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Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014

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We report the first detection of tetrodotoxins (TTX) in European bivalve shellfish. We demonstrate that TTX is present within the temperate waters of the United Kingdom, along the English Channel, and can accumulate in filter-feeding molluscs. The toxin is heat-stable and thus it cannot be eliminated during cooking. While quantified concentrations were low in comparison to published minimum lethal doses for humans, the results demonstrate that the risk to shellfish consumers should not be discarded.

Background

Tetrodotoxin (TTX) is the causative agent responsible for pufferfish/fugu poisoning, a fatal marine poisoning found predominantly in tropical regions. It is found mainly in the organs of fish from the *Tetraodontidae* family, as well as other marine species such as the blue-ringed octopus and gastropods [1]. The toxin and its structural analogues are thought to originate from a variety of marine bacteria, including *Vibrio* spp. [2].

Clinical effects include a range of neuromuscular symptoms such as paraesthesia of lips and tongue, dizziness and headache, together with gastrointestinal symptoms such as nausea, abdominal pain, diarrhoea and vomiting. Higher degree symptoms include ataxia, incoordination, cardiac arrhythmias, seizures and respiratory failure, leading to death [3]. To date, the only reported occurrences of TTX in bivalve molluscs (clams, cockles, mussels, oysters, scallops and others) have been in New Zealand clams [4] and in Japanese scallops [5]. In European seafood, the only reported occurrence was in 2007. It was detected in the course of a non-fatal human intoxication following consumption of the contaminated sea snail *Charonia lampas lampas* (a gastropod) harvested in Spain [6]. There has been no evidence for the accumulation of tetrodotoxin in bivalve molluscs grown within European waters to date, and the threat from this toxin is deemed negligible within the European Union. However, with *Vibrio* spp., reported to be associated with TTX production, detected in United Kingdom shellfish in 2010 [7], and evidence for increasing sea temperatures [8], we aimed to assess the potential for this toxin to accumulate in bivalves grown on the south coast of England, along the Channel.

Testing of bivalve shellfish samples

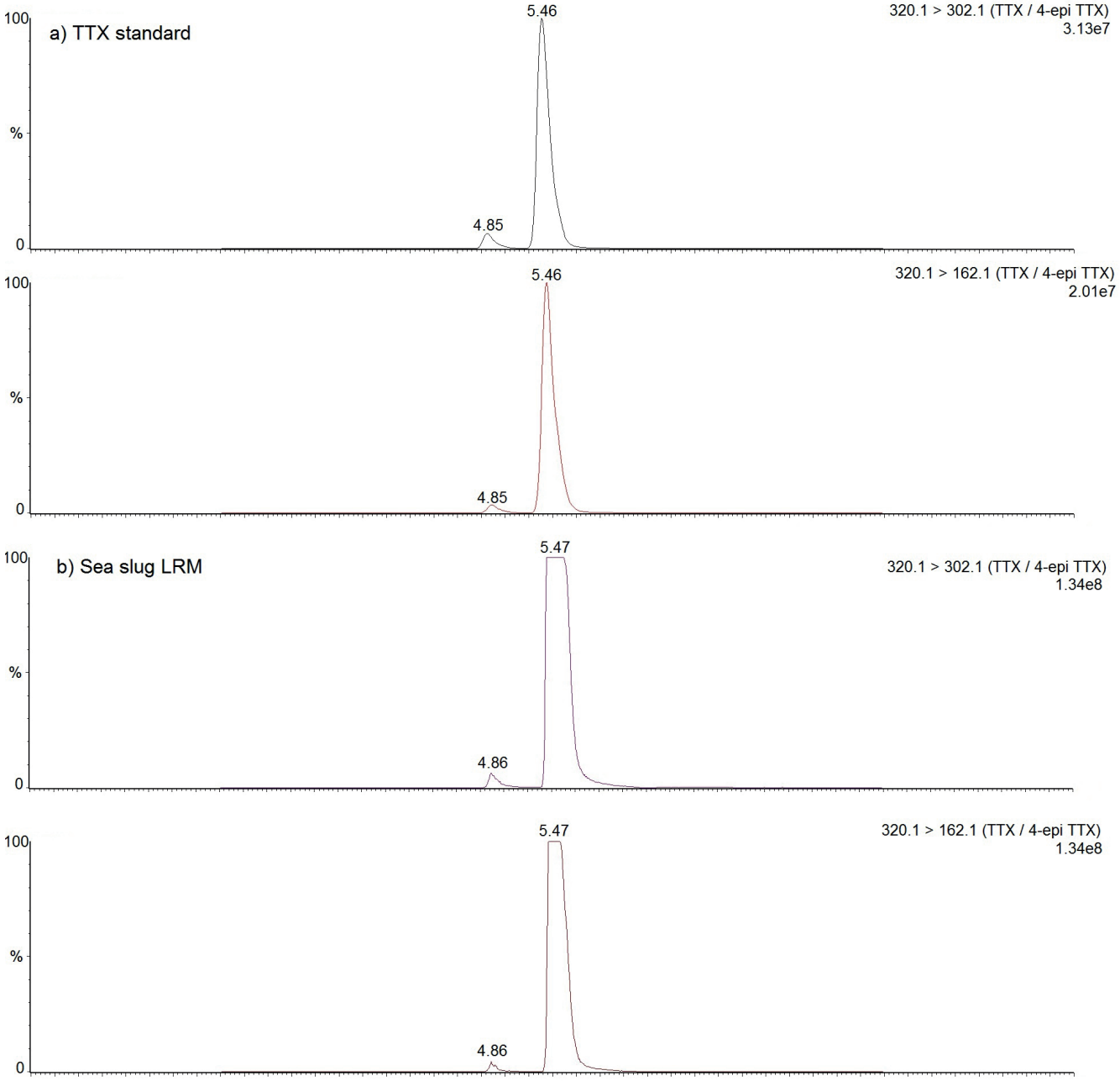
Twenty-nine shellfish samples (*Mytilus Edulis* and *Crassostrea gigas*), each comprising a minimum of 20 live animals, were harvested between February 2013 and October 2014 from two marine sites on the south coast of England. After shucking, shellfish tissue was prepared for bacterial pathogen detection as previously described [9] and the remainder frozen in storage before chemical analysis. TTXs were analysed in thawed, homogenised shellfish tissues following the methods described by McNabb et al. [4], with a modified shellfish extraction procedure based on [10] and incorporating additional TTX analogues taken from [11]. Hydrophilic interaction chromatography (HILIC) using an ultra performance liquid chromatograph (UPLC) with electrospray ionisation tandem quadrupole mass spectrometry (MS/MS) was used for detection of TTXs. The TTX standard was sourced from Enzo Life Sciences (Exeter, UK).

Two selected reaction monitoring (SRM) transitions were optimised for each of the seven tested toxins, enabling the quantification of toxin concentrations against an external TTX calibration. A Waters Acquity UPLC and Xevo TQ-S MS/MS were optimised for detection of TTX and six TTX congeners (4-epi TTX; 5,6,11-trideoxy TTX; 4,9-anhydro TTX; 11-nor TTX-6-ol; monodeoxy TTX; 11-oxo TTX) based on previous studies [4,11]. Semiquantitation of TTX analogues was conducted assuming a relative response factor of 1 to the parent TTX. A second HILIC-MS/MS method based on the detection of TTX dehydration products (C_9 base 2-amino-6-(hydroxymethyl)quinazolin-8-ol) following alkaline derivatisation, was used for additional confirmation [4].

Additionally, *Vibrio parahaemolyticus* isolated from six of the shellfish samples, and confirmed by PCR targeting species specific markers [9] were cultured in the

Figure A

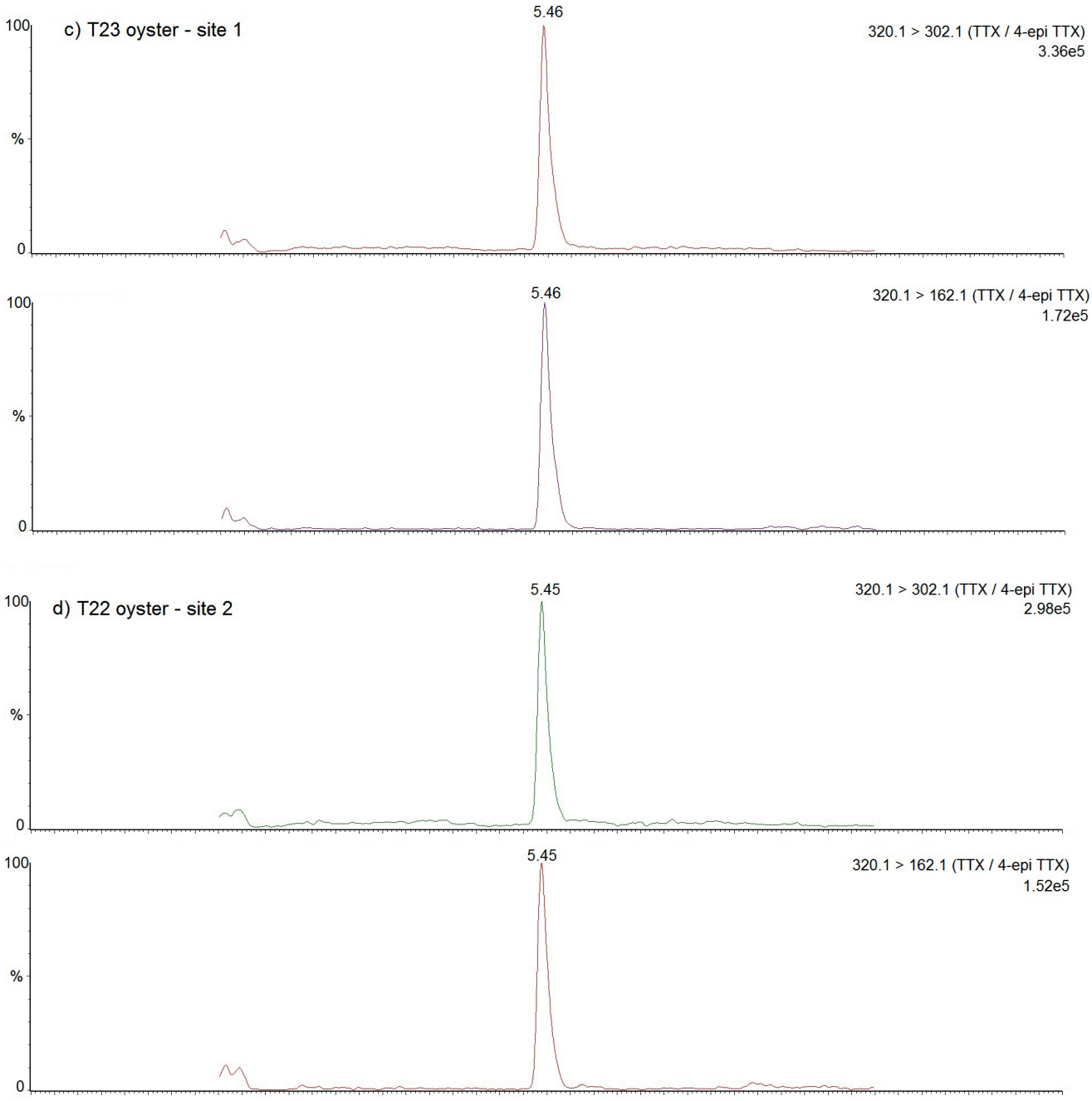
Selected reaction monitoring chromatograms obtained following the analysis of tetrodotoxin (TTX) in TTX calibration standard (a), laboratory reference material (b), T23 oyster (c), T22 oyster (d), culture APC6 (e)



LRM: laboratory reference material; TTX: tetrodotoxin.

Figure B

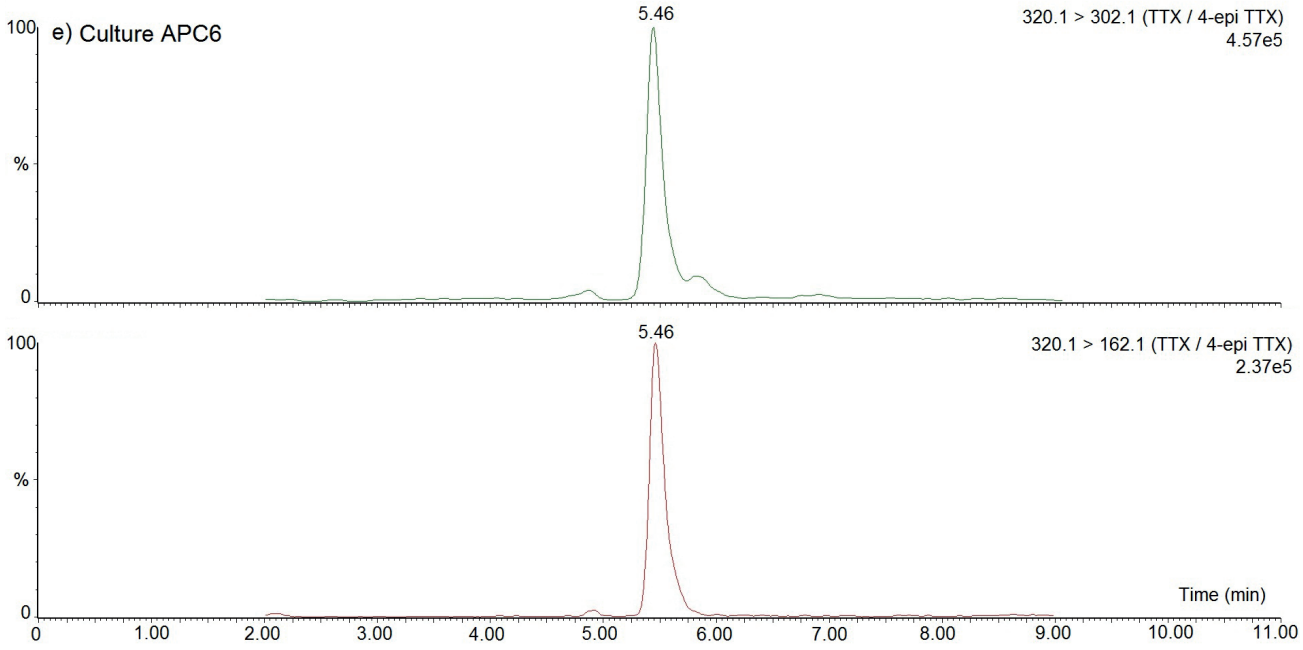
Selected reaction monitoring chromatograms obtained following the analysis of tetrodotoxin (TTX) in TTX calibration standard (a), laboratory reference material (b), T23 oyster (c), T22 oyster (d), culture APC6 (e)



LRM: laboratory reference material; TTX: tetrodotoxin.

Figure C

Selected reaction monitoring chromatograms obtained following the analysis of tetrodotoxin (TTX) in TTX calibration standard (a), laboratory reference material (b), T23 oyster (c), T22 oyster (d), culture APC6 (e)



LRM: laboratory reference material; TTX: tetrodotoxin.

laboratory and tested for TTXs. Cultures were centrifuged and the bacterial pellets extracted in 1% acetic acid before HILIC-MS/MS analysis. Analysis of all unknown samples was conducted alongside two sets of six-level calibration standards and a highly TTX-positive laboratory reference material (LRM) extract prepared from New Zealand Sea Slugs (*Pleurobranchaea maculata*) [4].

Results

Bivalve shellfish samples

Eleven of 29 shellfish samples were found to contain *V. parahaemolyticus* in the shellfish tissue, with one additional sample found to be positive for *V. cholerae*. TTX was detected in 14 of 29 samples, with detection confirmed through the presence of chromatographic peaks for both the primary (quantifier) and secondary (qualifier) SRM transitions, at the same retention time as the TTX standard calibrants and in the LRM (Figures A, B).

The mean primary to secondary SRM peak ratios were 1.87±0.13 (7%) for the TTX standards and 1.83±0.26 (14%) for the average of all TTX-positive samples.

4-epi TTX was identified in five out of 29 samples, notably those containing the highest TTX concentrations. 5,6,11-trideoxy TTX and 4,9-anhydro TTX were detected in 13 and one sample respectively, with detection confirmed with SRM peaks at the same retention time as those present in the LRM. Detection and semi-quantitation of the C_9 base product provided a further level of TTX confirmation in the five samples containing the highest concentrations of toxin. The absence of the C_9 base product in samples containing lower concentrations of TTX is thought to relate to differences in method sensitivity. TTX concentrations ranged from approximately the limit of quantitation (3 µg TTX/kg shellfish tissue) to a maximum of 120 µg/kg. TTX analogues were quantified at lower levels, typically 10–15% of the total TTX content (Table 1). The maximum summed concentration quantified of all TTX analogues was 137 µg TTX/kg in sample T23.

Tetrodotoxins in bacterial cultures

Eleven bacterial isolates were obtained from six different TTX-contaminated bivalve samples. These were cultured for two days, before being processed for TTX analysis. Ten of the cultures were *V. parahaemolyticus*, with the other isolate *V. cholerae*. TTX was detected in ten of the cultures (Figure C), at concentrations between 42 and 718 ng TTX/L of culture (Table 2), with TTX the only analogue detectable in any of the cultured samples.

Discussion

Our study reveals, to our best knowledge, the first detection of the causative agent of pufferfish/fugo

Table 1

Analysis of bivalve molluscs for *Vibrio parahaemolyticus* and tetrodotoxins, England, 2013–2014

Sample	Date of collection	Site info	Species	Vibrio	TTX[a]	4-epi TTX[a]	5,6,11-trideoxy TTX[a]	4,9-anhydro TTX[a]	C9 base of TTX[a]
T1	30 Oct 2013	Site 1	PO	ND	ND	ND	5.1	ND	ND
T2	17 Dec 2013	Site 1	PO	ND	ND	ND	2.8	ND	ND
T3	17 Dec 2013	Site 2	PO	ND	11	ND	ND	ND	ND
T4	26 Feb 2014	Site 1	PO	ND	ND	ND	4.4	ND	ND
T5	26 Nov 2014	Site 2	PO	ND	5.6	ND	ND	ND	ND
T6	29 Oct 2013	Site 2	PO	ND	4.4	ND	ND	ND	ND
T7	29 Jan 2014	Site 2	M	Y	3.0	ND	ND	ND	ND
T8	29 Jan 2014	Site 2	PO	Y	ND	ND	ND	ND	ND
T9	26 Feb 2014	Site 2	PO	ND	ND	ND	ND	ND	ND
T10	26 Feb 2014	Site 2	M	ND	ND	ND	ND	ND	ND
T11	17 Dec 2014	Site 2	PO	ND	ND	ND	ND	ND	ND
T12	29 Jan 2014	Site 1	PO	ND	ND	ND	2.4	ND	ND
T13	27 Aug 2013	Site 1	PO	ND	7.6	ND	ND	ND	ND
T14	25 Nov 2013	Site 1	PO	ND	ND	ND	2.8	ND	ND
T15	29 Feb 2013	Site 1	PO	Y	52	2.0	3.4	ND	37
T16	27 Aug 2013	Site 2	PO	Y	14	ND	4.3	ND	ND
T17	26 Feb 2014	Site 2	PO	Y	15	ND	1.3	ND	ND
T18	26 Feb 2014	Site 2	M	ND	ND	ND	ND	ND	ND
T19	31 Oct 2013	Site 2	PO	ND	ND	ND	ND	ND	ND
T20	29 Jul 2013	Site 2	PO	Y	14	0.4	3.1	ND	ND
T21	27 Aug 2013	Site 2	PO	ND	2.7	ND	ND	ND	ND
T22	17 Jun 2014	Site 1	PO	Y†	89	2.8	6.5	ND	76
T23	17 Jun 2014	Site 2	PO	Y	120	3.9	11	1.8	121
T24	17 Jun 2014	Site 2	M	Y	39	1.2	3.8	ND	28
T25	25 Nov 2013	Site 2	M	ND	ND	ND	ND	ND	ND
T26	29 Jul 2013	Site 2	PO	Y	15	ND	1.9	ND	22
APF1	15 Sep 2014	Site 2	PO	Y	ND	ND	ND	ND	ND
APF2	15 Sep 2014	Site 2	M	Y	ND	ND	ND	ND	ND
APF3	16 Sep 2014	Site 1	PO	ND	ND	ND	ND	ND	ND

M: mussels; ND: not detected; PO: Pacific oyster; TTX: Tetrodotoxin; Y: *Vibrio* spp. detected; Y†: *Vibrio cholera* detected.
[a] µg per kg shellfish tissue.

poisoning, TTX in bivalve molluscs, mussels and Pacific oysters harvested in Europe. It is also the first detection of TTX in any form within the marine waters of the UK. TTXs are not monitored routinely anywhere in the world for their presence in bivalves, given the absence of published data demonstrating a risk of TTX intoxication from bivalves. The findings reported here are notable given the established assumption that TTXs are associated either with pufferfish or with marine bacteria found exclusively in tropical and sub-tropical oceans and seas [3,6]. Here we provide new evidence for the presence of TTX in the temperate waters of the English Channel, thereby extending the range of known occurrences of these important toxins.

TTX was quantified against known standards, with confirmation in positive samples coming from the acquisition of two SRMs, toxin retention time checks and determination of SRM ion ratios. Further confirmation was achieved through detection of TTX C_9 base products. Toxin profiles in the bivalve shellfish were dominated by the parent toxin. With *Vibrio* cultures containing only TTX, the analogues may result from metabolism by shellfish, as opposed to direct bacterial products. The overall concentrations of TTX were lower than those quantified previously in a sample of the New Zealand bivalve *Paphies australis* [4]. Interestingly, here also the parent TTX was the only analogue detected in the bacterial culture samples.

Table 2

Analysis of bacterial cultures for tetrodotoxins, England, 2013–2014

Culture sample	Known pathogen	TTX ng/L in culture	Associated shellfish sample
APC 1	*Vibrio parahaemolyticus*	59	T23
APC 2	*V. parahaemolyticus*	67	T24
APC 3	*V. parahaemolyticus*	42	T26
APC 4	*V. cholerae*	84	T22
APC 5	*V. parahaemolyticus*	117	T17
APC 6	*V. parahaemolyticus*	718	T24
APC 7	*V. parahaemolyticus*	62	T23
APC10	*V. parahaemolyticus*	ND	T23
APC11	*V. parahaemolyticus*	103	T24
APC13	*V. parahaemolyticus*	84	T23
APC14	*V. parahaemolyticus*	116	T24

ND: not detected; TTX: tetrodotoxin.

The detection of TTX in all but one of the *V. parahaemolyticus* cultures isolated from bivalve molluscs may be significant, providing additional compelling evidence for the production of TTX by *Vibrio* spp. The detection of quantifiable levels of TTX in the bivalves in tandem with the detection of *Vibrio* spp., strengthens the possibility that the bacteria provide the source of the toxin detected in bivalve molluscs, however, further work in this area is clearly necessary. Interestingly, not all TTX-positive bivalves were found to contain *Vibrio* species, while three of the *Vibrio*-positive bivalve samples showed no TTX above the limit of detection. However, in the absence of quantitative data for *Vibrio*, these differences may relate to differences in method sensitivities.

Given the absence of any formal regulatory guidance of TTX in shellfish, the maximum concentration of 137 µg/kg TTX quantified here, equates to 17% of the maximum permitted level of saxitoxin (STX) equivalents (800 µg STX equivalents/ kg shellfish tissue), noting the similarity in biological activity between the two toxin groups. 137 µg/kg would also equate to a low level dose of toxin in comparison to the proposed minimum lethal dose (MLD) for TTX of between 0.5 to 2 mg [3]. Consumption of 500g of shellfish contaminated with 137 µg/kg of TTXs would equate to the intake of ca70 µg TTX, ca 14% of the proposed MLD if taken as 0.5 mg TTX for a 60 kg human [12]. However, this calculation does not incorporate any additional safety factors as applied by the European Food Standards Agency (EFSA) in their risk assessment methods, taking into account measurement or toxicity-related uncertainties [13], and/or the likely high variability of toxin content in bulk samples of shellfish across harvesting areas.

Consequently, while the human health risk determined from the samples analysed in this study is shown to be low, there is the potential for health impacts, particularly if the levels of TTX were significantly higher at other times or in other areas associated with shellfish harvesting. It is important to note that while bacterial pathogens may be eliminated in shellfish products following effective cooking, TTXs are heat stable and will thus not be destroyed in the food preparation process.

Given the evidence presented here for TTX occurrence in European bivalve molluscs, and the traditional occurrence of these toxins in warm tropical waters, an important question is whether this is linked to increasing sea surface temperatures. The frequency of extreme hot days has increased significantly in the last decade along the margins of the east Atlantic, most notably in the North Sea and English Channel. The frequency of extreme cold periods has also gone down and annual warming is seen to occur earlier in the year on average [8].

Conclusions

We reveal the presence, for the first time, of the neurotoxin tetrodotoxin in bivalve mollusc shellfish grown at two marine sites along the south coast of England. These toxins have previously been assumed not to occur in bivalve molluscs, particularly in temperate waters. Further, we found an association between the occurrence of TTX and marine *Vibrio* species both in bivalve molluscs and in bacterial cultures. Given the increasingly favourable conditions for *Vibrio* proliferation in European waters as sea surface temperatures will possibly rise in the coming decades, we suggest that the potential for occurrence of autochthonous marine bacteria such as *Vibrio* and TTXs in seafood grown in temperate areas should be more widely investigated.

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Conflicts of interest

None declared.

Authors' contributions

AT and AP designed the study. AP performed the sample preparation and bacterial analysis. CBA performed molecular confirmation of Vibrio strain. AS and AT extracted and SPE-cleaned the shellfish. AT performed HILIC-MS/MS quantitation of TTXs in shellfish extracts and bacterial cultures. AT, AP, DL and CBA discussed the results and participated in the writing.

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2012/13 influenza vaccine effectiveness against hospitalised influenza A(H1N1)pdm09, A(H3N2) and B: estimates from a European network of hospitals

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While influenza vaccines aim to decrease the incidence of severe influenza among high-risk groups, evidence of influenza vaccine effectiveness (IVE) among the influenza vaccine target population is sparse. We conducted a multicentre test-negative case–control study to estimate IVE against hospitalised laboratory-confirmed influenza in the target population in 18 hospitals in France, Italy, Lithuania and the Navarre and Valencia regions in Spain. All hospitalised patients aged ≥18 years, belonging to the target population presenting with influenza-like illness symptom onset within seven days were swabbed. Patients positive by reverse transcription polymerase chain reaction for influenza virus were cases and those negative were controls. Using logistic regression, we calculated IVE for each influenza virus subtype and adjusted it for month of symptom onset, study site, age and chronic conditions. Of the 1,972 patients included, 116 were positive for influenza A(H1N1)pdm09, 58 for A(H3N2) and 232 for influenza B. Adjusted IVE was 21.3% (95% confidence interval (CI): -25.2 to 50.6; n=1,628), 61.8% (95% CI: 26.8 to 80.0; n=557) and 43.1% (95% CI: 21.2 to 58.9; n=1,526) against influenza A(H1N1) pdm09, A(H3N2) and B respectively. Our results suggest that the 2012/13 IVE was moderate against influenza A(H3N2) and B and low against influenza A(H1N1) pdm09.

Background

Antigenic drifts of influenza viruses expose the population to new but related influenza variants on a regular basis [1]. On the basis of a yearly revised composition of seasonal influenza vaccines, the World Health Organization (WHO) considers annual Influenza vaccination as the most efficient measure against influenza [2]. Every year, the seasonal influenza vaccine licensure is obtained based on immunogenicity data [3]. While these immunogenicity data are thought to be valid for healthy adults [4], the development of correlates of protection suited to vulnerable populations is still to be achieved [5].

The population targeted for influenza vaccination in Europe includes those at increased risk of exposure to influenza virus as well as of developing severe disease, especially disease resulting in hospitalisation or death [6]. Target groups for vaccination usually include adults over 59 or 64 years of age and people of any age with certain underlying medical conditions [7,8]. Measuring influenza vaccine effectiveness (IVE) in each influenza season is important for the following reasons: to identify vaccines types and brands with low IVE; to decide on alternative preventive strategies if early estimates of IVE are low (e.g. preventive use of antivirals among vulnerable individuals); and to help decide on the next season's vaccine content. Repeated evidence of sub-optimal IVE among the population targeted for annual

Table 1

Generic protocol adaptations in each study site, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Protocol adaptation	France	Italy	Lithuania	Spain	
				Navarre	Valencia
Additonal staff for the study	Yes	Yes	No	No	Yes
Services	Emergency ward	Emergency ward internal medicine unit	Emergency ward Infectious disease hospital	All	Emergency ward
Vaccine status ascertainment	Patient	Patient or GP	Patient or GP	Register	Register and oral
Ascertainment of type of vaccine used	Ecological data	Individual data	Ecological data	Individual data	Individual data
Exclusion based on place of residence	No	No	No	Yes	Yes
Inclusion of patients unable to sign the consent form	Yes	Yes	No	Yes	Yes
Type of respiratory specimen	Nasal	Nasal and pharyngeal	One pharyngeal and two nasal	Nasal and pharyngeal	Nasal and pharyngeal
Data entry validation	Coordination team	Coordination team	Coordination team	Coordination team	Double entry for laboratory results Weekly quality checks
Study periods[a]					
Influenza A(H1N1)pdm09	Week 1, 2013	Week 2, 2013	Week 52, 2012	Week 7, 2013	Week 47, 2012
	Week 10, 2013	Week 8, 2013	Week 9, 2013	Week 11, 2013	Week 15, 2013
Influenza A(H3N2)	Week 52, 2012	Week 3, 2013	Week 3, 2013	Week 4, 2013	Week 9, 2013
	Week 14, 2013	Week 6, 2013	Week 13, 2013	Week 13, 2013	Week 12, 2013
Influenza B	Week 50, 2012	Week 5, 2013	Week 4, 2013	Week 50, 2012	Week 51, 2012
	Week 13, 2013	Week 9, 2013	Week 15, 2013	Week 11, 2013	Week 15, 2013

GP: general practitioner.

[a] The International Organization for Standardization's week numbers were used, to ensure consistency across study sites.

influenza vaccination would also further advocate the need for vaccines that are more effective in this population. Moreover, there are ongoing scientific debates about the effect of repeated vaccination on the immunological response induced by the seasonal influenza vaccine [9-11] and further evidence is needed.

In 2011, we launched a pilot study to estimate the IVE against laboratory-confirmed influenza hospitalisation using a network of hospitals in the European Union (EU) [12]. During the 2012/13 influenza season, co-circulation of influenza A(H1N1)pdm09, A(H3N2) and B/Victoria- and B/Yamagata-lineage viruses was reported in Europe [13]. The objective of the study presented here was to measure the 2012/13 seasonal IVE against hospitalisation with subtype-specific laboratory-confirmed influenza in a hospital network in four EU countries: France, Italy, Lithuania and Spain.

Methods

We conducted a case–control study using the test-negative design [14] in 18 hospitals located in five study sites: France (five hospitals), Italy (two), Lithuania (two), and the Navarre (four) and Valencia (five) regions in Spain. Each study site adapted a generic protocol [15] to the local context (Table 1).

Study population

The study population was all community-dwelling adults (18 years of age or older), belonging to the target groups for vaccination as defined locally [16-20], admitted to one of the participating hospitals with no contraindication for influenza vaccination. Patients were excluded if they had previously tested positive for influenza virus in the 2012/13 season or resided outside the hospital catchment area (for the 11 hospitals with known catchment area).

Study teams actively screened all patients admitted for potentially influenza-related conditions. These conditions included the following: acute myocardial infarction or acute coronary syndrome; heart failure; pneumonia and influenza; chronic pulmonary obstructive disease; myalgia; altered consciousness, convulsions, febrile-convulsions; respiratory abnormality; shortness of breath; respiratory or chest symptoms; acute cerebrovascular disease; sepsis; and systemic inflammatory response syndrome. Among them, study teams invited patients with an onset of influenza-like

Table 2

Definition of the categories of chronic conditions according to the variables collected, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Categories of chronic conditions	Chronic conditions	Study sites that collected the information
Cardiovascular disease	Cardiovascular disease[a]	FR, IT, LT, VA
	Heart disease	FR, IT, LT, NV, VA
	Stroke	FR, IT, LT, NV
	Transient ischemic attack	IT
	Peripheral arterial disease	IT, VA
Respiratory disease	Lung diseases[a]	FR, IT, LT, NV
	Asthma	IT, VA, LT
	Chronic obstructive pulmonary disease	IT, LT
	Emphysema	IT, LT
	Mucoviscidosis	FR, IT, LT
	Bronchitis	VA, LT
Metabolic and endocrine disorders	Diabetes	FR, IT, NV, VA
	Nutritional deficiency	FR, IT, LT
	Endocrine disease	FR, IT, LT, VA
Haematological disease or cancer	Haematological cancer	FR, IT, LT, NV
	Anaemia/spleen condition	FR, IT, LT, VA
	Drepanocytosis	FR, IT
	Cancer	FR, IT, LT, NV, VA
Immunodeficiency	Immunodeficiency	FR, IT, LT, NV, VA
	Rheumatological disease	FR, IT, LT, NV
Hepatic disease		FR, IT, LT, NV, VA
Renal disease		FR, IT, LT, NV, VA
Obesity[b]		FR, IT, LT, NV, VA
Neuromuscular disorder		FR, IT
Dementia		FR, IT, LT, NV, VA

FR: France; IT: Italy; LT: Lithuania; NV: Navarre, Spain; VA: Valencia, Spain.

[a] May include the conditions from the same category listed below.

[b] Defined as body mass index ≥30 kg/m².

illness (ILI) symptoms (one systemic and one respiratory symptom) within the past seven days to participate. Those accepting to participate were swabbed and tested for influenza. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect influenza viruses and to classify them as influenza A(H3N2), influenza A(H1N1)pdm2009 or influenza B. Patients positive for influenza were classified as cases of a given influenza type/subtype and those testing negative were controls.

We defined the study period as at least 15 days after the beginning of each site-specific seasonal influenza vaccination campaign until the end of the influenza season as declared by local influenza surveillance systems. For each of the influenza type/subtype analyses, we excluded the controls with onset of symptoms before the week of the first laboratory-confirmed case or after the week of the last laboratory-confirmed case. We used the International Organization for Standardization's week numbers [21] to ensure consistency across study sites.

We considered patients as vaccinated against seasonal influenza if they had received at least one dose of the 2012/13 influenza vaccine more than 14 days before onset of ILI symptoms. Patients not vaccinated or vaccinated less than 15 days before ILI onset were considered as unvaccinated.

Data collection

We collected data on the ILI episode, demographics, chronic diseases (Table 2), number of hospitalisations in the previous 12 months, number of consultations at the general practitioner (GP) in the previous three months, smoking status, vaccination against influenza in 2012/13 and 2011/12 and, for those aged 65 years and over, functional status before ILI onset using the Barthel score [22]. The data were gathered from hospital medical records, face-to-face interviews with the patient and/or patient's family and laboratory databases. The vaccination status was obtained from vaccination registers in two study sites, interview with the patients and/or patient's family in two sites and contact with the patient's physician in one site.

Table 3

Number of records received by the pooled analysis coordinator and included in the pooled analysis by study site, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Type of record	Number of records per study site					
	France[a]	Italy	Lithuania[b]	Navarre, Spain	Valencia, Spain	Total
Eligible records	433	84	184	93	1,535	**2,329**
Non-target groups for vaccination	78	14	96	18	102	**308**
Missing laboratory results	2	0	0	0	43	**45**
Unknown vaccination status	3	0	1	0	0	**4**
Total records used for the analyses	350	70	87	75	1,390	**1,972**
Influenza A(H1N1)pdm09						
Cases	20	10	20	9	57	**116**
Controls	213	39	24	24	1,213	**1,513**
Influenza A(H3N2)						
Cases	38	4	9	2	5	**58**
Controls	229	24	29	33	204	**519**
Influenza B						
Cases	62	13	25	17	115	**232**
Controls	219	31	28	45	971	**1,294**

[a] In France, one specimen of influenza A virus could not be subtyped.

[b] In Lithuania, one patient was coinfected with A(H3N2) and A(H1N1)pdm09 viruses.

Data analysis

Study sites transmitted anonymised datasets to the pooled analysis coordinator, through a password-secured web-based platform. We ran a complete case analysis, excluding records for which laboratory results, vaccination status or potential confounding variables were missing.

To test for heterogeneity between study sites, we used Cochran's Q-test and the I^2 index [23]. The Q-test provides a p value that indicates the presence or not of heterogeneity. The I^2 index quantifies the proportion of the variance attributable to differences between study sites. It is common to consider that I^2 around 25%, 50% and 75% indicate low, medium and high heterogeneity, respectively.

We conducted separate analyses for each type/subtype of influenza. We estimated the pooled IVE as 1 minus the odds ratio (OR) (expressed as a percentage) of being vaccinated in cases versus controls, using a one-stage method with study site as fixed effect in the model [24].

We assessed the presence of effect modification by comparing the time- and study site-adjusted OR (assuming that the test-negative design case–control study is a density case–control study implying adjustment for the time of symptom onset) across strata of characteristics using the homogeneity test. We considered a variable as a confounder when the percentage change between the unadjusted and adjusted OR was greater than 15%.

We conducted a multivariable logistic regression analysis. In addition to study site and month of symptom onset, we adjusted the models for the covariates identified as potential confounders in the stratified analysis as well as the presence of at least one underlying condition and the age that we modelled as a restricted cubic spline with four knots [25]. The likelihood ratio test was used to decide on the final models. We conducted stratified analyses by age group (less than 65 years, 65–79 years and 80 years and above).

To study the effect of previous influenza vaccination on laboratory-confirmed influenza, we conducted a stratified analysis using four vaccination status categories: vaccination in none of the seasons (2011/12 and 2012/13), 2012/13 vaccination only, 2011/12 vaccination only and vaccination in both seasons and computed and compared IVE for each of these categories using vaccination in none of the seasons as a reference.

We carried out sensitivity analyses excluding the weeks when less than 10% of the patients included were positive for influenza, excluding patients who received antivirals between the onset of symptoms and swabbing and by restricting the analysis to patients swabbed within four days of symptoms onset. To avoid the inclusion of patients with acute manifestation of chronic respiratory illnesses rather than respiratory infection, we restricted our analysis to patients with no underlying respiratory conditions.

We ran all analyses with Stata v12 (Stata Corp LP, College Station, TX, United States).

Table 4

Characteristics of influenza A(H1N1)pdm09 (n=116), influenza A(H3N2) (n=58) and influenza B (n=232) cases and corresponding test-negative controls included in the study, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13 (n=1,972)

Charactertistic	A(H1N1)pdm09		A(H3N2)		B	
	Controls[b] (n=1,513)	Cases (n=116)	Controls[c] (n=519)	Cases (n=58)	Controls[d] (n=1,294)	Cases (n=232)
	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]
Median age in years	77.0	63.0*	75.0	73.0	77.0	75.2
Age group in years						
18–64	339 (22.4)	60 (51.7)*	146 (28.1)	14 (24.1)	301 (23.3)	60 (25.9)
65–79	563 (37.2)	42 (36.2)*	175 (33.7)	22 (37.9)	473 (36.6)	92 (39.7)
80–103	611 (40.4)	14 (12.1)*	198 (38.2)	22 (37.9)	520 (40.2)	80 (34.5)
Sex						
Male	851 (56.2)	67 (57.8)	294 (56.6)	24 (41.4)*	718 (55.5)	108 (46.6)*
Vaccine status						
2012/13 seasonal influenza vaccination	866 (57.2)	39 (33.6)*	296 (57.0)	20 (34.5)*	734 (56.7)	88 (37.9)*
2011/12 seasonal influenza vaccination	835 (55.3)	37 (31.9)*	296 (57.5)	25 (44.6)	702 (54.5)	102 (44.5)*
Presence of comorbidities						
Metabolic and endocrine disorders	546 (36.1)	41 (35.3)	195 (37.6)	24 (41.4)	462 (35.7)	72 (31.0)
Cardiovascular disease	768 (50.8)	49 (42.2)	247 (47.6)	26 (44.8)	636 (49.1)	103 (44.6)
Renal disease	198 (13.1)	9 (7.8)	84 (16.2)	8 (13.8)	165 (12.8)	27 (11.7)
Respiratory disease	750 (49.6)	50 (43.5)	243 (46.8)	25 (43.1)	634 (49.0)	80 (34.6)*
Neuromuscular disorder	82 (5.6)	7 (8.0)	27 (5.9)	3 (6.4)	70 (5.7)	7 (3.7)
Hepatic disease	65 (4.3)	2 (1.7)	14 (2.7)	0 (0.0)	57 (4.4)	8 (3.5)
Immunodeficiency	102 (6.7)	8 (6.9)	40 (7.7)	5 (8.6)	87 (6.7)	16 (6.9)
Haematological disease or cancer	321 (21.7)	16 (14.5)	96 (19.2)	12 (21.8)	279 (21.6)	30 (13.0)*
Any chronic condition (of all chronic conditions collected in the study site)	1,404 (92.8)	106 (91.4)	473 (91.1)	52 (89.7)	1,195 (92.3)	192 (82.8)*
More than one chronic condition	1,013 (67.0)	62 (53.4)*	340 (65.5)	37 (63.8)	853 (65.9)	113 (48.7)*
Obesity[f]	423 (28.1)	26 (22.6)	127 (24.7)	10 (17.9)	359 (27.9)	54 (23.5)
Pregnancy	10 (0.7)	1 (1.3)	7 (2.0)	0 (0.0)	11 (1.0)	8 (4.7)*
Low functional status[g] (among patients ≥65 years)	232 (19.8)	9 (16.1)	5 (14.8)	4 (9.1)	187 (18.9)	34 (19.8)
Other potential confounders						
More than one GP visit in previous 3 months	738 (49.1)	46 (39.7)	261 (51.3)	26 (46.4)	649 (50.7)	109 (48.0)
Hospitalisations in previous 12 months	582 (38.5)	32 (27.6)*	205 (39.6)	22 (37.9)	502 (38.8)	70 (30.2)*
Smoker status						
Current	277 (18.3)	39 (33.6)*	108 (20.8)	13 (22.4)	243 (18.8)	32 (13.9)*
Former	580 (38.3)	35 (30.2)*	173 (33.4)	16 (27.6)	485 (37.5)	58 (25.1)*
Never	656 (43.4)	42 (36.2)*	237 (45.8)	29 (50.0)	565 (43.7)	141 (61.0)*
Potential for misclassification						
Swabbing delay<4 days	745 (49.2)	69 (59.5)*	233 (44.9)	24 (41.4)	621 (48.0)	90 (38.8)*
Antiviral treatment before swabbing	18 (1.2)	12 (10.4)*	17 (3.3)	5 (8.6)	18 (1.4)	17 (7.3)*

GP: general practitioner.

* p value for difference between cases and controls<0.05.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

[b] Comparisons were made with controls recruited between the week of the first case of influenza A(H1N1)pdm09 and the week of the last case of influenza A(H1N1)pdm09 (1,513 controls).

[c] Comparisons were made with controls recruited between the week of the first case of influenza A(H3N2) and the week of the last case of influenza A(H3N2) (519 controls).

[d] Comparisons were made with controls recruited between the week of the first case of influenza B and the week of the last case of influenza B (1,294 controls).

[e] Unless otherwise indicated.

[f] Defined as body mass index ≥30 kg/m².

[g] Determined using the Barthel score [22].

Table 5

Influenza vaccine effectiveness against influenza A(H1N1)pdm09, A(H3N2) and B, adjusted for various covariables by age group, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13

Groups assessed	A(H1N1)pdm09	A(H3N2)	B
All target groups			
Number of cases and controls	1,628	577	1,526
Number of cases; number of vaccinated cases	116; 39	58; 20	232; 88
Number of controls; number of vaccinated controls	1,512; 865	519; 296	1,294; 734
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site	47.0 (18.8 to 65.4)	54.4 (16.1 to 75.2)	46.5 (27.7 to 60.4)
Study site and month of symptom onset	45.7 (16.4 to 64.8)	53.0 (13.2 to 74.5)	44.3 (24.3 to 59.0)
Study site, month of symptom onset and age	20.9 (–25.3 to 50.1)	61.9 (27.2 to 80.1)	46.9 (26.8 to 61.5)
Study site, month of symptom onset, age and presence of chronic conditions	21.3 (–25.2 to 50.6)	61.8 (26.8 to 80.0)	43.1 (21.2 to 58.9)
Patients aged 18–64 years belonging to target groups			
Number of cases and controls	372[b]	143[c]	346[d]
Number of cases; number of vaccinated cases	60; 9	14; 3	60; 7
Number of controls; number of vaccinated controls	312; 105	129; 39	286; 91
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of onset	42.5 (–28.3 to 74.3)	26.1 (–215.9 to 82.7)	68.4 (25.7 to 86.6)
Study site, month of onset and presence of chronic conditions	41.8 (–30.7 to 74.1)	NA[c]	66.0 (19.3 to 85.7)
Patients aged 65–79 years			
Number of cases and controls	504[e]	181[f]	565
Number of cases; number of vaccinated cases	42; 18	22; 7	92; 40
Number of controls; number of vaccinated controls	462; 276	159; 91	473; 287
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of onset	44.2 (–9.0 to 71.4)	55.7 (–22.8 to 84.0)	37.3 (–2.1 to 61.5)
Study site, month of onset and presence of chronic conditions	43.8 (–10.7 to 71.5)	52.4 (–33.9 to 83.1)	28.2 (–18.9 to 56.6)
Patients aged 80–103 years			
Number of cases and controls	623[g]	216[h]	600
Number of cases; number of vaccinated cases	14; 12	22; 10	80; 41
Number of controls; number of vaccinated controls	609; 412	194; 147	520; 348
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of symptom onset	–171.7 (–1,170.7 to 41.9)	73.8 (30.0 to 90.2)	46.4 (9.6 to 68.2)
Study site, month of symptom onset and presence of chronic conditions	NA[g]	73.8 (29.9 to 90.2)	44.8 (6.7 to 67.4)

CI: confidence interval; NA: not applicable.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

[b] A total of 27 controls dropped because no cases in November among patients less than 65 years.

[c] A total of 17 controls dropped because no cases in December and April and in Italy among patients less than 65 years. No adjustment for chronic disease because all A(H3N2) cases aged less than 65 years had chronic conditions.

[d] A total of 15 controls dropped because no cases in April among patients less than 65 years.

[e] A total of 101 controls dropped because no cases in December among patients aged 65–79 years.

[f] A total of 16 controls dropped because no cases in December and in Navarre, Spain, among patients aged 65–79 years.

[g] Two controls dropped because no cases in Lithuania among patients aged 80 years and over. No adjustment for chronic disease because all A(H1N1)pdm09 cases aged 80 years and over had chronic conditions.

[h] Four controls dropped because no cases in April among patients aged 80 years and over.

Results

Overall, 2,329 eligible patients, of whom 2,021 belonged to the target groups for influenza vaccination, were recruited in the 18 study hospitals (Table 3). A total of 45 (2.2%) and four (0.2%) patients were excluded due to missing laboratory results and missing vaccination status, respectively. We included a total of 1,972 patients in the analysis: 1,390 from Valencia (177 cases), 350 from France (121 cases), 87 from Lithuania (53 cases), 75 from Navarre (28 cases) and 70 from Italy (27 cases).

Influenza A(H3N2), A(H1N1)pdm09 and B co-circulated in all study sites (Table 1). The study site having included patients for the longest period of time was Valencia (week 47, 2012 to 15, 2013) and for the shortest period was in Italy (week 2–8, 2013). The period of

Table 6

Crude and adjusted vaccine effectiveness against influenza A(H1N1)pdm09 (n=1,625), A(H3N2) (n=571) and B (n=1,518) by vaccination status, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13

Influenza type	Number of cases	Number of controls	Crude VE[b] (95% CI)	Adjusted VE[c] (95% CI)
A(H1N1)pdm09 (n=1,625)				
No vaccination in 2012/13 and 2011/12	71	539	–	–
2012/13 vaccination only	8	135	26.2 (–62.9 to 66.6)	6.2 (–110.4 to 58.2)
2011/12 vaccination only	6	108	39.8 (–47.1 to 75.4)	26.6 (–81.6 to 70.3)
2011/12 and 2012/13 vaccinations	31	727	52.8 (24.3 to 70.6)	27.9 (–20.5 to 56.9)
A(H3N2) (n=571)				
No vaccination in 2012/13 and 2011/12	30	183	–	–
2012/13 vaccination only	1	36	65.3 (–176.6 to 95.7)	68.3 (–157.2 to 96.1)
2011/12 vaccination only	6	40	5.1 (–156.4 to 64.9)	12.3 (–140.7 to 68.1)
2011/12 and 2012/13 vaccinations	19	256	49.2 (1.7 to 73.8)	59.6 (18.5 to 80.0)
B (n=1,518)				
No vaccination in 2012/13 and 2011/12	121	478	–	–
2012/13 vaccination only	6	109	69.5 (27.6 to 87.2)	68.3 (24.5 to 86.7)
2011/12 vaccination only	21	82	0.4 (–73.1 to 42.7)	–5.6 (–84.5 to 39.6)
2011/12 and 2012/13 vaccinations	81	620	39.3 (15.5 to 56.3)	37.3 (10.7 to 56.0)

CI: confidence interval; VE: vaccine effectiveness.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).
[b] Adjustment for study site and month of symptom onset.
[c] Adjustment for study site, month of symptom onset, age and comorbidities.

recruitment was the longest for A(H1N1)pdm2009 (21 weeks) and the shortest for A(H3N2) (15 weeks).

Of the 1,972 patients included in the pooled analysis, 116 patients tested positive for influenza A(H1N1)pdm09, 58 for A(H3N2) and 232 for influenza B. Two patients were coinfected with types A and B and one patient was coinfected with A(H3N2) and A(H1N1)pdm09. One specimen of influenza A could not be subtyped.

Influenza A(H1N1)pdm09 cases were younger (63 vs 77 years, p<0.05) than controls. A lower proportion of A(H1N1)pdm09 cases had more than one underlying condition (53.4% vs 67.0%, p<0.05), had been hospitalised in the previous year (27.6% vs 38.5%, p<0.05) and a higher proportion were current smokers (33.6% vs 18.3%, p<0.05) compared with controls (Table 4).

Influenza A(H3N2) cases and controls were similar for all characteristics except for the proportion of male patients (41.4% vs 56.6%, p<0.05).

Compared with controls, a lower proportion of influenza B cases had underlying conditions (82.8% vs 92.3%, p<0.05), had been hospitalised in the previous year (30.2% vs 38.8%, p<0.05) and were smokers (13.9% vs 18.8% of current smokers, p<0.05).

The 2012/13 vaccine coverage was 57.2% among all controls (all influenza-negative patients included in the study), 33.6% among A(H1N1)pdm09, 34.5% among A(H3N2) and 37.9% among influenza B cases (Table 4).

The p values associated with the Q-test and the I^2 index using models adjusted for age, month of symptom onset and chronic condition, testing for heterogeneity between study sites, were respectively 0.19 and 40.0% for A(H3N2), 0.10 and 48.3% for A(H1N1)pdm09 and 0.08 and 56.2% for influenza B.

The overall adjusted A(H1N1)pdm09 IVE was 21.3% (95% confidence interval (CI): –25.2 to 50.6; n=1,628); 41.8% (95% CI: –30.7 to 74.1; n=372) among the 18–64 year-old patients and 43.8% (95% CI: –10.7 to 71.5; n=504) among those aged 65–79 years. Among patients aged 80 years and older, there were 14 A(H1N1)pdm09 cases, including 12 vaccine failures (Table 5). Restricted to those aged less than 80 years-old, the adjusted IVE was 35.2% (95% CI: –9.1 to 61.5; n=1,004). Adjusted IVE against A(H1N1)pdm09 was 6.2% (95% CI: –110.4 to 58.2; n=753) among patients vaccinated in the 2012/13 season only, 26.6% (95% CI: –81.6 to 70.3; n=724) for those vaccinated in 2011/12 and 27.9% (95% CI: –20.5 to 56.9; n=1,368) for those vaccinated in both seasons (Table 6).

Table 7

Adjusted[a] vaccine effectiveness against influenza A(H3N2), influenza A(H1N1)pdm09 and B viruses according to various restrictions, hospital-based influenza vaccine effectiveness study, four European countries[b], 2012/13

Restriction	A(H1N1)pdm09		A(H3N2)		B	
	Total number/ number of cases	Adjusted VE (95% CI)	Total number/ number of cases	Adjusted VE (95% CI)	Total number/ number of cases	Adjusted VE (95% CI)
No restriction	1,628/116	21.3 (–25.2 to 50.6)	577/58	61.8 (26.8 to 80.0)	1,526/232	43.1 (21.2 to 58.9)
No antiviral treatment started between symptom onset and swabbing	1,598/104	18.6 (–30.7 to 49.3)	555/53	59.4 (21.7 to 79.0)	1,491/215	40.5 (17.3 to 57.2)
Swabbing delay≤4 days	1,147/88	14.9 (–47.1 to 50.8)	359/36	60.4 (10.0 to 82.5)	1,037/151	45.3 (18.8 to 63.2)
Weeks when ratio controls to cases was<9:1	1,019/109	29.8 (–15.1 to 57.2)	542/56	62.7 (27.5 to 80.8)	1,142/221	44.3 (21.6 to 60.4)
Patients with no chronic respiratory conditions	829/66	38.9 (–20.3 to 69.0)	304/33	57.8 (–4.3 to 82.9)	812/152	50.7 (24.1 to 68.0)

CI: confidence interval: VE: vaccine effectiveness.

[a] Adjustment for study site, month of symptom onset, presence of any chronic condition and age.

[b] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

The overall adjusted IVE against A(H3N2) was 61.8% (95% CI: 26.8 to 80.0; n=577) (Table 5). The adjusted IVE was 52.4% (95% CI: –33.9 to 83.1; n=181) among 65–79 years patients and 73.8% (95% CI: 29.9 to 90.2; n=216) among those 80 years and older. Among patients aged less than 65 years, all cases had chronic conditions. In this age group, the IVE adjusted for month of symptom onset and study site was 26.1% (95% CI: –215.9 to 82.7; n=143). Adjusted IVE was 68.3% (95% CI: –157.2 to 96.1; n=250) among patients vaccinated in 2012/13 only and 59.6% (95% CI: 18.5 to 80.0; n=488) among patients vaccinated in 2011/12 and 2012/13 (Table 6).

The overall adjusted IVE against influenza B was 43.1% (95% CI: 21.2 to 58.9; n=1,526), 28.2% (95% CI: –18.9 to 56.6; n=565) among patients aged 65–79 years and 66.0% (95% CI: 19.3 to 85.7; n=346) among those younger than 65 years (Table 5). Adjusted IVE against influenza B was 68.3% (95% CI: 24.5 to 86.7; n=714) among patients vaccinated in 2012/13 only and 37.3% (95% CI: 10.7 to 56.0; n=1,300) in those vaccinated in both seasons (Table 6).

There were few changes in the IVE when conducting the sensitivity analyses (Table 7). The IVE against A(H1N1) pdm09 was higher when restricted to patients with no chronic respiratory conditions (38.9% (95% CI: –20.3 to 69.0) vs 21.3% (95% CI: –25.2 to 50.6)).

Discussion

Our results suggest that in the population targeted for the influenza vaccination, the 2012/13 IVE for laboratory-confirmed hospitalised influenza was 21.3% against A(H1N1)pdm09, 61.8% against A(H3N2) and 43.1% against B.

The adaptation of a generic protocol by 18 European hospitals enabled us to pool data and obtain a sample of 1,972 hospitalised ILI patients targeted for influenza vaccination. In a season with co-circulation of the three viruses, this large sample size allowed us to compute type-/subtype-specific estimates of IVE against hospitalised influenza and to further attempt to stratify by age group. However, stratified analyses led to estimates with broad confidence intervals. Consequently, some results of the stratified analyses can only be used to generate hypotheses.

The test-negative design has been mainly discussed and validated for GP-based studies [26,27]. It is assumed that by restricting the study population to patients consulting for ILI, the health-seeking behaviour confounding effect (associated with propensity to get vaccinated and to go to the GP in case of influenza) is controlled for. Since in our study sites all people needing hospitalisation are likely to be hospitalised, we believe that confounding due to health-seeking behaviour is minimised.

In hospital-based studies, several outcomes could be used. If we were to measure IVE against influenza confirmed-severe acute respiratory infection (SARI), we would need to make sure that for both cases and controls a respiratory infection was the cause of admission. We have chosen a broader case definition and a more sensitive inclusion criteria to cover a larger part of the influenza disease burden. As a consequence, some of the ILI in the seven days before admission may correspond to an exacerbation of underlying respiratory conditions. This could lead to an overestimation of the

IVE. Restricting our analysis to patients with no underlying respiratory conditions provides similar results and does not support this hypothesis. Furthermore, we adjusted for the presence and number of previous hospitalisations for underlying conditions.

The inclusion of patients swabbed more than four days after symptoms onset or after antiviral treatment had started could have led to misclassification biases if viral clearance occurred before swabbing. However, analyses confined to patients swabbed within four days of symptom onset and to patients who did not receive antiviral treatment did not change the results.

Studies using the test-negative design may underestimate the IVE when the ratio of controls to cases is high, especially if the laboratory tests have low specificity [28]. In our study, all cases were confirmed by RT-PCR, which has high specificity [29]. In the analyses restricted to weeks when the control to case ratio was lower than 9:1 resulted in very similar IVE estimates.

The data quality was high with only 49/2,021 records with missing outcomes or exposures in the database. We believe that ascertainment of vaccination status through patient interviews in two of the five study sites has not introduced differential information bias as data were collected before laboratory testing.

Due to the small sample size in some study sites, the test of heterogeneity may have had no power to detect heterogeneity even if differences exist between study sites. Different IVE across study sites could be due to variations in circulating strains, different vaccines by study site or different measured and unmeasured confounding factors. Further typing of circulating strains would be valuable to discuss site-specific IVE with regard to the level of matching between vaccine and locally circulating strains. Different access to vaccination according to age and underlying condition and to hospitalisation [30] could partly explain variations in IVE across study sites. Finally, the presence of random errors cannot be ruled out due to low sample size by study site. A larger sample size would be needed to carry out a two-stage pooled analysis [24].

Our results suggest that, in people belonging to target groups for vaccination, the 2012/13 IVE varied by subtype and age group. However, we cannot exclude the possibility that the variability of IVE results by age group mainly reflects sample size limitations. Small stratum-specific sample sizes (and very small number of cases) lead to unstable results and do not allow for biological interpretation of age-specific results. Our results would suggest that IVE against A(H3N2) was higher among older age groups. This observation would be in contradiction to the principles of immune senescence. In addition to the sample-size limitations, and as discussed above, we cannot exclude a selection bias for our controls, which we adjusted for. However we used the same control group for the three subtypes and age-specific results vary by subtype. We consider that it is unlikely that confounding factors would differ by subtype.

When looking at the effect of repeated vaccination (over two consecutive seasons), similar patterns were observed for influenza A(H3N2) and B. The highest point estimate IVE was in patients vaccinated in 2012/13 only, the lowest in those vaccinated in 2011/12 only and intermediate among those vaccinated both seasons. Such findings are consistent with recent reports from the Unites States and Australia [9,10,31]. The 2011/12 vaccine included an A/Perth/16/2009(H3N2)-like virus and a B/Brisbane/60/2008-like virus, while the 2012/13 vaccine included an A/Victoria/361/2011(H3N2)-like virus and a B/Wisconsin/1/2010-like (Yamagata lineage). On the basis of European virological surveillance data [13], the main circulating strains during the 2012/13 season were an A/Victoria/361/2011(H3N2) (with some A/Texas/50/2012 circulation reported) and B/Wisconsin/1/2010-like (with some B/Estonia and B/Massachusets/2/2012 circulation reported). These data support the absence of protection by the 2011/12 seasonal vaccine on the 2012/13 circulating strains as they were not matched.

Some authors have discussed the hypothesis of attenuated immunological responses as a result of repeated vaccination. From a school-based study, Davies et al. [32] suggested that a natural infection in season 1 produces antibodies that have a larger potential to form high post-vaccination titres in season 2 than vaccine-induced antibodies. Smith et al. [33] hypothesised that large antigenic distances between vaccines in seasons 1 and 2, and between vaccine in season 1 and epidemic strain in season 2, significantly increase the risk of infection among repeated vaccinees compared with those receiving the vaccine in season 2 only. Considering the antigenic differences between the 2011/12 vaccine and the 2012/13 circulating strains, this hypothesis could explain our results, suggesting a higher IVE against influenza A(H3N2) and B among patients vaccinated in 2012/13 only compared with those vaccinated in 2011/12 and 2012/13. Further studies, including a longer history of vaccine uptake and natural infections would be of great value to better understand the effect of repeated vaccination on the immunological response to a new influenza seasonal vaccine and the level of clinical protection conferred to individuals.

Our results suggest a low IVE against A(H1N1)pdm09, especially among the elderly [34]. A total of 14 cases of influenza A(H1N1)pdm09 occurred among patients older than 80 years. While the majority of these cases (n=12) were vaccinated patients, small numbers make the IVE estimates hard to interpret in that age group. The IVE was similar for those vaccinated in 2011/12 only or in both seasons. There was no effect for those vaccinated in 2012/13 only. The recommended A/California/7/2009(H1N1)pdm09-like virus strain

was the same for the 2011/12 and 2012/13 vaccines and matched the 2012/13 circulating strains (some A/California/06/2009 also reported). Long-lasting immune response induced by trivalent inactivated vaccines was previously described [35] and some recent results suggest that frequent previous vaccinations may be effective for the current influenza season [11]. The absence of protection among patients vaccinated in 2012/13 only is difficult to understand and interpret; it may reflect the presence of associated (and unmeasured) negative confounders for which repeated vaccination may be a surrogate. In addition, other studies [36-38] suggest a decreasing effect in the season difficult to reconcile with a long-term effect between seasons. Considering the small sample size in some of the vaccination groups in our study, we cannot exclude the possibility that this observation is due to chance.

Increasing the number of study sites in this network would allow a sufficient sample size to be reached early enough in the season to prompt the use of alternative prevention measures if a low IVE against hospitalised cases is observed among the target group. Early estimates of IVE against hospitalised influenza are also a useful complement to guide the decision-making of WHO experts regarding the composition of the next season's vaccines. A larger sample size and good documentation of vaccine brands used would allow the computing of brand-specific IVE. To further study the effect of previous seasonal vaccination will require documenting past vaccination over several seasons. In addition, ways to measure past natural immunity may also be needed to better understand the complex immunity of influenza natural infection and vaccination.

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Conflict of interest

No conflict of interest. Sanofi Pasteur, GlaxoSmithKline, Sanofi Pasteur MSD supported the study. They had no role in study design, data collection, pooled analysis and publication.

Authors' contributions

Marc Rondy was involved in the original methodological design of the study (generic protocol). He coordinated the European hospital IVE network, undertook the statistical analysis on which the research article is based and led the writing of the research article. Alain Moren initiated the original methodological design of the study. He coordinated the European hospital IVE network and contributed to the writing of the research article. Odile Launay, Joan Puig-Barberà, Giedre Gefenaite, Jesús Castilla, Katleen de Gaetano Donati, Florence Galtier, Eelko Hak, Marcela Guevara and Simona Costanzo were responsible for the coordination of the study at the local level. They were in charge of the data collection and management. They read, contributed and approved the manuscript final version. The European hospital IVE network contributors were in charge of supervising the study at the hospital level and collected the data published in this research article.

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* Authors' correction

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Widespread implementation of EUCAST breakpoints for antibacterial susceptibility testing in Europe

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The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was established to harmonise clinical antimicrobial breakpoints and to define breakpoints for new agents in Europe. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) external quality assessment (EQA) exercises from 2009 to 2012, from the United Kingdom External Quality Assessment Scheme (UK NEQAS) from November 2009 to March 2013 and data collected by EUCAST through a questionnaire in the first quarter of 2013 were analysed to investigate implementation of EUCAST guidelines in Europe. A rapid change to use of EUCAST breakpoints was observed over time. Figures for implementation of EUCAST breakpoints at the end of the studied period were 61.2% from EARS-Net data and 73.2% from UK NEQAS data. Responses to the EUCAST questionnaire indicated that EUCAST breakpoints were used by over 50% of laboratories in 18 countries, by 10 to 50% of laboratories in eight countries and by less than 10% in seven countries. The EUCAST disk diffusion method was used by more than 50% of laboratories in 12 countries, by 10 to 50% of laboratories in ten countries and by less than 10% in eleven countries. EUCAST guidelines implementation is essential to ensure consistent clinical reporting of antimicrobial susceptibility results and antimicrobial resistance surveillance.

Background

The use of common clinical breakpoints for antimicrobial susceptibility testing is important both for consistent clinical reporting of antimicrobial susceptibility and for international surveillance of the antimicrobial susceptibility of microorganisms. The principal objective of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [1] is to harmonise antimicrobial breakpoints in Europe and to define breakpoints for new agents in collaboration with the European Medicines Agency (EMA) [2] following a standard operating procedure agreed between EUCAST and the EMA [3,4]. EUCAST was established by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) in 1997 [5]. The committee was restructured in the years 2001 and 2002 with the support and central involvement of the national breakpoint committees that were active in Europe, i.e. those in France, Germany, the Netherlands, Norway, Sweden and the United Kingdom, and has been in operation in its current form since 2002. EUCAST has a General Committee [6], which includes one representative of each country from Europe and any country outside Europe interested in being part of the EUCAST process.

ESCMID has remained the administrative, financial and scientific platform of EUCAST throughout. Principal financial support over the years has been from ESCMID, European Union (EU) grants, a grant from the European Centre for Disease Prevention and Control (ECDC) and currently through a framework contract with the ECDC.

Today, EUCAST is well established as the only pan-European antimicrobial breakpoint committee, with representatives throughout Europe and beyond. It is accepted as the European antimicrobial breakpoint committee by clinicians and clinical microbiologists, by

national breakpoint committees and medicines agencies in Europe, the ECDC, the EMA, the European Food Safety Authority (EFSA), the pharmaceutical industry and diagnostic companies with interests in antimicrobial susceptibility testing. Of note, the EUCAST clinical breakpoints apply to antimicrobial resistance case definition as reportable to the European Union (EU) surveillance network for communicable diseases [7].

The breakpoint harmonisation process for all major groups of antimicrobial agents and organisms was completed in 2008/09. Since then there has been rapid adoption of EUCAST breakpoints and methods in Europe. Complete data on uptake in all European laboratories are not available as in most countries there is no mechanism for collection of information on susceptibility testing guidelines followed. A combination of different data sources needs to be used to obtain this information.

Analysed data sources

Data presented here are taken from three different sources. Firstly, the external quality assessment (EQA) exercise that is part of the European Antimicrobial Resistance Surveillance Network (EARS-Net) [8] organised by ECDC though a framework contract with the UK National External Quality Assessment Scheme (UK NEQAS). Secondly, the international external quality assessment scheme run by UK NEQAS [9]. Thirdly, data collected by EUCAST in the first quarter of 2013 through a questionnaire on guidelines and methods used in different countries.

EARS-Net external quality assessment

The ECDC EARS-Net resistance surveillance programme collects data from all EU countries, two European Economic Area countries (Norway and Iceland) [7], plus Bosnia, Croatia (also EU since 1 July 2014), Israel and Turkey between 2009 and 2011 only. The number of participating laboratories in each country varies, with a total of between 766 and 817 laboratories from 28 to 30 countries participating in the annual EQA exercises between 2009 and 2012 [10–13]. As part of the EQA exercise information is collected on breakpoint guidelines followed and methods used.

UK NEQAS for antimicrobial susceptibility testing

The UK NEQAS EQA scheme [9] includes subscribing laboratories principally from European countries and, as with EARS-Net, the number of participating laboratories in each country is variable. However, the distribution of numbers of laboratories among countries differs from that of EARS-Net, with a total of between 632 and 656 laboratories participating in the EQA scheme between November 2009 and March 2013. In the UK NEQAS for antimicrobial susceptibility testing two organisms of a variety of species are distributed each month. The number of participating laboratories returning results varies with the organism and antimicrobial agent so for consistency the data are based on results returned for *E. coli* isolates tested against ciprofloxacin, one of the most widely tested combinations. For each organism distributed, information is collected on breakpoint guidelines followed and methods used.

EUCAST questionnaire on guidelines and methods used in different countries

In the first quarter of 2013, a questionnaire was distributed to all General Committee members with the objective of collecting information on whether EUCAST breakpoint guidelines were followed, adoption of the EUCAST disk diffusion method and whether the country has a national antimicrobial susceptibility committee (NAC) as recommended by EUCAST [14]. At that time there were 35 countries with national representatives on the EUCAST General Committee, Australia, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Latvia, Lithuania, Luxemburg, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom. In most countries there is no official requirement for laboratories to follow any particular breakpoint guidelines and a variety of methods is used. Also most countries have no mechanism for collecting precise information on guidelines and methods used, and this may be continually changing as individual laboratories make decisions to change susceptibility testing guidelines followed or methods used. Therefore, the General Committee representatives were asked to provide estimates of the proportions of laboratories falling into broad categories for use of EUCAST guidelines and the EUCAST disk diffusion method. The categories provided were below 10%, 10 to 50% and above 50% of laboratories.

Results

Data from EARS-Net (Table) show a decline in use of the Clinical and Laboratory Standards Institute (CLSI, United States) breakpoints from 67.5% in September 2009 to 38.4% in May 2012, and an increase in use of EUCAST breakpoints from 22.2% in 2009 to 61.2% in 2012. Some national guidelines such as the British Society for Antimicrobial Chemotherapy (BSAC, United Kingdom) [15] and the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM, France) [16] have adopted EUCAST MIC breakpoints and initially calibrated their own disk diffusion method to the EUCAST breakpoints, so they were using EUCAST-related methods and are therefore also counted as using EUCAST breakpoints. Both BSAC and CA-SFM are now in the process of changing to the EUCAST disk diffusion method.

Data from UK NEQAS EQA (Table) show a similar decline in use of CLSI breakpoints as seen in EARS-Net, from 58.5% in November 2009 to 26.8% in March 2013 and an increase in use of EUCAST breakpoints, from 36.1% to 73.2% over the same period. As with EARS-Net data, some national guidelines have adopted EUCAST

Table

Antimicrobial susceptibility testing guidelines used by laboratories participating in the EARS-Net EQA exercises, 2009–2012 and UK NEQAS for antimicrobial susceptibility testing, 2009–2013

Data source	Date, number of laboratories	Percentage of laboratories using indicated guidelines		
		CLSI	EUCAST and EUCAST-based	Other[a]/ combined/ not stated
EARS-Net EQA	September 2009 n = 775	67.5	22.2	10.3
	June 2010 n = 766	65.8	28.7	5.5
	May 2011 n = 817	46.8	47.6	5.6
	May 2012 n = 807	38.4	61.2	0.4
UK NEQAS EQA	November 2009 n = 651	58.8	36.1	5.1
	November 2010 n = 656	51.5	42.2	6.3
	November 2011 n = 643	36.8	58.6	4.6
	April 2012 n = 632	31.8	68.2	0
	March 2013 n = 650	26.8	73.2	0

CLSI: Clinical and Laboratory Standards Institute; EARS: European Antimicrobial Resistance Surveillance Network; EQA: external quality assessment; EUCAST: European Committee on Antimicrobial Susceptibility Testing; UK NEQAS: United Kingdom External Quality Assessment Scheme.

[a] Other guidelines are local methods not complying with EUCAST or CLSI recommendations.

MIC breakpoints and are therefore counted as using EUCAST breakpoints.

Questionnaires were completed by 33 of the 35 General Committee representatives. Countries with a NAC are shown in Figure 1. At the time of the survey, 25 of the responding countries had an established NAC, four were in the process of setting up a NAC and four had no NAC. Use of EUCAST breakpoint guidelines is shown in Figure 2. EUCAST breakpoints were used by more than 50% of laboratories in 18 countries, by 10 to 50% of laboratories in eight countries and by less than 10% in seven countries. Use of EUCAST disk diffusion method is shown in Figure 3. The EUCAST disk diffusion method was used by more than 50% of laboratories in 12 countries, by 10 to 50% of laboratories in ten countries and by less than 10% in eleven countries.

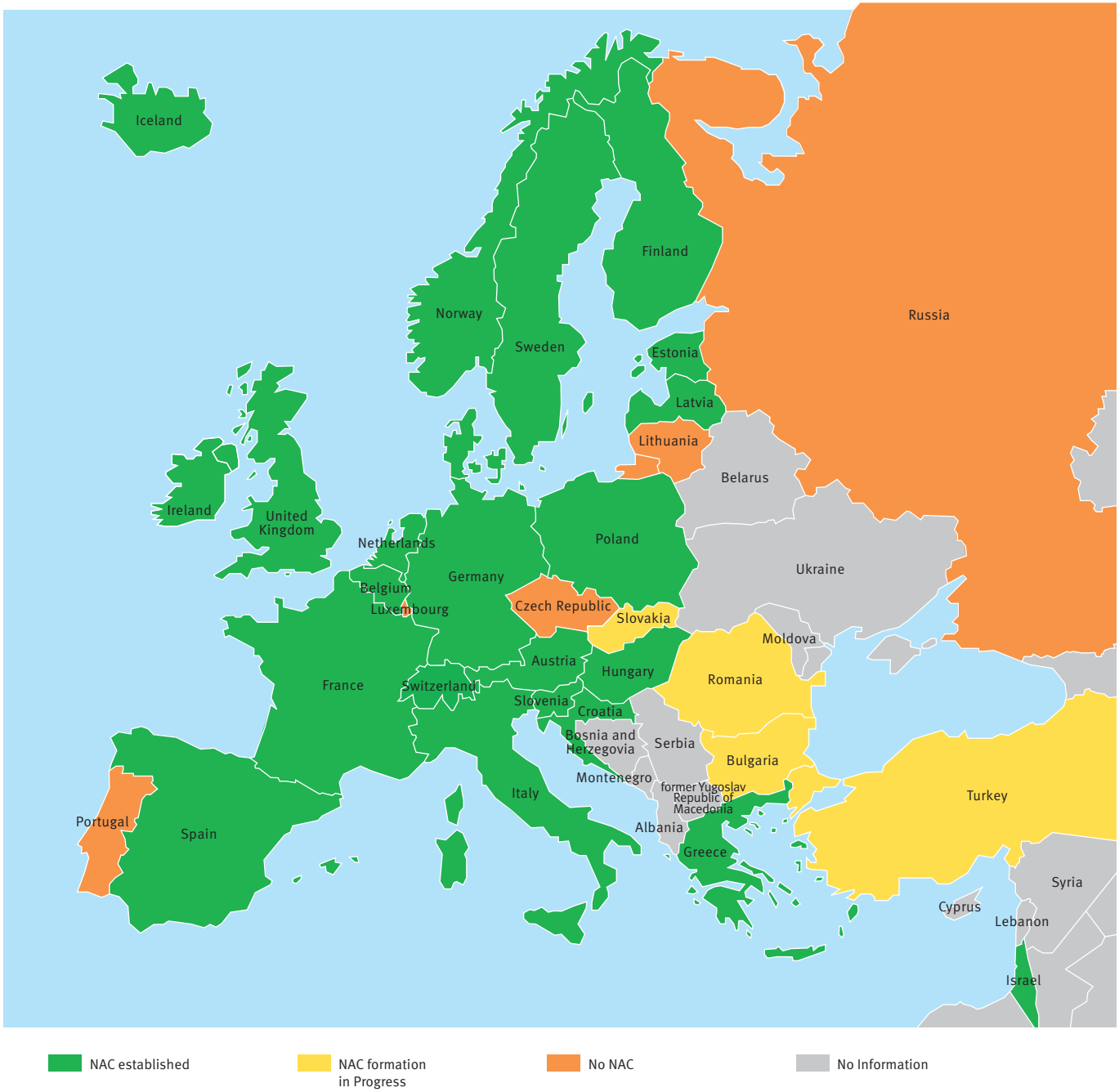
Discussion

Collection of reliable data on use of clinical breakpoint guidelines and methods for antimicrobial susceptibility testing in different countries is difficult because in most countries there is no national requirement to follow particular guidelines or methods, there are no mechanisms in place to collect such data, and the situation may change gradually over time as laboratories decide to change guidelines or methods. However, the findings from three independent data sources presented here consistently show that there has been widespread adoption of EUCAST breakpoints in recent years across clinical laboratories in the majority of European countries. The EQA exercises organised by EARS-Net and UK NEQAS include different but overlapping sets of laboratories covering most European countries. These EQA exercises show similar trends towards adoption of EUCAST breakpoints since 2009, with the UK NEQAS data indicating that over 70% of the laboratories providing data used EUCAST breakpoints in March 2013. The adoption of EUCAST guidelines has been mirrored by a decline in the use of CLSI breakpoints. This process has been fuelled by the adoption of EUCAST breakpoints by EMA in 2005 [3] as part of the official European process for marketing authorisation of antimicrobial agents, the adoption of EUCAST breakpoints by the European Commission Decision on case definition for surveillance of antimicrobial resistance in humans in 2012 [7], as well as the strong support by ESCMID and ECDC for use of EUCAST breakpoints for surveillance. Moreover, in some countries, the position taken by national societies of clinical microbiology and/or infectious diseases has had a positive impact.

The rate of adoption of EUCAST breakpoints has been variable in different countries, as illustrated by the results from the EUCAST survey early in 2013. While in just over half of the countries surveyed the majority of laboratories have adopted EUCAST breakpoints, in others the proportion of laboratories using EUCAST

Figure 1

Countries with National Antimicrobial Susceptibility Testing Committees, EUCAST survey 2013

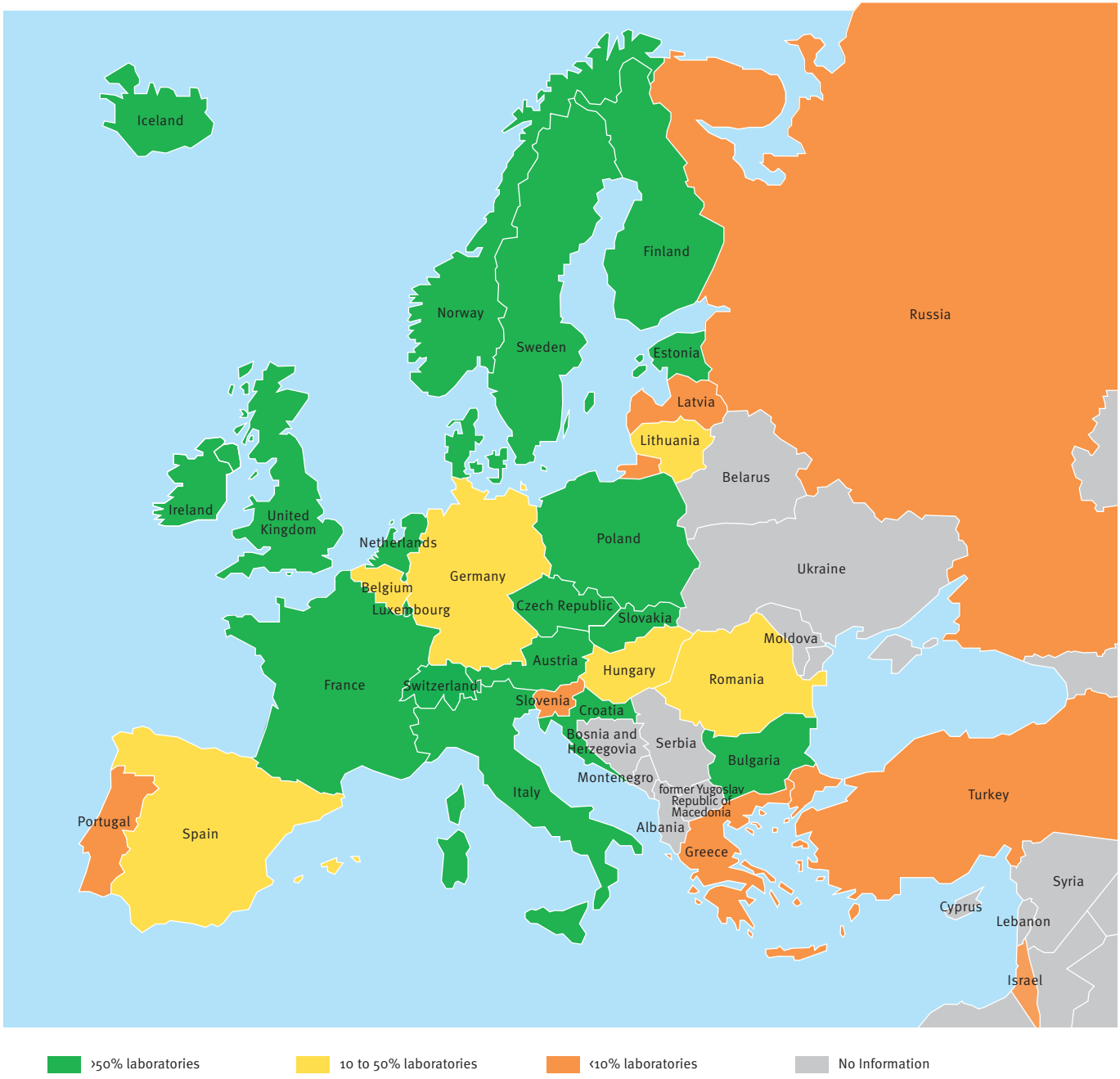


Australia (has NAC) is not on this map.

EUCAST: European Committee on Antimicrobial Susceptibility Testing, NAC: National Antimicrobial Susceptibility Testing Committee.

Figure 2

Use of EUCAST breakpoint guidelines in different countries, EUCAST survey 2013

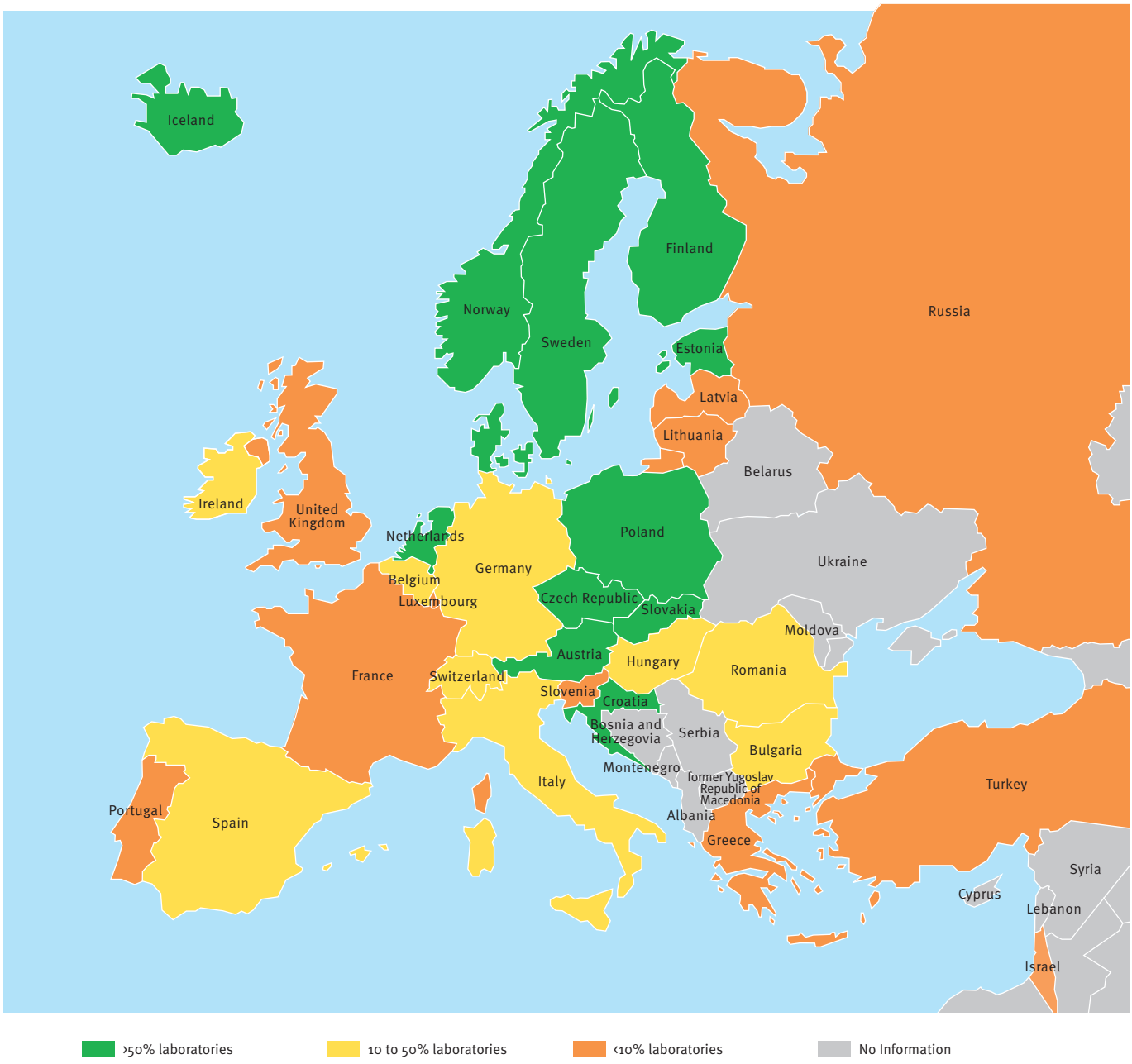


Australia (10 to 50% laboratories) is not on this map.

EUCAST: European Committee on Antimicrobial Susceptibility Testing.

Figure 3

Use of the EUCAST disk diffusion method in different countries, EUCAST survey 2013



Australia (10 to 50% laboratories) is not on this map.
EUCAST: European Committee on Antimicrobial Susceptibility Testing.

breakpoints is still small. It is expected that the uptake of guidelines will be gradual as laboratories make the decision to change breakpoints and incorporate breakpoints into local methods and information systems. The existence of a NAC to provide national guidance on antimicrobial susceptibility testing breakpoints and methods might have a substantial impact on laboratory practices. EUCAST has actively promoted the establishment of NACs in countries where no such group existed. The EUCAST survey shows that most countries now have a NAC or are in the process of setting up a NAC, and it is likely that these committees will positively influence the uptake of EUCAST guidelines. Furthermore, adoption of EUCAST breakpoints by public health microbiology national reference laboratories participating in ECDC-supported external quality assessment programmes will encourage alignment of testing practice across the EU. In addition, free access to EUCAST breakpoint documents via the internet and implementation of EUCAST breakpoints in automatic susceptibility testing devices facilitate the wide adoption of EUCAST guidelines.

Any standardised antimicrobial susceptibility testing method may be calibrated to EUCAST MIC breakpoints and national disk diffusion methods in France and the UK have been calibrated in this way [17,18]. However, there has been widespread demand for a EUCAST disk diffusion method and a EUCAST disk diffusion method was released in 2010 and published in 2014 [19]. The EUCAST 2013 survey has shown that, as with the uptake of EUCAST breakpoints, adoption of EUCAST disk diffusion method has been variable in different countries, but is used in a considerable proportion of laboratories in two thirds of surveyed countries. In many laboratories, the main antimicrobial susceptibility testing method is an automated system and delays in the implementation of EUCAST breakpoints in automated systems have delayed adoption of EUCAST breakpoints in some laboratories. However, the majority of EUCAST breakpoints are now implemented in automated systems [20] and laboratories can choose to use EUCAST breakpoints in their automated systems.

The information on uptake of EUCAST guidelines from EARS-Net and EUCAST relates only to clinical laboratories and the UK NEQAS EQA scheme includes greater than 95% of clinical laboratories. Information on guidelines followed in veterinary and food safety laboratories has not been surveyed by EUCAST but it would be useful to do so in collaboration with veterinary and food safety networks.

It is clear that there has been a rapid change to use of EUCAST breakpoints over the last few years and there are indications that this trend is continuing as EUCAST breakpoints are increasingly referred to in scientific communications. The wide adoption of EUCAST breakpoints will result in increased consistency of reporting of antimicrobial susceptibility testing results in different countries and better comparability of antimicrobial resistance surveillance data among countries. Annual monitoring of progress in implementation of EUCAST breakpoints across clinical and reference laboratories in Europe will be conducted jointly by EUCAST and ECDC as a key public health microbiology performance indicator.

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Conflicts of interest

Derek Brown is Scientific Secretary of EUCAST, chairs the UK NEQAS Specialist Advisory Group on Antimicrobial Susceptibility Testing and advises UK NEQAS on Antimicrobial Susceptibility Testing issues. Rafael Cantón is Chairman of EUCAST and Gunnar Kahlmeter is Clinical Data Coordinator of EUCAST. Derek Brown, Gunnar Kahlmeter, Luc Dubreuil, Sören Gatermann, Christian Giske, Alasdair MacGowan, Luis Martínez-Martínez, Johan Mouton, Robert Skov, Martin Steinbakk and Rafael Cantón are members of the EUCAST Steering Committee. Christine Walton is the organiser of the UK NEQAS for Antimicrobial Susceptibility Testing. Ole Heuer and Liselotte Diaz Högberg administer the ECDC EARS-Net programme and Marc Struelens managed the ECDC service contract with EUCAST.

Authors' contributions

Derek Brown, Rafael Cantón and Gunnar Kahlmeter led the preparation of this manuscript. All members of the EUCAST Steering Committee were involved in the EUCAST survey and reviewed the manuscript. Christine Walton led the UK NEQAS team that organised and, with Derek Brown, analysed the data from the UK NEQAS and EARS-Net EQA distributions and also reviewed this manuscript. Ole Heuer, Liselotte Diaz Högberg and Marc Struelens reviewed and contributed to this manuscript.

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Letter to the editor: Vaccinating healthcare workers: evidence and ethics

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To the editor:

In a recent issue of *Eurosurveillance*, Betsch argued that vaccination of healthcare workers (HCWs) will prevent transmission of pathogens from HCWs to patients and that HCW vaccination should be encouraged by correcting skewed impressions of risk and by an appeal to altruistic or 'pro-social' motivation [1]. Using hepatitis B and influenza vaccines as examples, Betsch noted there appeared to be less resistance to hepatitis B than to influenza vaccination, quoting a study of German medical students showing 87% vaccination coverage against hepatitis B compared with 35% against influenza [2].

While there are a number of accepted reasons that HCWs refuse vaccination [3] part of the explanation for this observation may also be the different perception of these two vaccines.

In the early trials of hepatitis B vaccine targeting at-risk seronegative human immunodeficiency virus (HIV) infected men, hepatitis B vaccine provided 92% protection [4]. This level of protection has been repeatedly confirmed and population-based vaccination programmes in the past 20 years have reduced the burden of hepatitis B in many previously highly endemic countries [5,6].

Inactivated influenza vaccines are less effective than hepatitis B vaccines. Evidence from contemporary meta-analyses of randomised controlled trials suggest point estimates of efficacy of influenza vaccines of 59% [7] and 52% to 65%, with the latter range depending on the degree of match between the circulating and vaccine strains [8]. These trials were performed in healthy adults, who would be generally representative of HCWs. A meta-analysis of observational case test-negative studies in older people – who are targeted for influenza vaccination in most countries with publicly funded programmes – suggested that inactivated influenza vaccines were of the order of 50% effective [9].

Annual vaccination is needed to provide immunity against influenza, but because the influenza vaccine is only partially effective, immunity will only be partial. Only three or four primary doses of hepatitis B vaccine are needed to confer probable lifelong immunity in most recipients [10].

Vaccinating HCW against hepatitis B will provide very good protection for both the HCW and subsequently the HCW's patients, with systems usually in place to identify the small proportion of people who fail to respond to vaccine. Unfortunately, we cannot reach the same conclusion about vaccinating HCW against influenza when the aim of vaccination is to protect patients from hospital-acquired influenza. Firstly, given that most HCWs can be considered as healthy working adults, a group for which the influenza vaccine has been shown to be only moderately effective, influenza vaccination does not guarantee immunity against influenza for the HCW [7,8]. Secondly, HCWs are not the only source of influenza for hospitalised patients. In a review of 28 published studies of influenza outbreaks in hospitals, HCWs were assessed as the outbreak source in 10 (35%) outbreaks, patients in six (22%) outbreaks and friends and visitors in six (22%) outbreaks. No source was identified for the remaining six (22%) outbreaks [11]. Lastly, there are no good quality studies to suggest vaccinating HCWs against influenza protects patients in hospitals from laboratory-confirmed influenza. Existing evidence on protection of patients is derived from cluster randomised trials or observational studies in nursing homes and is based on non-specific outcomes, such as prevention of all-cause mortality [12]. Non-specific outcomes have been shown to produce biased estimates of direct influenza vaccine effectiveness in this patient group [13]. Such biases may well be amplified when considering indirect protection of older patients through incomplete HCW vaccination.

Despite these shortcomings, however, one can mount an ethical argument for vaccinating HCWs who care for patients, Firstly, with only occasional exceptions, inactivated influenza vaccines are safe. Secondly, influenza vaccines may protect HCWs, their families and patients from influenza. Thirdly, HCWs have a duty of care to protect their patients. The ethical argument is

stronger when made in the context of a hospital respiratory infection prevention programme, which may also include respiratory precautions and appropriate sickness absence behaviour.

However, it may be more difficult to make an ethical argument for HCWs who do not have direct patient contact when vaccination would only be done to protect the HCW. In this case, encouraging or mandating vaccination may compromise the ethical principles of autonomy and bodily integrity. In this context, vaccination of HCWs is restricted to those with patient contact in the United Kingdom (UK) [14]. However, differential treatment of HCWs (with and without direct patient contact) can also introduce an ethical dilemma. Aristotle's principle of justice maintains that equals should be treated equally.

The evidence for vaccinating populations against hepatitis B is strong and there are vaccination programmes in many endemic and non-endemic countries. On the other hand, while there is good evidence that influenza vaccines provide modest protection to recipients in most years, there is no good evidence that vaccinating HCWs in hospitals will protect their patients from influenza. It has been frequently argued that the ethical reasons for vaccinating HCWs against influenza to protect their patients outweigh the lack of evidence of benefit. Yet the ethical argument is not straightforward, with different arguments able to be advanced for HCWs with and without direct patient contact. Not all jurisdictions adopt the same approach to HCW influenza vaccination as the UK. It is hardly surprising then that the debate continues. What remains surprising is some of the ethical and evidential arguments used in the debate.

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Conflict of interest

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Authors' contributions

HK is the sole author and takes responsibility for all views expressed.

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Author's reply: Vaccinating healthcare workers: ethics and strategic behaviour

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To the editor:

In the debate about vaccinating healthcare workers (HCWs) against influenza, the ethical argument stresses HCWs' social responsibility to be vaccinated against influenza to protect their patients. In his letter to the editor, Heath Kelly [1] highlights that this argument may be over-used, given that Cochrane reviews show that there is insufficient evidence that influenza vaccination in HCWs protects patients in hospitals from laboratory-confirmed influenza [2]. Besides this, it is argued that the ethical argument only holds for HCWs who are in contact with patients.

Analysing the vaccination decision that HCWs face as a social dilemma situation, as suggested in my original article [3], does not necessarily deliver the justification to put ethical pressure on HCWs. In short, due to indirect effects, vaccinations create positive externalities for other members of a society, because they reduce transmission. As vaccination itself can be costly in terms of time, effort, and potential side-effects, a rational strategy at the individual level may be to 'free-ride', i.e. omit vaccination and thus avoid the costs associated with vaccination while enjoying the benefits of herd immunity. This choice may, however, compromise the collective benefit, because herd immunity cannot be reached when too much free-riding takes place [3,4]. Thus, this analysis suggests that if there is an indirect effect of vaccination, this aspect will influence the individual decision. There is an increasing amount of evidence, that this is actually the case, i.e. that individuals are more inclined to get vaccinated if this benefits others – providing that their own costs are low, e.g. [3,5].

One crucial point in the analysis is the size of the indirect effect. The externalities of the individual decision vary according to the effectiveness of the vaccine. For influenza, theoretical vaccination coverage of 80% is required to establish herd immunity in the general population [6]. This may not be sufficient in hospital settings, where coverage may have to be higher instead. In fact, there is research suggesting that if 100% of HCW in nursing homes are vaccinated against influenza, the infections are reduced by 60% [7]. Thus, it is possible that for hospital situations vaccine coverage of HCWs higher than 80% or even up to 100% would be needed to attain positive effects.

One can consider two possible scenarios: If 100% coverage is necessary for herd immunity, free-riding is theoretically not possible. Nobody can opt-out without putting patients at risk. In such situations, ethical pressure seems necessary to ensure that full coverage is reached so as to provide the maximum possible protection of patients. To interrupt transmission chains, even HCW without direct contact to patients need to be immunised, assuming that such HCWs have contact with HCWs who directly work with patients. If a lower coverage of e.g. 80% is sufficient, however, 20% can free-ride without imposing a threat to herd immunity. In this situation social motives are likely to play a role: those who are either pro-socially oriented or whose social motives are activated should be more likely to get vaccinated [8]. Appeals to pro-sociality may also be effective here to reach an 80% uptake [9]. In order to examine if these different situations will impact behaviour and if pressure vs appeals will be suitable to reach the thresholds, controlled behavioural experiments should examine if awareness of the herd immunity threshold has an impact on HCWs' influenza vaccine uptake.

Differential treatment of HCWs with and without direct patient contact may pose additional problems. In economics, it is a well-known finding that free-riders in public goods dilemmas can nearly completely destroy cooperation [10] – it seems, that not only diseases are contagious, but that free-riding is contagious, too. Thus, those who do not contribute to the public good seem to undermine the trust in others' cooperation. From this point of view, it seems also advisable that there should be universal recommendations for HCWs rather than only for those who are in contact with patients – if evidence suggests that vaccinating HCWs is beneficial [11].

Conflict of interest

None declared.

Authors' contributions

CB wrote the letter.

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Call for papers for a special issue on impact of anthropogenic changes to water on human pathogens

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Eurosurveillance invites authors to submit papers for a special issue on the impact of anthropogenic changes to water on human pathogens and the epidemiology of infectious diseases and relevance for public health.

Water can act as a solvent for antimicrobials, antifungals, antivirals, pesticides, and heavy metals. The release of such substances in the water can lead to the development of respective resistance in pathogens or related vectors. The resistance can spread between pathogens (e.g. via plasmid exchange), but resistant pathogens can also be propagated further in the environment via currents, or food webs, allowing humans to be exposed in new ways.

The aim of this special issue is to provide examples relevant for European public health, on how anthropogenic changes to water affect epidemiology of human infectious disease and how these changes cause infections with pathogens exhibiting novel drug resistance and/or virulence patterns.

Topics of interest include, but are not limited to:

- emerging opportunistic fungal and bacterial infection acquired in the healthcare setting through contact with water and aerosols
- infections caused by organisms from ground water, drinking wells and water reservoir with resistance to antimicrobials due to increasing concentrations of such substances in these artificial water systems
- unusual human outbreaks due to ingestion of pathogens present in foods originating from aquatic environments affected by anthropogenic changes, or due to exposure to pathogens from such environments
- issues related to the detection and identification of cases and the proof of anthropogenic change to water as a cause.

The submission deadline is 15 April 2015. If you would like to submit a paper or ask for more information, please see our instructions for authors regarding article formats and contact the editorial team at eurosurveillance@ecdc.europa.eu.