.

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