Interim estimates of 2016/17 vaccine effectiveness against influenza A(H3N2), Canada, January 2017

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Using a test-negative design, the Canadian Sentinel Practitioner Surveillance Network (SPSN) assessed interim 2016/17 influenza vaccine effectiveness (VE) against dominant influenza A(H3N2) viruses considered antigenically matched to the clade 3C.2a vaccine strain. Sequence analysis revealed substantial heterogeneity in emerging 3C.2a1 variants by province and over time. Adjusted VE was 42% (95% confidence interval: 18–59%) overall, with variation by province. Interim virological and VE findings reported here warrant further investigation to inform potential vaccine reformulation.

The 2016/17 season in Canada has been characterised by dominant influenza A(H3N2) activity, increasing since late November 2016 but with regional variation in timing and intensity from west to east [1]. We assessed interim 2016/17 vaccine effectiveness (VE) against influenza A(H3N2) viruses collected through the Canadian Sentinel Practitioner Surveillance Network (SPSN). Detailed genetic characterisation of sentinel viruses was undertaken to assess the contribution of emerging clade 3C.2a1 variants and their potential impact on protection conferred by the clade 3C.2a vaccine, specifically the A/Hong Kong/4801/2014(H3N2)-like component.

Virological and vaccine effectiveness evaluation

As previously described [2,3], nasal/nasopharyngeal specimens collected from patients aged 1 year and older presenting within 7 days of influenza-like illness (ILI) onset to community-based sentinel practitioners in four provinces (Alberta, British Columbia, Ontario and Quebec) were included in the interim analysis. Epidemiological information was collected at the time of specimen collection using a standard questionnaire. Ethics review boards in each province approved the study.

Specimens collected between 1 November 2016 (week 44) and 21 January 2017 (week 3) were included in primary VE analysis, corresponding to the period during which influenza test positivity consistently exceeded 10% (Figure 1).

Influenza virus testing and influenza A subtyping were conducted using real-time RT-PCR assays validated for use at provincial reference laboratories, including in-house assays in Alberta [4] and British Columbia [5] and commercial assays in Ontario [6] and Quebec [7]. Sequencing of the haemagglutinin (HA) gene was attempted directly on all influenza A(H3N2)-positive patient specimens contributing to VE analysis that had sufficient viral load and that were available up to 21 January 2017 in order to determine clade designation and to identify mutations in established antigenic sites labelled A–E for H3N2 viruses [8,9].

VE was derived using a test-negative design [2,3]. Patients testing positive for influenza A(H3N2) were considered cases; those testing negative were considered controls. Patients who self-reported receiving at least one dose of influenza vaccine at least 2 weeks before ILI onset were considered vaccinated; those vaccinated less than 2 weeks before onset or who had

Influenza detections by type/subtype/clade and week of specimen collection, Canadian Sentinel Practitioner Surveillance Network, 2 October 2016–21 January 2017 (n = 1,096)[a]
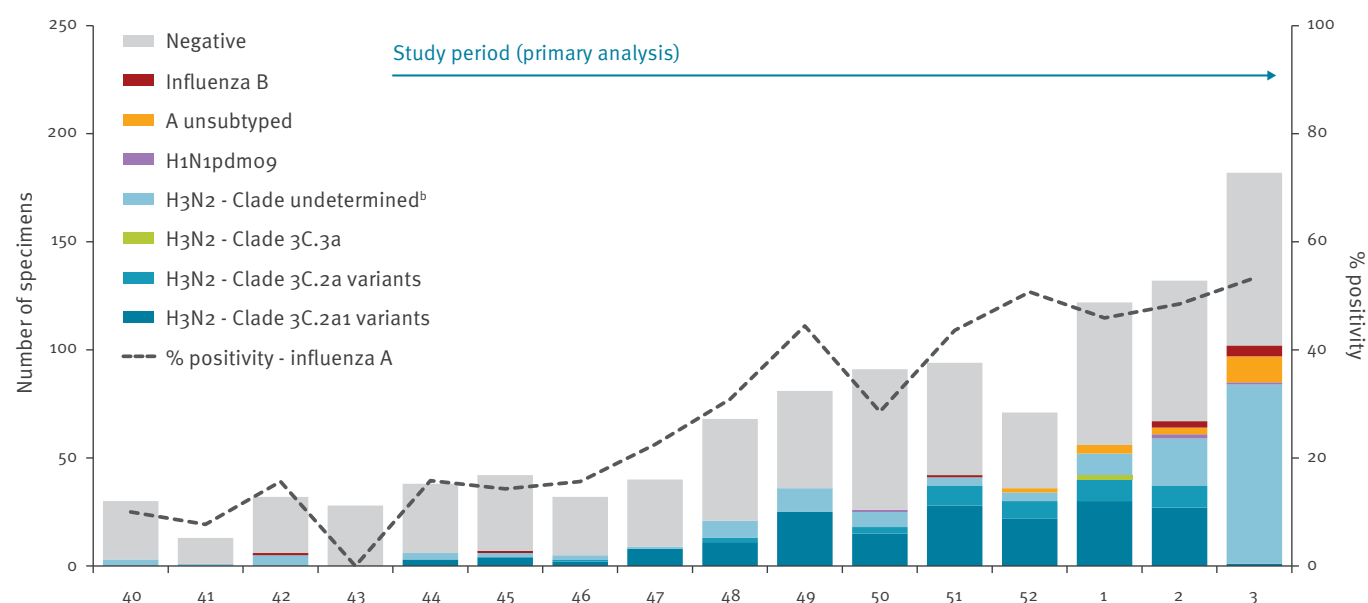
[a] Specimens were included if the patient: (i) met the influenza-like illness case definition (requiring fever and cough and at least one or more of sore throat, arthralgia, myalgia or prostration; fever was not required in elderly patients ≥ 65 years-old), (ii) had specimen collection ≤ 7 days after symptom onset, (iii) was ≥ 1-year-old at the time of onset, and (iv) had valid laboratory RT-PCR results. Specimens are displayed in the epidemic curve regardless of the patient's vaccination status or timing of vaccination. Missing specimen collection dates were imputed as the date the specimen was received and processed at the provincial laboratory minus two days, the average time between specimen collection date and laboratory received date among specimens with complete information for both values.

[b] Specimens with undetermined clade included those where sequencing was attempted but failed (n = 42) or sequencing was not attempted, e.g. those with insufficient viral load (n = 8), submitted after the start of the mid-season analysis on 21 January 2017 (n = 99), or excluded from primary vaccine effectiveness (VE) analysis (n = 23).

unknown vaccination status or timing were excluded. Patients who did not meet the ILI case definition, those with specimen collection more than 7 days since ILI onset or ILI onset date unknown and those with indeterminate RT-PCR results were also excluded. Odds ratios (OR) were estimated using a logistic regression model, adjusted for age group, province, time from onset to specimen collection and specimen collection date (grouped into 2-week intervals). VE was derived as (1−OR) × 100%, comparing influenza A(H3N2) test positivity between vaccinated and unvaccinated participants.

Virological and vaccine effectiveness findings

A total of 932 specimens met study inclusion criteria. Influenza viruses were detected in 396 (42%) specimens, including 387 (98%) influenza A and nine (2%) influenza B. Of the 374 (97%) influenza A viruses with available subtype information, almost all (n = 370; 99%) were A(H3N2); four A(H1N1)pdm09 viruses were detected. VE analyses are presented for A(H3N2) only, including 370 test-positive cases and 536 test-negative controls (n = 906 overall). Working-age adults 20−64-years-old comprised the majority (57%) of the study sample (Table 1).

Overall 24% of cases and 30% of controls were considered vaccinated (p=0.04), corresponding to an unadjusted VE of 27% (95% confidence interval (CI): 1−46) against medically attended influenza A(H3N2) illness (Table 2). After adjustment for relevant covariates, VE was 42% (95% CI: 18−59).

Genetic clade information was available for 221 of 263 (84%) influenza A(H3N2) sentinel specimens for which sequencing was attempted. The majority of viruses (176/221; 80%) clustered with the newly emerging clade 3C.2a1, defined by N171K +/− N121K mutations in site D, with most (165/176; 94%) having between one and three additional antigenic site mutations (Table 3). Other clade 3C.2a variants, each with two or three antigenic site mutations, comprised 43 (19%) sequenced influenza A(H3N2) specimens.

Considerable genetic heterogeneity was also observed among dominant but emerging clade 3C.2a1 variants by province and time (Figure 2).

In exploratory analyses, VE was highest and significantly protective in Alberta where an earlier epidemic start included a more limited range of clade 3C.2a1 variants dominated by N121K + R142G + I242V mutations

Distribution of clade 3C.2a1 variants by province[a] and week of specimen collection, Canadian Sentinel Practitioner Surveillance Network (SPSN), 1 November 2016–16 January 2017 (n = 176)
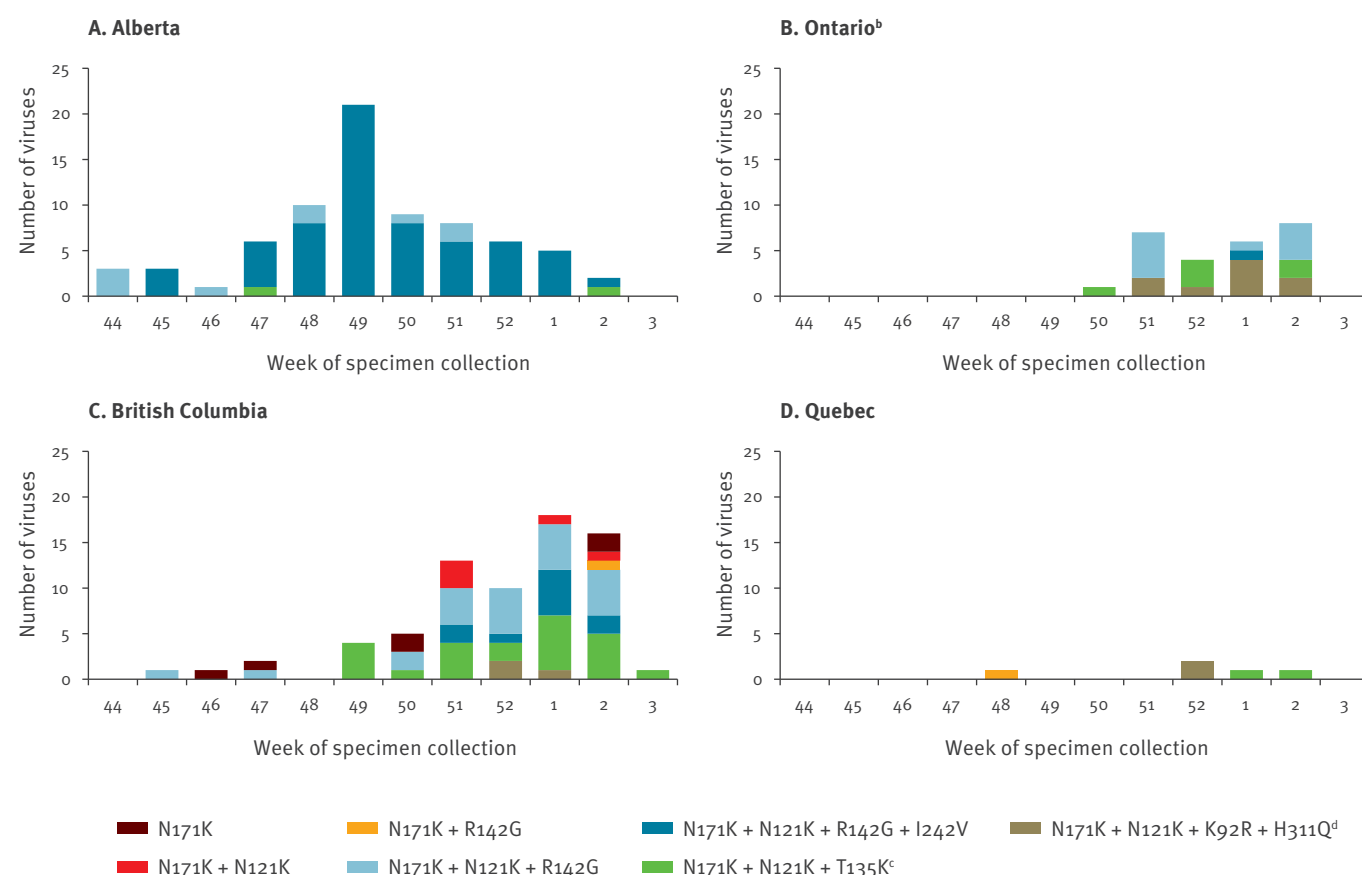
a Alberta and British Columbia are adjacent provinces located in western Canada; Ontario and Quebec are adjacent provinces located in central Canada, >2,500 km away from Alberta and British Columbia.

b Due to logistical issues, specimen collection did not begin until week 48 in Ontario.

c +/−R142G or T167S or I242M mutations.

d +/−Q197R mutation.

(Figure 2, Table 2). Conversely, in the adjacent westernmost province of British Columbia and also further east in the provinces of Ontario and Quebec in central Canada, delayed epidemic activity was associated with lower VE and greater diversity in circulating clade 3C.2a1 variants, although confidence intervals overlapped for all four provinces.

Discussion
Whereas the 2015/16 season was mild overall with late-season circulation of influenza A(H1N1)pdm09 viruses, the current 2016/17 season has been characterised to date by dominant influenza A(H3N2) activity, more comparable to the 2014/15 or 2012/13 seasons [1,10-12]. In the 2016/17 interim VE analysis reported here, we found overall vaccine protection of 42% (95% CI: 18−59) against medically-attended A(H3N2) illness, with variation by province that may reflect genetic

heterogeneity in circulating A(H3N2) variants. This overall estimate is consistent with a recent meta-analysis of global studies based on the test-negative design that reported a pooled VE, including both interim and end-of-season estimates, of 33% (95% CI: 26−39) against seasonal A(H3N2) viruses [13]. Early VE estimates for the 2016/17 season available from Finland and Sweden found significant protection of 20−30% against laboratory-confirmed influenza in adults 65 years and older [14]; however, methodological details and influenza virus characterisations are not available for these estimates, limiting their interpretation.

Although still suboptimal given the substantial disease burden associated with influenza A(H3N2) seasons [15,16], our mid-season VE estimate for 2016/17 is considerably higher than the last A(H3N2)-dominated season in 2014/15 during which no vaccine protection was

TABLE 1

Participant characteristics, interim vaccine effectiveness evaluation, Canadian Sentinel Practitioner Surveillance Network, 1 November 2016–21 January 2017 (n = 906)

Characteristic	Overall % (column)		Distribution by case status % (column)					Vaccinated % (row)						
			H3N2 cases		Negative controls		p value[a]	Overall		p value[a]	H3N2 cases		Negative controls	
	n	%	n	%	n	%		n	%		n	%	n	%
n % (row)	906	100	370	41	536	59	NA	246	27	NA	87	24	159	30
Age group (years)														
1–8	137	15	51	14	86	16		24	18		8	16	16	19
9–19	133	15	66	18	67	13		18	14		8	12	10	15
20–49	359	40	141	38	218	41		74	21		26	18	48	22
50–64	155	17	59	16	96	18	0.19	54	35	< 0.01	17	29	37	39
≥65	122	13	53	14	69	13		76	62		28	53	48	70
Median (range)	34 (1–97)		34 (1–91)		35 (1–97)		0.99	52.5 (1–97)		< 0.01	50 (1–90)		53 (1–97)	
Sex														
Female	524	58	205	56	319	60		154	29		44	21	110	34
Male	378	42	164	44	214	40	0.20	92	24	0.09	43	26	49	23
Unknown	4	NA	1	NA	3	NA	NA	0	NA	NA	0	NA	0	NA
Co-morbidity[b]														
No	664	80	270	81	394	79		147	22		49	18	98	25
Yes	166	20	63	19	103	21	0.52	77	46	< 0.01	28	44	49	48
Unknown	76	NA	37	NA	39	NA	NA	22	NA	NA	10	NA	12	NA
Province														
Alberta	278	31	110	30	168	31		71	26		20	18	51	30
British Columbia	327	36	134	36	193	36		92	28		37	28	55	29
Ontario	179	20	87	24	92	17	0.03	64	36	< 0.01	25	29	39	42
Quebec	122	13	39	11	83	15		19	16		5	13	14	17
Specimen collection interval from ILI onset (days)[c]														
≤4	687	76	316	85	371	69		174	25		70	22	104	28
5–7	219	24	54	15	165	31	< 0.01	72	33	0.03	17	31	55	33
Median (range)	3 (0–7)		3 (0–7)		3 (0–7)		< 0.01	3 (0–7)		0.03	3 (0–7)		3 (0–7)	
Specimen collection date (2-week interval)														
Weeks 44–45	64	7	10	3	54	10		4	6		0	0	4	7
Weeks 46–47	61	7	13	4	48	9		12	20		3	23	9	19
Weeks 48–49	139	15	54	15	85	16		31	22		12	22	19	22
Weeks 50–51	174	19	65	18	109	20		51	29		11	17	40	37
Weeks 52–1	184	20	86	23	98	18	< 0.01	58	32	< 0.01	24	28	34	35
Weeks 2–3	284	31	142	38	142	26		90	32		37	26	53	37

ILI: influenza-like illness; NA: not applicable.

[a] Differences between cases and controls and vaccinated and unvaccinated participants were compared using the chi-squared test or Wilcoxon rank-sum test.

[b] Includes chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization (NACI), including: heart, pulmonary (including asthma), renal, metabolic (such as diabetes), blood, cancer, or immunocompromising conditions, conditions that compromise management of respiratory secretions and increase risk of aspiration, or morbid obesity (body mass index ≥ 40).

[c] Missing specimen collection dates were imputed as the date the specimen was received and processed at the provincial laboratory minus two days, the average time between specimen collection date and laboratory received date among specimens with complete information for both values. Specimen collection interval was derived based on the number of days between ILI onset and the specified or imputed specimen collection date.

found [2,3]. In 2014/15, with unchanged vaccine components from the prior 2013/14 season and substantial antigenic drift in circulating viruses, negative interference from the prior season's vaccination may have contributed to the historically low VE observed [3,17]. While more than 80% of vaccinated participants in 2016/17 were also vaccinated in the prior 2015/16 season (data

not shown), higher VE than in 2014/15 was anticipated. This expectancy was in part based on the change in vaccine component from the prior 2015/16 season's A/Switzerland/9715293/2013(H3N2)-like (clade 3C.3a) virus to the A/Hong Kong/4801/2014(H3N2)-like (clade 3C.2a) vaccine strain [18]. The latter is also considered a better antigenic match to circulating viruses than was

TABLE 2

Interim vaccine effectiveness estimates for influenza A(H3N2), Canadian Sentinel Practitioner Surveillance Network, 1 November 2016–21 January 2017 (n = 906)

Model	n total	Cases		Controls		VE % (95% CI)
		n	% vaccinated	n	% vaccinated	
Primary analysis[a]						
Unadjusted	906	370	24	536	30	27 (1 to 46)
Individual covariate adjustment						
Age group (1–8, 9–19, 20–49, 50–64,≥65 years)						30 (4 to 50)
Province[b]						32 (7 to 50)
Specimen collection interval from ILI onset (≤4, 5–7 days)						23 (−5 to 44)
Specimen collection date (2-week interval)						38 (15 to 55)
Full covariate adjustment						
Adjusted						42 (18 to 59)
Restricted by province[c]						
Alberta						
Unadjusted	278	110	18	168	30	49 (8 to 72)
Adjusted						62 (26 to 80)
British Columbia						
Unadjusted	327	134	28	193	29	4 (−56 to 41)
Adjusted						28 (−30 to 60)
Ontario[d]						
Unadjusted	179	87	29	92	42	45 (−2 to 71)
Adjusted						27 (−60 to 66)
Quebec						
Unadjusted	122	39	13	83	17	28 (−118 to 76)
Adjusted						NE
All provinces excluding Alberta						
Unadjusted	628	260	26	368	29	16 (−19 to 42)
Adjusted[e]						34 (−1 to 57)

CI: confidence interval; ILI: influenza-like illness; NE: not estimated (insufficient sample size); VE: vaccine effectiveness.

[a] Analysis adjusted for age group, province, specimen collection interval from ILI onset and specimen collection date (2-week interval).

[b] Alberta, British Columbia, Ontario, Quebec.

[c] Analysis adjusted for age group, specimen collection interval and specimen collection date (2-week interval).

[d] Due to logistical issues, specimen collection for the 2016/17 season began late in Ontario. The study period for Ontario-specific VE analysis was defined as 12 December 2016 (week 50) to 21 January 2017 (week 3).

[e] Analysis adjusted for age group, province (British Columbia, Ontario, Quebec), specimen collection interval and specimen collection date (2-week interval).

the case in 2014/15 [18,19]. Specific evaluation of this hypothesis related to less pronounced effects of repeat vaccination for 2016/17 awaits end-of-season analyses.

Circulating influenza A(H3N2) viruses in Canada and elsewhere this season have continued to evolve, with an increasing proportion since June 2015 clustering with the newly emerging clade 3C.2a1 that is distinguished by the HA1 substitution N171K, often combined with N121K, both in antigenic site D [20,21]. These clade 3C.2a1 variants are considered antigenically similar to the egg-adapted clade 3C.2a vaccine strain based on haemagglutination inhibition (HI) assay [1,19]. However, recent A(H3N2) viruses continue to be difficult to characterise antigenically by HI assay [20]. A potential glycosylation motif present at positions 158–160 in all clade 3C.2a and descendant viruses has resulted in variable agglutination of erythrocytes; loss or partial loss of this glycosylation motif during cell-culture passage may enable HI characterisation of a subset of clade 3C.2a viruses but also limit the generalisability of antigenicity findings on that basis [20,22].

In sequencing analysis, we identified considerable diversity among circulating influenza A(H3N2) strains, including a mix of genetic variants that differed geographically and with time. The majority (80%) of A(H3N2) viruses included in our VE analysis belonged to the newly emerging clade 3C.2a1, but with continuing genetic evolution compared with the vaccine strain. Almost all (95%) 3C.2a1 viruses had both the N171K and N121K mutations in site D that distinguish this clade. About two-thirds had acquired an additional R142G (site A) mutation, also present in all clade 3C.3 viruses

TABLE 3

Clade distribution and antigenic site mutations for influenza A(H3N2) viruses contributing to interim vaccine effectiveness evaluation, Canadian Sentinel Practitioner Surveillance Network, 1 November 2016–16 January 2017 (n = 221)[a]

Clade	Clade-defining amino acid substitutions (antigenic site)[b,c]	Distribution by province, % (column)									
		Alberta (n = 81)		BC (n = 81)		Ontario (n = 48)		Quebec (n = 11)		Total (n = 221)	
		n	%	n	%	n	%	n	%	n	%
Clade 3C.2a	N145S (A)+N144S (A) (−CHO)+F159Y (B)+K160T (B) (+CHO)+N225D (RBS)+Q311H (C)	0	0	0	0	0	0	0	0	0	0
Clade 3C.2a variants	Clade 3C.2a+Q197K (B)+R261Q (E)	0	0	1	1	0	0	0	0	1	0
	Clade 3C.2a+T131K (A)+R142K (A)+R261Q (E)	6	7	3	4	21	44	2	18	32	14
	Clade 3C.2a+N121K (D)+S144K (A) +/−S219Y (D)	1	1	6	7	1	2	2	18	10	5
3C.2a subtotal		7	9	10	12	22	46	4	36	43	19
Clade 3C.2a1	Clade 3C.2a+N171K (D)	0	0	6	7	0	0	0	0	6	3
Clade 3C.2a1 variants	Clade 3C.2a+N171K (D)+N121K (D)	0	0	5	6	0	0	0	0	5	2
	Clade 3C.2a+N171K (D)+R142G (A)	0	0	1	1	0	0	1	9	2	1
	Clade 3C.2a+N171K (D)+N121K (D)+R142G (A)	9	11	23	28	10	21	0	0	42	19
	Clade 3C.2a+N171K (D)+N121K (D)+R142G (A)+I242V (D)	63	78	10	12	1	2	0	0	74	33
	Clade 3C.2a+N171K (D)+N121K (D)+T135K (A) (−CHO) +/−R142G (A) or T167S (D) or I242M (D)	2	2	23	28	6	13	2	18	33	15
	Clade 3C.2a+N171K (D)+N121K (D)+K92R (E)+H311Q (C) +/−Q197R (B)	0	0	3	4	9	19	2	18	14	6
3C.2a1 subtotal		74	91	71	88	26	54	5	45	176	80
Clade 3C.3a	T128A (B) (−CHO)+R142G (A)+N145S (A)+A138S (A)+F159S (B)+N225D (RBS)	0	0	0	0	0	0	2	18	2	1

BC: British Columbia; CHO: carbon-hydrogen-oxygen (glycosylation motif); RBS: receptor binding site.

[a] Sequencing was attempted on all influenza A(H3N2) sentinel specimens contributing to VE analysis that had sufficient viral load and that were available up to 21 January 2017, with the last included collection date 16 January 2017. Genetic clade information was available for 221 of 263 (84%) viruses for which sequencing was attempted. Sequencing was not attempted on influenza A(H3N2) specimens with insufficient viral load (i.e. high CT value in the RT-PCR assay; n=8) or those submitted after 21 January 2017 (n=99).

[b] Letters A through E refer to established antigenic sites in influenza A(H3N2) viruses [8,9]. RBS refers to the receptor binding site. Substitutions indicated with −CHO refer to mutations resulting in the loss of a potential glycosylation site; those indicated with +CHO refer to mutations resulting in the gain of a potential glycosylation site.

[c] Additional substitutions in the egg-adapted high-growth reassortant vaccine strain are not considered here.

and the majority of clade 3C.2a variants detected in this study, with or without an I242V mutation (site D). The clinical implications of accumulated antigenic site D mutations, representing a shift away from the heavily glycosylated but immunodominant sites A and B, requires further investigation [8,23]. Another 3C.2a1 variant, detected more frequently in the later study period but comprising only 15% of study viruses overall, had an additional T135K mutation in site A. T135K is associated with loss of a potential glycosylation site at positions 133–135 that has otherwise been present in all descendant A(H3N2) viruses since A/Sydney/5/1997 [24]. Changes in glycosylation motifs may be relevant to antigenicity, viral fitness and/or pathogenicity [24-26]. The ecological correlation between greater genetic diversity and lower VE by geographic region warrants further investigation in other countries, as well as end-of-season analyses.

Limitations of this analysis include the observational study design for which residual bias and confounding cannot be ruled out, and the small sample size resulting in wide confidence intervals, particularly in subgroup analyses. Although interim estimates are generally considered a reliable predictor of final estimates, this reliability depends in part upon the stage of the epidemic and virus evolution, and contributing virological and participant profiles, at the time of the mid- and end-of-season analyses [27]. Of particular note, Alberta had an earlier start to the influenza season and findings may not reflect the full diversity or distribution of evolved variants or VE estimates for the remainder of the season. Given the high specificity of RT-PCR assays for influenza viruses, differences in diagnostic test characteristics between provinces are unlikely to have influenced VE findings [28]. VE estimates are subject to change and are provided here only for influenza A(H3N2); if feasible, VE against other types/subtypes, as well as clade-specific VE, will be explored and compared with findings from other settings in end-of-season analyses.

Conclusion

We report interim VE of ca 40% for the 2016/17 influenza A(H3N2) epidemic in Canada, which is higher than in 2014/15 and consistent with expected but suboptimal VE estimates for influenza A(H3N2) more generally. Given that a substantial proportion of vaccinated people

may remain unprotected against influenza A(H3N2) illness, other adjunct measures should be considered to minimise associated morbidity and mortality, particularly among high-risk individuals. Continued evolution in circulating 3C.2a variants and their derivatives, and the impact on vaccine protection, warrants ongoing monitoring to inform potential vaccine reformulation.

GenBank Accession Numbers

KY583507 – KY583727.

Conflict of interest

Within 36 months of manuscript submission, GDS has received grants unrelated to influenza from GSK and Pfizer and travel reimbursement to attend an ad hoc advisory board meeting of GSK also unrelated to influenza; he has provided paid expert testimony in a grievance against a vaccinate-or-mask healthcare worker influenza vaccination policy for the Ontario Nurse Association. JBG has received research grants from GlaxoSmithKline Inc. and Hoffman-La Roche Ltd to study antiviral resistance in influenza, and from Pfizer Inc. to conduct microbiological surveillance of Streptococcus pneumoniae. MK has received research grants from Roche, Merck, Siemens, Hologic, and Boerhinger Ingelheim for unrelated studies. Other authors have no conflicts of interest to declare.

Authors' contributions

Principal investigators (epidemiological): DMS (National and British Columbia); JAD (Alberta); ALW (Ontario); and GDS (Québec). Principal investigator (laboratory): AJ and MK (British Columbia); SD (Alberta); JBG (Ontario); HC (Québec); and NB and YL (National Microbiology Laboratory). Virus sequencing: SS. Data analysis: CC and DMS (epidemiological); RB (statistical support); SS (molecular). Preparation of first draft: CC and DMS. Draft revision and approval: all.

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Trends in carbapenemase-producing Enterobacteriaceae, France, 2012 to 2014

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In 2014, a total of 2,976 Enterobacteriaceae isolates with decreased susceptibility to carbapenems were received at the French Associated National Reference Center for Antibiotic Resistance (NRC) and were characterised for their molecular resistance mechanism to carbapenems and compared with results obtained during 2012 and 2013.The overall number of enterobacterial isolates with decreased susceptibility to carbapenems received at the NRC rapidly increased (more than twofold in two years) with a growing proportion of carbapenemase producers (23.1% in 2012 vs 28.6% in 2013 vs 36.2% in 2014). Between 2012 and 2014, the main carbapenemase type was OXA-48, with an increase in OXA-48 variants (mostly OXA-181) and NDM producers, whereas the number KPC producers decreased. We identified a potential spread of OXA-181 producers in the tropical region of Africa. Finally, OXA-48 and OXA-48-related enzymes remained the predominant carbapenemases in France. The number of carbapenemase-producing *Escherischia coli* isolates was multiplied by fivefold between 2012 and 2014, suggesting a possible dissemination in the community.

Introduction

During the last decade, Gram-negative isolates, in particular Enterobacteriaceae, with a decreased susceptibility to carbapenems have been increasingly reported in Europe [1,2]. In Enterobacteriaceae, decreased susceptibility to carbapenems may be due to (i) a beta-lactamase with significant hydrolytic activity towards carbapenems, i.e. a carbapenemase, or (ii) a combination of overexpression of beta-lactamases possessing a weak carbapenemase activity towards carbapenems, i.e. extended spectrum beta-lactamase and/or cephalosporinase, with a decreased outer-membrane permeability or efflux overexpression [1]. The most clinically-relevant carbapenemases encountered in Enterobacteriaceae belong to either Ambler class A (mostly KPC-type) [3], or Ambler class B (metallo-beta-lactamases (MBLs)) such as IMP-, VIM- and NDM-types) [1,4] or Ambler Class D (OXA-48-like enzymes) [5].

According to the results of the European Survey on Carbapenemase-producing Enterobacteriaceae (EUSCAPE) survey [6], four European countries (Greece, Italy, Malta, Turkey) are facing a situation where carbapenemase-producing Enterobacteriaceae (CPE) are endemic. However, endemicity is associated with different types of carbapenemases in different countries: in Greece VIM and KPC, in Italy KPC and in Malta and Turkey OXA-48. Although most European countries have reported an increase in the spread of CPE, once again, strong geographical differences exist in terms of the carbapenemase types involved. KPC producing Gram-negative bacteria were mostly reported in Italy and Greece. OXA-48 producers were more widespread in some western European countries (Belgium, France, Spain) and in Romania and Turkey in the eastern part of the continent. VIM producers were endemic in Greece and interregional spread has been described in Italy, Spain and Hungary. Finally, NDM-producing Enterobacteriaceae were found to be more prevalent in central and eastern Europe (e.g. Poland, Romania). A precise identification of carbapenemase production and type is important for (i) the follow up of the spread of carbapenemase producers (ii) the timely identification of outbreaks and their prevention and (iii) the choice of treatment with novel drugs such as ceftazidime/avibactam active against producers of Ambler class A and D but not on class B carbapenemases [7].
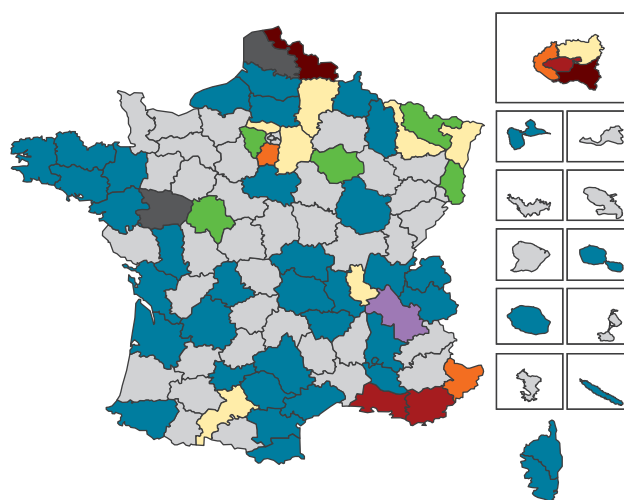
FIGURE 1

Geographic distribution of A. Isolates received at the French Associated National Reference Center for Antibiotic Resistance B. Carbapenemase-producing Enterobacteriaceae C. OXA-48-like-, NDM-, VIM-, KPC-, IMP- and IMI-producers, France, 2012–2014
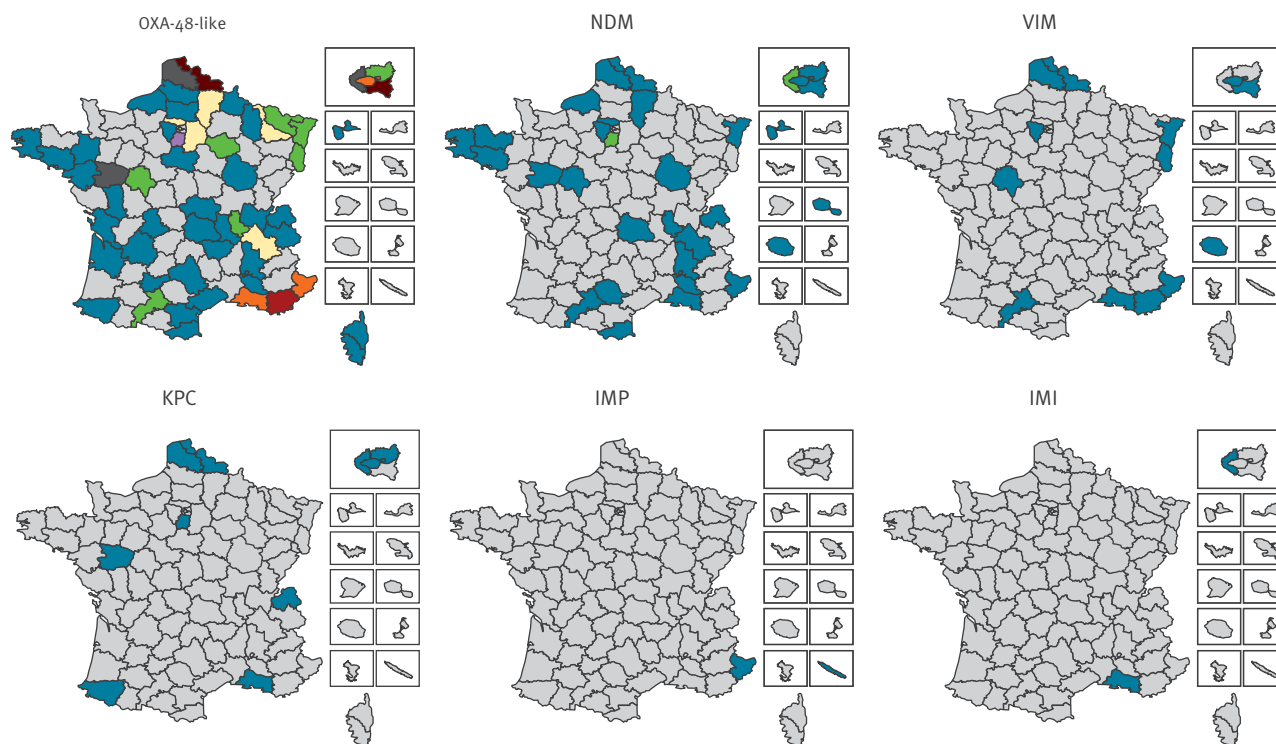
A. Isolates received at the French Associated National Reference Center
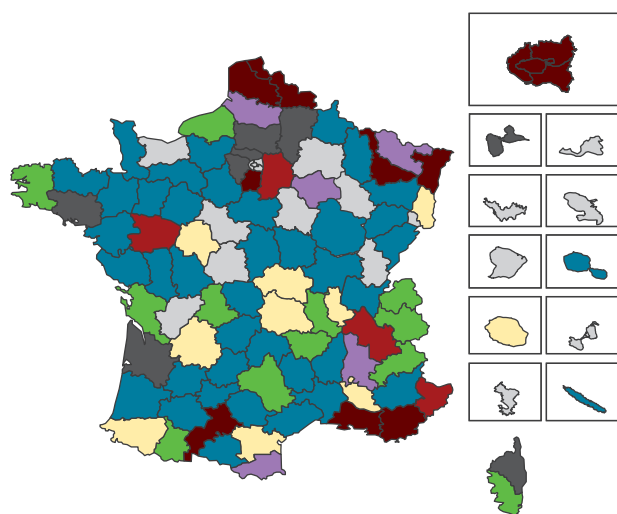
B. Carbapenemase-producing Enterobacteriaceae

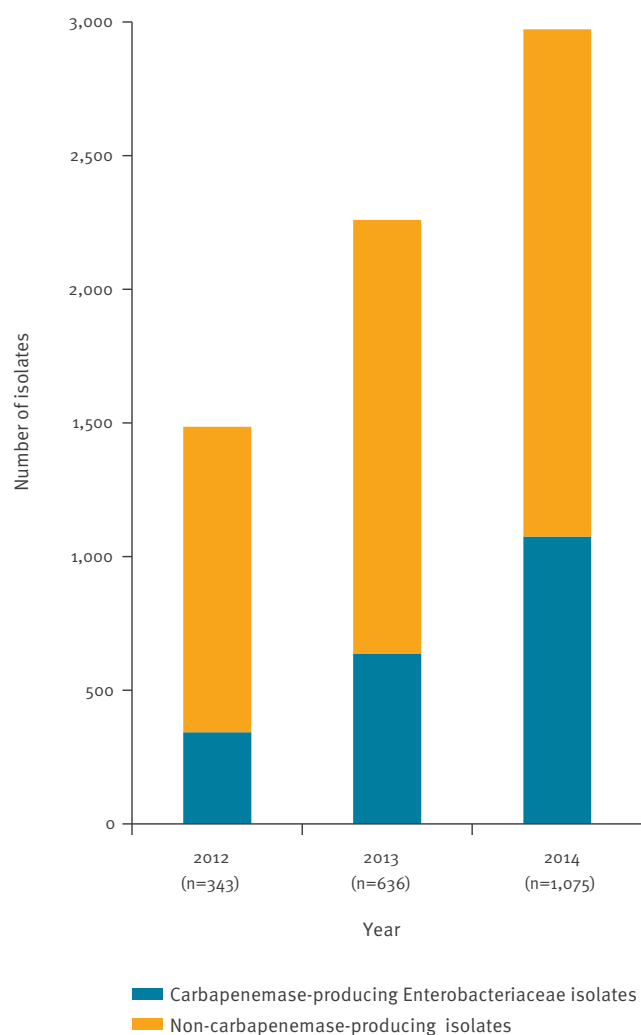**C. OXA-48-like-, NDM-, VIM-, KPC-, IMP- and IMI-producers**

OXA-48-like

NDM

VIM

KPC

IMP

IMI

Number of isolates

0   1–9   10–19   20–29   30–39   40–49   50–59   60–69   ≥ 70

The number of carbapenemase-producing Enterobacteriaceae isolates identified is indicated below each year on the x-axis.

Here, we assessed the epidemiology of Enterobacteriaceae with decreased susceptibility to carbapenems in France and analysed its evolution between 2012 and 2014.

Methods

Specimen collection

From January 2012 to December 2014, 6,682 enterobacterial isolates (1,485 in 2012; 2,225 in 2013 and 2,972 in 2014) were received and tested for carbapenem activity at the French Associated National Reference Center for Antibiotic Resistance (NRC) in Le Kremlin-Bicêtre. Isolates were submitted from the whole of France, including French overseas territories. They were recovered from both clinical and screening specimens, and sent on a voluntary basis by any type of laboratory
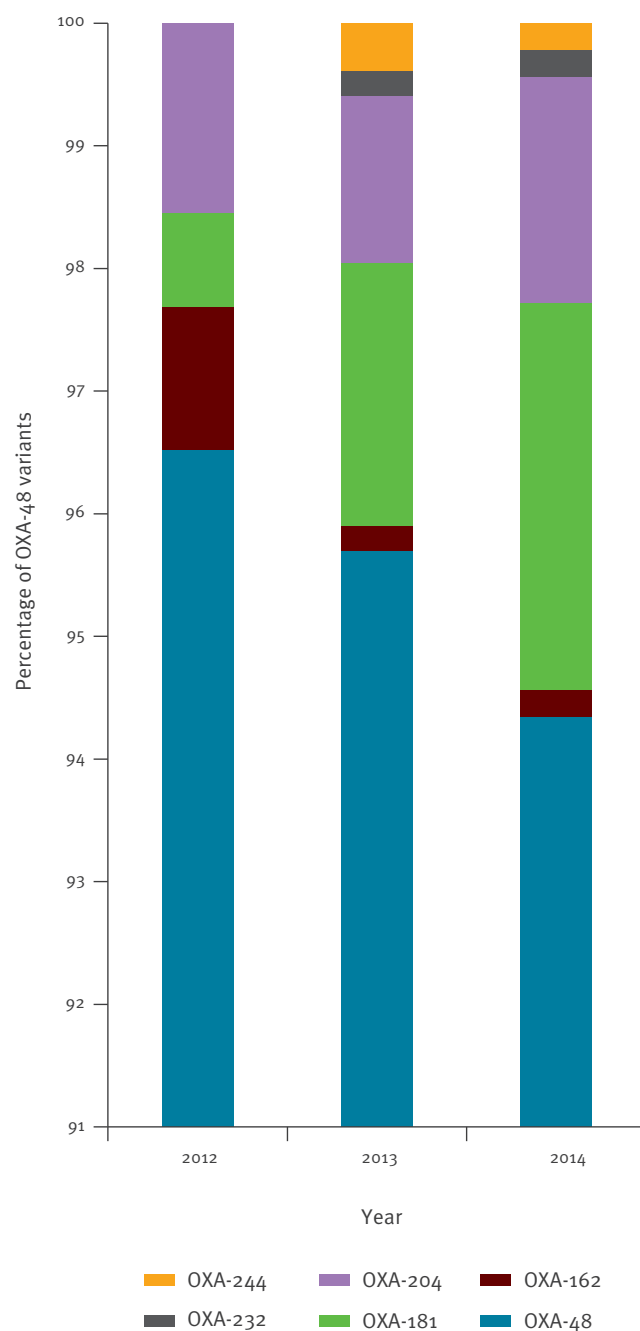
(n = 486) related to any health facility such as private and public hospitals, nursing homes, and community laboratories (Figure 1A).

Isolates with reduced susceptibility to carbapenems (ertapenem, meropenem, or imipenem) according to the Antibiogram Committee of the French Society of Microbiology (CA-SFM) (i.e. inhibition diameter < 22 mm, < 22 mm and < 25 mm for meropenem, imipenem or ertapenem respectively by disc diffusion) [8,9] were investigated for carbapenemase activity.

Known geographic origin of possible acquisition of infections and/or colonisations with carbapenemase-producing Enterobacteriaceae, France, 2014 (n=140)
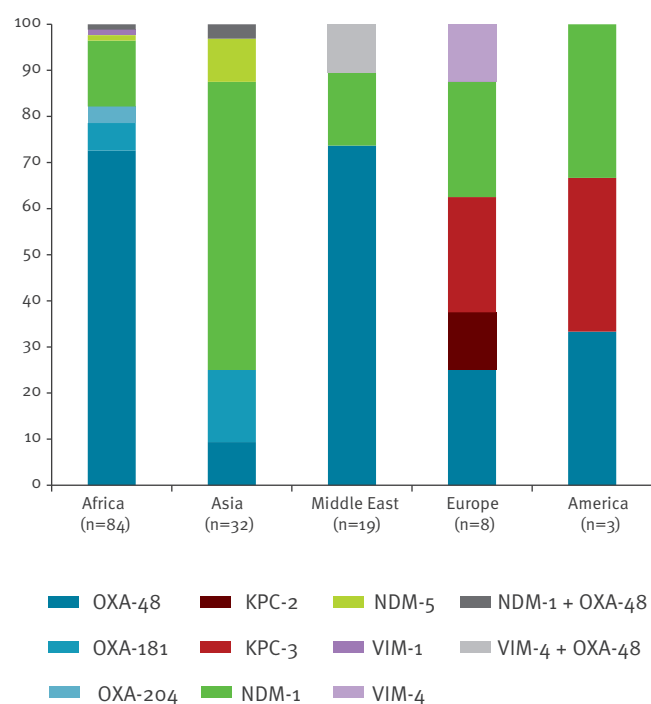
Only patients for whom travel to a foreign country was known, were included.

With each strain, provision of critical information was compulsory, such as the origin of specimens (screening rectal sample or any type of clinical sample), date of isolation, information regarding patient's travel abroad in the year preceding the strain isolation (if yes, the country was recorded), the type of laboratory (hospital, community laboratory).

Duplicated isolates from the same patient were excluded from the study. If different species or different carbapenemase types were recovered from the same patient, the corresponding isolates were taken into consideration individually. Isolates were also re-identified at the NRC using a MALDI-TOF spectrometric technique (Maldi-Biotyper, Bruker Daltonique SA, Wissembourg, France). Most of them were *Klebsiella pneumoniae* (36%), *Enterobacter cloacae* (33.3%) and *Escherichia coli* (15%).

Carbapenemase detection and molecular identification

The carbapenemase production was detected using the biochemical-based technique, the Carba NP test, as previously described [10]. Carbapenemase gene screening was performed by PCR aimed at identifying the *bla*KPC, *bla*NDM, *bla*VIM, *bla*IMP, *bla*IMI and *bla*OXA-48-like genes [11]. In case of positive PCR, sequencing of the full-length gene was performed. A decreased susceptibility to carbapenems due to (i) an outer-membrane permeability defect, (ii) an overexpression of a cephalosporinase (chromosome-encoded or plasmid-acquired) associated with outer-membrane permeability defect, (iii) an extended spectrum beta-lactamase (ESBL) production associated with outer-membrane permeability defect or (iv) association of an ESBL and overexpression of a cephalosporinase outer-membrane permeability defect were suspected when the Carba NP test and PCR screening results were negative [12].

Results

Epidemiology of carbapenemase-producing Enterobacteriaceae

According to EUCAST breakpoints [13], most of the isolates (99.6%, n = 6,655) received at the NRC were non-susceptible to at least one of the three carbapenems tested (imipenem, meropenem, ertapenem). The number of enterobacterial isolates with decreased susceptibility to carbapenems received at the NRC increased from 1,485 in 2012 to 2,225 in 2013 and 2,972 in 2014. The percentage of CPE among the Enterobacteriaceae with decreased susceptibility to carbapenems rose from 23.1% in 2012 to 28.6% in 2013 and 36.2% in 2014 (Figure 2) [12].

In 2014, carbapenemases were OXA-48- (85.6%), NDM- (8.5%), VIM- (2.7%), KPC- (1.8%), and IMI-like enzymes (0.3%) (Table 1).

From 2012 to 2014, carbapenemase producers were recovered from patients hospitalised and/or living in three main regions: the north, the south-east and the Paris area (Figure 1B), mostly following the global geographic distribution of OXA-48-like producers (Figure 1C). NDM producers seemed to be randomly scattered across France. Of note, all CPE, except one, recovered on Réunion island, a French overseas department and region in the Indian Ocean, were of the NDM type (Figure 1C). Finally, all CPE recovered in French New Caledonia were of the IMP type (Figure 1C). The number of OXA-48-like producers and NDM producers constantly increased from 2012 to 2014 (256, 512 and 920 OXA-48-like producers and 27, 61 and 91 NDM producers in 2012, 2013 and 2014, respectively). Contrary to this, the number of KPC producers decreased over the same period of time (39, 29 and 19 KPC producers in 2012, 2013 and 2014, respectively). One of the most relevant features observed between 2012 and 2014 is the increased diversity of OXA-48-like producers, which is mostly related to the identification of the OXA-181 variant (Figure 3).

In addition, in 2014, an OXA-48-like variant with decreased susceptibility to carbapenems but devoid of any carbapenemase activity, OXA-405 was evidenced [14].

As previously observed, most of the carbapenemase-producing Enterobacteriaceae obtained in 2014 were

A. Known geographic origin of possible acquisition of infections or colonisation with NDM- and VIM-producing Enterobacteriaceae (n=44) B. KPC-producing Enterobacteriaceae (n=4) C. OXA-48-like- producing Enterobacteriaceae (n=94), France, 2014

A. Patients infected or colonised with NDM- and VIM-producing Enterobacteriaceae

B. Patients infected or colonised with KPC-producing Enterobacteriaceae

C. Patients infected or colonised with OXA-48-like-producing Enterobacteriaceae
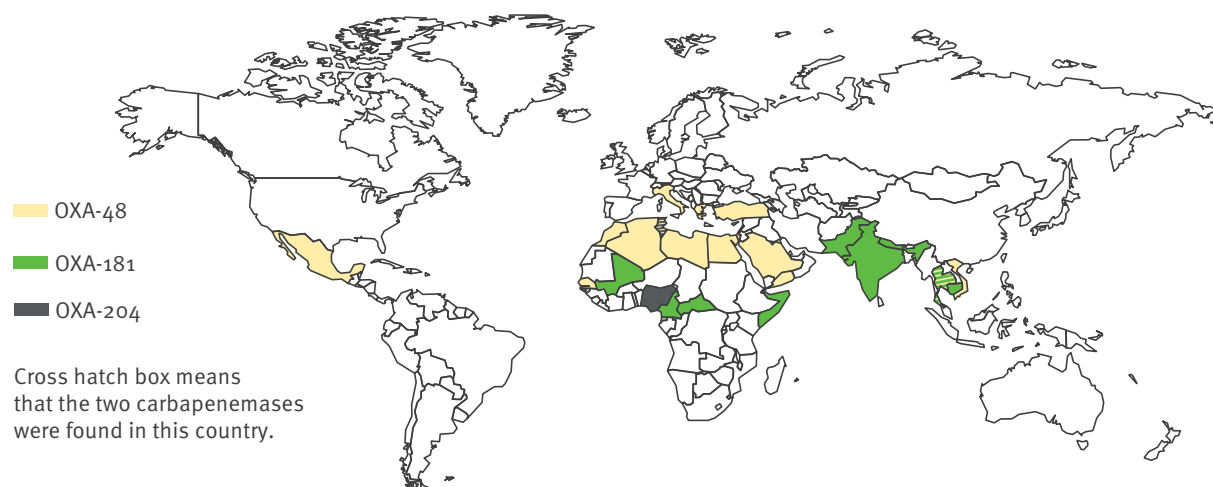
Distribution of carbapenemase types identified among carbapenemase-producing Enterobacteriaceae, France, 2014 (n = 1,075)

Type of carbapenemase	n	%
OXA-48-like	920	85.6
KPC	19	1.8
NDM	91	8.5
VIM	29	2.7
IMP	3	0.3
IMI	3	0.3
OXA-48-like + NDM	7	0.7
OXA-48-like + VIM	2	0.2
NDM + VIM	1	0.1
Total	**1,075**	100

Distribution of carbapenemase-producing Enterobacteriaceae by bacterial species, France 2014 (n = 1,075)

Enterobacterial species	n	%
Klebsiella pneumoniae	614	57.1
K. oxytoca	19	1.8
Escherichia coli	256	23.8
Enterobacter cloacae	106	9.9
E. aerogenes	13	1.2
Other *Enterobacter* spp.	4	0.4
Citrobacter freundii	38	3.5
C. koseri	9	0.8
Other *Citrobacter* spp.	1	0.1
Serratia spp.	7	0.7
Proteus mirabilis	1	0.1
Other *Proteae* (*Proteus* spp.. *Providentia* spp.)	1	0.1
Morganella morganii	4	0.4
Other	2	0.2
Total	**1,075**	100

nosocomially acquired bacterial species, including *K. pneumoniae* (57.1%), *E. cloacae* (9.9%) and *Citrobacter freundii* (3.5%) (Table 2 and Table 3) [12].

However, the number of carbapenemase-producers among carbapenem non susceptible *E. coli* isolates rose from 28.2 to 51.8% from 2012 to 2014. Of note, 46 patients were colonised with multiple CPE isolates (representing 106 isolates). In 93.5% (99/106) of the cases, the OXA-48 carbapenemase was identified reflecting the well-known de-repressed transfer properties of the incL/M OXA-48 prototype plasmid [15].

Mechanisms of decreased susceptibility to carbapenems in non-carbapenemase-producing Enterobacteriaceae

In the absence of carbapenemase production, the decreased susceptibility to carbapenems was mostly explained by a decreased outer-membrane permeability associated with the expression of an ESBL in *K. pneumoniae* (61.8%) and *E. coli* (50.4%). Overexpression of a chromosome-encoded cephalosporinase was mainly involved in natural producers of cephalosporinase that were *Enterobacter* spp. (61.6%), *Citrobacter* spp. (64.8%) and *Serratia* spp. (55.6%) (Table 3). Of note, for these non-carbapenemase producers, decreased susceptibility to ertapenem but retained susceptibility to imipenem and meropenem is frequently observed.

Colonisation vs infection with carbapenemase-producing Enterobacteriaceae

Among the 1,075 CPE identified in 2014, 643 (59.8%) were from rectal swabs i.e. colonisation and 377 (35.1%) from infection samples (Table 4). These two ratios remained the same since 2012 and were identical regardless of the carbapenemase type [12]. The most frequent clinical samples were urinary samples (68.2%) (Table 4).

Carbapenemase-producing Enterobacteriaceae colonisation and travel abroad

Although epidemiological data were sometimes not well documented, a possible importation from abroad was established for 13.2% (140/1,075) of patients colonised or infected with a CPE in 2014, (27.6% (94/341) in 2012 and 22.8% (145/636) in 2013 (Figure 4).

From 2012 to 2014, the identification of NDM-producing isolates was often linked to the Indian sub-continent (2012: 17/21; 2013: 17/30, 2014:23/42) where NDM-producers are endemic [12]. In addition, identification of NDM-producers was also observed with travel association from North African (n = 13) and Middle Eastern countries (n = 3) (Figure 5A. KPC producers for which data were available (n=3) were mostly recovered from patients previously hospitalised in endemic countries for KPCs such as Greece (n = 1), Italy (n = 2) and the United States (US) (n = 1) (Figure 5B) [3]. Finally when a link with a foreign country was established (10.2% (n = 94) of the cases for OXA-48-like), OXA-48-like producers were mostly recovered from patients with travel history to Africa and the Middle East (Figure 5C) corresponding to the known spread those CPE in these regions. The 52 OXA-48 variants, and mostly OXA-181 variants (n = 29), were identified from patients returning from the Indian subcontinent (n = 3), South-Eastern Asia (n = 2), and from the tropical region of Africa (n = 5) (Figure 5C).

Discussion

Carbapenem-resistance in enterobacterial isolates from France were rising about twofold over a three year period with a growing impact of CPE among the Enterobacteriaceae with decreased susceptibility to

Table 3

Distribution of carbapenemase and non-carbapenemase-producing isolates by enterobacterial species, France, 2014

Enterobacterial species	Total number of isolates	Carbapenemase-producing Enterobacteriacease										Non-carbapenemase-producing Enterobacteriacease					
		CPE	OXA-48-like	KPC	NDM	VIM	IMP	IMI	OXA-48-like+NDM	OXA-48-like+VIM	NDM+VIM	Non CPE	Case	ESBL	ESBL+Case	Imper.	Susceptible to carbapenems[a]
		n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Klebsiella spp.	1,180	633	552	17	51	9	1	0	3	0	0	547	68	338	24	113	4
Escherichia coli	490	256	220	1	28	2	0	0	4	0	1	234	52	118	17	45	2
Enterobacter spp.	1,073	123	101	1	6	9	1	3	0	2	0	950	585	18	326	21	0
Citrobacter spp.	139	48	39	0	1	7	1	0	0	0	0	91	59	2	26	4	0
Serratia spp.	25	7	5	0	0	2	0	0	0	0	0	18	10	2	0	5	1
Other species	65	8	3	0	5	0	0	0	0	0	0	57	22	6	3	22	4

Case: cephalosporinase; CPE: carbapenemase-producing Enterobacteriaceae; ESBL: extended spectrum beta-lactamase; Iperm: impermeability.
[a] Susceptible isolates after confirmation of minimum inhibitory concentration values [13].

Table 4

Distribution of specimens from which carbapenemase-producing Enterobacteriaceae were identified, France, 2014

Carbapenemase	Samples from which carbapenemase-producing Enterobacteriaceae were recovered:									Total
	Screening samples (Colonisation)	Infections samples							Information not available	
		Urine	Blood	Respiratory tract	Abscess	Wound	Other	Total infections		
OXA-48-like	556	224	20	29	8	17	24	322	42	920
KPC	12	1	1	2	1	0	1	6	1	19
NDM	50	24	3	1	0	3	5	36	5	91
VIM	15	7	0	2	0	0	2	11	3	29
IMP	1	1	0	0	0	0	0	1	1	3
IMI	3	0	0	0	0	0	0	0	0	3
Multiple carbapenemases	6	0	1	0	0	0	0	1	3	10
Total	**643**	**257**	**25**	**34**	**9**	**20**	**32**	**377**	**55**	**1,075**

carbapenems. The increased number of identified CPE mirrored the increasing number of reported nosocomial outbreaks due to CPE in France [16]. However, the resistant isolates were sent by French laboratories to the NRC on a voluntary basis, making their exact prevalence rate unpredictable. For 80% of CPE episodes, sporadic cases or several cases related by an identified chain of transmission, documented by the French Public Health Agency, one or more isolates were characterised by the NRC [17].

The analysis of the 6,682 strains with decreased susceptibility to carbapenems between 2012 and 2014 highlights several features. The three main species with a decreased susceptibility to carbapenems were *K. pneumoniae*, *Enterobacter* spp. and *E. coli*. When compared to 2012, the number of CPE identified in *E. coli* was five times higher in 2014 [12]. This observation hints towards a possible future endemic spread of carbapenemase-producing *E. coli* in the community as previously observed for ESBL-producing *E. coli*.

Overall, the main carbapenemase type identified was OXA-48 as observed in several countries in western Europe (e.g. Belgium, France, Spain) [6]. We suggest that the OXA-48 dissemination is likely the result of strong relationships and population movement between North African countries, where OXA-48 producers are endemic, and France or Belgium and Spain [18-22]. A growing diversity of OXA-48-like variants was identified with OXA-181 most frequently reported. Although the occurrence of OXA-181 is known in the Indian subcontinent and South-Eastern Asia, colonisation with OXA-181 producers in the tropical region of Africa might be more important than expected.

The spread of OXA-181 producers might have previously been missed since one of the most widespread, for example in France, molecular commercial assay for the screening of CPE named Xpert Carba-R performed on the GeneXpert (Cepheid, Sunnyvale, CA, US), did not detect OXA-181 and OXA-232 variants until recently [23,24]. This failure was corrected in the 2015 version

of the test that is now available on the market [25,26]. This example underlines that CPE screening should not be limited to molecular tests. Several tests were recently developed for the detection of carbapenem hydrolysis activity such as (i) biochemical test (the Carba NP test and its derivatives RAPIDEC Carba NP, Rapid CARB Screen, blue Carba) [10,27,28], (ii) MALDI-TOF based techniques [29], as well as electrochemical assays (the BYG test) [30]. In addition, in the context of such high prevalence of OXA-48 (ca 80% of the total CPE), rapid immunochromatographic tests able to detect all known OXA-48-like carbapenemases (OXA-48 K-SeTs from Coris BioConcept, BioRad), might be of interest [31,32].

Although the origin of colonisation with a CPE producer was not always documented, it is likely that acquisition abroad is fuelling the growing number of CPE identified in France.

Taken together, our results may indicate that the spread of OXA-48 like and NDM-like producers may soon become difficult to control due to their silent spread in community-acquired *E. coli* as suggested as early as in 2012 [33,34]. Contrary to this, spread of KPC producers that were still identified mostly in *K. pneumoniae*, remained confined to nosocomial settings and should thus still be largely controllable. As exemplified in the public hospitals in Paris (AP-HP), a large regional multi-hospital institution, prevention of outbreaks due to CPE may remain possible when CPE is still mostly a nosocomial problem [16]. Based on our own experience and the results of this study, we advocate for a systematic screening of at-risk patients to identify carriers of CPE. Early screening of patients colonised with CPE is the pre-requisite for the rapid implementation of strict hygiene measures based on isolation of colonised patients and cohorting to prevent and control nosocomial outbreaks.

Conflict of interest

None declared.

Authors' contributions

LD and PN wrote the manuscript. LD and GC performed the experiments and recorded the data. VP contributed to the revision of the manuscript.

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Establishment of a voluntary electronic *Chlamydia trachomatis* laboratory surveillance system in Germany, 2008 to 2014

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Chlamydia trachomatis (CT) infections are not reportable in Germany and limited data on prevalence are available. CT screening has been offered free of charge to pregnant women since 1995 and to all women under 25 years since 2008. For symptomatic women and men, diagnostic testing is covered by statutory health insurance. We describe the establishment of a nationwide, laboratory-based, voluntary sentinel that electronically collects information on all performed CT tests with test results, test reason and patient information. The sentinel represents one third of all performed CT tests in Germany. In the period from 2008 to 2014, 3,877,588 CT tests were reported, 93% in women. Women aged 20–24 years and men aged 25–29 years were the most frequently tested age groups. The overall proportion of positive tests (PPT) among women was 3.9% and among men 11.0%. The highest PPT among women was in the age groups 15–19 (6.8%) and 20–24 years (5.9%), and among men in the age groups 20–24 (19.2%), 15–19 (15.4%) and 25–29 years (14.8%). The PPT for CT was high among women and men younger than 25 years. Prevention is urgently needed. Monitoring of CT infection in Germany should be continued.

Introduction

Infections with *Chlamydia trachomatis* (CT) rank among the most frequent sexually transmitted infections (STI) in Europe and worldwide [1,2]. According to European data, the most affected age groups are women aged 15–24 years and men aged 20–24 years [2,3].The CT infection may be asymptomatic and can, if not detected and treated, result in complications such as pelvic inflammatory disease,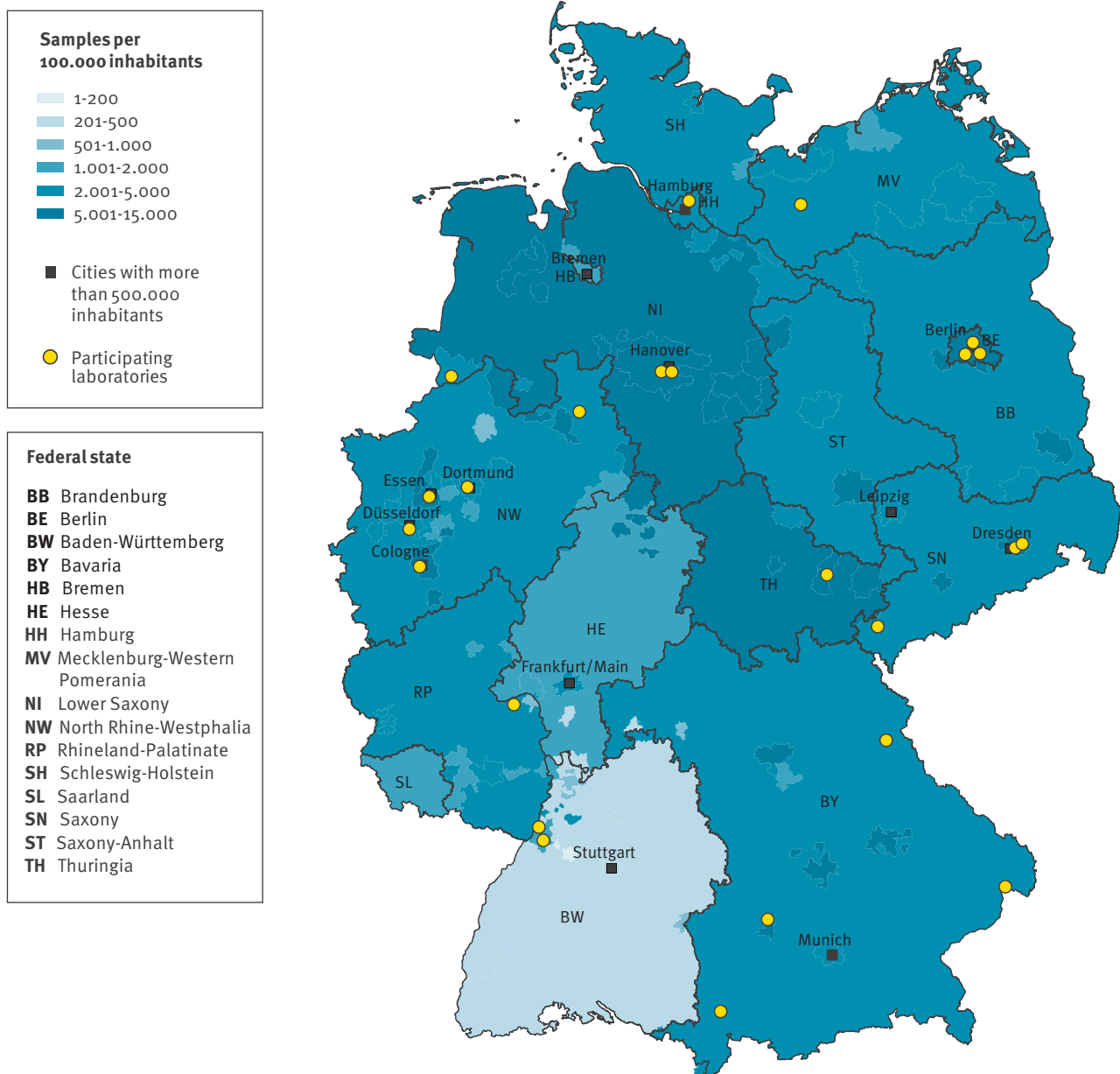 chronic abdominal pain, ectopic pregnancy, tubal sterility, a higher risk of adverse pregnancy outcomes for women and of epididymitis for men [3-10]. Evidence whether CT screening can prevent these complications is, however, controversial [3].

CT infections are not reportable in Germany, except for one federal state (Saxony), where we observed a continuous increase from 40.8 reported CT infections per 100,000 population in 2004 to 101.0 in 2012 [11]. However, only detected infections are reported. The true incidence in the population might be higher owing to the large proportion of asymptomatic infections that might remain undetected. In population-wide studies in Germany performed between 2003 and 2006, we observed a prevalence of up to 4.5% among women aged 17–19 years and 4.9% among men aged 25–29 years [12-14]. Between 2003 and 2009, data on CT were collected through the STI sentinel surveillance system from 247 sites (mainly local municipality counselling centres for STI, followed by STI outpatient clinics, general practitioners and other specialists) situated all over Germany but not representative of the general population in Germany. CT was the most frequently diagnosed STI, with a positivity of 6.0% among performed tests [15-17]. Sixty-seven per cent of the diagnosed CT infections were among women, many of them working as sex workers who attended the free-of-charge local municipality counselling clinics. The median age of infected women was 25 years and of men 31 years [15,16].

Health insurance in Germany is compulsory and individuals are covered either by statutory health insurance (ca 90%) or private health insurance. Private

FIGURE 1

Number of reported *Chlamydia trachomatis* tests per 100,000, by federal state, Germany, 2008–14 (n = 3,220,628)

Samples per 100.000 inhabitants

- 1-200
- 201-500
- 501-1.000
- 1.001-2.000
- 2.001-5.000
- 5.001-15.000

■ Cities with more than 500.000 inhabitants

○ Participating laboratories

Federal state

BB Brandenburg
BE Berlin
BW Baden-Württemberg
BY Bavaria
HB Bremen
HE Hesse
HH Hamburg
MV Mecklenburg-Western Pomerania
NI Lower Saxony
NW North Rhine-Westphalia
RP Rhineland-Palatinate
SH Schleswig-Holstein
SL Saarland
SN Saxony
**ST** Saxony-Anhalt
TH Thuringia

health insurance is available only to some segments of the population [18].

Patients in Germany can freely choose their medical practitioner, i.e. not based on place of residence. Laboratories do not have a defined catchment area, thus, there are laboratories serving only surrounding areas as well as laboratories receiving samples from all over Germany.

Since 1995, opportunistic CT screening for pregnant women with statutory insurance has been in place, and in 2008, yearly CT screening for sexually active women under the age of 25 years with statutory insurance, as well as a CT test before planned abortion, was introduced in Germany [19]. Up until now, there have been no CT screening programmes for men. Health insurance

companies can reimburse men and women for the costs of testing if they report specific symptoms or unspecific symptoms together with risk behaviour or if a sex partner has been tested positive for CT (diagnostic testing). Otherwise, the CT test can be requested and paid by the patient.

CT has been classified in the highest priority group of pathogens in Germany [20]. However, data on the proportion of positive tests (PPT) in different age groups and regions are limited. Furthermore, there are no data on the frequency of the different test indications for CT in women and on the coverage of the screening programme for women younger than 25 years. Except for Saxony, there is no information on the CT infection trend over time.

Figure 2

Proportion of reported *Chlamydia trachomatis* tests by age group, test reason and sex, Germany, 2008–14
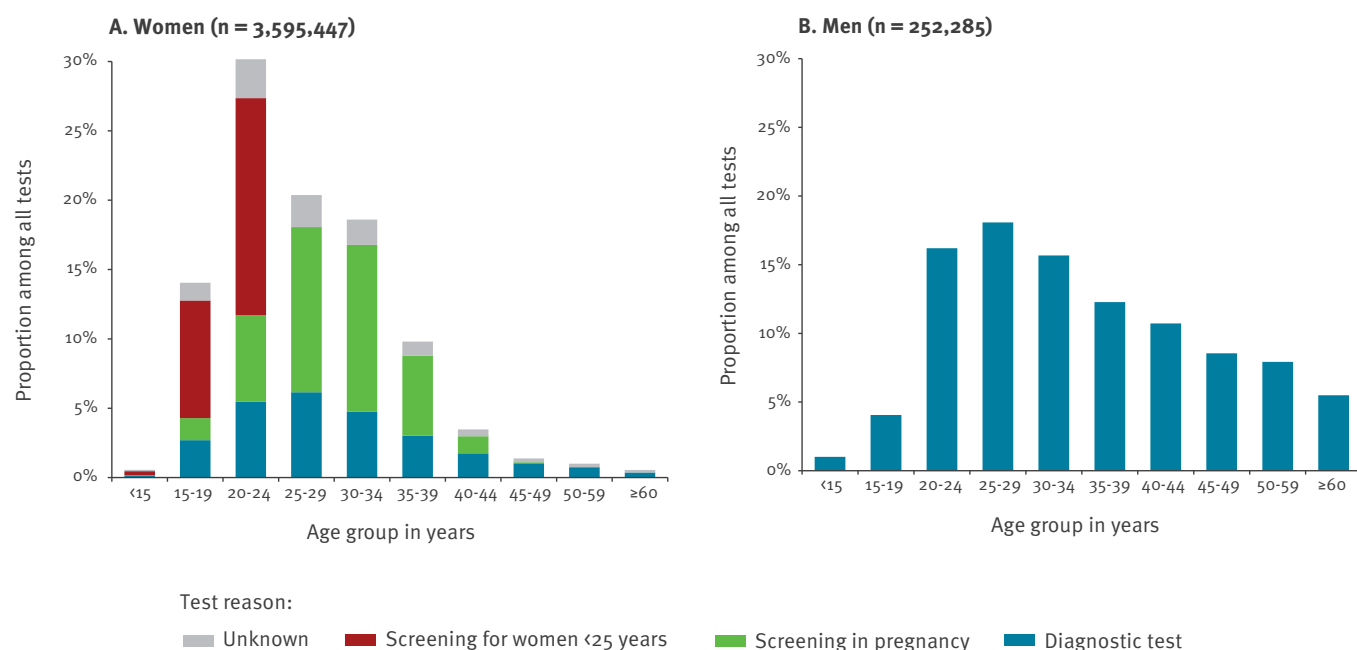
To close this knowledge gap, we introduced a new laboratory-based CT surveillance system, the 'CT laboratory sentinel' in Germany in 2010. The aim of the CT laboratory sentinel was to monitor CT testing data and infections in Germany and to evaluate the newly introduced CT screening for women under 25 years of age, in order to develop public health recommendations for targeted prevention measures.

Before the laboratory-based CT-surveillance was set up, all laboratories testing for CT in Germany were mapped [21]. Of 1,504 contacted facilities, 725 (48%) responded to a questionnaire; 143 reported that they performed CT diagnostics and of those 143, 60 reported that they would be interested in reporting data [21].

In this paper, we report on how the CT laboratory sentinel was established and present the first results.

Methods

Establishment of the *Chlamydia trachomatis* laboratory sentinel
In September 2010, we started implementing a voluntary laboratory-based sentinel system in Germany for electronic and, where possible, automated collection of information that is routinely available in laboratories on CT tests. Mapping of the laboratories performing CT diagnostics [21] provided us with a list of laboratories that expressed interest in participation and with information on the number of CT tests per quarter and catchment area. We recruited laboratories based on the number of performed CT tests and on the size of the catchment area. Our aim was to recruit laboratories performing many CT tests and to reach equally good

geographical distribution in each federal state. After review of the geographical distribution and coverage in our sample, we decided to recruit additional laboratories with catchment areas from underrepresented regions.

Through the CT laboratory sentinel, we collected retrospective (back to 2008) and continuous data on the performed CT tests up to 31 December 2014. Data were reported on a quarterly basis. The laboratories indicated that not all variables could be selected from their data systems or the selection would be very time consuming. To keep the effort reasonable, we defined a standard common set of mandatory and optional variables. Mandatory variables were sample and patient identification number, date of laboratory testing, test result, sex, and year of birth. Optional variables included date of sampling, the first three digits of the standard five-digit postal code of the patient, the first three digits of the postal code of the submitting medical practitioner, month of birth, reason for testing or billing codes (used for invoicing health insurance), pregnancy status, tested material, health insurance status (statutory or private) and method of testing.

If the three-digit postal code of the patient was not available, we used the three-digit postal code of the submitting medical practitioner. We generated information on the test reason from the reported reason for testing or respective billing codes. Samples from female patients, who were tested because of symptoms or suspicion of infection, were categorised as 'Diagnostic testing'. Samples from female patients who were tested during pregnancy or before a planned abortion were categorised as 'Screening in pregnancy'.

Proportion of positive *Chlamydia trachomatis* tests with 95% confidence intervals, by sex, age group and test reason, Germany, 2008–14, among women (n = 3,577,935) and men (n = 249,857)
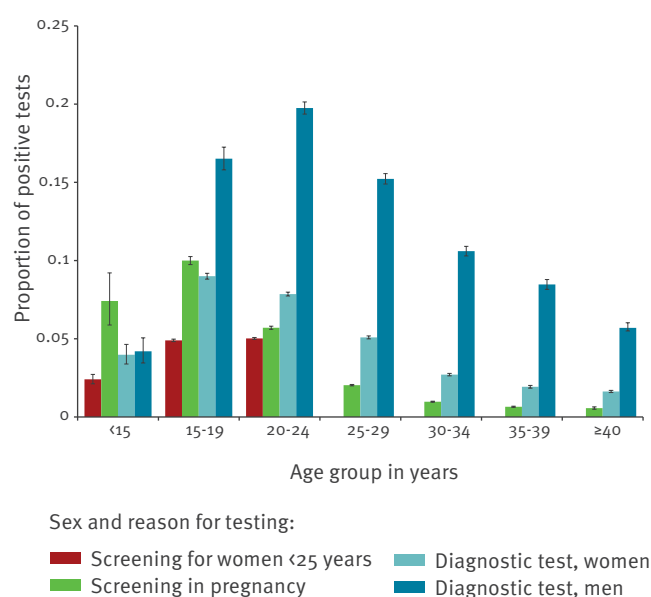
Women who were screened as part of the screening for under 25 years of age were categorised as 'Screening for women under 25'.

On the basis of the year of birth, we calculated patient age at the time of testing. Laboratories reported the CT tests for the complete time period, or less if reporting for the complete time period was not possible.

Data were transmitted electronically. The data were sent to us either via email as extensible markup language (.XML) files, comma-separated values (.CSV) or Excel spreadsheet (.XLS) files or in XML format via secure sockets layer (SSL)-encrypted Internet connection to a web service. After performing predefined automated plausibility checks, the received data were combined in a structured query language (SQL) database. Unplausible variables were set to missing. The sample and patient numbers were MD5-encrypted and transmitted as 32-digit hash codes. Decryption of this code was not possible. If patients were tested more than once at the same laboratory, the 32-digit hash code enabled us to assign data from several samples over time to one patient. However, samples from the same patient tested in different laboratories could not be assigned to the same patient. If laboratories used different input data (for example, surname and date of birth in one quarter and name plus surname and date of birth in the subsequent quarter) to generate the 32-digit hash codes, we were not able to trace those patient numbers over time. In order to understand if person-related analysis, such as testing frequency and time intervals between tests, is possible for this way

of data collection, we proved the traceability of the patient identification numbers by laboratory over time. There was no financial compensation for laboratories to participate in the study. The data collection protocol was confirmed by the data protection officer at the Robert Koch Institute, Berlin. Additional approval from an ethics committee was not deemed necessary, as no patient-identifying data were collected.

Data analysis

We analysed all CT tests available for the time period between 1 January 2008 and 31 December 2014. For the reported CT tests, we calculated counts and proportions of the available and missing variables. We calculated the duration of the reporting period by laboratory as well as counts and proportions of the CT tests by laboratory.

We defined coverage as the proportion of CT tests from individuals with statutory insurance collected through the sentinel among all CT tests from individuals with statutory insurance. The National Association of Statutory Health Insurance provided us with data on all performed CT tests from individuals with statutory insurance for the years 2011 and 2012. We are not able to individually link patients or tests in the two data sources. Instead, we first calculated the proportion of individuals with statutory insurance among the CT tests with available information on health insurance status. Then, we extrapolated this proportion to all CT tests collected within the laboratory sentinel in the years 2011 and 2012 and calculated the total number of CT tests from individuals with statutory insurance. Finally, we assessed the coverage of the laboratory sentinel by comparing the total number of CT tests from persons with statutory insurance in Germany and from persons with statutory insurance collected in the CT laboratory sentinel. We assumed that the coverage of CT tests for privately insured persons was similar.

The geographical distribution of the reported CT tests based on the postal codes was described as the number of CT tests per 100,000 population by federal state in Germany.

We described CT tests in the laboratory sentinel and the PPT by age group, sex, reason for testing (diagnostic testing because of symptoms, screening in pregnancy or screening for women under 25 years of age) and tested material (for men).

Results

Participating laboratories and collected data

Of the 60 laboratories selected for recruitment, 24 agreed to participate and have been reporting data to the CT laboratory sentinel. The reasons for refusing to participate were: data selection in the requested format was not possible (n = 10), too much effort was required (n = 12), CT samples were forwarded to a partner/alliance laboratory or organisational changes

Proportion of positive *Chlamydia trachomatis* tests with 95% confidence intervals among men, by tested material, Germany, 2008–14 (n = 174,346)
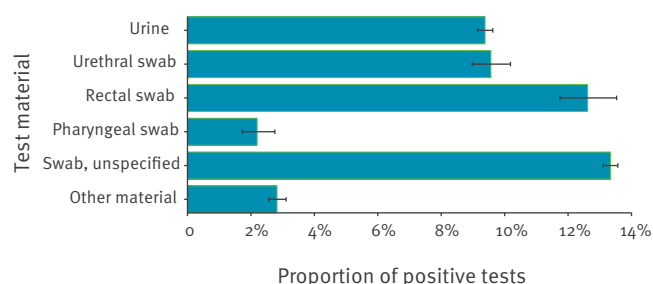
(n = 7), refusal without a specific reason (n = 4), other reasons (n = 3). Three of the laboratories refusing to participate were large laboratories with a nationwide catchment area. Currently, two laboratories are reporting data by using the web service; three send the data as XML, five as CSV and 14 as Excel spreadsheets via email.

By 24 November 2015, a total of 3,877,588 CT tests had been reported for the period from 1 January 2008 to 31 December 2014. A total of 15 laboratories have reported data for each quarter of the entire study period. A further nine laboratories have reported data for a minimum of 1 month and a maximum of 4 years and 7 months (Table 1).

Information on the mandatory variables was missing in less than 1% of all reported CT tests and on optional variables between 13% and 80% (Table 2). Patient number was consistently coded and therefore traceable over the entire reporting time in 15 laboratories, consistently coded only for part of the time in seven laboratories, and two laboratories did not report patient identification numbers (Table 1).

Coverage

In total, 91.1% and 78.1% of CT tests with information on health insurance were attributable to, respectively, the women and men with statutory health insurance. We estimated that 34.3% of all CT tests performed among statutorily insured persons in Germany were reported to the CT laboratory sentinel. These estimates varied by federal state from 4.4% in Baden-Wurttemberg to 60.9% in Thuringia (Table 3). The coverage was 34.6 for CT tests among women and 28.7% for CT tests among men (Table 3).

Regional distribution

The number of reported CT tests with information on the three-digit postal code for the entire period per 100,000 of the population varied by region between 141 and 14,901 (Figure 1). Based on information on the catchment areas provided from laboratories that did not report information on postal code, ca 50%

of CT tests with missing postal codes would be from Saxony, around 40% from the western part of the country (Bremen, Lower Saxony, North Rhine-Westphalia, Hesse, Rhineland-Palatinate, Saarland and Baden-Württemberg) and the rest from Berlin.

CT testing

Of the total of 3,877,588 reported CT tests for the period 2008 to 2014, 92.8% (3,599,821) were done in women and 6.6% (255,634) in men. Among women with information on age (3,595,447), the most frequently tested age groups were women aged 20–24 years, and among men (252,285) those aged 25–29 years, followed by those aged 20–24 years and 30–34 years. The proportion of CT tests by age group among men and women are reported in Figure 2.

Reason for testing in women

Among CT tests in women with information on the reason for testing, 41.9% were attributable to screening in pregnancy, 26.9% to screening of women under 25 years of age and 28.7% to diagnostic tests (Figure 2).

Tested material in men

Among CT tests in men with information on tested specimen, 49.0% were unspecified swabs, 32.5% urine, 5.3% urethral, 3.1% rectal and 1.9% pharyngeal swabs. In 8.2% of tests, other materials were tested.

Proportion of positive tests

Among tests with valid test results (n = 3,827,792), 3.9% (95% confidence interval (CI): 3.9–4.0) of tests among women and 11.0% (95% CI: 10.9–11.2) of tests among men were positive. PPT varied by federal state from 3.0% in Saarland to 6.8% in Mecklenburg-Western Pomerania among women and 9.0% in Saarland to 17.0% in Mecklenburg-Western Pomerania among men.

The PPT among women differed by reason for testing and age (Figure 3). Overall, the highest PPT was observed among women aged 15–19 years and 20–24 years (Figure 3). The PPT when screening women under 25 years was 4.9% in 15–19 and 5.0% in 20–24 year-olds. While screening tests in pregnancy and diagnostic testing revealed, respectively, a PPT of 10.0% and 9.0% among 15–19 year-olds and 5.7% and 7.9% among 20–24 year-olds, PPT among pregnant women decreased to 2.0% among women 25–29 years of age and was < 1% in those 30 years and older. The PPT in diagnostic tests also decreased with increasing age (Figure 3).

Among men, the highest PPT was observed among the age groups 20–24 years (19.2%), 15–19 years (15.4%) and 25–29 years (14.8%). The PPT among women and men decreased with age (Figure 3).

Among men, the PPT was higher in rectal (12.3%) and in unspecified swabs (13.4%) (Figure 4).

Table 1

Number of reported *Chlamydia trachomatis* tests, proportion of tests in women, data reporting period, catchment area and patient traceability period by laboratory, Germany 2008–2014 (n = 3,877,588)

Laboratory	Reported CT tests n[a]	Tests in Women %	Reporting period (number of CT tests)							Patient traceability period
			2008	2009	2010	2011	2012	2013	2014	
1	1,629,040	98.0	78,770	183,085	211,571	244,480	268,823	309,423	332,888	Partly
2	450,368	93.0	49,184	59,628	66,939	63,678	63,383	70,182	77,374	Complete
3	342,929	91.1	41,451	51,327	51,190	50,430	48,649	50,946	48,936	Complete
4	268,248	86.2	15,821	33,004	34,360	39,379	47,893	47,990	49,801	Partly
5	200,204	90.4	15,366	27,885	29,839	29,859	31,142	33,842	32,271	Complete
6	132,726	92.4	ND	ND	ND	ND	ND	282	132,444	Partly
7	126,170	96.6	2,609	19,884	19,836	20,892	21,919	21,663	19,367	Complete
8	95,022	74.0	8,964	10,618	11,548	12,336	14,261	15,805	21,490	Complete
9	92,824	80.2	12,162	12,882	11,529	11,746	11,998	15,684	16,823	Partly
10	83,715	92.3	8,338	11,924	12,025	12,973	12,282	13,236	12,937	Complete
11	64,806	94.9	ND	ND	ND	ND	ND	29,906	34,900	Patient number missing
12	64,133	96.6	7,705	8,449	8,894	8,937	9,482	10,118	10,548	Complete
13	59,098	99.9	5,834	14,592	15,013	14,583	9,076			Complete
14	51,755	90.7	6,524	7,371	7,128	7,857	7,715	7,460	7,700	Complete
15	49,839	84.1	3,219	7,594	7,452	7,533	7,162	7,926	8,953	Complete
16	45,708	94.7	ND	ND	ND	ND	15,816	14,951	14,941	Partly
17	39,969	70.3	ND	ND	ND	ND	64	20,017	19,888	Complete
18	29,573	90.9	ND	ND	ND	7,849	7,296	7,251	7,177	Partly
19	28,636	32.9	3,569	3,541	3,549	3,849	4,573	4,782	4,773	Complete
20	12,398	63.9	1,587	1,437	1,411	1,769	1,822	2,065	2,307	Complete
21	8,105	66.8	766	895	995	1,198	1,053	1,260	1,938	Complete
22	1,590	69.7	ND	ND	ND	422	397	391	380	Partly
23	564	97.2	ND	ND	ND	554	10	ND	ND	Patient number missing
24	168	92.3	ND	ND	ND	ND	168	ND	ND	Complete
Total	3,877,588	92.8	261,869	454,116	493,279	540,324	584,984	685,180	857,836	

CT: Chlamydia trachomatis; ND: no data reported.

[a] Laboratories sorted by number of reported CT tests.

Discussion

We established a CT laboratory sentinel in Germany that electronically collects data that are routinely available in laboratories on performed CT tests; the CT laboratory sentinel serves as a surveillance system. In the period from 2008 to 2014, we reached good coverage and collected a large number of samples representing one third of all performed CT tests in Germany, together with epidemiological information and data on testing. In total, 24 laboratories reported data on a voluntary basis; for the majority of the data, we had information for a complete time period (January 2008 to December 2014). Completeness of the five mandatory variables was more than 99%, while completeness of the eight optional variables varied by laboratory and variable.

We estimate that we have collected 34% of all CT tests of individuals with statutory health insurance in the CT laboratory sentinel. This was possible because we were able to recruit some very large laboratories. Although this estimate is based on data from 2011 and 2012, we

assume that we have reached at least the same coverage in the following years 2013 and 2014. We also assume that the coverage of CT tests from individuals with private health insurance was similar. The coverage was slightly better for statutorily insured women than men. The reason for this is unclear. One possible explanation may be that statutorily insured men are being tested at specialist HIV centres or at centres targeting men who have sex with men (MSM), and that these centres might be cooperating with local laboratories not included in the sentinel.

We were able to collect data from samples from all over Germany. Baden-Wurttemberg contributed the lowest number of reported CT tests per 100,000 population and also reached the lowest coverage compared with the other federal states. A substantial proportion of the CT tests with missing information on postal codes was reported from one laboratory with a catchment area in Baden-Wurttemberg, Hesse and Rhineland-Palatinate. We therefore assume that the geographical

Number of *Chlamydia trachomatis* records with information on collected variables, and number and proportion of records with unknown, missing or implausible information, Germany, 2008–2014 (n = 3,877,588)

Variable	Type of variable	Available	Unknown, missing or unplausible	
		n	n	%
Sample number	Mandatory	3,877,588	0	0.0
Patient number	Mandatory	3,877,588	0	0.0
Test result	Mandatory	3,856,972	20,616	0.5
Sex	Mandatory	3,855,455	22,133	0.6
Year of birth	Mandatory	3,859,684	17,904	0.5
Month of birth	Optional	3,313,251	564,337	17.0
Three-digit postal code[a]	Optional	3,220,557	657,031	20.4
Date of sampling	Optional	776,349	3,101,239	80.0
Test reason	Optional	3,496,011	381,577	9.8
Pregnancy status	Optional	2,954,620	922,968	23.8
Tested material	Optional	3,378,347	499,241	12.9
Method of testing	Optional	2,126,643	1,750,945	45.2
Health insurance status	Optional	1,207,750	2,669,838	68.9

[a] For 3,097,980 records (96.2%), patient three-digit postal codes were reported, and for 122,577 records (3.8%), postal codes of practitioners were reported.

distribution of tested persons in these federal states or neighbouring areas is better than that estimated based on the postal codes. To obtain better regional data and better coverage of the CT tests from men, we are recruiting further laboratories for participation. An update of the laboratory mapping exercise would be desirable to indicate further potential laboratories covering Baden-Wurttemberg that were not reached in the first mapping.

In several laboratories, the number of performed CT test has increased over the years. Based on information provided from laboratories, a substantial part of the observed increase can be attributed to merging or expansion of the laboratories. However, we are not able to quantify this. We believe that there has been a real increase in CT testing activity in Germany. Further analysis of the statutory insurance registry can clarify if the number of performed CT tests has risen since 2008.

The majority of the reported CT tests were from women, as CT screening is offered to women under 25 years of age and pregnant women. Women aged 20–24 years were by far the most frequently tested age group, followed by women aged 25–34 years. Men aged 20–34 years were most frequently tested compared with other age groups. We also observed the highest PPT in age groups with the highest test frequency. PPT among both men and women was high among tests from younger people and decreased with age. In order to analyse the PPT variation by region further, sociodemographic information is necessary.

We observed the highest PPT among women and men aged 15–24 years, which is similar to several population-based studies in Europe [12-14,22-28]. National chlamydia testing data with information on denominator from England and Norway report PPT of, respectively, 7.8% and 11.5% among 15–24 year-old women and of 10.0% and 17.1% among 15–24 year-old men [27,28]. Opportunities for testing free of charge, especially for men, are scarce in Germany, comparison with England and Norway [27,28]. This impacts testing rates, the groups tested and the PPT.

The PPT was high among very young women screened during pregnancy (these data include also CT tests before abortion). This might be explained by a young age at first sexual intercourse, which several studies have linked to having more partners, more diverse sexual experiences, less frequent use of condoms, and increased risk for bacterial STI, pregnancy and abortion [29]. The PPT among CT tests in pregnancy decreased with increasing age and was less than 1% among women older than 30 years. Our data suggest that it is more rational to screen younger pregnant women, especially those under 25 years of age, than older ones. Furthermore, it is likely that with the given PPT in older pregnant women, some tests may be false positive and will lead to unnecessary treatment. With the current data collected in the laboratory sentinel, we cannot determine what proportion of positive CT tests can be explained by risk behaviour, such as new or multiple sexual partners, other STI or history of sex work. Testing groups with higher prevalence is more effective in terms of detection rate. Age- and risk behaviour-indicated screening in pregnancy in Germany instead of screening of all pregnant women should be further discussed. A cost–benefit analysis taking into account estimates of age-specific adverse health outcomes in

Proportion of *Chlamydia trachomatis* tests from men and women with statutory health insurance (n = 2,964,346), collected in the Chlamydia trachomatis laboratory sentinel, by federal state, Germany, 2011–12 (n = 1,016,231)

Federal state (total population[a])	Proportion of CT tests collected through the sentinel (%)			
	Women	Men	Unknown	Total
Baden-Wurttemberg (n = 10,786,227)	4.3	6.3	18.4	4.4
Bavaria (n = 12,595,891)	29.6	16.1	19.2	28.9
Berlin (n = 3,501,872)	57.9	53.2	177	57.3
Brandenburg (n = 2,495,635)	37.2	8.8	3.5	35.7
Bremen (n = 661,301)	12.2	1.9	0.0	11.5
Hamburg (n = 1,798,836)	15.3	2.4	0.7	13.5
Hesse (n = 6,092,126)	19.7	22.9	44.6	20.0
Lower Saxony (n = 7,913,502)	24.7	12.2	1.4	23.8
Mecklenburg-Western Pomerania (n = 1,634,734)	53.9	25.9	17.7	52.4
North Rhine-Westphalia (n = 17,841,956)	39.6	34.9	66.4	39.4
Rhineland-Palatinate (n = 3,999,117)	29.8	18.9	1170	29.5
Saarland (n = 1,013,352)	19.6	8.8	90.5	19.0
Saxony (n = 4,137,051)	27.7	35.4	49.0	28.4
Saxony-Anhalt (n = 2,313,280)	36.1	6.9	1.4	33.8
Schleswig-Holstein (n = 2,837,641)	22.6	3.3	5.5	21.1
Thuringia (n = 2,221,222)	60.1	82.6	41.5	60.9
Total	**34.6**	**28.7**	**177.3**	**34.3**

CT: Chlamydia trachomatis.

[a] Population by federal state in 2011 (Source: German Federal Statistical Office).

pregnancy due to chlamydia infection would facilitate these discussions.

PPT was higher in men than in women also when comparing only diagnostic CT tests. This was not unexpected, as we only reported on CT tests performed among men presenting with symptoms. The PPT in rectal swabs compared with urethral samples was high. Therefore we believe that a substantial proportion of positive CT tests among men might be attributable to MSM. Although almost half of the samples tested were unspecified swabs, we believe based on the PPT that a substantial proportion can be attributable to rectal swabs. Among MSM screened for STI in Germany, a CT prevalence of 9.4% (95% CI: 7.1–12.0) has been previously reported [30].

The majority of countries in the European Union and European Economic Area have a system for reporting and monitoring diagnosed CT cases at the population level [31]. These are however limited to infections that have been diagnosed and reported. The CT detection rates are influenced by populations tested and testing volume [31]. The CT laboratory sentinel provides information on both positive and negative test results which allows us to calculate the PPT and monitor it over time.

The limitations of this study are that the laboratories did not have an equal chance to be included in the sentinel, as we were selecting laboratories based on the interest to participate, number of performed CT tests

and catchment area. There may be other large laboratories that were not reached in the mapping phase [21] and thus not considered for the laboratory sentinel. Although we evaluated our data for coverage at least once per year and selected for recruitment additional laboratories with catchment areas in regions underrepresented in the sentinel, we could not obtain an even coverage in all regions. Few laboratories reported the optional variables, which could have resulted in a selection bias in these data. However, owing to the large number of reported CT tests, analyses describing these variables are still possible. Efforts are continuing to improve completeness of the optional variables. We are unable to collect more detailed epidemiological information such as route of transmission and symptoms through the CT laboratory sentinel. Usually, laboratories in Germany have only very limited epidemiological information and there is no legal basis to collect these data. Laboratories that have more information need to treat this information confidentially.

Conclusion

The implementation of our CT laboratory sentinel has shown that it is feasible in Germany to collect, electronically and continuously, readily available data from laboratories with a reasonable effort that can for now be used instead of mandatory surveillance. We managed to collect a large amount of data from all regions in Germany that represented around one third of all performed CT tests. In contrast to mandatory surveillance, the CT laboratory sentinel collects information

on all performed CT tests which allows analysis of PPT over time. In addition, regularly conducted population-based prevalence surveys, although costly, could help determine the true prevalence of CT infection in the population and evaluate prevention strategies.

A large PPT among young men and women and low awareness of CT in Germany [32] support the need for further prevention efforts. The CT laboratory sentinel should continue to collect data and expand the base of participating laboratories in order to monitor and describe CT infection in Germany and guide public health strategies. The participating laboratories should be continuously evaluated and the coverage and representation of different groups tested should be improved.

Conflict of interest

None declared.

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Approaches for quantifying antimicrobial consumption per animal species based on national sales data: a Swiss example, 2006 to 2013

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Antimicrobial use in animals is known to contribute to the global burden of antimicrobial resistance. Therefore, it is critical to monitor antimicrobial sales for livestock and pets. Despite the availability of veterinary antimicrobial sales data in most European countries, surveillance currently lacks consumption monitoring at the animal species level. In this study, alternative methods were investigated for stratifying antimicrobial sales per species using Swiss data (2006–2013). Three approaches were considered: (i) Equal Distribution (ED) allocated antimicrobial sales evenly across all species each product was licensed for; (ii) Biomass Distribution (BMD) stratified antimicrobial consumption, weighting the representativeness of each species' total biomass; and (iii) Longitudinal Study Extrapolation (LSE) assigned antimicrobial sales per species based on a field study describing prescription patterns in Switzerland. LSE is expected to provide the best estimates because it relies on field data. Given the Swiss example, BMD appears to be a reliable method when prescription data are not available, whereas ED seems to underestimate consumption in species with larger populations and higher treatment intensity. These methods represent a valuable tool for improving the monitoring systems of veterinary antimicrobial consumption across Europe.

Introduction

Antimicrobial resistance has been gaining momentum as one of the most important topics within the public health sphere [1]. Part of the antimicrobial resistance burden for public health lies on the use of antimicrobials for veterinary purposes. Results from several studies have suggested that antimicrobial exposure in livestock is contributing to the emergence, selection and spread of antimicrobial resistant bacteria [2-4]. In addition, it is known that the use of antimicrobials in pets influences the resistance patterns found in those animals [5]. The subsequent spread of resistant bacteria from animals to humans can occur through multiple potential routes.

Monitoring systems in veterinary medicine can provide useful insights into temporal trends of antimicrobial consumption and ensure compliance with prudent usage practices, programmes or regulations. Furthermore, they can assist in identifying the most efficient interventions for optimising antimicrobial usage. When combined with antimicrobial resistance data, quantification of antimicrobial usage can be useful not only in identifying risk factors for the emergence of resistance, but also in describing temporal associations between antimicrobial usage and resistance [6,7]. Finally, monitoring systems can be a source of highly informative data for boosting research on the complex topic of emergence, selection and spread of antimicrobial resistance. Thus, monitoring antimicrobial consumption in livestock and companion animals is undoubtedly an important tool in the battle against antimicrobial resistance.

Research on the veterinary use of antimicrobials has focused on livestock species because their populations are larger and their antimicrobial consumption is higher than that of pet animals. Recognition of the importance of quantifying antimicrobial use in livestock emerged more than a decade ago [8] and the European Commission and the European Medicines Agency (EMA) have also emphasised the importance of monitoring antimicrobial use [9-11]. There is no binding European Union (EU) legislation with respect to the implementation of such monitoring programmes at national level and it is up to each country to define its strategy.

In Switzerland, a non-EU country, the legal basis for sales data collection was defined in Article 35 of the Federal Ordinance on Veterinary Medicinal Products, enacted in September 2004 [12].

The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project, initiated in 2010 by the EMA, has contributed considerably to the collection of standardised data on veterinary consumption in Europe [13]. ESVAC reports are published annually and are currently based on data provided by 26 countries, including Switzerland [14].

Prompted by the European Commission's Action plan against the rising threats from Antimicrobial Resistance [10], ESVAC published guidance for data collection on antimicrobial consumption at the species level [15]. Furthermore, international guidelines such as the World Organisation for Animal Health's Terrestrial Animal Health Code [7] and Integrated Surveillance of Antimicrobial Resistance: Guidance from a WHO Advisory Group [16], mention usage data at the species level as an important aspect that should be considered in monitoring systems. Data at the species and the production type level (such as dairy or beef cattle; broilers or laying hens; breeding, farrowing or fattening units) provide a better estimate of the antimicrobial exposure in each population and are therefore much more informative than overall sales data.

In mid-2000, Denmark implemented an automated system for nationwide collection of antimicrobial prescription data for production animals (pigs, poultry, cattle, sheep, goats, fish and mink) [6]. Systems providing data at the farm level can be used to identify high consumers and therefore implement benchmarking systems based on usage by individual farms or practitioners [17-19]. However, such systems can be very demanding in terms of resources and infrastructure [16] which might hinder their establishment.

In the absence of automated data collection schemes, alternatives need to be explored. ESVAC suggests that estimates of antimicrobial usage per species can be obtained through cross-sectional or longitudinal studies or based on data from marketing authorisation holders [15]. Some of these strategies have already been applied. In the Netherlands, longitudinal data on antimicrobial usage were collected from a sample of farms [20]. In France, antimicrobial consumption per animal species has been calculated based on estimates of marketing authorisation holders since 2009 [21].

In Switzerland, antimicrobial sales data have been obtained yearly at national level since 2004 by requesting the number of packages sold per product from the marketing authorisation holders [22]. However, this strategy does not enable quantification of antimicrobial consumption at the species level.

There is no standardised method for quantifying the distribution of antimicrobial sales per animal species. The choice of the method also depends on data availability. It is therefore of interest to compare different possible methods and observe how results vary.

The aim of this study was to propose and compare alternative methods for estimating the antimicrobial consumption in pet and livestock animal species or groups of species in Switzerland by combining sales data with (i) summary of product characteristics; (ii) summary of product characteristics and animal demographic data; (iii) prescription data from a longitudinal study.

Methods

Antimicrobial sales data and product information

Based on information obtained from marketing authorisation holders, the number of antimicrobial packages sold per product is converted into the corresponding amount of active ingredient. The results are published in the ARCH-Vet report (the official annual report on sales of antibiotics in veterinary medicine and antibiotic resistance monitoring of livestock in Switzerland) by the Federal Food Safety and Veterinary Office (FSVO) [23].

The FSVO granted the authors access to the detailed antimicrobial sales database. The models developed in this study were fed with sales data from the period 2006–2013. Results from these models are a proxy for antimicrobial consumption.

Antimicrobial products were categorised into two groups: monospecies products (authorised for a single species) and multispecies products (licensed for multiple species). This stratification was done by extracting from the Swiss Veterinary Drug Compendium data on the species each product is licensed for [24]. The following species or groups of related species (hereafter referred to as 'species') were considered: pigs, cattle, poultry, small ruminants (goats and sheep grouped together), horses and pets (cats and dogs grouped together). Poultry is roughly equivalent to the number of chickens because turkey or waterfowl production in Switzerland is negligible. It should also be noted that, in Switzerland, most horses are kept for leisure and only a small number enter the food chain.

Rabbits and fish were excluded as their population sizes are comparatively small, and therefore these groups are expected to represent a negligible contribution to the consumption of antimicrobials in Switzerland.

Animal demographic data

The national total biomass of each species was calculated from 2006 to 2013 using the population correction unit (PCU) method. PCU is a technical unit of measurement. One PCU is equivalent to 1 kg of

biomass of livestock and slaughtered animals [13]. For livestock, theoretical weights at the most likely time for treatment were based on ESVAC recommendations [15]. For cats and dogs we used 5 kg and 20 kg bodyweight, respectively, as these are accepted standards for drug regulatory agencies [25].

When possible, sources of demographic data used for the ESVAC report were consulted [26]. For pets, demographic data were collected from the Société pour l'alimentation des animaux familiers (Swiss Society for Pet Nutrition) [27,28]. For the years where no data were available (2009, 2011, 2013), the mean of the previous and the following year was used.

Field data on antimicrobial prescription patterns

Regula et al. (2009) assessed the prescription patterns of veterinarians in Switzerland for the period 2004–2005 [29]. Eight veterinary practices, representing 1.5% of all veterinary clinics in Switzerland (with a total of 15 veterinarians), were selected based on the proportion of owners keeping livestock and the use of electronic databases for disease and prescription records. Cattle, pigs, sheep, goats, horses, dogs and cats were included in this study. The proportion of animals at risk of being treated in the field study relative to the total number of animals in the country varied across the different animal species. To take this into account, the total amount of active ingredient prescribed was divided by the percentage of animals of each species at risk of being treated. The number of animals at risk of being treated during the field study were calculated as follows: (i) for horses, the number of owners in the practice records was used as a proxy for the number of animals (ii) for pets, the number of owners in the practice records was multiplied by the mean number of pets per household in Switzerland [27,28]; (iii) for pigs and cattle, veterinarians enrolled in the study provided estimates of the number of animals on the farms they visited; (iv) the number of small ruminants at risk of being treated was calculated based on the number of cattle at risk of being treated. We assumed that the ratio of cattle to small ruminants in the field study was the same as at the national level [26].

Field data were used to estimate the distribution of antimicrobial consumption by different species. These estimates were used to calculate mode values of Program Evaluation and Review Technique (PERT) distributions used in the Longitudinal Study Extrapolation (LSE) model described in detail below.

Distribution of antimicrobial sales per species

Three different methods were used to extrapolate antimicrobial usage per species from sales data: Equal Distribution (ED), Biomass Distribution (BMD) and Longitudinal Study Extrapolation (LSE). Each method was exemplified using Swiss data, allowing for the calculation of estimates of antimicrobial consumption for several animal species from 2006 to 2013. Data

analyses were performed using R statistical software [30].

Consumption estimates are presented in mg per PCU when referring to total national consumption and in mg per kg of biomass when describing the consumption by specific animal species.

Equal Distribution

ED assumed that antimicrobial consumption was equal for each species a product was licensed for. Thus, the amount of antimicrobial product used by a species in a given year was calculated as follows:

$$C_{p_a, spec_a, y_a} = \frac{S_{y_a}}{\sum spec_n}$$

C: Consumption estimate; pa: a given product; ya: a given year; speca: a given species for which a product is licensed; specn: all the species for which a product is licensed; S: amount of product in sales.

The model was developed on a product basis. Calculated amounts of active ingredient belonging to the same antimicrobial class were summed for each year and animal species.

Biomass Distribution

In this method, the amount of product sold (in 2006–2013) was distributed proportionally to the relative importance of a species' total biomass at a national level. The analysis was done for each product individually, taking into account the animal species the product is licensed for and the corresponding annual biomass values. The calculation for every product was performed as follows:

$$C_{p_a, spec_a, y_a} = S_{y_a} * \frac{BM_{spec_a, y_a}}{\sum BM_{spec_n, y_a}}$$

C: Consumption estimate; pa: a given product; ya: a given year; speca: a given species a product is licensed for; specn: all the species a product is licensed for; S: amount of product in sales; BM: biomass.

Finally, the results were summed up for every combination of animal species, antimicrobial class and year.

Longitudinal Study Extrapolation

In this approach, estimates of the antimicrobial sales repartition per species (i.e. the amount of antimicrobials sold for use by each species) were derived from a Monte Carlo simulation, using PERT distributions to model the uncertainty of the data derived from the longitudinal study. This type of beta distribution is generated from three values: minimum (Min), mode and

maximum (Max). PERT distributions were created for every combination of antimicrobial class, year and animal species. The values in these distributions ranged from 0 to 1 and represented proportions of the total amount of sales for the respective antimicrobial class in a given year.

Min and Max were calculated by combining sales data with information from the Swiss Veterinary Drug Compendium [24]. Min was estimated by summing the amounts of monospecies products sold for each combination of antimicrobial class, animal species and year. Max was calculated as the sum of the amounts sold of all the products (monospecies and multispecies products) of a certain antimicrobial class licensed for a specific species, in a specific year.

Both Min and Max values were converted into a proportion of the total amount of antimicrobial for the same combination of antimicrobial class and year. In summary, Min and Max were calculated as follows:

$$Min_{spec_a,y_a,AMC_a} = \frac{\sum Mono_{spec_a,y_a,AMC_a}}{S_{y_a,AMC_a}}$$

$$Max_{spec_a,y_a,AMC_a} = \frac{\sum Mono_{spec_a,y_a,AMC_a} + \sum Multi_{spec_a,y_a,AMC_a}}{S_{y_a,AMC_a}}$$

Min: minimum of the PERT distribution; Max: maximum of the PERT distribution; Mono: monospecies products; Multi: multispecies products; speca: a given species a product is licensed for; ya: a given year; AMCa: a given antimicrobial class; S: amount of product in sales.

Mode values of the PERT distributions were based on data from the field study on antimicrobial prescription patterns in Switzerland [29]. Specifically, the total amount of active ingredient from each antimicrobial class prescribed for each species was divided by the total amount of authorised products for that same species and antimicrobial class. This value was then used to estimate a mode value between the Min and the Max. The mode for each combination of species, antimicrobial class and year was calculated as follows:

$$Mode_{spec_a,y_a,AMC_a}$$
$$= (\frac{F_{Mono\,spec_a,AMC_a} + F_{Multi\,spec_a,AMC_a}}{F_{Mono\,spec_n,AMC_a} + F_{Multi\,spec_n,AMC_a}}) * (Max_{spec_a,y_a,AMC_a}$$
$$- Min_{spec_a,y_a,AMC_a}) + Min_{spec_a,y_a,AMC_a}$$

Mode: mode of the PERT distribution; F: amount of antimicrobial from the field study; Min: minimum of the PERT distribution; Max: maximum of the PERT distribution; Mono: monospecies products; Multi: multispecies products; speca: a given species a product is licensed

for; ya: a given year; AMCa: a given antimicrobial class; specn:: all the species a product is licensed for.

Poultry was not included in the field study, and therefore mode values for the PERT distributions of this species group were calculated as the mean value of the Min and Max for each year.

The mode values were standardised so that they added up to 1 for each combination of antimicrobial class and year. For this, the mode values were recalculated proportionally to their species distribution in the longitudinal study.

The simulation model was then run 10,000 times, using the R package 'mc2d' [31], and the mean of the results of each iteration was calculated. This result represented the proportion of the total sales of a certain antimicrobial class in a given year that was sold for consumption by a given animal species. Due to the stochasticity of the model and the skewness of some distributions (particularly when the mode value was close to the Min or Max), the sum of the repartition per species was often different from 100% for each year/antimicrobial class combination. For that reason, the repartition values were standardised proportionally to each species estimates. The 95% credibility intervals for each estimate were calculated using the R package 'stats' [30]. Finally, these values were used to estimate, for each antimicrobial class and year, the amount of antibiotics sold for use by each of the animal species.

For the three models, results are presented at an antimicrobial class level as the total amount of antimicrobials sold in kg and as mg of active ingredient sold per kg biomass.

Due to confidentiality reasons, no results disclosing the sales of individual products or marketing authorisation holders can be presented. Thus, sales at an antimicrobial class level are not shown for some species.

Results

Descriptive statistics: veterinary antimicrobial sales data from Switzerland, 2006–2013
From 2006 to 2008 there was an increase in the sale of veterinary antimicrobial products from 67,423 kg of active ingredient to 72,300 kg. Starting in 2008, a steady decrease in sales was observed, resulting in a total reduction of 26.2% by 2013.

The amount of monospecies products sold throughout the study period ranged from 24.9% (2011) to 30.1% (2006) of the total amount of antimicrobial product sold. Multispecies products authorised for two species represented 51.6–56.4% of the total amount of antimicrobial sold in each of the 8 years considered.

When the total sales were converted into mg per PCU, it was observed that variations in animal demographics

FIGURE 1

Distribution of total antimicrobial sales per species according to the three different approaches. Switzerland, 2006–2013
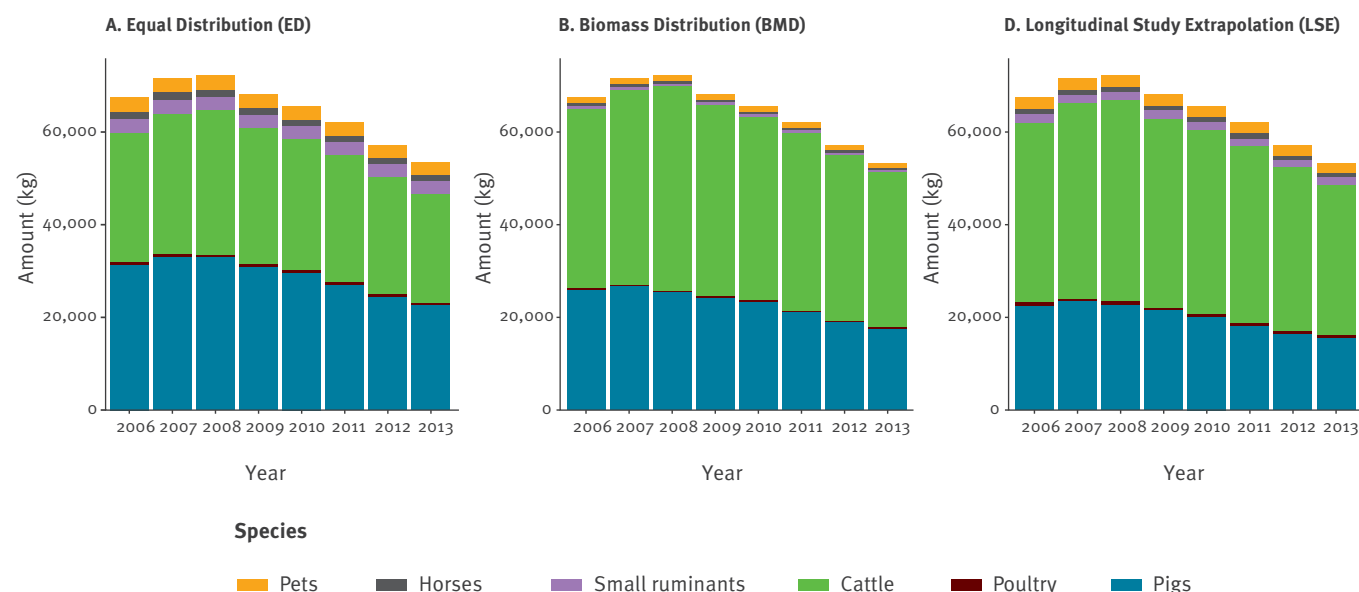
Equal Distribution (ED): equal sales repartition by the species the products are licensed for.

Biomass Distribution (BMD): sales repartition based on the relative total biomass of the species in the country.

Longitudinal Study Extrapolation (LSE): sales repartition based on results from a previous field study. Pets are defined as cats and dogs, while small ruminants include goats and sheep. Horses are included as a leisure/sports species.

did not influence the antimicrobial consumption pattern. Sales per PCU peaked in 2008 (87.9 mg/PCU); in 2013, the sale of veterinary antimicrobial products reached a minimum of 64.5 mg/PCU (Table 1).

PCU: population correction unit.

'Others' includes amphenicols, quinolones (other than fluoroquinolones), lincosamides, pleuromutilins

Sulfonamides, tetracyclines and penicillins were the antimicrobial classes sold the most throughout the years. Their contribution to the total sales ranged from 81.7% to 82.3% of the total mg per PCU. In parallel, sulfonamides and tetracyclines were the antimicrobial classes that contributed most to the observed decrease in antimicrobial consumption from 2008 to 2013, with decreases of 12.5 and 6.3 mg per PCU, respectively.

Equal Distribution
ED estimated that most of the antimicrobials (min–max, 2006–2013) were sold for use in pigs (42.6–46.4%) and cattle (41.4–44.1%). Over the years, a decrease in the sale of antimicrobials for pigs was observed. However, the proportion of antimicrobials sold for cattle increased, despite a reduction in the total amount of antimicrobials sold for this species (Figure 1).
The estimates for poultry revealed a negligible contribution to the overall sales (0.9–1.1%).

When using mg of antimicrobial per kg biomass (mg/BM) as a consumption metric, pets (145.4–179.5 mg/BM) and pigs (110.0–160.0 mg/BM) were the species that seemed to be under the greatest antimicrobial pressure. Antimicrobials for cattle ranged from 48.8 to 64.5 mg/BM (Table 2).

Biomass Distribution
Estimates from this model highlighted cattle as the species for which most antimicrobials were sold, with an increase in percentage from 57.6% in 2006 to 62.7% in 2013. Despite this result, the total amount of antimicrobials sold for cattle decreased from 38,809 kg to 33,446 kg in the same period. For pigs, percentages ranged from 38.7% in 2006 to 33.1% in 2013. For other species, the repartition estimates varied as follows: pets 1.7–1.9%; small ruminants 0.8–0.9%; horses 0.8–0.9%; poultry 0.3–0.9%.

Using mg/BM as a consumption metric, the values for the three main livestock species were between 86.3–124.4 mg/BM for swine, 69.3–90.9 mg/BM for cattle and 4.3–6.2 mg/BM for poultry (Table 2).

Longitudinal Study Extrapolation
With the exception of small ruminants (for which antimicrobial sales were relatively stable throughout the years), the LSE model calculated a reduction in antimicrobial consumption over time for all species. Total consumption of antimicrobials by cattle as

FIGURE 2

Relative distribution of the consumption of different antimicrobial classes for pigs and cattle, Switzerland, 2006–2013

A. Pigs

B. Cattle

Antimicrobial class

- Aminoglycosides
- Macrolides
- Penicillins
- Tetracyclines
- Cephalosporins/fluoroquinolones
- Others
- Sulfonamides/trimethoprim
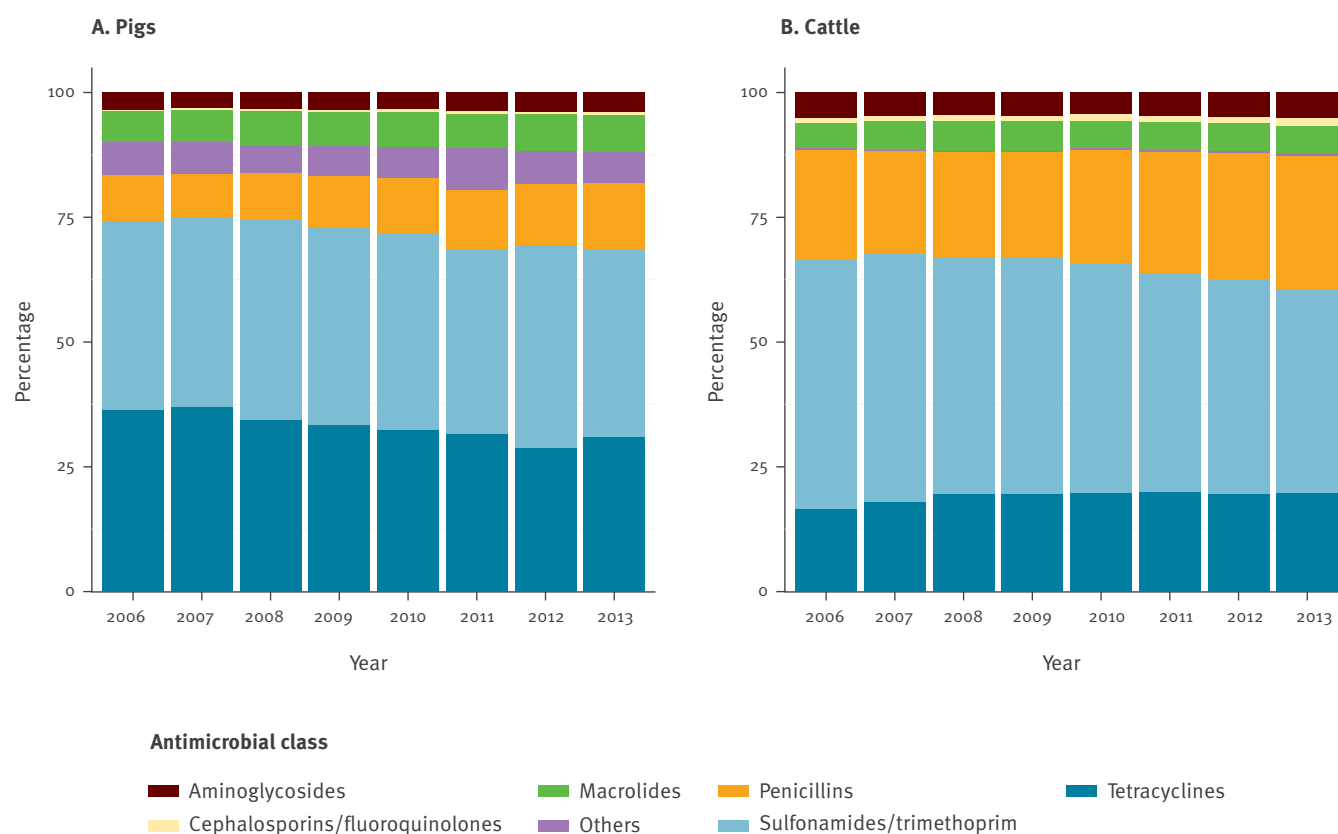
The estimates were obtained through the Longitudinal Study Extrapolation (LSE) approach: sales repartition based on results from a previous field study. 'Others' includes amphenicols, quinolones (other than fluoroquinolones), lincosamides, pleuromutilins and polymixins.

a percentage of consumption by all species was lowest in 2006, at 57.6% (33.2–72.8%) (mean (minimum of the 95% credibility interval–maximum of the 95% credibility interval)) and highest in 2012, at 61.8% (35.7–78.3%). The consumption of antimicrobials by pigs went in the opposite direction, with a minimum of 28.8% (12.4–55.1%) in 2012 and a maximum of 33.4% (17.9–58.1%) in 2006.

Despite some differences in terms of the relative proportion of consumption of different antimicrobial classes (Figure 2), estimates for pigs and cattle decreased over time in terms of mg/BM: the estimated consumption by cattle dropped from 81.6 mg/BM (47.1–103.3 mg/BM) in 2006 to 67.4 mg/BM (38.7–85.4 mg/BM) in 2013; for pigs, consumption estimates went down from 102.5 mg/BM (54.9–178.6 mg/BM) to 76.4 mg/BM (34.0–143.8 mg/BM) in the same time period.

For cattle, sulfonamides were the antimicrobial class that contributed the most to this decrease; for pigs, tetracyclines and sulfonamides were the classes for which consumption reduced the most (Figure 3).

Discussion

We compared different methods for stratifying antimicrobial sales data per animal species in Switzerland. This research follows the premise of the ESVAC project regarding the need to develop quantification methods of antimicrobial consumption at the species level [15].

The observed decrease of 26.2% in antimicrobial sales from 2008 to 2013 is most likely related to several concomitant reasons. Increased awareness by farmers and veterinarians about the issue of antimicrobial usage and resistance, due to several educational programmes by the FSVO, including the StAR programme [32], might have played a role in this decline. Viral diseases can lead to the use of antimicrobials to treat secondary infections [33,34]. For this reason, the implementation of the Bovine Viral Diarrhoea eradication programme and the commercialisation of Porcine Circovirus-2 vaccines might also have contributed to this reduction.

With regards to the results of the models, ED seemed to overestimate antimicrobial consumption for all species except cattle. ED does not consider variation in the levels of consumption by different species and does not take into account animal demographics, which might explain this overestimation. Indeed, it is likely

Estimated antimicrobial consumption of different antimicrobial classes for pigs and cattle, Switzerland, 2006–2013
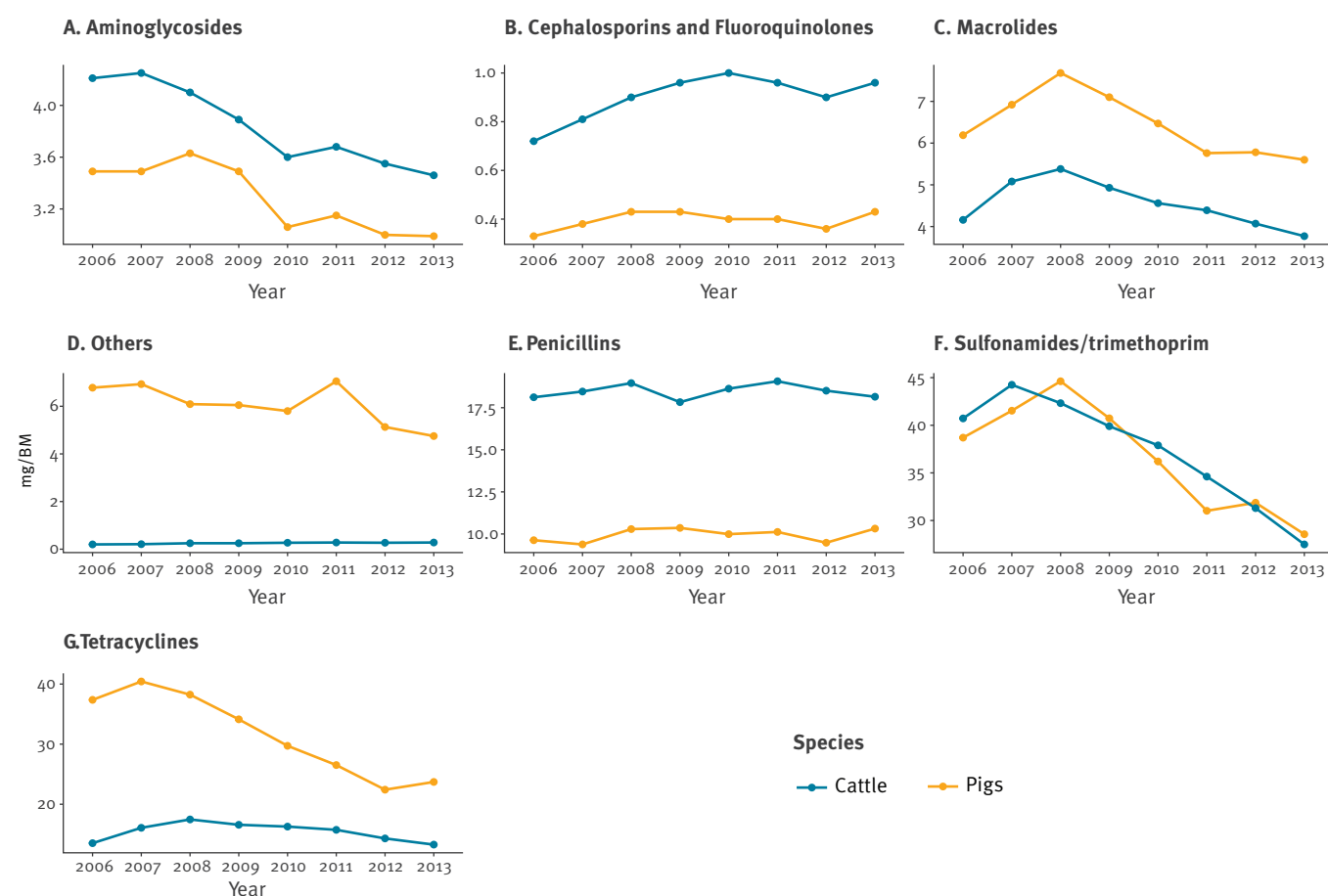
Results are presented in mg per kg of biomass (mg/BM). The estimates were obtained through the Longitudinal Study Extrapolation approach (LSE): sales repartition based on results from a previous field study. 'Others' includes amphenicols, quinolones (other than fluoroquinolones), lincosamides, pleuromutilins and polymixins.

that differences exist in the species repartition for multispecies products, especially in those shared between livestock and pet animals. Moreover, in Switzerland, the total cattle biomass was higher than for other species [35], largely due to the large number of dairy cattle. Given that this method did not take into account the existing number of animals in the country, it probably underestimated the usage for species with a higher total biomass and overestimated the usage for species with a lower total biomass.

The differences in the species total biomass values substantially influenced the BMD estimates, as biomass is the main driver for the repartition of sales data when using this approach. It was therefore not surprising that estimates of antimicrobial consumption by cattle were higher than those from ED. Concomitantly, extrapolated consumption by species with a lower biomass but high treatment intensity might have been underestimated.

For cattle and pigs, estimates from BMD and LSE were similar throughout the study period. The maximum difference between the two approaches was 16.2 mg/BM for pigs and 1.9 mg/BM for cattle. For the other species, LSE estimates tended to lie between ED and BMD results. The BMD approach seems to be a useful method when field data are not available. Nevertheless, extrapolation of this method to other countries should be done with care, as it is highly dependent on animal demographics.

The LSE method calculated a steady decrease in antimicrobial consumption throughout the studied years for most of the species. Small ruminants were an exception and were associated with a minor increase over the study period, from 44.9 mg/BM (2.1–130.5 mg/BM) to 45.9 mg/BM (2.0–133.7 mg/BM). Nevertheless, it should be highlighted that the uncertainty around these estimates is large.

In poultry, a steep decrease in the estimates of antimicrobial consumption (from 21.0 mg/BM (6.9–34.9 mg/

Table 1

Sales (mg per population correction unit) for different antimicrobial classes in Switzerland, 2006–2013

Veterinary antimicrobial sales in mg per PCU								
	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Aminoglycosides	4.6	4.6	4.5	4.3	3.9	4.0	3.9	3.8
Cephalosporins	0.6	0.6	0.6	0.7	0.7	0.7	0.7	0.6
Fluoroquinolones	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.5
Macrolides	4.4	4.9	5.2	4.9	4.6	4.2	4.0	3.8
Penicillins	15.9	15.9	16.6	15.8	16.1	16.4	15.9	15.8
Polymyxins	2.3	2.0	1.9	1.9	1.8	1.7	1.3	1.0
Sulfonamides/trimethroprim	35.8	38.2	37.7	35.1	32.9	29.6	27.6	24.3
Tetracycline	18.5	20.5	20.3	18.8	17.7	16.5	14.5	14.1
Others	0.4	0.6	0.5	0.5	0.5	0.9	0.7	0.7
Total	82.9	87.8	87.9	82.5	78.6	74.5	68.7	64.5

BM) to 13.9 mg/BM (4.8 – 22.6 mg/BM)) was observed from 2006 to 2007. This change might be a model artefact and not a true reduction in antimicrobial consumption. As field data were unavailable for poultry, mode values of PERT distributions might not have been very accurate, especially for those antimicrobial classes where the difference between the Min and the Max was more accentuated. In those cases, it is likely that the mode used to represent poultry's antimicrobial consumption was overestimated. In addition, this steep decrease might be partly related to the discontinuation of some products licensed for poultry between 2006 and 2008.

Pigs showed the largest decrease in antimicrobial consumption. We estimated that the pig producing industry had a particularly large antimicrobial consumption in the beginning of the study, and thus more opportunities to reduce usage were available, which might partially explain this steep decrease. Furthermore, the use of vaccines against Porcine Circovirus-2 and porcine proliferative enteritis (*Lawsonia intracellularis*) might also have played a role in the reduction of antimicrobial sales for use in pigs.

The wide use of Porcine Circovirus-2 vaccination in Switzerland might be associated with a lower prevalence of respiratory disease [36]. Tetracyclines are the main class used to treat respiratory disease in pigs [37]. We investigated whether LSE was able to capture this decline in specific antimicrobial classes. Indeed, tetracycline consumption showed a decline by 38.0% in pigs (larger than the decline for any other species) between 2008 and 2013.

On an antimicrobial class level, differences were observed between the classes used to treat pigs and cattle. Despite the general decrease in the usage of most antimicrobial classes, a slight increase in the consumption of cephalosporins and fluoroquinolones was estimated for cattle and pigs.

One of the most relevant benefits of having antimicrobial consumption estimates at the species level relates to the possibility of analysing them together with the resistance patterns from the national monitoring system. In Switzerland, indicator (*Escherichiacoli* and *Enterococcus* spp.) and zoonotic (*Salmonella* spp. and *Campylobacter* spp.) isolates from the three main livestock species (cattle, pigs and poultry) are collected every year. Regarding zoonotic bacteria, a general decrease in resistance was observed in Salmonella throughout the study period. This is in line with the reduction in antimicrobial consumption in the same period. For *Campylobacter jejuni* collected from poultry, a rise in the prevalence of ciprofloxacin resistant isolates was observed, from 12.0% in 2006 to 41.4% in 2013. In the same period, an increase in the consumption of fluoroquinolones was observed. This is of particular relevance for public health given that fluoroquinolones are the treatment of choice for severe Campylobacter infections. With respect to *Campylobacter coli* from pigs, a fairly stable level of resistant isolates was found [38]; the level of antimicrobial consumption did not seem to influence the resistance pattern observed.

Concerning the indicator bacteria, it is interesting to note that an increase of ciprofloxacin-resistant *E.coli* isolates was observed for poultry, which was more pronounced than that for other species. On the other hand, streptomycin resistance in *E. coli* was higher for cattle and pigs when compared with broilers. This might be related to the lack of licensed aminoglycoside products for use in Swiss poultry production [38].

It is important to highlight that when assessing temporal associations between antimicrobial usage data and resistance, other factors need to be taken into account, such as cross- and co-resistance, as well as the emergence and selection of specific clones. A comprehensive analysis of these temporal patterns and the effect

TABLE 2

Antimicrobial consumption (mg per kg of biomass) for different animal species according to the three approaches in Switzerland, 2006–2013

Antimicrobial consumption in mg per kg of biomass																		
Year	Pigs			Poultry			Cattle			Small ruminants			Horses			Pets		
	ED	BMD	LSE	ED	BMD	LSE	ED	BMD	LSE	ED	BMD	LSE	ED	BMD	LSE	ED	BMD	LSE
2006	142.6	118.7	102.5 (54.9–178.6)	20.7	6.2	21.0 (6.9–34.9)	58.7	81.6	81.6 (47.1–103.3)	69.6	14.1	44.9 (2.1–130.5)	70.6	25.5	48.4 (15.7–116.0)	178.1	68.7	142.1 (58.7–300.7)
2007	153.6	124.6	109.1 (56.7–192.3)	13.4	5.1	13.9 (4.8–22.6)	64.2	89.1	89.1 (51.3–113.2)	72.0	14.4	45.3 (2.0–133.2)	72.8	27.7	49.3 (17.0–115.8)	174.6	70.9	139.0 (59.3–291.5)
2008	160.0	124.4	111.0 (54.0–201.3)	12.6	5.2	13.9 (5.0–22.6)	64.5	90.9	89.4 (50.8–113.7)	71.4	14.4	45.9 (2.2–133.9)	66.3	25.8	47.2 (16.4–110.1)	179.5	70.8	145.3 (60.9–302.5)
2009	147.5	115.9	102.3 (49.8–186.4)	12.4	5.1	12.7 (4.4–20.7)	60.6	85.2	84.3 (48.0–107.2)	70.9	13.8	44.9 (2.1–130.5)	61.9	21.8	42.9 (14.3–103.1)	175.1	70.7	141.9 (60.9–294.3)
2010	135.0	107.1	91.6 (42.1–170.2)	12.6	5.2	12.8 (4.5–21.2)	58.7	81.9	82.2 (46.0–104.7)	70.4	13.2	45.4 (2.1–132.5)	60.1	23.2	40.8 (15.1–94.3)	178.0	69.4	144.9 (61.0–304.6)
2011	124.6	97.8	84.0 (36.6–160.3)	12.9	5.6	12.2 (4.3–21.3)	56.8	79.4	78.7 (44.7–101.0)	70.6	13.2	45.0 (2.1–131.7)	62.3	24.2	49.3 (24.0–102.7)	168.0	67.0	137.3 (59.2–281.7)
2012	115.9	90.3	78.0 (33.7–149.4)	10.1	4.6	9.0 (3.2–15.4)	52.3	73.9	72.9 (42.2–92.4)	72.4	13.4	46.5 (2.1–134.6)	60.5	22.5	40.5 (13.3–97.3)	156.9	60.6	126.7 (52.6–263.9)
2013	111.0	86.3	76.4 (34.0–143.8)	8.8	4.3	8.2 (2.8–14.3)	48.8	69.3	67.4 (38.7–85.4)	71.9	13.3	45.9 (2.0–133.7)	54.3	19.6	36.6 (10.9–90.5)	145.4	57.3	117.3 (47.1–248.0)

BMD: biomass distribution; ED: equal distribution; LSE: longitudinal study extrapolation

Results for the three approaches:

ED: equal sales repartition by the species the products are licensed for.

BMD: sales repartition based on the relative total biomass of the species in the country.

LSE: sales repartition based on results from a previous field study) presented in mg per kg biomass. Results from LSE are presented as: mean (minimum 95% credibility interval – maximum 95% credibility interval). Goats and sheep were grouped together as 'small ruminants'; cats and dogs were grouped together as 'pets'. The species 'horses' is mainly represented by leisure animals

of antimicrobial consumption on the resistance of animal isolates should be performed.

Although it is not possible to validate the models, we are convinced that the LSE approach provided the best estimates. In this approach, input data for the model are derived from a longitudinal field study. These data are closer to the actual usage of antimicrobials than sales data and are therefore more likely to reflect reality. Nonetheless, the LSE method also presents some potential bias. In the first place, the field data that fed the model were from the period 2004–2005. Consumption patterns may have changed since then. However, product repartition values are not expected to vary greatly from year to year. In Switzerland, marketing authorisation holders update their repartition estimates every 5 years. These estimates are used in the Periodic Safety Update Reports (PSURs) for calculating the incidence of adverse reactions. Nonetheless, we recommend performing field studies more frequently when applying this method to yearly monitoring. Another uncertainty might arise from the number of animals at risk of being treated in the field study, which was estimated from the participating veterinarians or calculated based on the number of farms/owners. This may have introduced some bias into the extrapolation of the field study results to a national level. When applying this method, it is advisable to have accurate estimates of the number of animals at risk of being treated in the field study. In addition to the methods presented, data for antimicrobial sales repartition per species might be obtained by asking the marketing authorisation holders [21]. It has not yet been possible to apply this valuable approach in Switzerland due to data limitations. Likewise, repartition estimates from PSURs can also provide a basis for sales stratification.

We presented three methods for extrapolating antimicrobial consumption per animal species from sales data. These approaches could be of use for countries which have not implemented detailed monitoring systems and which base their schemes on overall sales data. The best model choice in a given situation will depend on data availability. Results must always be interpreted in the light of data availability and country characteristics, and the limitations of each model must be considered. We shall also highlight that having consumption data per species enables the calculation of treatment incidence metrics, which better describe exposure to antimicrobials than mg per PCU. The LSE approach might also be of relevance for monitoring systems that rely on compliance of the people prescribing and administering antimicrobials to animals. In cases of imperfect compliance, a model that repartitions total sales data per species allows comparison of recorded vs expected amounts used. This might be very useful for targeted interventions to improve data quality of the monitoring system.

Conflict of interest

None declared.

Authors' contributions

LPC analysed the data, interpreted the results and wrote the manuscript. CM provided expertise on the topic and assisted on the sales database management and interpretation. AC and GM contributed to the conception of the methods. GSR and IM designed the study. Furthermore, IM assisted the first author (LPC) in all steps of the study. The manuscript was revised by all the authors.

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